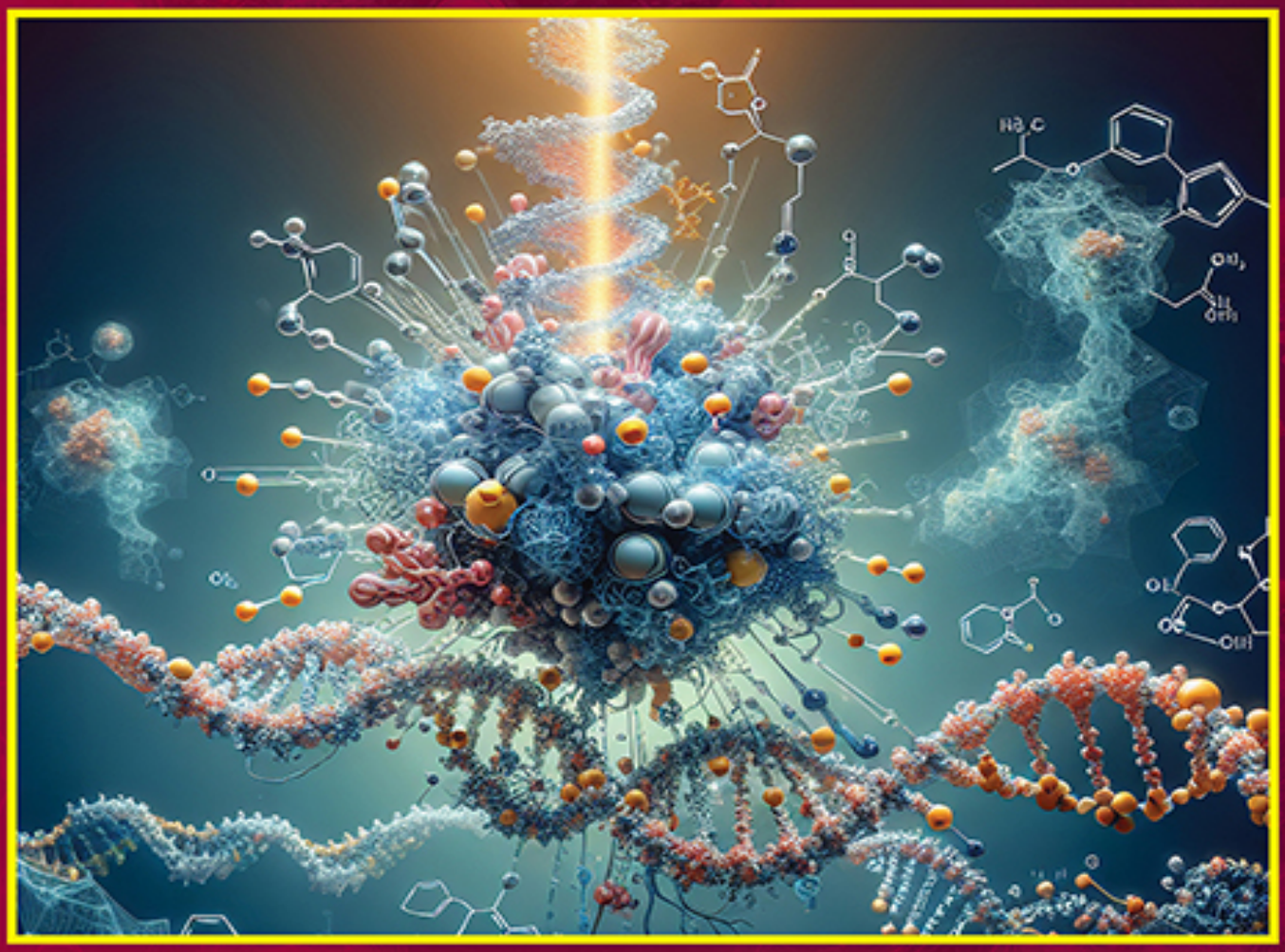
DRUGS AND THE PHARMACEUTICAL SCIENCES

POLYPEPTIDE INNOVATIONS

ADVANCEMENTS IN DELIVERY AND TARGETING FOR PRECISION MEDICINE



Edited by
Ahmed A.H. Abdellatif

CRC Press
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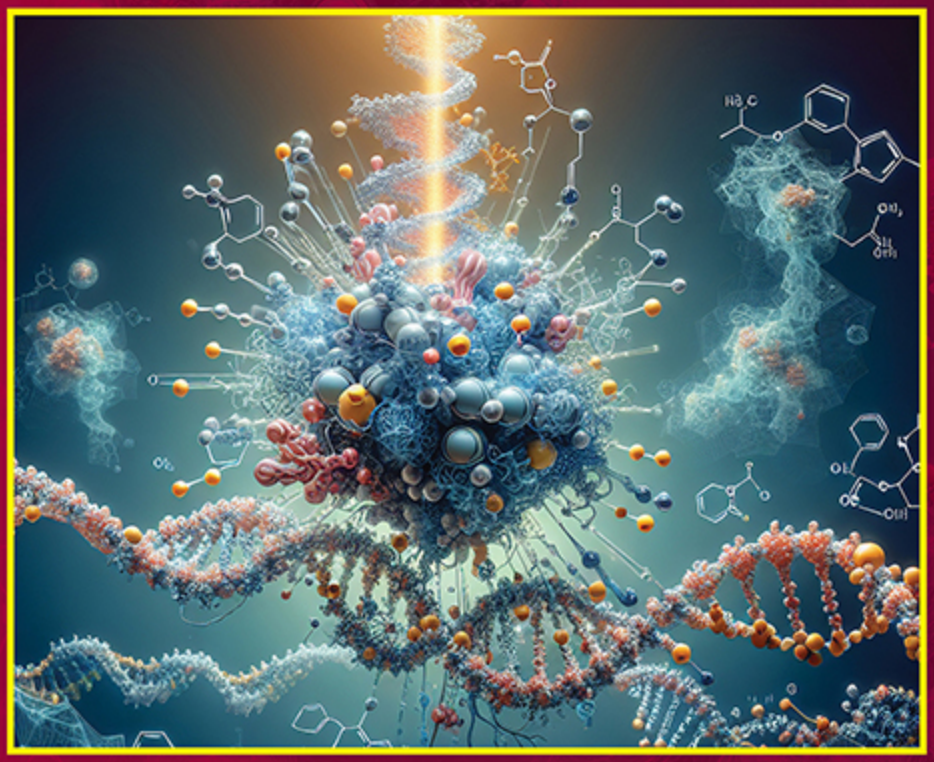

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Polypeptide Innovations

Polypeptides are essential biomolecules for physiological and therapeutic purposes, and this book addresses the complex relationships between polypeptides and biological systems. It examines transport and targeting processes to reveal the latest polypeptide precision medicine advances. A complete overview of polypeptides and their creative medical uses, particularly in distribution, targeting, and therapy is presented, as well as polypeptides' fundamental ideas and nanomedicine innovations which are important in biological processes and therapeutics. An extensive list of references and case studies provides the readers with additional resources to deepen their knowledge on the subject.

- Addresses the complex relationships between polypeptides and biological systems.
- This unique text can be used on pharmacology, medicinal chemistry, and biomedical engineering courses, providing the latest research and case studies.
- Provides a complete guide to polypeptide synthesis and formulations and their applications in disease treatment.
- Essential for business training programs that teach cutting-edge science and companies investing in nanotechnology and biomedical research.

- Covers the latest research and clinical trials, making it a valuable resource for researchers, clinicians, and students.

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Polypeptide Innovations

Advancements in Delivery and Targeting for Precision Medicine

Edited by

Ahmed A.H. Abdellatif

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Preface

The rapid growth of polypeptides in the last two decades has led to a great evolution in the field of the pharmaceutical industry. Different types of polypeptides and peptide-based therapeutic activities play a crucial role in precision medicine through improving the healthcare process. Polypeptides can reach different organs and sites in the body, facilitating the process of specific targeting or active targeting, making it very potent, and decreasing the side effects. The science of nanotechnology, active targeting through specific receptors, molecular targeting, and various types of engineered formulations have significantly expanded their clinical potential, transforming the landscape of drug delivery and disease management.

Furthermore, the polypeptides play a crucial role in the communications of cells, the targeting therapy, physiological regulations, and the immune regulations throughout the body. However, there are many challenges facing the clinical applications of polypeptides, such as poor stability, the rapid effect of enzymatic degradation, the restricted bioavailability, and the delivery barriers. Many techniques can help improve these categories, such as nanoformulations, smart biomaterials, and advanced targeting formulations to target sites. These smart techniques can help prolong the circulation time, controlled release, and improve their stability.

This book was prepared to provide a brief exploration of all of these challenges and how to overcome. It provides a complete set of principles of polypeptide delivery, design, structures, stability problems, clinical

translations, active and passive targeting, and regulatory concerns. All chapters focus on the theoretical and practical understanding of polypeptide therapy and clinical applications.

Special emphasis is placed on:

- Nano-formulations of polypeptides as smart delivery systems
- Intracellular and extracellular transport strategies
- Therapeutic glycoprotein manufacturing
- Stability problem and degradation mechanisms
- Precision medicine and clinical applications
- Regulatory concern and translational challenges

This book is considered a multidisciplinary perspective for pharmaceutics, pharmaceutical technology, pharmacology, nanotechnology, and biomedical engineering. All contributors in this book are experts in the field of polypeptide drug delivery. There is a great deal of support from Qassim University, KSA, in writing this book, through the supply of references and collaborations, which reflects the global and collaborative nature of modern pharmaceutical innovation.

This book is considered a source for all researchers and scientists with a pharmaceutical, medical, and biotechnological background, undergraduate and postgraduate students, clinicians, and industrial professionals. We hope this book serves as a reference text for new, high-quality, and promising research in the development of safe and effective peptide-based delivery and therapy. Also, there is a hope that polypeptide innovations represent a pivotal step toward a more targeted, efficient, and patient-centered therapeutic future.

Ahmed A.H. Abdellatif

About the Editor

Ahmed A.H. Abdellatif is a distinguished academic and researcher currently serving as a Professor of Pharmaceutics at the College of Pharmacy, Qassim University, Saudi Arabia. He concurrently holds the position of Professor of Pharmaceutics and Pharmaceutical Technology at Al-Azhar University in Assiut, Egypt. With an h-index reflecting extensive scholarly impact, he is recognized among the world's top 2% of scientists according to the Stanford University citation analysis. He earned his PhD in Pharmaceutical Technology (Dr. rer. nat.) from Regensburg University, Germany, in 2013. Beyond his teaching and research roles, he serves as an Associate Editor-in-Chief for *Drug Development and Industrial Pharmacy* (Taylor & Francis).

His research focuses on the cutting edge of nanomedicine and targeted drug delivery systems. He has secured significant funding for numerous high-impact projects, totaling millions in research grants. His key areas of expertise include:

- **Targeted Nanotherapy:** Development of gold, silver, and polymeric nanoparticles for active targeting of cancer, including colon and lung cancers.
- **Green Nanotechnology:** Synthesis of nanoparticles using natural extracts (e.g., Trigonella foenum-graecum, Thymus vulgaris) for wound healing and antimicrobial applications.
- **Smart Delivery Systems:** Innovation in thermosensitive chitosan hydrogels and injectable polymers for controlled parenteral delivery of anticancer agents like Cetuximab and 5-Fluorouracil.
- **Biotechnology & RNA Therapeutics:** Research into non-coding RNA-directed therapeutics and MCT4 knockdown for aggressive breast cancer treatment.

Dr. Abdellatif is a prolific author with a vast portfolio of more than 130 publications in prestigious journals such as *Science, Nanoscale Advances,* and *AAPS PharmSciTech*. He served as an Academic Editor for the *Journal of Biomedical Research International* and is a frequent organizer and coordinator for international pharmaceutical conferences. He has also been instrumental in academic accreditation and strategic research planning at Qassim University.

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1 Introduction to Polypeptides

The Definitions, Structures, and Ways of Synthesis

Ahmed A.H. Abdellatif, Ehab Saad Elkhayat, and Mahmoud A.H. Mostafa

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1.1 INTRODUCTION

Polypeptide (PPT) drug delivery has gained extensive attention in the field of the pharma industry due to the existence of PPT-based therapies in the global market (Rossino et al., 2023). PPT is a chain of amino acids connected by peptide bonds and can be used in the treatment of different diseases such as cancer, hypertension, diabetes, and other body diseases (Rossino et al., 2023; Yang et al., 2023). Amino acids are linked together to produce an amide. The formed peptide bond is formed through the binding of the carboxyl group of one amino acid with the amine group of the other amino acid (Ando et al., 1998). The significance of PPTs in the realm of biology cannot be overstated. From their role as the basic units of protein synthesis to their multifaceted functions in cellular processes, PPTs serve as

the foundation upon which the edifice of life is constructed (Ivanova et al., 2014).

PPT drugs have shown tremendous potential in treating a wide range of diseases, but delivering them to a specific target in the body has been a particular challenge; various strategies have been explored, with the use of liposomes and other drug delivery vehicles being the most promising approach (Li et al., 2023; Rossino et al., 2023).

1.2 POLYPEPTIDE DEFINITION AND STRUCTURE

1.2.1 Definitions

Peptides are sequences of amino acids arranged in a specific manner to be conjugated together through the formation of peptide bonds. PPTs have different ranges of molecular weights, starting from 10,000 Da. They are arranged to form proteins; they are considered a block of protein (Butterworth, 2005; Ge et al., 2023; Gold, 2019). Furthermore, peptides belong to another chemical class of organic polymers and oligomers, nucleic acids, oligosaccharides, and polysaccharides. Moreover, they can bind to ligands such as coenzymes, cofactors, and DNA or RNA (Ardejani & Orner, 2013).

The amino acids included in the peptide are called residues. Water molecules are released as amide bonds form, indicating peptide formation chemical complexes (Figure 1.1A). The peptides have also N-terminus (amine group) and C-terminal (carboxyl group) residues. This structure is necessary for understanding the behavior of peptides and their interactions in existing biological systems. Chains of less than twenty amino acids are called oligopeptides. They include dipeptides, tripeptides, and tetrapeptides,

each with unique properties and potential biological activities (Varlas et al., 2021) (Figure 1.1B).

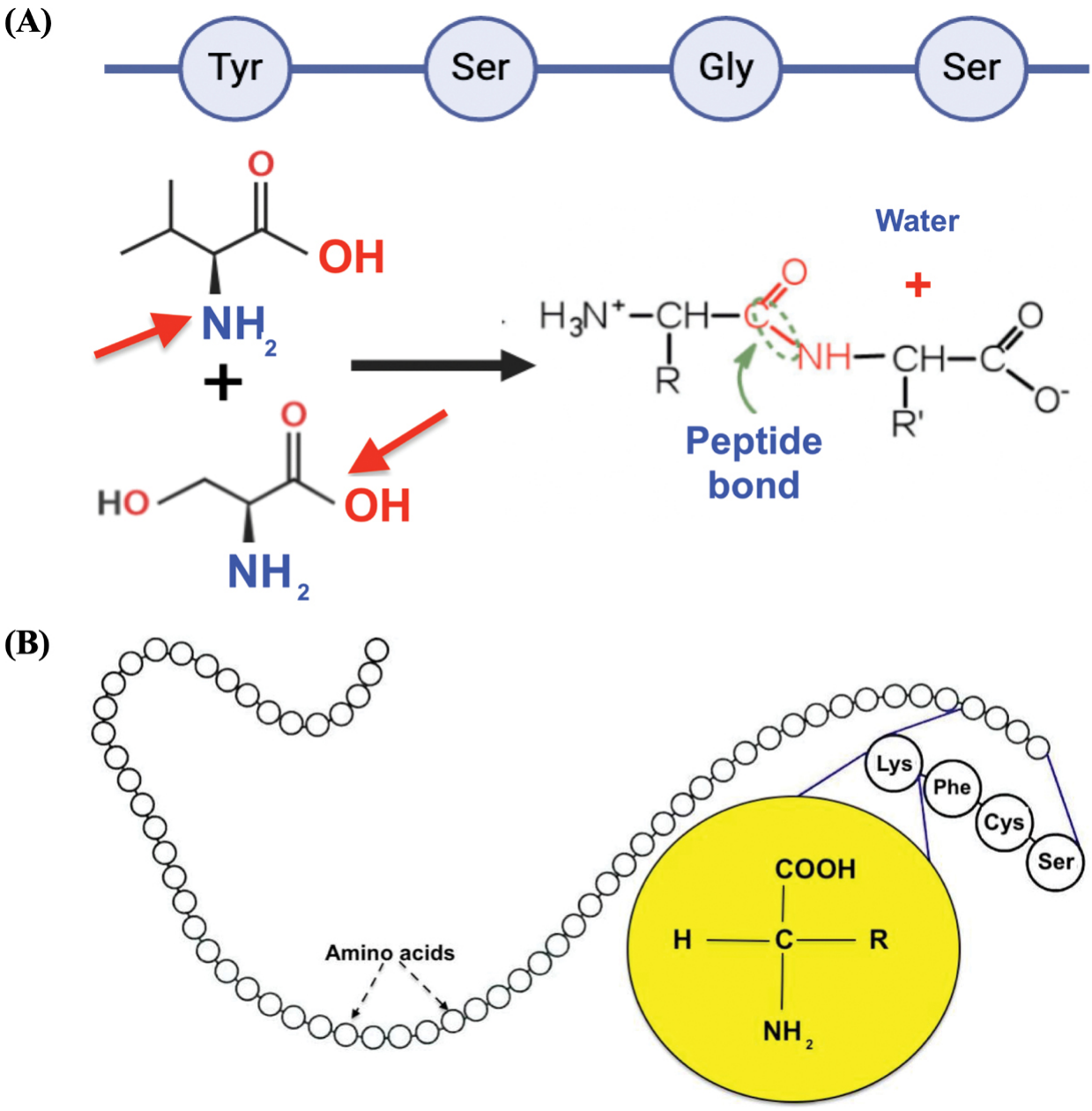

FIGURE 1.1 Illustration of PPTs and proteins linked by peptide bonds. (a) Peptide synthesis begins with activating the carboxyl group of one amino acid and the amino group of another. (b) Amino acids are joined together, and a chain of amino acids called peptides is formed. When a peptide chain reaches a certain length, usually only about 50 amino acids, it is called a PPT. ⏎

1.2.2 Structure of polypeptides

Each amino acid is composed of a central carbon atom linked to a hydrogen atom, an amino group, and a carboxyl group which gives them a variable side chain known as the R-group. Over 20 different naturally occurring amino acids, the PPT shows a highly combinatorial sequence of combinations and permutations, giving them the authority to display the array of functional proteins (Figure 1.1B) (Andrés et al., 2024; Oladiji et al., 2024). The amino acids are joined together by peptide bonds for PPTs or proteins, in which the amino (NH_2) can bind to the carboxyl acid (COOH) group of another amino acid, leading to PPT or protein synthesis (Bernkop-Schnurch & Gilge, 2000).

1.2.3 SPPS: Solid-Phase Peptide Synthesis

An approach that is applied for the manufacture of peptides is called SPPS, which is also referred to by its acronym, SPPS. Solid material such as resin is used, which helps in encompassing the amino acids to come together. The peptides can be synthesized and regulated through efficient and scalable technology. You can do this by using this method (Merrifield, 2002; Palomo, 2014). Peptides and proteins are chemically synthesized by sequentially adding amino acid residues to a developing peptide chain covalently linked to a solid support (resin). SPPS, developed by Robert Bruce Merrifield in the 1960s, revolutionized peptide chemistry and earned Merrifield the 1984 Nobel Prize in Chemistry (Hansen & Oddo, 2015).

1.2.3.1 Steps of Producing a Peptide Using SPPS

The first amino acid was attached to the resin. The C-terminal amino acid is covalently attached to the resin. Typically, a linker molecule cleaves the peptide from the resin. Common resins include polystyrene (Wang,

Merrifield) and polyethylene glycol (PEG) (Figure 1.2) (Tickler & Wade, 2007). Deprotection of amino group was proceeded through removal of the Nα-protecting group. Resin-bound amino acids have a temporary protecting group, such as 9-fluorenylmethyloxycarbonyl (Fmoc) or t-butyloxycarbonyl (Boc), to protect the α-amino group (Nα). In each cycle, this protective group is eliminated to release the amino group for coupling. Fmoc deprotection usually uses piperidine (Hansen & Oddo, 2015).

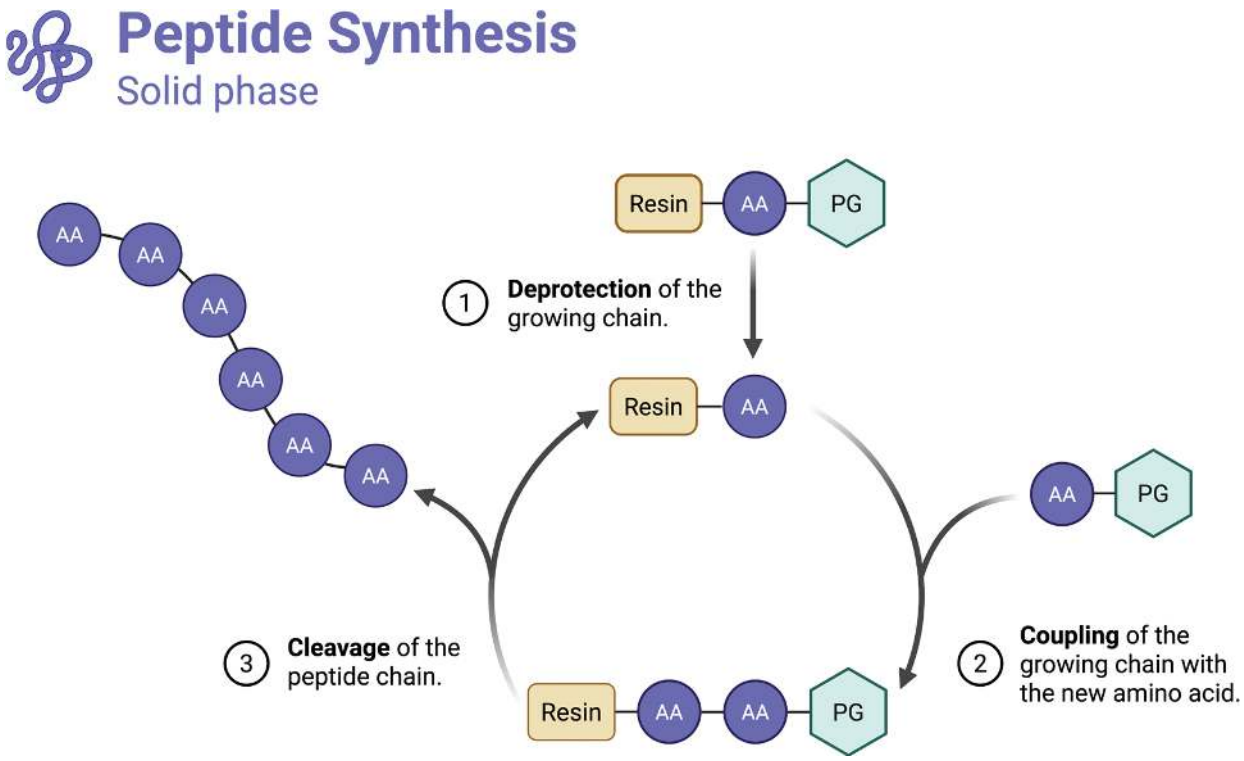

FIGURE 1.2 Solid-phase peptide synthesis is shown in the diagram. The main steps are deprotecting the growing chain: Removing resin-attached amino acid protective groups. Coupling: Adding an amino acid to prolong a peptide chain. (Cleavage: Created in BioRender. https://BioRender.com/zxh8v7x.) ⏎

After shielding the α-amino group and side chain with suitable groups, the next amino acid in the sequence is activated and connected to the free

amino group of the resin-bound amino acid. Couplers like DIC or HBTU activate the compound. Two amino acids form a peptide bond during coupling. It is common to cap unreacted amino groups after coupling. It inhibits deletion sequences (peptides missing one or more amino acids). Capping often uses acetic anhydride or other acylating chemicals (Cherkupally et al., 2014). Repeat the deprotection and coupling. Repeat steps 2–4 for each amino acid, adding them one by one to the peptide chain. After assembling the peptide sequence, it is split from the resin, and side-chain protective groups are removed. Most cleavage is done with strong acids like trifluoroacetic acid. Methods of purification include reversed-phase high-performance liquid chromatography (RP-HPLC) to eliminate contaminants from the crude peptide (Behrendt et al., 2016).

1.2.4 Liquid-Phase Peptide Synthesis (LPPS)

Liquid-phase peptide synthesis (LPPS) is cheaper than other techniques for synthesizing peptides with fewer amino acids. Higher-quality peptides can be obtained using LPPS in certain circumstances, particularly when using purification techniques. LPPS offers additional response conditions and protective group selection flexibility. LPPS has drawbacks such as being laborious and time-consuming due to the need for purification after each reaction step. LPPS is harder to automate than SPPS. LPPS may be inefficient and difficult to synthesize lengthy peptides (Sharma et al., 2022).

However, SPPS is preferred for most peptide synthesis as it is speedy, efficient, and automated. While LPPS is preferred for the shorter peptides, as it is precise in reaction conditions or purification techniques (Jones et al., 2008; Merrifield, 2002).

1.2.5 Amino Acids: the Simple Units of Protein Building

Amino acids are single units of protein building blocks; they are vital to all biological activities. Amino acids vary in their qualities and features that affect their biological functions. The simplest amino acid, glycine (Gly), has a non-polar side chain and weighs 75.1 g/mol. This simplicity gives it distinctive features like a pKa1 of 2.34 and an isoelectric point (pl) of 6. Gly's characteristics make it essential for protein structure and function (Jiang et al., 2025; Jimenez, 2020). The molecular weight (Mol. Wt), formulae, dissociation constants, and isoelectric points of 20 amino acids are examined in Table 1.1 to understand their significance and applications.

TABLE 1.1

The Properties and Characteristics of Amino Acids, Including Their Mol Wt, Mol Formulas, the Dissociation Constant, and pH at the Isoelectric Point (Lide, 1991)

Name	Abbreviations	M. Formula	M. Wt	pKa[a]	pKb[b]	pKx[c]	pI[d]
Glycine	Gly/G	$C_2H_5NO_2$	75.1	2.34	9.6	–	≅ 6
Alanine	Ala/A	$C_3H_7NO_2$	89.1	2.34	9.7	–	6.00
Serine	Ser/S	$C_3H_7NO_3$	105.1	2.21	9.2	–	5.7
Proline	Pro/P	$C_5H_9NO_2$	115.1	≅ 2	10.6	–	6.3
Valine	Val/V	$C_5H_{11}NO_2$	117.2	2.3	9.6	–	5.9
Threonine	Thr/T	$C_4H_9NO_3$	119.12	2.1	9.1	–	5.6
Cysteine	Cys/C	$C_3H_7NO_2S$	121.2	≅ 2	10.3	8.2	5.1
Hydroxyproline	Hyp/O	$C_5H_9NO_3$	131.1	1.82	9.7	–	–
Isoleucine	Ile/I	$C_6H_{13}NO_2$	131.2	2.36	9.6	–	≅ 6
Leucine	Leu/L	$C_6H_{13}NO_2$	131.2	2.36	9.6	–	≅ 6
Asparagine	Asn/N	$C_4H_8N_2O_3$	132.1	≅ 2	8.8	–	5.4
Aspartic acid	Asp/D	$C_4H_7NO_4$	133.1	1.88	9.6	3.7	2.8
Pyroglutamic	Glp/U	$C_5H_7NO_3$	139.1	–	–	–	5.7

Name	Abbreviations	M. Formula	M. Wt	pKa[a]	pKb[b]	pKx[c]	pI[d]
Glutamine	Gln/Q	$C_5H_{10}N_2O_3$	146.2	2.2	9.1	–	5.6
Lysine	Lys/K	$C_6H_{14}N_2O_2$	146.2	2.2	8.9	10.5	9.7
Glutamic acid	Glu/E	$C_5H_9NO_4$	147.1	2.2	9.7	4.3	3.2
Methionine	Met/M	$C_5H_{11}NO_2S$	149.2	2.3	9.2	–	5.7
Histidine	His/H	$C_6H_9N_3O_2$	155.2	1.8	9.2	6.00	7.6
Phenylalanine	Phe/F	$C_9H_{11}NO_2$	165.2	1.8	9.1	–	5.5
Arginine	Arg/R	$C_6H_{14}N_4O_2$	174.2	2.2	9.1	12.5	10.8
Tyrosine	Tyr/Y	$C_9H_{11}NO_3$	181.2	2.2	9.1	10.1	5.7
Tryptophan	Trp/W	$C_{11}H_{12}N_2O_2$	204.2	2.8	9.4	–	5.9

[a]pKa is the (- log) of the -COOH dissociation constant. ⏎

[b]pKb is the (- log) of the -NH_3 dissociation constant. ⏎

[c]pKx is the (- log) of the dissociation constant for any other group in the molecule. ⏎

[d]pI is the recorded pH at the isoelectric point. ⏎

Tryptophan is a larger amino acid of M. Wt of 204.22 g/mol and pKa values of 2.8 and 9.4, which is essential for human mood. The dissociation constants pKa and pKb are also important for amino acid ionization in different pH media. At the physiological pH, lysine (Lys) is reported to be a positive charge carrier with pK_{a1} of 2.2–2.8 and pK_{b2} of 8.8–9. These charges have effects on the protein interactions and cellular communication, emphasizing their acidic and basic individualities' functional relevance (Boca et al., 2024; Hoglund et al., 2019; Jenkins et al., 2016). Figure 1.3 shows some examples of different types of amino acids.

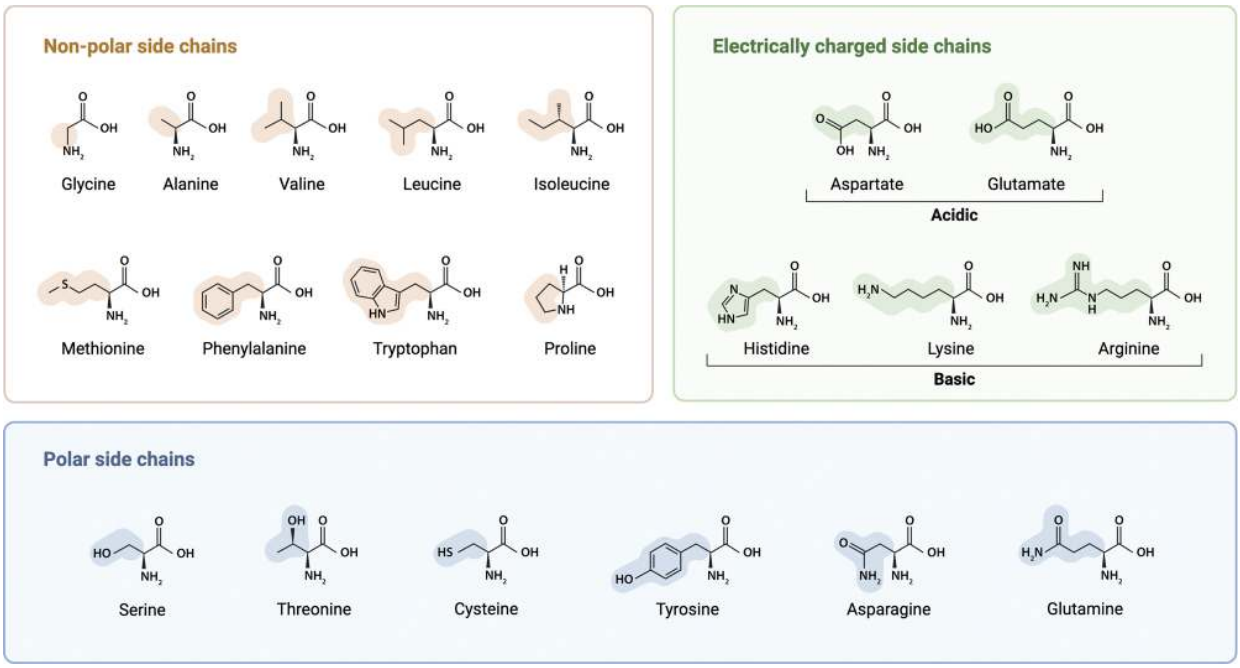

FIGURE 1.3 Detailed amino acid mapping shows how peptide charge, hydrophobicity, and polarity affect neuropeptide function. Pituitary peptides have abundances of basic residues for receptor binding affinity and acidic and polar residues for structural stability and specificity. These peptides' conserved characteristics display their evolutionary adaptation for precise neuroendocrine signaling. (Created in BioRender. https://BioRender.com/rjxjm0y.) ⏎

Glutamine (Gln) is considered a nitrogen donor for protein with a molecular weight of 146.2 g/mol. Its pK_{a1} is 2.2, while the isoelectric point is 5.6 that make balance in acidic and basic properties, permitting it to have a good function in many biochemical environments. Cysteine (Cys) is a sulfur-containing amino acid. It forms disulfide bonds to stabilize proteins with an identified shape. The mol. Wt of cysteine is 121.2 g/mol; it has a pK_{a1} of 2, and it has an isoelectric point of 5.1. Thiol (-SH) groups are part in proteins building block which provide different redox conditions. Thiol is necessary for joining the different amino acid building units that may be used in metabolism. The isoelectric points (pl) of amino acids are signs of

how well they dissolve and how they interact with other molecules. At neutral pH, the negative charge amino acids like aspartic acid (Asp) have pl of 2.8, while the positive amino acids such as arginine (Arg) have pl of 10.8 (Cooper, 1983; Jenkins et al., 2016; Stein et al., 2009). Table 1.1 shows the lists of a large variety of amino acids, the abbreviations, pKa^1, pKb^2, pKx^3, the pH at the pl, the mol wt of the amino acids, and their molecular formulae.

1.2.6 The Composition of Amino Acids and Their Associated Charge Characteristics

Thyrotropin-releasing hormone (TRH), gonadotropin-releasing hormone (GnRH), and somatostatin 14 (SST) have a balance of polar uncharged and basic residues. However, there are a few acidic amino acids (Glu) that are associated with the N-terminal end of TRH and GnRH. His, Arg, and Lys are examples of basic residues that have been identified, and their presence suggests that they play a role in receptor binding through electrostatic interactions. The capacity of GnRH to bind GnRH receptors with a high degree of specificity is supported by the fact that it possesses some polar residues (Ser, Tyr, and Gly) in addition to a considerable number of basic residues (His and Arg) (Millar, 2005). TRH, which is made up of three amino acids (His, Pro, and Thr), is an essential component in the process of controlling the release of prolactin and thyroid-stimulating hormone (TSH) (Pirahanchi et al., 2025; Wang et al., 2025) (Figure 1.4). GnRH is made up of 10 amino acids, some of which are hydrophobic and others of which are polar. This composition enables it to interact efficiently with the gonadotropin-releasing hormone receptor (Baca-Alonso & Quintanar, 2024; Hassanein et al., 2024; Millar, 2005), as shown in Figure 1.4. Moreover, SST is composed of 14 amino acids and is responsible for inhibiting the release

of GH. It can perform a variety of physiological functions, such as modulating neurotransmission and inhibiting the secretion of hormones via the gastrointestinal tract (Abdellatif et al., 2016, 2018) (Figure 1.4).

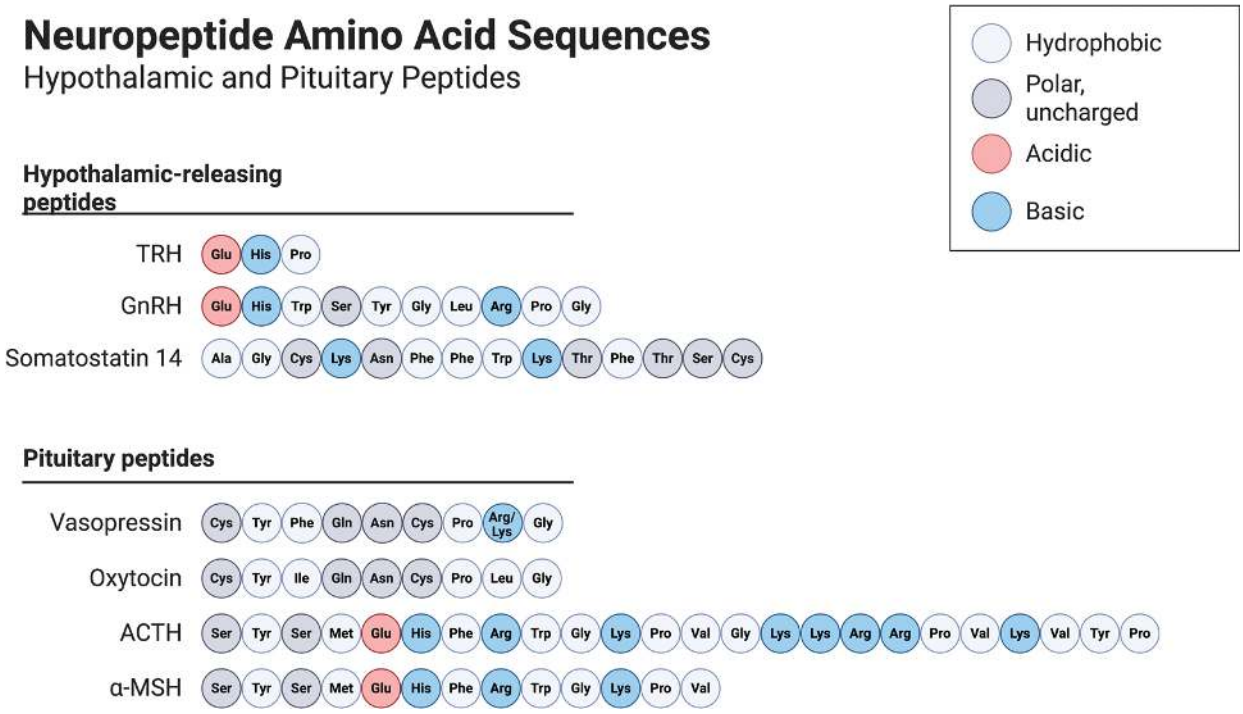

FIGURE 1.4 Different types and shapes of PPT in various types of NPs drug delivery. (Created in BioRender. https://BioRender.com/8qehv20.) ⏎

1.2.7 Calculations of Protein

When considering a protein that contains several amino acids, the mol wt is necessary to take into consideration (Freeman & Hamilton, 2005). To calculate the molecular weight of a protein, follow these steps:

i. Determine the sequence of amino acids in the protein. Further, count the number of each type of amino acid that is existing sequence.

ii. The molecular weight of certain amino acids should be recorded using the average mol wt of the 20 amino acids that are considered standard.

iii. Calculate the total weight of protein by multiplying the number of each amino acid by the mol wt appropriate for that amino acid and then adding the obtained results together:

$$\text{M. Wt.} = \sum (\text{Number of each amino acids} \times \text{Weight amino acid})$$

iv. Subtract water weight; during the process of peptide bond formation, water molecules are released when proteins are produced. For a protein with n (number) amino acids, then subtract (n−1)×18.02 Da(n−1)×18.02 Da (the molecular weight of water) from the total weight.

1.2.7.1 Example Calculation

For a peptide sequence with five amino acids: Ala, Gly, Lys, Ser, and Tyr, the calculation would be:

Count of Each Amino acid	Calculate	Subtract Water Weight
• **Ala: 1** • **Gly: 1** • **Lys: 1** • **Ser: 1** • **Tyr: 1**	• Total Weight = (1 × 89.09) + (1 × 75.07) + (1 × 146.19) + (1 × 105.09) + (1 × 181.19) = 596.63 Da	• Molecular Weight = 596.63 − (5 − 1) × 18.02 = 596.63 − 72.08 = 524.55 Da

1.3 CONCLUSION

PPTs participate in a wide range of biological activities and play a significant part in the delivery of drugs. They also influence other biological processes. PPTs are defined as polymers of amino acids that fold into complicated three-dimensional structures for a range of biological uses. The development of PPT-based drug delivery systems is currently confronted with several modern issues. Some of these challenges include the stability of peptide drugs, bioavailability, and customized dispersion. The solutions to these problems are necessary for the development of treatments. The two fundamental approaches to the production of PPTs are known as SPPS and LPPS. Other topics that are covered in this chapter include amino acids, which are the fundamental building blocks of PPTs. Because of the impact that they have on the structure and function of PPT chains, these fundamental components are necessary for the proper operation of proteins.

REFERENCES

Abdellatif, A. A., Zayed, G., El-Bakry, A., Zaky, A., Saleem, I. Y., & Tawfeek, H. M. (2016). Novel Gold Nanoparticles Coated With Somatostatin as a Potential Delivery System for Targeting Somatostatin Receptors. *Drug Dev Ind Pharm*, *42*(11), 1782–1791. https://doi.org/10.3109/03639045.2016.1173052⏎

Abdellatif, A. A. H., Aldalaen, S. M., Faisal, W., & Tawfeek, H. M. (2018). Somatostatin Receptors as a New Active Targeting Sites for Nanoparticles. *Saudi Pharm J*, *26*(7), 1051–1059. https://doi.org/10.1016/j.jsps.2018.05.014⏎

Ando, I., Kameda, T., & Asakawa, N. (1998). Polypeptides. In *Solid State NMR of Polymers* (pp. 819–851). https://doi.org/10.1016/s0167-6881(98)80030-x⏎

Andrés, C. M. C., Pérez de la Lastra, J. M., Bustamante Munguira, E., Juan, C. A., Plou, F. J., & Pérez Lebeña, E. (2024). Electrophilic Compounds in the Human Diet and Their Role in the Induction of the Transcription Factor NRF2. *Int J Mol Sci*, *25*(6), 3521.⏎

Ardejani, M. S., & Orner, B. P. (2013). Materials Science. Obey the Peptide Assembly Rules. *Science*, *340*(6132), 561–562. https://doi.org/10.1126/science.1237708⏎

Baca-Alonso, J. J. A., & Quintanar, J. L. (2024). The Effect of Gonadotropin-Releasing Hormone on the Nervous System. *Neuro Endocrinol Lett*, *45*(3), 188–196. https://www.ncbi.nlm.nih.gov/pubmed/39146565⏎

Behrendt, R., White, P., & Offer, J. (2016). Advances in Fmoc Solid-Phase Peptide Synthesis. *J Pept Sci*, *22*(1), 4–27. https://doi.org/10.1002/psc.2836⏎

Bernkop-Schnurch, A., & Gilge, B. (2000). Anionic Mucoadhesive Polymers as Auxiliary Agents for the Peroral Administration of (poly)peptide Drugs: Influence of the Gastric Juice. *Drug Dev Ind Pharm*, *26*(2), 107–113. https://doi.org/10.1081/ddc-100100335⏎

Boca, R., Imrich, R., Stofko, J., Vranovicova, B., & Rajnak, C. (2024). Molecular Properties of Linear Amino Acids in Water. *Amino Acids*, *56*(1), 5. https://doi.org/10.1007/s00726-023-03365-3⏎

Butterworth, P. (2005). *Lehninger: Principles of Biochemistry* (4th ed., p. 1119). D. L. Nelson and M. C. Cox. (plus 17 pp glossary), ISBN 0-7167-4339-6 (2004). In: Wiley Online Library.⏎

Cherkupally, P., Acosta, G. A., Ramesh, S., De la Torre, B. G., Govender, T., Kruger, H. G., & Albericio, F. (2014). Solid-Phase Peptide Synthesis (SPPS), C-Terminal vs. Side-Chain Anchoring: A Reality or a Myth. *Amino Acids*, *46*(8), 1827–1838. https://doi.org/10.1007/s00726-014-1746-7⏎

Cooper, A. J. (1983). Biochemistry of Sulfur-containing Amino Acids. *Annu Rev Biochem*, *52*, 187–222. https://doi.org/10.1146/annurev.bi.52.070183.001155⏎

Freeman, S., & Hamilton, H. (2005). *Biological science/Scott Freeman; Contributors, Healy Hamilton ... [et al.]* (2nd ed.). Table of contents http://www.loc.gov/catdir/toc/ecip053/2004027312.html⏎

Ge, K., Olson, D., & Sanner, M. F. (2023). Docking Peptides into HIV/FIV Protease with Deep Learning and Focused Peptide Docking Methods. *arXiv preprint arXiv:2310.09529*.⏎

Gold, V. (2019). *The IUPAC Compendium of Chemical Terminology*. https://doi.org/10.1351/goldbook⏎

Lide, D. R. (Ed.) (1991). *CRC Handbook of Chemistry and Physics* (72nd ed.).⏎

Hansen, P. R., & Oddo, A. (2015). Fmoc Solid-Phase Peptide Synthesis. *Methods Mol Biol*, *1348*, 33–50. https://doi.org/10.1007/978-1-4939-2999-3_5⏎

Hassanein, E. M., Szelenyi, Z., & Szenci, O. (2024). Gonadotropin-Releasing Hormone (GnRH) and Its Agonists in Bovine Reproduction I: Structure, Biosynthesis, Physiological Effects, and Its Role in Estrous Synchronization. *Animals (Basel)*, *14*(10). https://doi.org/10.3390/ani14101473⏎

Hoglund, E., Overli, O., & Winberg, S. (2019). Tryptophan Metabolic Pathways and Brain Serotonergic Activity: A Comparative Review. *Front Endocrinol (Lausanne)*, *10*, 158. https://doi.org/10.3389/fendo.2019.00158⏎

Ivanova, E. P., Bazaka, K., & Crawford, R. J. (2014). Advanced Synthetic Polymer Biomaterials Derived from Organic Sources. In *New Functional Biomaterials for Medicine and Healthcare* (pp. 71–99). https://doi.org/10.1533/9781782422662.71⏎

Jenkins, T. A., Nguyen, J. C., Polglaze, K. E., & Bertrand, P. P. (2016). Influence of Tryptophan and Serotonin on Mood and Cognition With a Possible Role of the Gut-Brain Axis. *Nutrients*, *8*(1). https://doi.org/10.3390/nu8010056⏎

Jiang, C., Tan, X., Jin, J., & Wang, P. (2025). The Molecular Basis of Amino Acids Sensing. *Adv Sci (Weinh)*, e2501889. https://doi.org/10.1002/advs.202501889⏎

Jimenez, E. C. (2020). D-Amino Acids in Peptides from Animals, Including Human: Occurrence, Structure, Bioactivity and Pharmacology. *Curr Protein Pept Sci*, *21*(6), 622–637. https://doi.org/10.2174/1389203721666200426233758⏎

Jones, M. A., Notta, J. K., Cobbold, M., Palendira, M., Hislop, A. D., Wilkie, J., & Snaith, J. S. (2008). Synthesis and Ex Vivo Profiling of Chemically Modified Cytomegalovirus CMVpp65 Epitopes. *J Pept Sci*, *14*(3), 313–320. https://doi.org/10.1002/psc.930⏎

Li, J., Wang, Q., Xia, G., Adilijiang, N., Li, Y., Hou, Z., Fan, Z., & Li, J. (2023). Recent Advances in Targeted Drug Delivery Strategy for Enhancing Oncotherapy. *Pharmaceutics*, *15*(9). https://doi.org/10.3390/pharmaceutics15092233⏎

Merrifield, R. B. (2002). Solid Phase Peptide Synthesis. I. The Synthesis of a Tetrapeptide. *J Am Chem Soc*, *85*(14), 2149–2154. https://doi.org/10.1021/ja00897a025⏎

Millar, R. P. (2005). GnRHs and GnRH Receptors. *Anim Reprod Sci*, *88*(1–2), 5–28.⏎

Oladiji, A. T., Oladele, J. O., & Ajayi, E. I. (Eds.) (2024). *Nutrition and Diet in Health*.⏎

Palomo, J. M. (2014). Solid-Phase Peptide Synthesis: an Overview Focused on the Preparation of Biologically Relevant Peptides. *RSC Adv*, *4*(62), 32658–32672. https://doi.org/10.1039/c4ra02458c⏎

Pirahanchi, Y., Toro, F., & Jialal, I. (2025). Physiology, Thyroid Stimulating Hormone. In *StatPearls*. https://www.ncbi.nlm.nih.gov/pubmed/29763025⏎

Rossino, G., Marchese, E., Galli, G., Verde, F., Finizio, M., Serra, M., Linciano, P., & Collina, S. (2023). Peptides as Therapeutic Agents: Challenges and Opportunities in the Green Transition Era. *Molecules*, *28*(20). https://doi.org/10.3390/molecules28207165⏎

Sharma, A., Kumar, A., de la Torre, B. G., & Albericio, F. (2022). Liquid-Phase Peptide Synthesis (LPPS): A Third Wave for the Preparation of Peptides. *Chem Rev*, *122*(16), 13516–13546. https://doi.org/10.1021/acs.chemrev.2c00132⏎

Stein, J., Boehles, H. J., Blumenstein, I., Goeters, C., & Schulz, R., & Working group for developing the guidelines for parenteral nutrition of The German Association for Nutritional, M. (2009). Amino Acids - Guidelines on Parenteral Nutrition, Chapter 4. *Ger Med Sci*, *7*, Doc24. https://doi.org/10.3205/000083⏎

Tickler, A. K., & Wade, J. D. (2007). Overview of Solid Phase Synthesis of "Difficult Peptide" Sequences. *Curr Protoc Protein Sci*, *50*, Unit 18. https://doi.org/10.1002/0471140864.ps1808s50⏎

Varlas, S., Maitland, G. L., & Derry, M. J. (2021). Protein-, (Poly)peptide-, and Amino Acid-Based Nanostructures Prepared via Polymerization-Induced Self-Assembly. *Polymers (Basel)*, *13*(16). https://doi.org/10.3390/polym13162603⏎

Wang, Z., Wang, H., & Sun, H. (2025). Thyroid-Stimulating Hormone: An Important Target for the Prevention of Nonalcoholic Fatty Liver Disease. *Physiol Res*, *74*(2), 175–187. https://doi.org/10.33549/physiolres.935453⏎

Yang, S., Wang, M., Wang, T., Sun, M., Huang, H., Shi, X., Duan, S., Wu, Y., Zhu, J., & Liu, F. (2023). Self-assembled Short Peptides: Recent Advances and Strategies for Potential

Pharmaceutical Applications. *Mater Today Bio*, *20*, 100644. https://doi.org/10.1016/j.mtbio.2023.100644⏎

2 Polypeptides Delivery

Nanoformulations and Applications

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2.1 INTRODUCTION

Nanoformulations are considered effective delivery systems contributing to the digestion of polypeptide-based therapeutics (PPTs), which makes them more stable, soluble, and specific to targets. Moreover, they protect the PPT molecules against decomposition or dissociation in the unfavorable biological conditions (Agbana et al., 2020; Jackson et al., 2022). The localization ability at the desired location is an exceptionally promising characteristic of the nanoemulsions and other forms of nanocarriers when it comes to nanosystems being the delivery vectors due to the capacity to perform both active and passive targeting (de Miguel et al., 2015; Gao et al., 2020; Qiu et al., 2020). PPTs may also be hidden in nanoparticles (NPs) and are effectively avoided in contact with harmful environmental perturbations. Surface engineering of NPs, including charge modulation, adjustment of hydrophilicity, and display of ligands, may be optimized with their use, and

each of them holds tremendous therapeutic consequences (Agbana et al., 2020).

Multiple nanocarrier classes have been developed for PPT delivery, including liposomes, polymeric NPs, and micelles. Beyond parenteral routes, transdermal and inhalation strategies have been evaluated to broaden administration options for PPTs; however, effects on adherence and clinical outcomes remain context-dependent and should be interpreted with attention to indication and study design (Hebel et al., 2022; Szunerits et al., 2022). NPs loaded with PPT have become an attractive form and a likely preferred current mode of targeted oncological systems and therapy, which became feasible because of their ability to deliver therapeutic agents to tumor cells selectively without promoting off-target effects and reducing systemic toxicity (Feng et al., 2021; Lin et al., 2019; Zhang et al., 2024).

Furthermore, the nanocarrier's shapes, cavity size, and morphology can impact the stability during storage and systemic management. The fibrous and irregular shape can improve their mechanical strength compared to the spherical formulations, making them appropriate for long-term storage, stability, and sustained release. The adaptability of NPs' morphologies opens the gates to generate different drug delivery strategies for oral delivery, topical, parenteral, targeted PPTs delivery, and sustained PPTs release (Abbasi et al., 2023) (Figure 2.1).

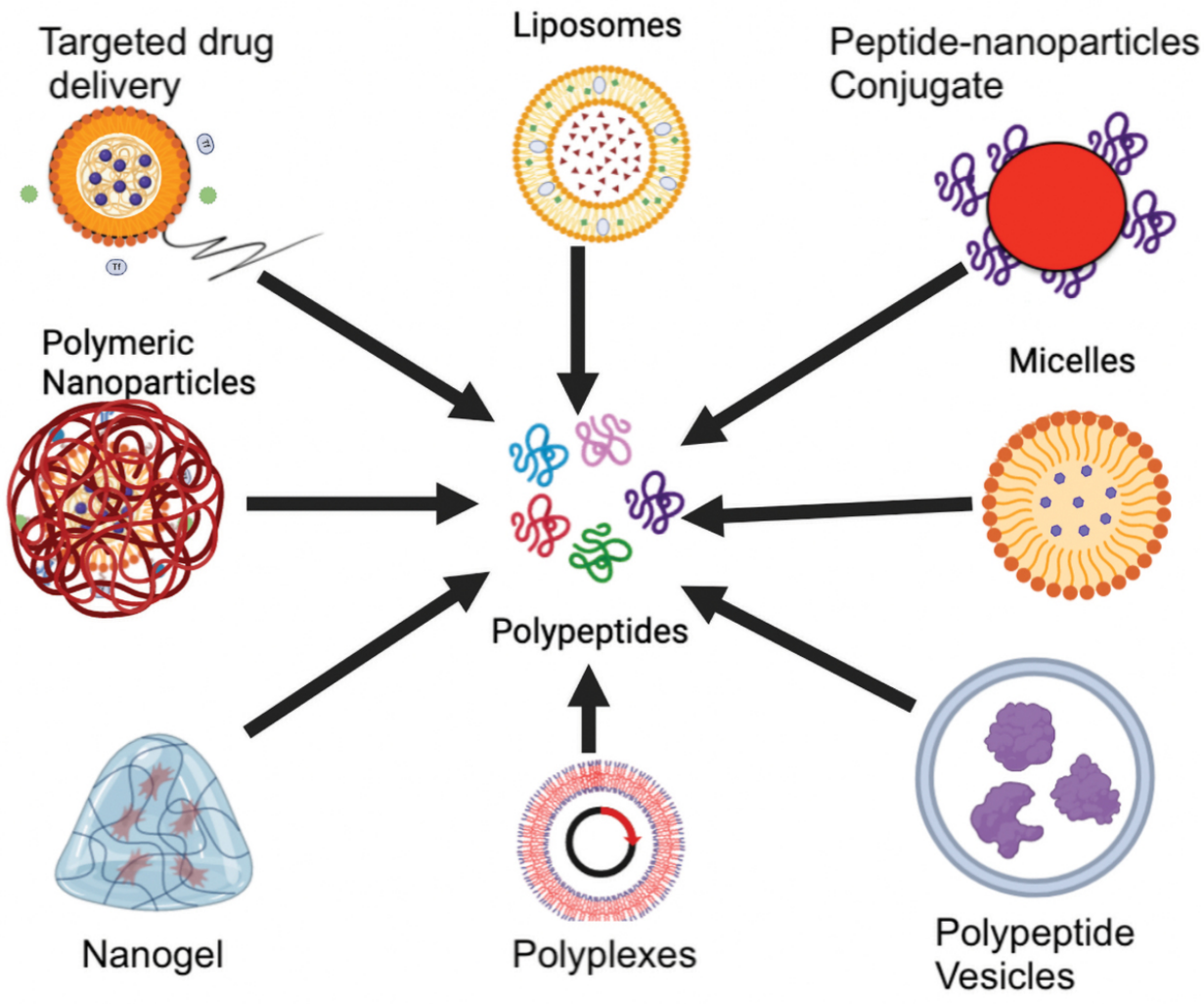

FIGURE 2.1 Illustration of the various types of PPTs that are used in NPs for the delivery of PPTs. ⏎

Nevertheless, in practice, nanocarrier optimization requires deliberate compromise between colloidal stability, targeting performance, and scalable manufacturability, constraints that frequently dictate whether a system progresses beyond preclinical evaluation.

2.1.1 Diversity of Polypeptide-Based NP Morphology

The morphology of PPT-NPs varies because of physical and chemical factors. The nanocarrier morphology depends on the polypeptide chemical

composition, molecular weight, and hydrophobic-hydrophilic balance (Hehir & Cameron, 2014; Judge et al., 2022). Among the described nanoscale morphologies are micelles, polymersomes, nanosuspensions, vesicles, nanocapsules, nanogels, and nanospheres (NS). The secondary structure of PPTs, i.e., the β-sheet/β-layer and α-helical content, determines the NP assembly and supramolecular morphology since it results from intra- and intermolecular interactions involving the putative hydrogen bonds, electrostatic interactions, and hydrophobic contacts between side-functioning groups of PPT amino acids (De Santis & Ryadnov, 2015; Stepanova et al., 2023). Additionally, the parameters for preparing or entrapping the nanocarriers into random-coil PPTs can be varied (Stepanova et al., 2023), such as pH, ionic strength, and temperature.

Lipophilic PPTs can also form NPs through non-covalent modification with two oppositely charged polymers via hydrophobic interactions or hydrogen bonds between homopolypeptides and copolymers (Zhao et al., 2014). There is a need to investigate the dissimilar shapes and properties of PPT-based NPs, which enable us to understand their prospective uses in drug delivery, biomedical imaging, and other domains (Azuar et al., 2021; Stepanova et al., 2023).

The ability of nanocarrier-based PPT to be self-assembled by different types of amino acids into several sizes is considered a fundamental benefit for the nanoformulations (Stepanova et al., 2023). Moreover, the shape of fabricated PPT-based NPs also affects diverse biological activities such as drug excretion, systemic circulation, and uptake in the cell. Reported results imply that rod-shaped NPs have a longer systemic circulation time compared to the round-shaped NPs, which can be explained by the fact that they do not become targets of phagocytic ingestion by the reticuloendothelial system. This property allows selective delivery of pharmacology because it provides

the opportunity to accumulate NPs in tumors or in specific tissue locations (Tesauro et al., 2019).

We must note that despite these advantages, shape-dependent improvements in circulation and uptake are not universal and may vary substantially with surface chemistry, protein corona formation, and biological environment.

2.2 POLYPEPTIDE-LOADED MICELLES

The micelles constructed from hydrophilic block-copolymers are considered to have a suitable core-shell crucial to PPT. Self-assembly occurs because block-PPTs or hybrid block copolymers' hydrophobic blocks do not dissolve in water. The hydrophobic block is the "core," while the hydrophilic block is the "shell" or "corona" of amphiphilic block copolymers (De Santis & Ryadnov, 2015). At the critical micelle concentration (CMC), micelles are generated spontaneously. Hydrophobic units, polymer molecular weight, and fragment molar concentration determine the amphiphilic system CMC. Polymeric micelles have a CMC lower than that of small amphiphilic micelles, making them more stable in water-soluble PPT (Hehir & Cameron, 2014; Stepanova et al., 2023). Micelles' hydrodynamic diameter is 1–200nm, and the polydispersity indices (PDIs) are 0.01–0.3 (Skoulas et al., 2017; Stepanova et al., 2023). This variety of properties allows creating PPT-based micelles that can be customized for drug delivery purposes. These amphiphilic block copolymers, herein, surround hydrophobic pharmaceuticals and thus form a stable and effective delivery system. Polymeric micelles have a number of benefits as a drug delivery system; they are more stable in a liquid state (aqueous) and the CMCs of polymeric micelles are lower compared to traditional amphiphilic surfactants (Skoulas et al., 2017). Hydrophilic PPT-based spherical micelles have gained a new

role in drug delivery, imaging, and other biomedical applications, while their self-assembled character offers flexibility of use (Abdellatif et al., 2016, 2024).

Table 2.1 shows the structure of micelles in copolymers made of amphiphilic polymers of PPT. They present the main parameters which are used to compare the micellar characteristics and uses, including a number-average molecular weight of 19400 Copolymer, CMC190290L of 190290L, hydrodynamic diameter (DH) at 0.0712um, and a low PDI.

TABLE 2.1

Promising Examples of Spherical Micelles Formed from Amphiphilic PPT-Based Copolymers

Block Copolymer	Copolymer M_n	CMC	D_H (nm)	PDI	Ref.
PSer-b-PPhe		4 mg/L	110–240	0.14–0.23	(Zhao et al., 2014)
PEG-b-PLeu			70–80		(Hua et al., 2021)
PEG-S-S-PLeu	6230–6840	2.8 mg/L	160		(Ren et al., 2011)
PLeu-b-PEG-b-PLeu	8000	3–4 mg/L	180–210		(Ding et al., 2015)
PMAG-b-PPhe	19,400		190–290	0.07–0.12	(Levit et al., 2018)
PLys-b-PCL	9520	0.3–0.8 mg/L	30–60		(He-Wei et al., 2015)
PLys-b-PPhe	6100–10,500		490–670	0.07–0.29	(Zhou et al., 2018)

Block Copolymer	Copolymer M_n	CMC	D_H (nm)	PDI	Ref.
Elastin-like block-copolymers		4–8 μM	30–40; 100–200		(Stepanova et al., 2023)
PAsp-b-PLA	28,000–35,500	63–360 mg/L (depending on pH)	30–40		(Arimura et al., 2005)
PMAG-b-P(Glu(OBzl))	7000–29,600		180–260	0.17–0.24	(Levit et al., 2020)
PEG-b-P(Glu-co-Phe)	7760	20 mg/L	120–140		(Lv et al., 2013)
PEG-g-PLys-b-Phe	6500	6 mg/L	80		(Suo et al., 2016)
PEO-b-PTyr	7700	50 mg/L	28		(Van Domeselaar et al., 2003)
PEO-b-PLeu	6900	14 mg/L	24		(Van Domeselaar et al., 2003)
PEO-b-PAsp(OBzl)	16,300	18 mg/L	20–130 (depending on conditions)		(La et al., 1996)
P(Lys-co-Lys-g-PEG)-b-PPhe	1900	2.1 mg/L	260	0.22	(Wong et al., 2023)

Abbreviations: PDI, polydispersity index; D_H, hydrodynamic diameter; CMC, critical micelle concentration.

The examined properties indicate the suitability of the material when used in the pharmaceutical industry; the homogeneity of the size distribution of the micelles and the resulting PDI are important ratios to assess the homogeneity of the PPT containing nanoformulations (Levit et al., 2018). A smaller value of PDI refers to a more homogeneous distribution, which is

beneficial in drug delivery, where it is important to be able to use particles of uniform size to be effective therapeutically (Abdellatif & Tawfeek, 2018). The CMC is used to indicate the concentration at which the copolymer begins to self-assemble into a micelle, and low CMC values mean that the copolymer can be used at lower concentrations, which is desirable in drug-delivery systems (Tawfeek et al., 2017). To determine the stability and biomolecule interactions, the hydrodynamic diameter of the micelles (in the form of a sphere in the solution) needs to be measured. High DH values could have an impact on the circulation time of micelles in the *in vivo* environment and their ability to encapsulate and deliver therapeutic agents. These copolymers are self-assembled and respond to the pH which allows them to be controlled to release drugs at various pH conditions. A wide range of biomedical uses has been studied concerning amphiphilic PPT-based copolymer spherical micelles; their biocompatibility and adjustable characteristics have potential in overcoming some of the toughest issues in the healthcare and biotechnological innovation sector (Arimura et al., 2005, Asayama et al., 2008).

Compared to their spherical analogs, cylindrical micelles are usually formed using block copolymers, which contain a crystallizable hydrophobic block, i.e., poly(e-caprolactone) (PCL), poly-L-lactide, a hydrophobic block, is the core of the micelle. Besides, amphiphilic elastin-like polypeptide tracts (ELPs) have been functionalized and loaded into cylindrical micelles (Stepanova et al., 2023). Zhang et al. (2018) established the creation of water-based cylindrical-shaped micelles of palmitoylated-PPT triblock amphiphiles. The architecture of PPTs absorbed in the cylindrical micelles is also pH sensitive, and this is an interesting observation: upon changing the pH of a system, the PPTs change into two sheets, but the micellar structure stays the same.

PEG-b-PGlu(OBzl) are copolymer-based sphere in the shape of micelles. When a graft copolymer is introduced, i.e., replaced by the block copolymer with a graft one with 20–50 wt% PEG-g-PGlu(OBzl), the morphological transition is observed to be bending from spheres to cylinders.

Such a morphological transition has potential applications in diverse applications with special emphasis made on drug delivery. Cylindrical micelles have a larger load per unit surface area and are a more thermodynamically favorable type (Li et al., 2006).

Outside of modulating micellar morphology, graft copolymers can be used to improve stability and efficacy of drug delivery systems; graft copolymers allow the production of derivatives that maximize the volume of the payload and delay the circulating half-life of therapeutic agents; in turn, that overcomes issues in medicinal delivery (Hakkinen et al., 2022; Stepanova et al., 2023). Turning spherical micelles into cylindrical micelles is a huge discovery in nanoscience and nanotechnology. The potential of creating stimulus-responsive nanosystems based on the ability to prepare stimulus-sensitive nanostructures with highly controlled morphology presents new opportunities to form the next generation of materials with wide industrial use (Sun et al., 2019).

Although advantageous, stability in micelles and retention of the payload are often impaired during physiological dilution, and in dynamic conditions used *in vivo*, highlighting a persistent barrier to translators to clinical reliability.

2.3 NANOGEL-PPT AS DRUG TARGETING

The interest in nanogels has been high due to their large water content, biocompatibility, and drug encapsulation ability (Soni et al., 2016). This can be an efficacious drug carrier in the form of a pH- and temperature-sensitive

gel, like a chitosan-based nanohydrogel encapsulated with PPT molecules (Salehi et al., 2023). PPTs in the nanogel matrix are not vulnerable to degradation and, therefore, enhanced stability and circulatory persistence are obtained. Nanogel carriers may be accurately designed to achieve controlled behavior, which extends the therapeutic efficacy and reduces adverse effects (Eckmann et al., 2014; Stepanova et al., 2023). Additionally, nanogels may be functionalized to accomplish targeting of particular tissues, cellular compartments, or intracellular locations, which will increase the specificity of drug delivery and reduce off-target effects (Keskin et al., 2021).

PPTs loaded nanogel can be used to target the tumor areas, thus sparing healthy tissues. As a result, nanogel-based PPTs can be used in inflammatory conditions to reach specific immune cells or inflamed tissues, and this is an effective therapy with less adverse effect profiles (Qiao et al., 2020; Tian et al., 2022). By encapsulation and protection of these delicate constituents, pharmaceutical preparations become more stable and bioavailable, overcoming the most common difficulties in drug delivery and eliminating the need for dependence on the provision of organic matter (Tian et al., 2022; Yang et al., 2024).

2.3.1 Enhancing Drug Delivery of PPTs

PPT vesicles are amphiphilic NPs with an aqueous core surrounded by polymeric shells. They can be formed as a result of the aggregation of polymers. These membranes assemble on their own in the aqueous media by hydrophobic interactions (Kamps et al., 2010). Practically, polymers designed to create two-dimensional membranes create vesicles of rather smaller sizes. It is necessary to state that the mechanical testing limitations have led to the introduction of the generalized term vesicle that includes polymeric vesicles and other similar structures (Gouveia et al., 2023).

2.3.2 Polypeptide NS in Drug Delivery

NS are polymeric structures (solid) (Begines et al., 2020) that cover proapoptotic agent pantoprazole (PPT) to avoid its degradation and untimely discharge (Thang et al., 2023). NSs have a high loading capacity of PPT and allows the administration of a large dose of this drug with the confined volume that allows reducing the dosage and avoids the negative side effects (Patra et al., 2018).

Functionalizing NS with particular cues is able to execute targeted drug delivery to particular tissues or cellular compartments, which can reduce off-target effects and increase the therapeutic index of the drug (Yusuf et al., 2023). In addition, surface modification methods allow a covalent conjugate of ligands or targeting moieties, which allows targeted pathological tissue (Li et al., 2023; Yusuf et al., 2023). Moreover, biocompatible formulations of PPT eliminate the shortcomings of these systems for long-term drug delivery (Feng et al., 2022). Furthermore, the active transport of PPTs and PPT-coated NS by M-cells in the specialized transport regions of the epithelium is a substantial breakthrough in drug delivery research. The drug delivery environment is about to undergo a massive transformation that can aid in the treatment of numerous patients needing accurate and effective treatment options (Porta et al., 2000). This advantage is often displayed simplistically. It is narrower and more contextualized than any schematic depicted.

2.4 POLYPLEXES

Polyplexes are structures that originate as polycations and polyanions, and recent studies have shown that they are useful as vehicles in the delivery of a wide range of therapeutic agents. Polyplexes are utilized to wrap and protect peptide-based therapeutic agents (PPTs) which are subject to enzymatic

degradation, and at the same time increase cell permeability (Figure 2.2) (Stepanova et al., 2023). Of special interest is the use of PPTs as payloads in peptide-based drug delivery systems, much of the available repertoire of bioactivities, and varied applications (Hashimoto et al., 2004; Stepanova et al., 2023).

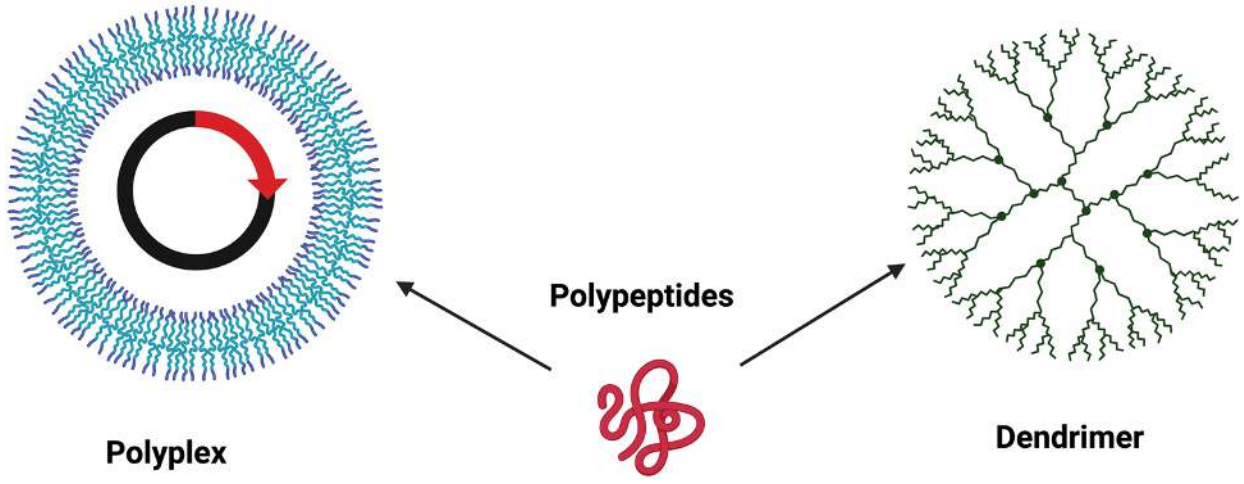

FIGURE 2.2 Illustrations of (a) Polyplexes and dendrimers loaded with PPTs are a promising approach in drug delivery, with far-reaching implications for the future of medicine. (b) Illustration of an active targeted drug delivery system, enhancing drug delivery to the specific sites. (Created in BioRender. https://BioRender.com/3cqzchx) ⏎

Polyplexes overcome the limitations of conventional concentrations of drugs; they stimulate the generation of complex PPTs in physiological conditions, increase the systemic circulation, and accumulate in target cells or tissues (Zhao et al., 2024). With its inherently designability, polyplexes make the creation of novel formulations that avoid the limitations of traditional regimens of drug delivery easier, which increases the effectiveness of therapy and minimizes side effects. The further adoption of polyplex-based systems of PPT delivery to pharmaceutical industry will

probably trigger the boost of novelty and the appearance of advanced therapeutics (Chang et al., 2021; Evans et al., 2015).

2.5 DENDRIMERS IN POLYPEPTIDE DRUG DELIVERY

The use of dendrimers in drug delivery into the body has also gained significant academic interest, and this can be explained by their hyperbranched, compartmentalized structure and their monodisperse nature. Dendrimers are branched polymers that offer a highly promising level of drug delivery due to their tunable physicochemical characteristics (Figure 2.2) (Wu et al., 2011). Dendrimers may be designed with dimensions on the order of 15 nm, which makes them especially effective in the use of the nanoscale of drug delivery. Their formation through a spherically oriented polymerization process creates internal cavities, which is a structural attribute that allows high loading capacity, particularly in high-generation dendrimers that possess more than 64 surface functional groups, unlike the lower-generation polymer systems. These properties, therefore, increase the attractiveness of dendrimers as drug delivery systems, specifically in targeted therapeutic solutions. One obvious benefit of dendrimers is that they are amenable to chemical modification; terminal groups can be easily conjugated with biocompatible groups and thus reduce cytotoxicity and increase permeability. Such a versatile character in functionality increases their prospects as delivery vectors since customizable changes at the surfaces allow targeting therapeutic agents to specific biological locations accurately. In addition, the design of dendrimers can be able to take into account the encapsulation and/or complexation methods, which can further enhance their appeal as vectors to deliver several agents simultaneously to distinct target tissues. Dendrimers have been utilized in the delivery of various biological macromolecules, including nucleic acids, chemotherapeutics, vaccines, and

mono- or copolymers which include chitin, polyethyleneimine, polyamidoamine, and poly(propyleneimine) (Hsu et al., 2017; Palmerston Mendes et al., 2017).

2.6 POLYPEPTIDE APPLICATION

2.6.1 Polypeptides: The Future of Disease Treatment

Other molecular backbones that have high potential in the treatment of diseases are peptide-derived therapeutics (PDTs), among other protein-based platforms. Both academia and nanotechnology, biotechnology, and pharmaceutical sciences in general are also showing interest in PDTs (Margulis et al., 2013; Stepanova et al., 2023). PDTs can also be engineered to attack certain diseases through disease-specific mechanisms of action and hence can be implemented in numerous different diseases. PDTs have been demonstrated to be useful in the treatment of cancers, immune system diseases, and infectious diseases (Figure 2.3) (Stepanova et al., 2023).

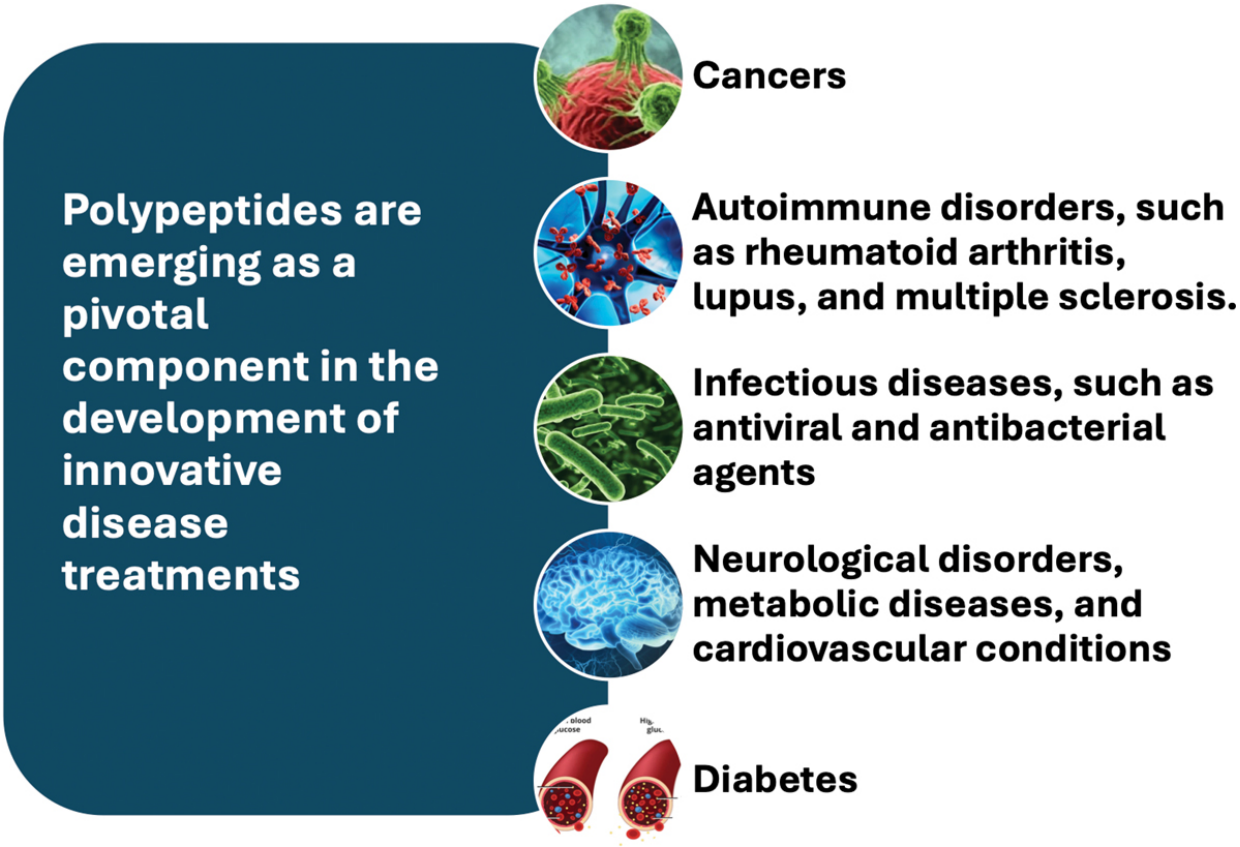

FIGURE 2.3 PPTs have many applications and have shown great promise in treating many diseases. Cancer, Diabetes, Cardiovascular diseases, Infectious diseases, Autoimmune disorders, Neurological disorders, and Rare genetic disorders. ⏎

PDTs have application in the treatment of cancer through chemotherapy. The finding of PDTs, which can be used to treat or prevent the development of cancers, opens the prospects of finding a treatment that is less toxic than the conventional means of chemotherapy. The wide range of possible forms of PDTs make the type of treatment that PDTs represent a very versatile one, and enable the creation of highly effective and customized cancer treatments by having them conjugated with the cancer biomarker of a patient (Jiang et al., 2022; Li et al., 2023). PDTs have also been shown to have improved immunomodulatory effects. Uncontrolled immune reaction against self-cells

and tissues is pathophysiologically adverse. PDTs can be effective in treating autoimmune diseases, including multiple sclerosis, autoimmune diseases like systemic lupus erythematosus, and rheumatoid arthritis specifically, because they have the potential to restore tolerance (Eggleton, 2003; Wada et al., 2021). Ongoing clinical trials are looking into the application of PDTs in the management of diabetes mellitus. Incretin hormone mimicking agents are peptides derived from agents with the capability to improve the glycemic control and pancreatic activity in diabetes patients with type 2 diabetes (Eggleton, 2003; Wada et al., 2021).

Additionally, research is being done on PPT's potential for treating diabetes. Peptide-based drugs that mimic the action of incretin hormones have shown promise in regulating blood sugar levels and improving pancreatic function in type 2 diabetes (Smith et al., 2022; Westermark & Westermark, 2013). PPT-based medications are used to treat a number of hereditary illnesses, including congenital aneurysms. PDT cure can also be used to treat hereditary disorders such as congenital aneurysm by replacing the faulty or missing proteins that cause such disorders (Levran et al., 2012).

2.6.2 Layer-by-Layer (LBL) NPs Capped Polypeptides: A Cutting-Edge Approach to Targeting Diseases

One of the promising plans is the LBL metal-NP-capped polypeptide polymers (PPTs) (Correa et al., 2016). The LBL architecture has specific PPTs that are designed to detect damaged cellular structures. These NP assemblies are likely to make a significant enhancement in therapeutic efficacy with reduced adverse effects, and allow therapeutic agents to be selectively released in areas of cell damage (Dacoba et al., 2020; Shao & Zhang, 2017; Xing et al., 2016).

The LBL-NP-capped PPTs could be designed to transport therapeutic agents of various sizes, including small-molecule drugs, nucleic acids, and imaging probes. This platform, in turn, provides numerous treatment approaches to all types of pathology malignancies, infectious diseases, and inflammatory disorders (Tan et al., 2018). One of the most interesting aspects about this technology is that it can be customized. The personalization of treatments by intervening with the composition of the NPs and the overlying PPTs is achievable, enabling the targeted disease biomarkers or genetic responses (Shangkum et al., 2018; Xing et al., 2016).

The special architecture is also able to circumvent the biological barriers and route further to the deeper parts of the tissue and therefore deliver drugs and imaging reagents to the desired site of action. Its approach is suggested to aid in solving some critical issues in drug delivery (Xing et al., 2016). Additionally, LBL-NPs make it possible to monitor and non-invasively detect diseases (Hasanzadeh et al., 2018; Lee et al., 2024). The effective disease diagnostic instruments can be imaging agents embedded in the LBL-NP that help to reveal and monitor the diseased tissues, therefore, enabling clinicians to identify the beginning of the disease and its progression throughout treatment (Lee et al., 2024; Ulanova et al., 2023).

LBL method of assembly entails consecutive layers of different polyelectrolytes with different charges deposited onto a substrate under a controlled laboratory working setup. This system allows designers to stack various items layer by layer until they are finished deciding what they want in that order.

When applied to drug delivery systems, this method has various advantages, including modulating the release of encapsulated drugs and stabilizing them (Blacklock et al., 2007; Jan et al., 2011). When incorporated into NP assemblies, PPTs can take advantage of their natural biocompatibility and biodegradability. One of the most interesting

possibilities of using LBL-NPs that are capped with PPTs for drug delivery is that the customizable structure can be used for the controlled release of beneficial therapeutic agents (Figure 2.4A, B, and C) (Pauling & Corey, 1951; Zhou et al., 2012).

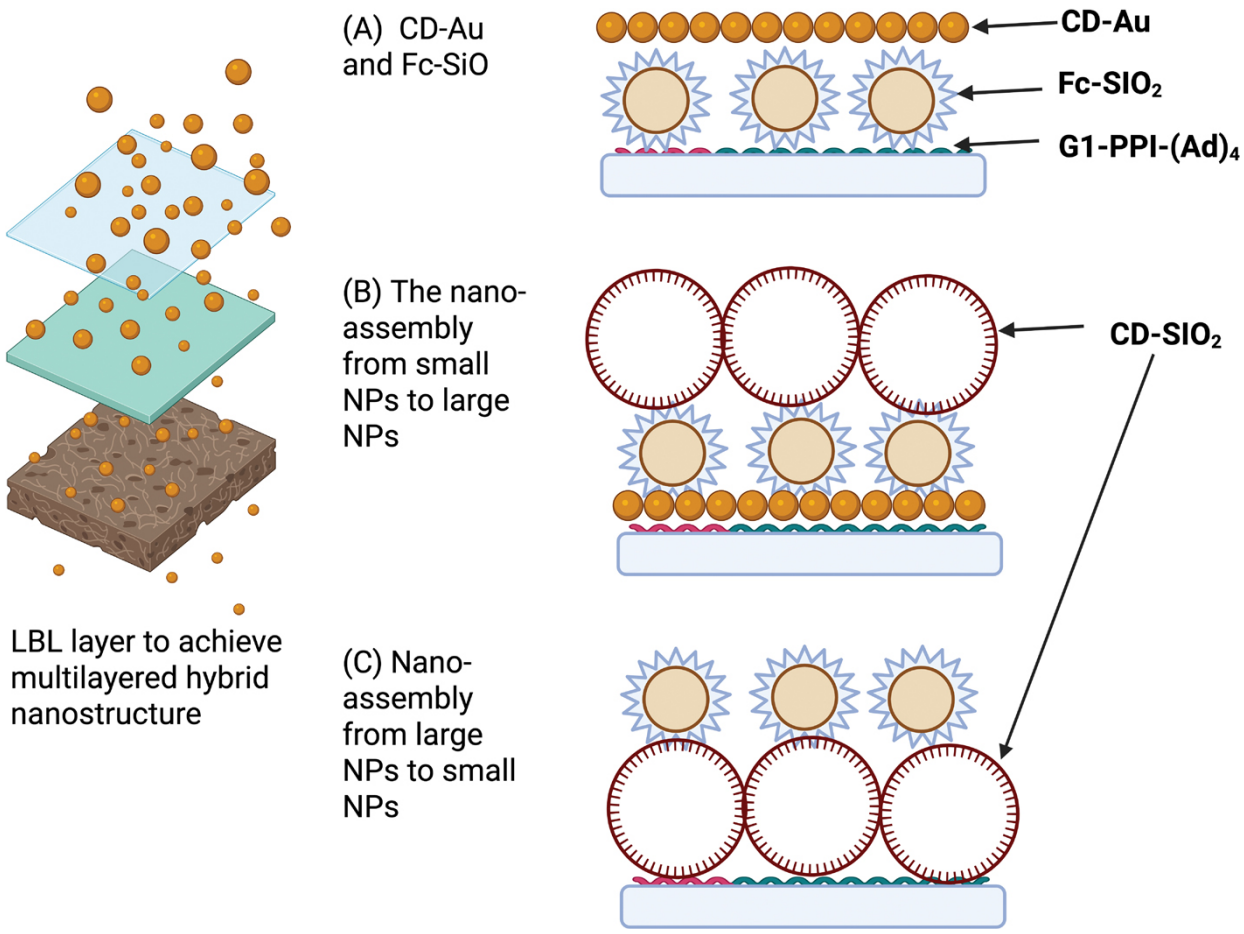

FIGURE 2.4 LBL-NPs capped and loaded PPTs, altering the sequence and composition of the deposited layers. (a) Applying LBL-NPs is practically capped with PPTs, where PPTs are inside. (b) Where the PPTs were inside the layers and the outer layers are larger NPs (c). The first layer is PPT as the surface of NPs, which can directly target the nearest organ. (Created in BioRender. https://BioRender.com/6npmsno) ⏎

Furthermore, PEI-conjugated dendrimers can also facilitate the transport and delivery of DNA, RNA, mRNA, and siRNA (Jhaveri et al., 2014). It can be loaded or capped within lipophilic tails on PEI dendrimer-tagged mRNA or linked with a PEGylated lipid carrier. Chahal et al. have formulated polymeric NPs with good immunity against different viruses such as H1N1 influenza, *Toxoplasma gondii*, Ebola, and Zika (Chahal et al., 2017). It was reported that protamine-RNA LPR was developed and loaded in liposomes to target herpes simplex virus (1-thymidine kinase). The lipophilic PNPs could also be used as a carrier to inhibit tumors in a human lung cancer (Wang et al., 2023).

2.7 CONCLUSION

Finally, PPT administration is a significant ethical challenge in the pharmaceutical industry of the globe; however, NP-based preparations have become a breakthrough solution to this problem. Certain nanocarrier systems, such as polymeric NPs, liposomes, and micelles, are highly flexible, therefore providing the PPTs with high stability and bioavailability and promoting their targeted deposition at anatomically relevant locations. These sophisticated formulae not only prolong systemic circulation and ease cellular uptake but also provide the benefit of accurate kinetics of drug release, which is extremely beneficial in chronic or sustained-release therapies. There is a strong potential for nanoformulated PPTs in various clinical fields, including treating neurological pathologies, metabolic diseases, and cancer. The therapeutic efficacy of nanoformulations can be optimized, and at the same time, systemic toxicity can be reduced by the strategic customization of nanoformulations.

This technological paradigm covers a medical need that has been previous and has not been met which has been refractory. Further

optimization of delivery systems and long-term development of PPT are likely to bring in new advancements in delivery methods, hence broadening the therapeutic bases of the polypeptide-based agents, improving patient outcomes, and increasing the overall therapeutic capabilities of these agent types.

REFERENCES

Abbasi, R., Shineh, G., Mobaraki, M., Doughty, S., & Tayebi, L. (2023). Structural parameters of nanoparticles affecting their toxicity for biomedical applications: A review. *J Nanopart Res*, *25*(3), 43. https://doi.org/10.1007/s11051-023-05690-w⏎

Abdellatif, A. A., Zayed, G., El-Bakry, A., Zaky, A., Saleem, I. Y., & Tawfeek, H. M. (2016). Novel gold nanoparticles coated with somatostatin as a potential delivery system for targeting somatostatin receptors. *Drug Dev Ind Pharm*, *42*(11), 1782–1791. https://doi.org/10.3109/03639045.2016.1173052⏎

Abdellatif, A. A. H., Alshubrumi, A. S., & Younis, M. A. (2024). Targeted nanoparticles: The smart way for the treatment of colorectal cancer. *AAPS PharmSciTech*, *25*(1), 23. https://doi.org/10.1208/s12249-024-02734-9⏎

Abdellatif, A. A. H., & Tawfeek, H. M. (2018). Development and evaluation of fluorescent gold nanoparticles. *Drug Dev Ind Pharm*, *44*(10), 1679–1684. https://doi.org/10.1080/03639045.2018.1483400⏎

Agbana, P., Lee, M. J., Rychahou, P., Kim, K. B., & Bae, Y. (2020). Ternary polypeptide nanoparticles with improved encapsulation, sustained release, and enhanced in vitro efficacy of carfilzomib. *Pharm Res*, *37*(11), 213. https://doi.org/10.1007/s11095-020-02922-9⏎

Arimura, H., Ohya, Y., & Ouchi, T. (2005). Formation of core-shell type biodegradable polymeric micelles from amphiphilic poly(aspartic acid)-block-polylactide diblock copolymer. *Biomacromolecules*, *6*(2), 720–725. https://doi.org/10.1021/bm0494491⏎

Asayama, S., Sudo, M., Nagaoka, S., & Kawakami, H. (2008). Carboxymethyl poly(L-histidine) as a new pH-sensitive polypeptide to enhance polyplex gene delivery. *Mol Pharm*, *5*(5), 898–901. https://doi.org/10.1021/mp800094b⏎

Azuar, A., Li, Z., Shibu, M. A., Zhao, L., Luo, Y., Shalash, A. O., Khalil, Z. G., Capon, R. J., Hussein, W. M., Toth, I., & Skwarczynski, M. (2021). Poly(hydrophobic amino acid)-based self-adjuvanting nanoparticles for group A streptococcus vaccine delivery. *J Med Chem*, *64*(5), 2648–2658. https://doi.org/10.1021/acs.jmedchem.0c01660⏎

Begines, B., Ortiz, T., Perez-Aranda, M., Martinez, G., Merinero, M., Arguelles-Arias, F., & Alcudia, A. (2020). Polymeric nanoparticles for drug delivery: Recent developments and future prospects. *Nanomaterials (Basel)*, *10*(7). https://doi.org/10.3390/nano10071403⏎

Blacklock, J., Handa, H., Soundara Manickam, D., Mao, G., Mukhopadhyay, A., & Oupicky, D. (2007). Disassembly of layer-by-layer films of plasmid DNA and reducible TAT polypeptide. *Biomaterials*, *28*(1), 117–124. https://doi.org/10.1016/j.biomaterials.2006.08.035⏎

Chahal, S., Wei, P., Moua, P., Park, S. P., Kwon, J., Patel, A., Vu, A. T., Catolico, J. A., Tsai, Y. F., Shaheen, N., Chu, T. T., Tam, V., Khan, Z. E., Joo, H. H., Xue, L., Lin-Cereghino, J., Tsai J. W., Lin-Cereghino, G. P. (2017). Structural characterization of the α-mating factor prepro-peptide for secretion of recombinant proteins in Pichia pastoris. *Gene*, *598*, 50–62. https://doi.org/10.1016/j.gene.2016.10.040⏎

Chang, Y. H., Lin, M. W., Chien, M. C., Ke, G. M., Wu, I. E., Lin, R. L., Lin, C. Y., & Hu, Y. C. (2021). Polyplex nanomicelle delivery of self-amplifying RNA vaccine. *J Control Release*, *338*, 694–704. https://doi.org/10.1016/j.jconrel.2021.09.008⏎

Chehelgerdi, M., Chehelgerdi, M., Allela, O. Q. B., Pecho, R. D. C., Jayasankar, N., Rao, D. P., Thamaraikani, T., Vasanthan, M., Viktor, P., Lakshmaiya, N., Saadh, M. J., Amajd, A., Abo-Zaid, M. A., Castillo-Acobo, R. Y., Ismail, A. H., Amin, A. H., & Akhavan-Sigari, R. (2023). Progressing nanotechnology to improve targeted cancer treatment: Overcoming hurdles in its clinical implementation. *Mol Cancer*, *22*(1), 169. https://doi.org/10.1186/s12943-023-01865-0

Correa, S., Choi, K. Y., Dreaden, E. C., Renggli, K., Shi, A., Gu, L., Shopsowitz, K. E., Quadir, M. A., Ben-Akiva, E., & Hammond, P. T. (2016). Highly scalable, closed-loop synthesis of drug-loaded,

layer-by-layer nanoparticles. *Adv Funct Mater*, *26*(7), 991–1003. https://doi.org/10.1002/adfm.201504385⏎

Dacoba, T. G., Anfray, C., Mainini, F., Allavena, P., Alonso, M. J., Torres Andon, F., & Crecente-Campo, J. (2020). Arginine-based poly(I:C)-loaded nanocomplexes for the polarization of macrophages toward M1-antitumoral effectors. *Front Immunol*, *11*, 1412. https://doi.org/10.3389/fimmu.2020.01412⏎

de Miguel, L., Popa, I., Noiray, M., Caudron, E., Arpinati, L., Desmaele, D., Cebrian-Torrejon, G., Domenech-Carbo, A., & Ponchel, G. (2015). Osteotropic polypeptide nanoparticles with dual hydroxyapatite binding properties and controlled cisplatin delivery. *Pharm Res*, *32*(5), 1794–1803. https://doi.org/10.1007/s11095-014-1576-z⏎

De Santis, E., & Ryadnov, M. G. (2015). Peptide self-assembly for nanomaterials: The old new kid on the block. *Chem Soc Rev*, *44*(22), 8288–8300. https://doi.org/10.1039/c5cs00470e⏎

Ding, J., Li, C., Zhang, Y., Xu, W., Wang, J., & Chen, X. (2015). Chirality-mediated polypeptide micelles for regulated drug delivery. *Acta Biomater*, *11*, 346–355. https://doi.org/10.1016/j.actbio.2014.09.043⏎

Eckmann, D. M., Composto, R. J., Tsourkas, A., & Muzykantov, V. R. (2014). Nanogel carrier design for targeted drug delivery. *J Mater Chem B*, *2*(46), 8085–8097. https://doi.org/10.1039/C4TB01141D⏎

Eggleton, P. (2003). Stress protein-polypeptide complexes acting as autoimmune triggers. *Clin Exp Immunol*, *134*(1), 6–8. https://doi.org/10.1046/j.1365-2249.2003.02263.x⏎

Evans, B. C., Hocking, K. M., Kilchrist, K. V., Wise, E. S., Brophy, C. M., & Duvall, C. L. (2015). Endosomolytic nano-polyplex platform technology for cytosolic peptide delivery to inhibit pathological vasoconstriction. *ACS Nano*, *9*(6), 5893–5907. https://doi.org/10.1021/acsnano.5b00491⏎

Feng, H., Fabrizi, J., Li, J., & Mayer, C. (2022). Syntheses of polypeptides and their biomedical application for anti-tumor drug delivery. *Int J Mol Sci*, *23*(9). https://doi.org/10.3390/ijms23095042⏎

Feng, X., Xu, W., Liu, J., Li, D., Li, G., Ding, J., & Chen, X. (2021). Polypeptide nanoformulation-induced immunogenic cell death and remission of immunosuppression for enhanced chemoimmunotherapy. *Sci Bull (Beijing)*, *66*(4), 362–373. https://doi.org/10.1016/j.scib.2020.07.013⏎

Gao, Z., Zhang, Z., Guo, J., Hao, J., Zhang, P., & Cui, J. (2020). Polypeptide nanoparticles with pH-sheddable PEGylation for improved drug delivery. *Langmuir*, *36*(45), 13656–13662. https://doi.org/10.1021/acs.langmuir.0c02532⏎

Gouveia, M. G., Wesseler, J. P., Ramaekers, J., Weder, C., Scholten, P. B. V., & Bruns, N. (2023). Polymersome-based protein drug delivery - quo vadis? *Chem Soc Rev*, *52*(2), 728–778. https://doi.org/10.1039/d2cs00106c⏎

Hakkinen, S., Tanaka, J., Garcia Maset, R., Hall, S. C. L., Huband, S., Rho, J. Y., Song, Q., & Perrier, S. (2022). Polymerisation-induced self-assembly of graft copolymers. *Angew Chem Int Ed Engl*, *61*(44), e202210518. https://doi.org/10.1002/anie.202210518⏎

Hasanzadeh, M., Babaie, P., Jouyban-Gharamaleki, V., & Jouyban, A. (2018). The use of chitosan as a bioactive polysaccharide in non-invasive detection of malondialdehyde biomarker in human exhaled breath condensate: A new platform towards diagnosis of some lung disease. *Int J Biol Macromol*, *120*(Pt B), 2482–2492. https://doi.org/10.1016/j.ijbiomac.2018.09.018⏎

Hashimoto, T., Murakami, A., & Yamaoka, T. (2004). An enhanced site-specific transcription efficiency of DNA/polypeptide-vector polyplexes. *Nucleic Acids Symp Ser*, *48*(1), 235–236. https://doi.org/10.1093/nass/48.1.235⏎

Hebel, M., Gacanin, J., Luckerath, T., Ng, D. Y. W., & Weil, T. (2022). Controlling polymer morphologies by intramolecular and intermolecular dynamic covalent iron(III)/Catechol complexation-from polypeptide single chain nanoparticles to hydrogels. *Macromol Rapid Commun*, *43*(12), e2100413. https://doi.org/10.1002/marc.202100413⏎

Hehir, S., & Cameron, N. R. (2014). Recent advances in drug delivery systems based on polypeptides prepared fromN-carboxyanhydrides. *Polym Int*, *63*(6), 943–954. https://doi.org/10.1002/pi.4710⏎

He-Wei, S., Rong, X., Yong-Jun, Z., Qian, Z., Shi-Yu, Z., Xiao-Yan, H., & Yi, Z. (2015). Study on synthesis of star poly(ε-caprolactone)-b-polylysine copolymer, characterization and its Micelles' properties. *Br J Appl Sci Technol*, *8*(4), 341–347. https://doi.org/10.9734/bjast/2015/17180⏎

Hsu, H. J., Bugno, J., Lee, S., Hong, S. J. W. I. R. N., & Nanobiotechnology. (2017). Dendrimer-based nanocarriers: a versatile platform for drug delivery. *Wiley Interdiscip Rev Nanomed Nanobiotechnol*, *9*(1), e1409.

Hua, C., Zhang, Y., & Liu, Y. (2021). Enhanced anticancer efficacy of chemotherapy by amphiphilic Y-shaped polypeptide micelles. *Front Bioeng Biotechnol*, *9*, 817143. https://doi.org/10.3389/fbioe.2021.817143⏎

Jackson, S., Agbana, P., Kim, K. B., & Bae, Y. (2022). Effects of organic acids on drug release from ternary polypeptide nanoparticles entrapping carfilzomib. *J Pharm Sci*, *111*(4), 1172–1177. https://doi.org/10.1016/j.xphs.2021.09.022⏎

Jan, J. S., Chuang, T. H., Chen, P. J., & Teng, H. (2011). Layer-by-layer polypeptide macromolecular assemblies-mediated synthesis of mesoporous silica and gold nanoparticle/mesoporous silica tubular nanostructures. *Langmuir*, *27*(6), 2834–2843. https://doi.org/10.1021/la103923c⏎

Jhaveri, A. M., & Torchilin, V. P. (2014). Multifunctional polymeric micelles for delivery of drugs and siRNA. *Front Pharmacol*, *5*, 77. https://doi.org/10.3389/fphar.2014.00077⏎

Jiang, A., Guan, X., He, L., & Guan, X. (2022). Engineered elastin-like polypeptides: An efficient platform for enhanced cancer treatment. *Front Pharmacol*, *13*, 1113079. https://doi.org/10.3389/fphar.2022.1113079⏎

Judge, N., Pavlović, D., Moldenhauer, E., Clarke, P., Brannigan, R., & Heise, A. (2022). Influence of the block copolypeptide surfactant structure on the size of polypeptide nanoparticles obtained by mini emulsion polymerisation. *Polymer Chemistry*, *13*(19), 2822–2830. https://doi.org/10.1039/d2py00331g⏎

Kamps, A. C., Sanchez-Gaytan, B. L., Hickey, R. J., Clarke, N., Fryd, M., & Park, S. J. (2010). Nanoparticle-directed self-assembly of amphiphilic block copolymers. *Langmuir*, *26*(17), 14345–14350. https://doi.org/10.1021/la1025558⏎

Keskin, D., Zu, G., Forson, A. M., Tromp, L., Sjollema, J., & van Rijn, P. (2021). Nanogels: A novel approach in antimicrobial delivery systems and antimicrobial coatings. *Bioact Mater*, *6*(10), 3634–3657. https://doi.org/10.1016/j.bioactmat.2021.03.004

La, S. B., Okano, T., & Kataoka, K. (1996). Preparation and characterization of the micelle-forming polymeric drug indomethacin-incorporated poly(ethylene oxide)-poly(beta-benzyl l-aspartate) block copolymer micelles. *J Pharm Sci*, *85*(1), 85–90. https://doi.org/10.1021/js950204r

Lee, G. Y., Li, A. A., Moon, I., Katritsis, D., Pantos, Y., Stingo, F., Fabbrico, D., Molinaro, R., Taraballi, F., Tao, W., & Corbo, C. (2024). Protein corona sensor array nanosystem for detection of coronary artery disease. *Small*, *20*(10), e2306168. https://doi.org/10.1002/smll.202306168

Levit, M., Zashikhina, N., Dobrodumov, A., Kashina, A., Tarasenko, I., Panarin, E., Fiorucci, S., Korzhikova-Vlakh, E., & Tennikova, T. (2018). Synthesis and characterization of well-defined poly(2-deoxy-2-methacrylamido-d-glucose) and its biopotential block copolymers via RAFT and ROP polymerization. *Eur Poly J*, *105*, 26–37. https://doi.org/10.1016/j.eurpolymj.2018.05.018

Levit, M., Zashikhina, N., Vdovchenko, A., Dobrodumov, A., Zakharova, N., Kashina, A., Ruhl, E., Lavrentieva, A., Scheper, T., Tennikova, T., & Korzhikova-Vlakh, E. (2020). Bio-inspired amphiphilic block-copolymers based on synthetic glycopolymer and poly(Amino acid) as potential drug delivery systems. *Polymers (Basel)*, *12*(1). https://doi.org/10.3390/polym12010183

Levran, O., Peles, E., Hamon, S., Randesi, M., Zhao, C., Zhang, B., Adelson, M., & Kreek, M. J. (2012). Nerve growth factor beta polypeptide (NGFB) genetic variability: Association with the methadone dose required for effective maintenance treatment. *Pharmacogenomics J*, *12*(4), 319–327. https://doi.org/10.1038/tpj.2011.6

Li, J., Wang, Q., Xia, G., Adilijiang, N., Li, Y., Hou, Z., Fan, Z., & Li, J. (2023). Recent advances in targeted drug delivery strategy for enhancing oncotherapy. *Pharmaceutics*, *15*(9). https://doi.org/10.3390/pharmaceutics15092233

Li, Q. Z., Zhou, Z. R., Hu, C. Y., Li, X. B., Chang, Y. Z., Liu, Y., Wang, Y. L., & Zhou, X. W. (2023). Recent advances of bioactive proteins/polypeptides in the treatment of breast cancer. *Food Sci Biotechnol*, *32*(3), 265–282. https://doi.org/10.1007/s10068-022-01233-6

Li, T., Lin, J., Chen, T., & Zhang, S. (2006). Polymeric micelles formed by polypeptide graft copolymer and its mixtures with polypeptide block copolymer. *Polymer*, *47*(13), 4485–4489. https://doi.org/10.1016/j.polymer.2006.04.011

Lin, W., Ma, G., Yuan, Z., Qian, H., Xu, L., Sidransky, E., & Chen, S. (2019). Development of zwitterionic polypeptide nanoformulation with high doxorubicin loading content for targeted drug delivery. *Langmuir*, *35*(5), 1273–1283. https://doi.org/10.1021/acs.langmuir.8b00851

Lv, S., Li, M., Tang, Z., Song, W., Sun, H., Liu, H., & Chen, X. (2013). Doxorubicin-loaded amphiphilic polypeptide-based nanoparticles as an efficient drug delivery system for cancer therapy. *Acta Biomater*, *9*(12), 9330–9342. https://doi.org/10.1016/j.actbio.2013.08.015

Margulis, B. A., Vigont, V., Lazarev, V. F., Kaznacheyeva, E. V., & Guzhova, I. V. (2013). Pharmacological protein targets in polyglutamine diseases: Mutant polypeptides and their interactors. *FEBS Lett*, *587*(13), 1997–2007. https://doi.org/10.1016/j.febslet.2013.05.022

Palmerston Mendes, L., Pan, J., & Torchilin, V. P. J. M. (2017). Dendrimers as nanocarriers for nucleic acid and drug delivery in cancer therapy. *Molecules*, *22*(9), 1401.

Patra, J. K., Das, G., Fraceto, L. F., Campos, E. V. R., Rodriguez-Torres, M. D. P., Acosta-Torres, L. S., Diaz-Torres, L. A., Grillo, R., Swamy, M. K., Sharma, S., Habtemariam, S., & Shin, H. S. (2018). Nano based drug delivery systems: Recent developments and future prospects. *J Nanobiotechnology*, *16*(1), 71. https://doi.org/10.1186/s12951-018-0392-8

Pauling, L., & Corey, R. B. (1951). The pleated sheet, a new layer configuration of polypeptide chains. *Proc Natl Acad Sci U S A*, *37*(5), 251–256. https://doi.org/10.1073/pnas.37.5.251

Porta, C., Dossena, S., Rossi, V., Pinza, M., & Cremaschi, D. (2000). Rabbit nasal mucosa: Nanospheres coated with polypeptides bound to specific anti-polypeptide IgG are better transported than nanospheres coated with polypeptides or IgG alone. *Biochim Biophys Acta*, *1466*(1–2), 115–124. https://doi.org/10.1016/s0005-2736(00)00182-6

Qiao, Y., Liu, X., Zhou, X., Zhang, H., Zhang, W., Xiao, W., Pan, G., Cui, W., Santos, H. A., & Shi, Q. (2020). Gelatin templated polypeptide co-cross-linked hydrogel for bone regeneration. *Adv Healthc Mater*, *9*(1), e1901239. https://doi.org/10.1002/adhm.201901239

Qiu, R., Sun, D., Bai, Y., Li, J., & Wang, L. (2020). Application of tumor-targeting peptide-decorated polypeptide nanoparticles with doxorubicin to treat osteosarcoma. *Drug Deliv*, *27*(1), 1704–1717. https://doi.org/10.1080/10717544.2020.1856221⏎

Ren, T.-B., Xia, W.-J., Dong, H.-Q., & Li, Y.-Y. (2011). Sheddable micelles based on disulfide-linked hybrid PEG-polypeptide copolymer for intracellular drug delivery. *Polymer*, *52*(16), 3580–3586. https://doi.org/10.1016/j.polymer.2011.06.013⏎

Salehi, S., Naghib, S. M., Garshasbi, H. R., Ghorbanzadeh, S., & Zhang, W. (2023). Smart stimuli-responsive injectable gels and hydrogels for drug delivery and tissue engineering applications: A review. *Front Bioeng Biotechnol*, *11*, 1104126. https://doi.org/10.3389/fbioe.2023.1104126⏎

Shangkum, G. Y., Chammingkwan, P., Trinh, D. X., & Taniike, T. (2018). Design of a semi-continuous selective layer based on deposition of UiO-66 nanoparticles for nanofiltration. *Membranes (Basel)*, *8*(4). https://doi.org/10.3390/membranes8040129⏎

Shao, X., & Zhang, X. (2017). Design of five-layer gold nanoparticles self-assembled in a liquid open tubular column for ultrasensitive nano-LC-MS/MS proteomic analysis of 80 living cells. *Proteomics*, *17*(8). https://doi.org/10.1002/pmic.201600463⏎

Skoulas, D., Christakopoulos, P., Stavroulaki, D., Santorinaios, K., Athanasiou, V., & Iatrou, H. (2017). Micelles formed by polypeptide containing polymers synthesized via N-carboxy anhydrides and their application for cancer treatment. *Polymers (Basel)*, *9*(6). https://doi.org/10.3390/polym9060208⏎

Smith, A. A., Moore, K. B. E., Ambs, P. M., Saraswati, A. P., & Fortin, J. S. (2022). Recent advances in the discovery of therapeutics to curtail islet amyloid polypeptide aggregation for type 2 diabetes treatment. *Adv Biol (Weinh)*, *6*(10), e2101301. https://doi.org/10.1002/adbi.202101301⏎

Soni, K. S., Desale, S. S., & Bronich, T. K. (2016). Nanogels: An overview of properties, biomedical applications and obstacles to clinical translation. *J Control Release*, *240*, 109–126. https://doi.org/10.1016/j.jconrel.2015.11.009⏎

Stepanova, M., Nikiforov, A., Tennikova, T., & Korzhikova-Vlakh, E. (2023). Polypeptide-based systems: From synthesis to application in drug delivery. *Pharmaceutics*, *15*(11).

https://doi.org/10.3390/pharmaceutics15112641⏎

Sun, H., Choi, W., Zang, N., Battistella, C., Thompson, M. P., Cao, W., Zhou, X., Forman, C., & Gianneschi, N. C. (2019). Bioactive peptide brush polymers via photoinduced reversible-deactivation radical polymerization. *Angew Chem Int Ed Engl*, *58*(48), 17359–17364. https://doi.org/10.1002/anie.201908634⏎

Suo, A., Qian, J., Zhang, Y., Liu, R., Xu, W., & Wang, H. (2016). Comb-like amphiphilic polypeptide-based copolymer nanomicelles for co-delivery of doxorubicin and P-gp siRNA into MCF-7 cells. *Mater Sci Eng C Mater Biol Appl*, *62*, 564–573. https://doi.org/10.1016/j.msec.2016.02.007⏎

Szunerits, S., Abderrahmani, A., & Boukherroub, R. (2022). Nanoparticles and nanocolloidal carbon: Will they be the next antidiabetic class that targets fibrillation and aggregation of human islet amyloid polypeptide in type 2 diabetes? *Acc Chem Res*, *55*(20), 2869–2881. https://doi.org/10.1021/acs.accounts.2c00415⏎

Tan, Y. F., Lee, Y. S., Seet, L. F., Ng, K. W., Wong, T. T., & Venkatraman, S. (2018). Design and in vitro release study of siRNA loaded layer by layer nanoparticles with sustained gene silencing effect. *Expert Opin Drug Deliv*, *15*(10), 937–949. https://doi.org/10.1080/17425247.2018.1518426⏎

Tawfeek, H. M., Abdellatif, A. A. H., Dennison, T. J., Mohammed, A. R., Sadiq, Y., & Saleem, I. Y. (2017). Colonic delivery of indometacin loaded PGA-co-PDL microparticles coated with Eudragit L100-55 from fast disintegrating tablets. *Int J Pharm*, *531*(1), 80–89. https://doi.org/10.1016/j.ijpharm.2017.08.069⏎

Tesauro, D., Accardo, A., Diaferia, C., Milano, V., Guillon, J., Ronga, L., & Rossi, F. (2019). Peptide-based drug-delivery systems in biotechnological applications: Recent advances and perspectives. *Molecules*, *24*(2). https://doi.org/10.3390/molecules24020351⏎

Thang, N. H., Chien, T. B., & Cuong, D. X. (2023). Polymer-based hydrogels applied in drug delivery: An overview. *Gels*, *9*(7). https://doi.org/10.3390/gels9070523⏎

Tian, A., Yi, X., & Sun, N. (2022). Application of mesenchymal stem cells combined with nano-polypeptide hydrogel in tissue engineering blood vessel. *Regen Ther*, *21*, 277–281.

https://doi.org/10.1016/j.reth.2022.07.009⏎

Ulanova, M., Gloag, L., Bongers, A., Kim, C. K., Duong, H. T. K., Kim, H. N., Gooding, J. J., Tilley, R. D., Biazik, J., Wen, W., Sachdev, P. S., & Braidy, N. (2023). Evaluation of dimercaptosuccinic acid-coated iron nanoparticles immunotargeted to amyloid Beta as MRI contrast agents for the diagnosis of Alzheimer's disease. *Cells*, *12*(18). https://doi.org/10.3390/cells12182279⏎

Van Domeselaar, G. H., Kwon, G. S., Andrew, L. C., & Wishart, D. S. (2003). Application of solid phase peptide synthesis to engineering PEO–peptide block copolymers for drug delivery. *Colloids Surf B Biointerfaces*, *30*(4), 323–334. https://doi.org/10.1016/s0927-7765(03)00125-5⏎

Wada, Y., Nakajima, S., Kushima, R., Takemura, S., Mori, N., Hasegawa, H., Nakayama, T., Mukaisho, K. I., Yoshida, A., Umano, S., Yamamoto, K., Sugihara, H., & Murakami, K. (2021). Pyloric, pseudopyloric, and spasmolytic polypeptide-expressing metaplasias in autoimmune gastritis: A case series of 22 Japanese patients. *Virchows Arch*, *479*(1), 169–178. https://doi.org/10.1007/s00428-021-03033-5⏎

Wang, Q., Atluri, K., Tiwari, A. K., & Babu, R. J. (2023). Exploring the application of micellar drug delivery systems in cancer nanomedicine. *Pharmaceuticals (Basel)*, *16*(3). https://doi.org/10.3390/ph16030433⏎

Westermark, G. T., & Westermark, P. (2013). Islet amyloid polypeptide and diabetes. *Curr Protein Pept Sci*, *14*(4), 330–337. https://doi.org/10.2174/13892037113149990050⏎

Wong, K. H., Guo, Z., Jiang, D., Zhou, X., Lin, L., Zhao, D., & Chen, M. (2023). Linear-like polypeptide-based micelle with pH-sensitive detachable PEG to deliver dimeric camptothecin for cancer therapy. *Asian J Pharm Sci*, *18*(1), 100773. https://doi.org/10.1016/j.ajps.2022.100773⏎

Wu, H. M., Pan, S. R., Chen, M. W., Wu, Y., Wang, C., Wen, Y. T., Zeng, X., & Wu, C. B. (2011). A serum-resistant polyamidoamine-based polypeptide dendrimer for gene transfection. *Biomaterials*, *32*(6), 1619–1634. https://doi.org/10.1016/j.biomaterials.2010.09.045⏎

Xing, R., Jiao, T., Ma, K., Ma, G., Mohwald, H., & Yan, X. (2016). Regulating cell apoptosis on layer-by-layer assembled multilayers of photosensitizer-coupled polypeptides and gold nanoparticles. *Sci Rep*, *6*, 26506. https://doi.org/10.1038/srep26506⏎

Yang, J., Wang, Z., Liang, X., Wang, W., & Wang, S. (2024). Multifunctional polypeptide-based hydrogel bio-adhesives with pro-healing activities and their working principles. *Adv Colloid Interface Sci*, *327*, 103155. https://doi.org/10.1016/j.cis.2024.103155⏎

Yusuf, A., Almotairy, A. R. Z., Henidi, H., Alshehri, O. Y., & Aldughaim, M. S. (2023). Nanoparticles as drug delivery systems: A review of the implication of Nanoparticles' physicochemical properties on responses in biological systems. *Polymers (Basel)*, *15*(7). https://doi.org/10.3390/polym15071596⏎

Zhang, R., Morton, L. D., Smith, J. D., Gallazzi, F., White, T. A., & Ulery, B. D. (2018). Instructive design of triblock peptide amphiphiles for structurally complex micelle fabrication. *ACS Biomater Sci Eng*, *4*(7), 2330–2339. https://doi.org/10.1021/acsbiomaterials.8b00300⏎

Zhang, Y., Zhang, H., Wang, Y., Ji, Y., Wang, F., & He, P. (2024). A novel Cu(II) loaded polypeptide SO(2) prodrug nanoformulation combining chemodynamic and gas anticancer therapies. *Macromol Biosci*, *24*(4), e2300429. https://doi.org/10.1002/mabi.202300429⏎

Zhao, B., Zhang, X., Bickle, M. S., Fu, S., Li, Q., & Zhang, F. (2024). Development of polypeptide-based materials toward messenger RNA delivery. *Nanoscale*, *16*(5), 2250–2264. https://doi.org/10.1039/d3nr05635j⏎

Zhao, L., Li, N., Wang, K., Shi, C., Zhang, L., & Luan, Y. (2014). A review of polypeptide-based polymersomes. *Biomaterials*, *35*(4), 1284–1301. https://doi.org/10.1016/j.biomaterials.2013.10.063⏎

Zhao, Z., Wang, Y., Han, J., Wang, K., Yang, D., Yang, Y., Du, Q., Song, Y., & Yin, X. (2014). Self-assembled micelles of amphiphilic poly(L-phenylalanine)-b-poly(L-serine) polypeptides for tumor-targeted delivery. *Int J Nanomedicine*, *9*, 5849–5862. https://doi.org/10.2147/IJN.S73111

Zhou, D., Xiao, H., Meng, F., Zhou, S., Guo, J., Li, X., Jing, X., & Huang, Y. (2012). Layer-by-layer assembled polypeptide capsules for platinum-based pro-drug delivery. *Bioconjug Chem*, *23*(12), 2335–2343. https://doi.org/10.1021/bc300144e⏎

Zhou, X., Su, X., & Zhou, C. (2018). Preparation of diblock amphiphilic polypeptide nanoparticles for medical applications. *Eur Poly J*, *100*, 132–136. https://doi.org/10.1016/j.eurpolymj.2018.01.025⏎

3 Design and Characterization of Polypeptide Nanocarriers

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3.1 INTRODUCTION

Drug delivery systems rely heavily on the nanocarriers to increase bioavailability, as well as targeting. Polypeptide (PPT) is one of the most common types of nanocarriers due to having many properties, such as biocompatibility, biodegradability, and adaptability (Abdellatif et al., 2020b, 2016). PPT nanocarriers can increase bioavailability, stability, and solubility of therapeutic agents. As ligands, they can be tailored to reach sick cells, thus enhancing the effectiveness of the therapeutic process, and reducing off-target effects at the same time (Peer et al., 2020; Allen and Cullis, 2013). Due to their flexibility, PPT is able to self-assemble to form micelles, vesicles, and nanoparticles to deliver the maximum drug due to their flexibility, their physical and chemical characteristics. It was this propertied that made these nanocarriers efficient in therapeutic uses (Stepanova et al., 2023). Nanocarriers may be prepared in many ways, one of them being the solvent displacement technique which is generally employed in the

production of NPs through precipitation and the addition of PPTs. The polymer and PPT are combined and then inserted into a big volume of a non-solvent in this procedure and stirred in a continuous process. The precipitation method can be used to separate the formulated NPs as the solvent diffuses rapidly into the non-solvent leading to the development of NP-loaded PPTs. The simplicity, cost-effectiveness, and industry scale are defined by this approach (Thwala et al., 2018).

This chapter discusses the design of polypeptide nanocarrier formulations. The formulation design, PPT selection, conjugation, and how self-assembly nanocarriers are created. The generated nanocarriers' size, shape, stability, and drug release characteristics are also discussed. PPTs nanocarriers are significant in the advancement of therapeutic targeting due to their prospective uses in cancer treatment, gene transfer, and vaccination regimens.

3.1.1 Selection of Polypeptides

The proper selection of PPTs in the early stages of producing nanocarriers has received a lot of interest in the field of PPT protein synthesis. The structure, sequence, and content of amino acids are among the most significant factors to consider. Both natural PPTs include peptides derived from proteins (e.g., albumin and elastin), and manufactured PPTs are commonly used (Wang et al., 2024; Birshtein et al., 1974).

Binding affinity, solubility, and rate of degradation are related to the amino acid sequence. Specific sequences allow nanocarriers to incorporate recognition patterns that preferentially bind to the receptors of target cells. The sequence dictates whether the polypeptide produces a stable complexation with pharmaceutical drugs or not (Yang et al., 2023; Wang et al., 2021).

The amino acid composition of a polypeptide determines its hydrophobicity, charge, and flexibility. Charged or polar amino acids improve the compound's water solubility because hydrophobic residues promote the self-assembly of nanostructures. To maximize medication loading and release, these qualities must be balanced (Betush et al., 2018).

3.1.2 Preparation of Nanoparticle Loaded with Polypeptides

Numerous techniques are reported to formulate NPs loaded with PPTs (Figure 3.1). One of these techniques is emulsification-solvent evaporation. In this process, the polymer and PPT are dissolved in an organic solvent to create a water-in-oil emulsion, then sonicated to lower the droplet size, and then, the oily phase (organic solvent) must be evaporated. Using this technique, hydrophilic PPTs can be encapsulated in hydrophobic polymeric NPs (He et al., 2007). Another widely used technique is nanoprecipitation, in which the polymer and PPT are rapidly combined with a non-solvent under circumstances, causing NPs to naturally form. This technique avoids hazardous organic solvents, making it more ecologically friendly (Donia et al., 2020; Huang et al., 2012).

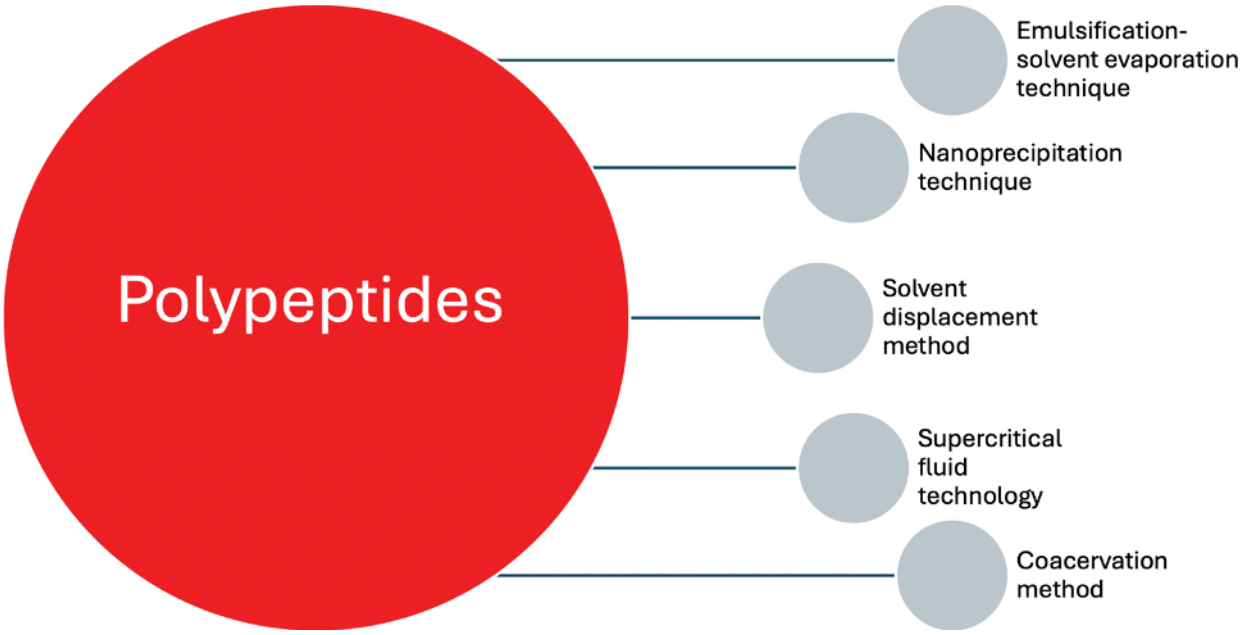

FIGURE 3.1 Various methods, such as emulsification-solvent evaporation, nanoprecipitation, solvent displacement, supercritical fluid technology, and coacervation. ⏎

Supercritical fluid technology has also received a lot of attention since it produces PPT-rich nanoparticles. In this technology, polymer is dissolved by super critical carbon dioxide and PPT, which in turn expands very rapidly to create NPs. This strategy possesses a number of advantages, a solvent-free type of processing, a full ability to control the size of particles, and less damage to the PPT payload (Xu et al., 2022; Zhu et al., 2021). Also, the coacervation technique can make NPs full of PPTs. In this method, a complex coacervate is produced through the separation of two oppositely ionized polymers, one of which is the PPT. The coacervate thus formed is solidified to form NPs, which encapsulates of the PPT. The technique is common because it is easy to use and has the ability to encapsulate sensitive PPTs (Rapaka and Urry, 1978).

3.2 CONJUGATION STRATEGIES

The goal of drug delivery is to improve and augment therapeutic efficacy by actively or passively delivering to the target site in the body, hence reducing the drug's off-target accumulation. This can be accomplished by improving the drug's accumulation at the target sites, lowering drug toxicity, and improving its pharmacokinetics (PKs). Also, the patient acceptance and compliance resulted from this type of medication improvement. The identification of delivery challenges related to each class of therapy has spurred innovation in delivery technology and methods (Vargason et al., 2021).

3.2.1 Classes of Therapeutic and Delivery Challenges

Small-molecule drugs, such as chemotherapeutics, antibiotics, and steroids, have been used as pharmaceuticals since the late 1800s due to their ability to rapidly diffuse through biological fluids and cell membranes (Schmidt et al., 2008). However, 90% of preclinical drug candidates are low-solubility compounds. Strategies to overcome low bioavailability include improving drug solubility by modulating the local microenvironment, such as using pH modifiers for small molecules with pH-dependent solubility. Other strategies involve altering the physicochemical properties of small molecules for improved solubilization, diffusion, or absorption (Vargason et al., 2021; Kempf et al., 1998).

Basic research on drug transport through the vasculature and into tissues or cells led to PK and PD principles. As the association between PK/PD characteristics and small molecule efficacy, duration of action, and toxicity became obvious, early attempts concentrated on regulating dose and dosing regimen (frequency and infusion rate) to optimize medication efficacy. These fundamental PK experiments and clinical trials lay the groundwork for controlled-release drug delivery systems that use dissolution, diffusion,

osmosis, and ion exchange to generate predictable drug release kinetics over a set duration (Vargason et al., 2021).

Early studies found that poly(ethylene glycol) (PEG) coatings extended particle circulation half-life and increased particle retention at tumor locations. The first nanoparticle treatment approved by the FDA (in 1995) was PEGylated liposomal doxorubicin (Doxil). The difficulty of site-selective delivery has been addressed by substantial preclinical nanoparticle research. After controlling the solubility, dosage, and other delivery characteristics for small compounds, the delivery systems have been applied to all therapeutic classes (Vargason et al., 2021; Barenholz, 2012).

Proteins and peptides. Biologically developed peptides, which are around 2–50 amino acids, and proteins, which consist of around 50 or more amino acids, have excellent selectivity for target sites. Unlike many small compounds, peptides and proteins have better potency and lower toxicity due to their vast size and varied tertiary structures, which promote protein pocket interaction (Ge et al., 2025; Vargason et al., 2021; Lau and Dunn, 2018; Bruno et al., 2013). The increased clinical use of peptides and proteins has led to unique challenges in their delivery due to their complex structure, poor stability, and sensitivity to proteases, temperature, pH, and the rapid diffusion of stabilizing excipients (Vargason et al., 2021). Peptides and proteins can activate the immune system, leading to rapid drug clearance and immunogenicity-driven adverse events. Furthermore, the synthetic and humanized peptide analogues can improve drug-like properties by incorporating non-natural amino acids or coupling with chemical moieties (Abdellatif et al., 2020, 2018, 2016). Moreover, the half-life, stability, receptor affinity, and toxicity of synthetic or humanized peptide analogues can also be improved (Abdellatif et al., 2020; Abdellatif, 2019). The most effective methods to reduce protein immunogenicity and to lengthen its half-life. By shielding immunogenic epitopes and increasing the drug's

hydrodynamic diameter, PEG reduces renal clearance and extends the circulation half-life (Goncalves and Caliceti, 2024; Abdellatif et al., 2022; Dozier and Distefano, 2015; Jevsevar et al., 2010). Another technique involves the injection of protease inhibitors, which interfere with the degradation of the peptide or protein in physiological fluids. This strategy involves the microenvironmental manipulation of the organism (Brown et al., 2019). Peptides and proteins' large size limits their penetration into biological barriers, leading to the development of penetration enhancers, such as sodium N-[8-(2-hydroxybenzoyl) amino caprylate] (SNAC), which improve gastric pH and transcellular absorption (Suzuki et al., 2020).

Antibodies dominate therapies, with over 500 clinical studies and 70 US approvals (Vargason et al., 2021). Antibodies neutralize target antigens, preventing unwanted cell processes. Antibodies can interact specifically with target cells, such as host immune cells. These processes can initiate phagocytosis, antibody-dependent cytotoxicity, or complement-dependent cytotoxicity, triggering cell death (Shih and Lindley, 2006; Smith, 2003).

Antibodies' unique features can lead to adverse effects such as rashes and autoimmune diseases. Advances in antibody manufacturing allowed the production of daclizumab, a humanized therapeutic antibody for multiple sclerosis, and adalimumab, an anti-TNF drug for rheumatoid arthritis (Bradbury et al., 2011; Mccafferty et al., 1990). PEGylation can improve the antibody half-life and immunogenicity, which can improve the clinical approval of some drugs, such as certolizumab pegol, for chronic inflammatory conditions (Chapman, 2002).

Antibody-drug conjugates (ADCs) have been made since antibodies are now a way to treat diseases. Using these conjugates, which mix antibodies with powerful tiny molecules, makes it possible to deliver drugs to specific places and boost the immune system (Bouazzaoui and Abdellatif, 2024; Bouazzaoui et al., 2021). For instance, DM1, a cytotoxic chemical, is

combined with humanized mAb trastuzumab in Kadcyla. ADCs have made it easier for tiny compounds such as calicheamicins, auristatins, and maytansinoids to be used in clinical settings (Beck et al., 2017; Alley et al., 2010).

NPs helps to deliver drugs via their surface structure and size manipulation. Using NPs to coat, encapsulate, overlay, and image medications results in intelligent antiviral systems (Abd Ellah et al., 2020, 2019). Theranostic nanoparticles (NPs) have been created to target COVID-19 with various anticoagulants, vaccinations, siRNA, and peptides (Itani et al., 2020). These NPs fall into three categories: liposomes, polymeric nanoparticles, and dendrimers. They can be organic, inorganic, or virus-like (Abdellatif et al., 2018).

NPs can target viral receptors by combining them with ligands, which may include hormones, peptides, or antibodies (Abdellatif, 2020; Abdellatif et al., 2020b). This conjugation results in the elimination (Abd Ellah et al., 2020). Smart targeting can be used to distribute polypeptides using inorganic nanostructures, such as quantum dots, gold, and silica nanoparticles that carry the anticoagulant on their surfaces or nanocarriers such as polymeric micelles, liposomes, and hydrogels (Lombardo et al., 2019). Vaccines boost the immune system against pathogens, with a messenger RNA (lipid nanoparticle-mRNA) vaccine are estimated based on studies of SARS-CoV and MERS. Conjugated NPs aim to provide more defense than a vaccine alone, sometimes stronger than natural infection, and with fewer health implications (Chan, 2020).

Formulation of liposomes (LNPs) can easily transport vaccines, RNA/DNA, and antibodies to target sites via cell entrance. LNPs have exterior catatonic membranes (Theobald, 2020). Solid LNPs have drawbacks like cell toxicity, liver and spleen accumulation, low hydrophilic drug payloads, and the reticuloendothelial system's ability to clear liposomes.

Scientists are exploring alternatives to deliver enough nucleic acid without unwanted side effects, addressing the challenge of delivering enough nucleic acid (Ghasemiyeh and Mohammadi-Samani, 2018). N4 Pharma has developed a new silica nanoparticle technology to produce cancer and viral vaccines using mRNA and pDNA. The technology, licensed by Queensland University, reduces daily dosage levels from three to one. The nanosilica can be functionalized with polyethylene to protect nucleic acids from nuclease enzymes, allowing for foreign/target protein synthesis and activating the immune system. This technology is used to deliver vaccines and medicines (Theobald, 2020).

3.3 ADVANCED DELIVERY TECHNOLOGIES

3.3.1 Selection of the Route of Administration

Oligonucleotide therapeutics are primarily developed through topical and local delivery, with most FDA-approved products being delivered locally. Only two FDA-approved products, patisiran and vutrisiran, are intravenously delivered, highlighting the challenges of systemic delivery. Patisiran is complexed within LNPs for liver delivery, while vutrisiran uses enhanced stabilization chemistry and GalNAc for liver targeting (FDA). Oligonucleotide-based drugs are administered via intravenous (IV), subcutaneous (SC), or intrathecal (IT) routes, depending on the intended application and target organ. FDA-approved drugs like Patisiran (Adams et al., 2018), Spinraza (Hoy, 2017), and Mipomersen (Wong and Goldberg, 2014) are delivered IV, SC, and IT. However, there are no FDA-approved locally delivered oligonucleotides for human diseases. Local routes of NAT administration are being investigated as potential alternatives (NIH)).

IV administration remains dominant in in vivo studies on animal models, despite local delivery advantages, due to technical feasibility, rapid results, and uniform distribution of dose to target organ cells (Younis et al., 2020). Despite IV administration being known to cause adverse effects in off-target organs and systemic effects like weight loss, there is increasing evidence supporting local administration in cancer treatment. Garbuzenko and co-workers conducted an *in vivo* comparative study between IV and IT delivery of ASO, siRNA, and a molecular drug, Doxorubicin, via PEGylated liposomes (Garbuzenko et al., 2009). Local intratracheal infusion of ASO and siRNA dramatically suppressed tumor development, demonstrating the successful targeting of nanoparticles (Garbuzenko et al., 2009). A delivery method for CpG oligodeoxynucleotide is an oligonucleotide that is utilized in immunotherapy for the treatment of cancer. It was observed that the release of cytokines was prolonged when CpG was complexed with polymeric nanoparticles and administered to mice using orotracheal instillation. Only a reduction in tumor growth was observed in mice that were treated with soluble CpG, whereas local administration of CpG nanoparticles resulted in a survival rate of one hundred percent. Following the initial dose, there was a noticeable weight reduction; however, this effect was only temporary (Perry et al., 2020).

3.3.2 Chemically Modified Oligonucleotides

Therapeutic oligonucleotides like ASO, siRNA, miRNA, and aptamers were developed from natural nucleotides. However, chemically modified nucleotides have improved NAT performance and overcome their limits. To improve pharmacokinetics and pharmacodynamics, sugar backbone or nitrogenous base changes can be made (Bost et al., 2021). Chemical modification protects oligonucleotides from binding, nucleases, and

degradation and extends their shelf life. Peptides, lipids, and aptamers can enhance selectivity and promote complexation with nanocarriers such as LNPs, polymeric nanoparticles, or biomimetic vectors (Metelev and Oretskaya, 2021; Khvorova and Watts, 2017). Example: Short interfering ribonucleic neutral molecules (siRNNs) are produced by chemically modifying siRNA at the nitrogenous base level by adding neutralizing, bioreversible phosphotriester groups. Cytoplasmic thioesterases can convert these stable, neutral siRNA molecules into native wild-type anionic siRNA molecules, allowing for in vivo distribution without the need for transfection vectors or carriers (Hamil and Dowdy, 2016). Another basic modification is phosphorothioate (PS), which replaces the oxygen atom that does not bridge the phosphate backbone with sulfur. This alteration improves oligonucleotide stability against nucleases and pharmacokinetics via binding to plasma proteins. This reduces renal clearance speed. The hydrophobicity of phosphorothioated oligonucleotides increases their cellular absorption rate compared to wild-type ones (Kher et al., 2011).

3.3.3 Nanocarriers

Oligonucleotides must be used to deliver target transcripts to cells and tissues. New delivery strategies are being developed to protect oligonucleotides in transit and boost cell targeting without decreasing cell entrance or intracellular bioavailability. If nanocarriers infiltrate cells, NATs should escape endosomes and enter the cytoplasm (Nakamura et al., 2022; Khalil et al., 2020). As a non-viral delivery method, LNPs, polymeric nanoparticles, biomimetic nanoparticles, and extracellular vesicles have exhibited good *in vivo* results in intravenous, subcutaneous, and intratracheal injections (Table 3.1).

TABLE 3.1

Nanoparticle-Delivered Oligonucleotide Therapies for Lung Cancer Preclinical Research

Oligonucleotide/polypeptides	Nanocarrier	References
miR-155 inhibitor	PEA-NPs	(Yang et al., 2018)
ASO	LNPs	(Cheng et al., 2018)
C274 and B1826 CpG ODNs on phosphorothioate	Liposomes	(Loira-Pastoriza et al., 2021)
CpG ODNs	Polymeric nanoparticles	(Perry et al., 2020)
ASO-G3139-GAP chemically modified	LNPs	(Cheng et al., 2017)
Targeting ligand EGFR aptamer	PLGA	(Zhang et al., 2020)
ASO	Liposomes	(Garbuzenko et al., 2010)
Phosphorothioate-linked antimiR-21	LNPs	(Yung et al., 2016)

Abbreviations: PEA, poly(ester amine); EGFR, epidermal growth factor receptor; MRP1, multidrug resistance-associated factor.

LNPs have become the highest standard for non-viral gene delivery vectors after the success of COVID-19 LNP vaccines. They are fast to make, have abundant precursor lipids, and resemble biological and cellular membranes (Abdellatif et al., 2023; Younis et al., 2023). Microfluidic mixing technology has evolved as a replacement for traditional preparation methods over the course of the last ten years. This technology has made it possible to produce LNPs in a scalable manner, with a high degree of homogeneity, particle size control, high loading capacity, and high encapsulation efficiency of nucleic acid cargos (Younis et al., 2021). The composition and physico-chemical properties of LNPs are altered to

manipulate their endogenous protein corona after in vivo administration, which recruits endogenous transport mechanisms for highly efficient and selective delivery to target cells. Thus, simple, affordable, and scalable delivery systems can replace ligand-based targeting techniques (Younis et al., 2023). Multifunctional envelope-type nanodevice (MEND) based on pH-sensitive cationic lipid YSK05 and modified with GALA peptide to target lung endothelium was synthesized. An aggressive mouse model of metastatic lung cancer showed substantial tumor elimination after loading MEND with siRNA targeting CD31 (Abd Elwakil et al., 2019).

Polymers are used to create nanoparticles as they are versatile, biodegradable, and easy to synthesize. Cationic polymers are promising for non-viral gene delivery systems because they are easily conjugated with genetic material. The entirety of these parameters, among others, includes the. Their ability is determined by their molecular structure, molecular weight and degree of surface charge. to be loaded with nucleic acids (Younis et al., 2022a). It was found out that an *in vivo,* polymeric NPs made of biodegradable PEA and hyaluronic acid can decrease the development of tumors formed in mice which received miR-155 inhibitors (Yang et al., 2018). Also, EGFR-targeting aptamer functionalized PLGA NPs were delivered. as an anticancer drug, homoharringtonine, with preference to lung cancer, showing the threat of oligonucleotides in tumor-targeting drug delivery (Zhang et al., 2020). Additionally, chitosan NPs were found to have a capacity to deliver miR-34a in cancer therapy and improve targeted anti-cancer activity. These systems portray the plasticity of chitosan as a polymer to. carrying anticancer drugs (Ragelle et al., 2016, 2014). Furthermore, the chitosan HCL has also showed great prospects as a carrier in enhancing drug delivery and absorption in the management of lung cancer (Nafee et al., 2007). Research has proven that PLGA nanoparticles are coated with chitosan hydrochloride, which facilitates the uptake of the medication in the

A549, and Calu3 cells as they reduced the cell survival of A549 (Taetz et al., 2009; Nafee et al., 2007).

The concept of biomimetic drug delivery systems emerged due to the transition that was made to overcome nature obstacles to learning nature and imitating nature paths. Thus, biomimetic nanoparticles are a novel mode of delivery of biologically active substances to tumors. Their ability to mimic the endogenous molecules reduces the activation of innate-immune systems and enhances local delivery of active drugs (Nakamura et al., 2022; Alhasan et al., 2022; Sheikhpour et al., 2017). The study by Vázquez-Ríos et al. (2019) provided proof-of-concept for biomimetic nanosystems (EMNs). The EMNs were designed to resemble A549-derived exosomes and functionalized with specific proteins. F-EMNs showed similar characteristics to natural exosomes, faster preparation, and higher batch-to-batch reproducibility. Hyaluronic acid (HA) has been extensively studied as a biomimicry material due to its unique ability to bind and activate the CD44 receptor, which is upregulated in several cancers (Mattheolabakis et al., 2015). There are various categories of medical and delivery methods. A total of five generations of therapy micro, small, molecules, peptides and proteins, antibodies, nucleic acids, and cell therapies (living cells) each has its own unique delivery issues (Figure 3.2A). Each of these generations of therapy has its own unique collection of delivery issues. The modification of the drug or of its surroundings (Figure 3.2B) and the engineering of drug delivery systems (Figure 3.2C) are two of the delivery strategies that have been developed because of these issues to increase the therapeutic function of the drug. Regardless of the type of treatment being administered, drug delivery systems have incorporated one or more mechanisms for modifying the drug composition or adjusting the surrounding environment.

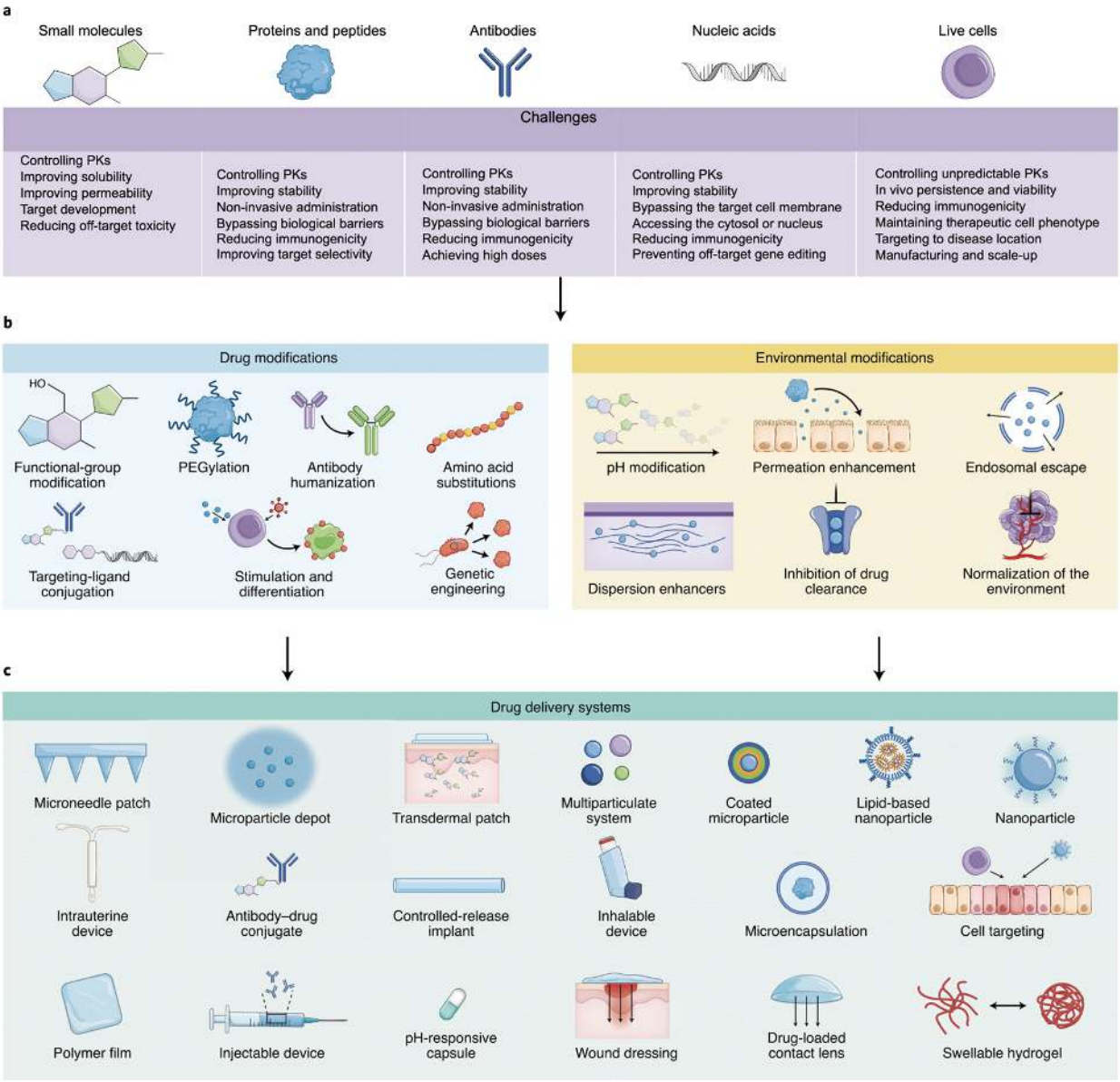

FIGURE 3.2 The five generations of therapy—micro, tiny, molecules, peptides and proteins, antibodies, nucleic acids, and cell therapies (living cells)—have different delivery issues (a). These issues have led to the development of drug and environment adaptation (b) and drug delivery system engineering (c) for improved therapeutic function. (This figure was published after approval from Vargason et al. (2021).) ⏎

3.4 POLYPEPTIDE CONJUGATES

These conjugates have shown a lot of potential to transform targeted drug. delivery, opening new possibilities of treating many ailments (Alshangiti et al., 2023). A conjugate of PPT is a type of molecule whereby a PPT chain is combined with another one to be used as a pharmacological activity or as a target moiety. Their biocompatibility, stability, and their variety functions (Darge et al., 2019). Bioengineering these molecules with pharmaceuticals or targeting agents enables the development of the PPT methods that convey certain therapeutic agents to the intended location, reducing off-target action and enhancing overall therapy. By combining purposeful fragments with conjugates, e.g., antibodies or peptides recognizing particular, these systems can be used to specifically home to target tissues of sick cells using biomarkers found on their surface, as healthy cells are inhibited to survive (Tewabe et al., 2021). With its capacity to target cytotoxic agents to cancer cells without damaging normal cells, this personalized method can have vast cancer treatment possibilities. Patients can report Much improved consequences and reduced side effects of conventional chemotherapy medication. The following are several reasons why colleagues ought to view the aforementioned case study as notable (Chehelgerdi et al., 2023; Tewabe et al., 2021).

Further, the conjugation of PPT with other polymers can also be used to promote the pharmacokinetic. This is the profile of the drug being administered and increases its stay in the body circulation (Glassman and Muzykantov, 2019). It develops sustained release preparations of long-term drug discharge that can lead to improved patient adherence and make it less frequent, in this way augmenting the care of chronic diseases (Ezike et al., 2023). We can create targeted molecular imaging reagents that detect markers of biological processes or disease in the body through the conjugation of imaging of contrast agents to PPTs. The administration of the drugs may be particularly useful in clinical decision making and monitoring

of treatment, and this attribute has important implications in such areas of study as molecular imaging involving an accurate detection and localization of infectious lesions (Pan et al., 2024; Tan et al., 2023; Liu et al., 2020). A key goal is optimize conjugates to ensure stability, biodegradability, and low immunogenicity. Moreover, a large amount of preclinical and clinical research will be required to determine the safety and efficacy of these new combinations prior to their application in practice (Clauss et al., 2023; Gong et al., 2022).

3.4.1 Active Targeting of Polypeptide Nanoparticles

It is believed that the active targeting of PPT-NPs could be a possible approach to delivering the transfer of medication and PPT to body tissues or areas. The synthesized PPT-NPs are taken into consideration. The attractive drug delivery system due to their bioavailability, biocompatibility. biodegradability, and programmable surface characteristics (Meszaros et al., 2023; Wu and Angelova, 2023). They are not accumulated passively in target tissues and therefore must be accumulated deliberately methods of enhancing the effectiveness of treatment (Costa et al., 2019; Sun et al., 2018).

PPT-NPs are ligand functionalized to selectively bind to certain cell receptors or overexpressed molecules. This strategy has the potential to increase the accumulation of NPs at the target location, enhancing the efficiency of drug delivery and decreasing off-target effects (Xia et al., 2018b). The flexibility of the ligand conjugation of PPTs is advantageous to active targeting. PPT-NPs may be conjugated and antibodies, peptides, aptamers, or small molecules using conjugation chemistry. This is the flexibility of PPT that enables the targeting strategy to be tailored to aim at

cell properties and the desired mode of action (Meszaros et al., 2023; Weber et al., 2021).

In addition, the prepared pH-sensitive PPT-NPs are applicable in the tumor which is acidic microenvironment targeted drug release, enhancing therapeutic effect, and reducing their toxicity (Xia et al., 2018a; Han et al., 2015). It was also discovered that the developed nanoformulation includes PPT which is actively targeted to cancer, inflammatory diseases, and infectious disorders. Killing tumor cells and sparing tissue damage has the potential to enhance safety and efficacy of cancer treatment (Liu et al., 2018; Matsumoto et al., 2014). Development of PPT-NPs operative in targeting, imaging, or stimuli responsive drug release may offer customized and specific treatment. This method can increase NP to enhance the deposition of the drug at the target site, enhance the delivery of drugs, and reduce off-target side effects. In active targeting, the most advantageous fact is that they are applicable in ligand conjugation (Meszaros et al., 2023; Weber et al., 2021). Active targeting PPT active targeting is broad existence of possible drug delivery areas, such as cancer, inflammatory diseases, and a number of disease areas, including infectious diseases. The safety and efficiency of the ability to target tumor cells would be of great benefit to the therapy of cancer injury of normal tissue (Liu et al., 2018; Matsumoto et al., 2014). Additionally, precision PPT-NPs developed based on dynamic targeting and combine it with therapy make this possible imaging capabilities. Ligand density optimization onto the surface of formulated NPs, creation of beneficial conjugation strategies and systematically predicting pharmacokinetics and biodistribution. Some of the challenges that should be overcome include preclinical and clinical backgrounds in active targeting of PPT to target cells (Sun et al., 2019). Costa et al. (2019) stated that a significant development in customized drug delivery is the ability to bind extrinsic moieties site-specifically without cross-reactivity. By combining an

artificial amino acid with nanobody-targeted, elastin-like PPT-NPs, novel therapeutic delivery options are made possible. Research indicates that compared to free medications, these techniques increase kidney accumulation, pharmacokinetics, biodistribution, and plasma half-life. This concentration was greater in the kidneys than in other organs, indicating transport unique to the kidneys (Abdellatif et al., 2021).

Also, it was reported that PPTs and advanced carriers improve kidney medication delivery in multiple ways. Medication targeting kidneys may improve kidney-related therapy outcomes by increasing drug concentration at the intended site of action. Drug distribution should minimize systemic side effects, and PPTs and sophisticated carriers may help achieve this goal. By targeting drugs to the kidneys, off-target effects in other organs are decreased, potentially enhancing treatment safety (Yang et al., 2021; Xue et al., 2020) (Figure 3.3). Additionally, employing cutting-edge carriers, including liposomes, chitosan, PVD, and G3c12 peptides, offers chances to get around obstacles related to traditional drug delivery techniques. These carriers can preserve drugs during renal transport and improve their stability and bioavailability, also modulating medication release, resulting in extended therapeutic effects and reduced dose (Abdellatif et al., 2021).

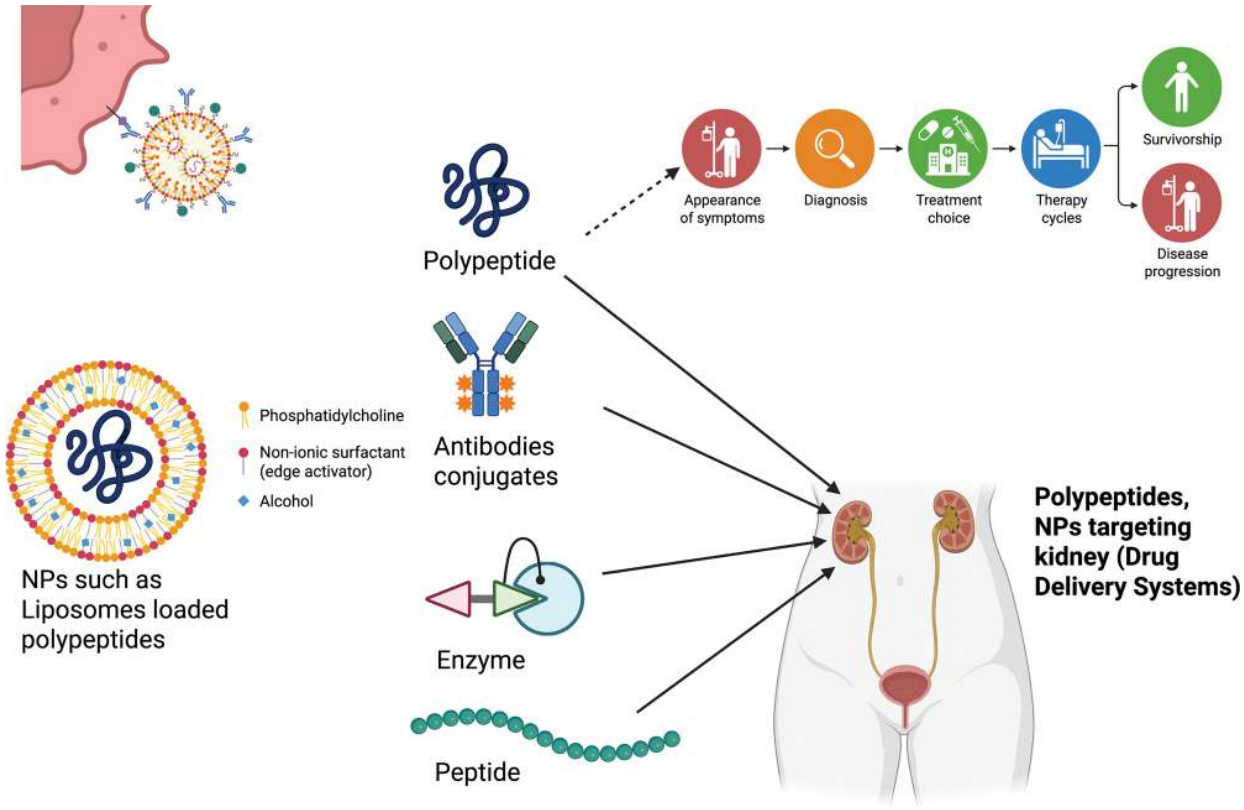

FIGURE 3.3 The PPTs and NPs internalize into cells, the different types of mechanisms of PPTs and NPs, and the endocytosis process of PPTs and NPs into cells. ⏎

3.5 INTERNALIZATION OF NANOPARTICLES IN MAMMALIAN CELLS: RATIONAL MECHANISMS AND IMPLICATIONS

There is a need to develop effective drug delivery strategies (Figure 3.3). NPs can be incorporated into cells, such as phagocytosis, macropinocytosis, clathrin-mediated endocytosis, caveolae-mediated endocytosis, and clathrin-caveolae-independent endocytosis (Verma et al., 2008; Mayor and Pagano, 2007; Conner and Schmid, 2003). Also, receptor-mediated endocytosis, where a ligand binds to its receptor, has NP internalization and plays an essential role.

For instance, studies have demonstrated that gold NPs (AuNPs) with varying sizes and shapes were internalized into HeLa cells via receptor-mediated endocytosis (Chithrani et al., 2006). Additionally, the internalization of folate conjugated to folic-PEG-coated-Qdots was internalized by folate receptors (Song et al., 2009). Furthermore, specific receptors can be targeted for NP internalization. Qdots conjugated with octreotide (OCT) were used to target somatostatin receptors (SSTRs) (Abdellatif, 2018). Also, AuNPs physically coated OCT through the van der Waals attraction forces were intended to target SSTRs subtype 2 with a great potential for receptor-specific targeting (Abdellatif et al., 2015). Studies have also investigated the internalization of AuNPs-somatostatin (SST) via electrostatic attraction through somatostatin receptors (Abdellatif et al., 2016). These findings emphasize the necessity of understanding NP internalization receptors and mechanisms for optimal drug delivery. Average endocytic vesicle sizes in phagocytosis and pinocytosis pathways for particle internalization are also shown (Mao et al., 2013). Phagocytes can take up large particles (or NP aggregates), opsonized NPs, or particles with specific ligand alterations via phagocytosis. The accepted mechanism for NPs internalization in a non-phagocytic mammalian cell is mostly through pinocytosis or direct diffusion (Gomes Dos Reis et al., 2020).

Moreover, with many different surface modifications, particles can be taken up via specific (receptor-mediated) endocytosis or nonspecific endocytosis (Mao et al., 2013). Receptor-mediated endocytosis permits the import of extracellular large molecules (Figure 3.3) (Kelf et al., 2010; Delehanty et al., 2009). In this process, specialized membrane micro-domains engulf the plasma membrane inwards, forming clathrin or caveolin-coated pits (Kelf et al., 2010). However, the specific uptake of NPs via surface receptors can be increased either by direct interactions between coated particles and receptors or via ligands attached to NPs (Kelf et al.,

2010; Hild et al., 2008; Osaki et al., 2004). Phagocytes can take up large particles, opsonized NPs, or particles with specific ligand alterations via phagocytosis. On the other hand, in non-phagocytic mammalian cells, NPs are primarily internalized through pinocytosis or direct diffusion processes (Li et al., 2024; Lima et al., 2022).

Surface alteration is also a significant phenomenon of NP internalization. NPs can be taken up by nonspecific endocytosis or specific (receptor-mediated) endocytosis. Receptor-mediated endocytosis is a cellular process that can import extracellular macromolecules by special membrane microdomains and loading pits with a coating of clathrin or pit coating with a coating of caveolin. This direct interfaces between coated particles and special absorption can be increased on NPs ligand- or receptor-mediated (Kelf et al., 2010; Delehanty et al., 2009). Delivering receptor-mediated endocytosis is updated by NPs to create the signaling of target diagnostics and therapeutics for the therapy of a high variety of disorders, including cancer and rheumatoid arthritis (Abdellatif, 2019).

3.5.1 Internalization of Polypeptide in Cells

The proposed biological mechanism in which PPT enters the plasma membrane of the endocytosis route is known as the mechanism of the cells (Figure 3.4A). This may take place in several ways, including clathrin-mediated endocytosis, caveolae-mediated endocytosis, and macropinocytosis, among others. These internal transduction pathways permit access of PPTs to cells. The biological functions are realized in (Figure 3.4B) (Hinuma et al., 2022; Carpentier, 1984). Some PPTs contain cell-penetrating peptide (CPP) sequences as well that enable them to get into the cell invade and enter into the cytoplasm. Mediation of PPT entry tocells may be done with these CPPs responding to lipids and membrane proteins.

This enables the PPTs to circumvent intracellular pathways (Xie et al., 2020). Understanding PPT internalization allows us to build and create cell-penetrating PPTs to improve peptide-based medicinal delivery and efficacy. This can transform drug delivery and improve cancer, neurological, and metabolic illness treatment (Rossino et al., 2023; Papadopoulos and Lennartsson, 2018).

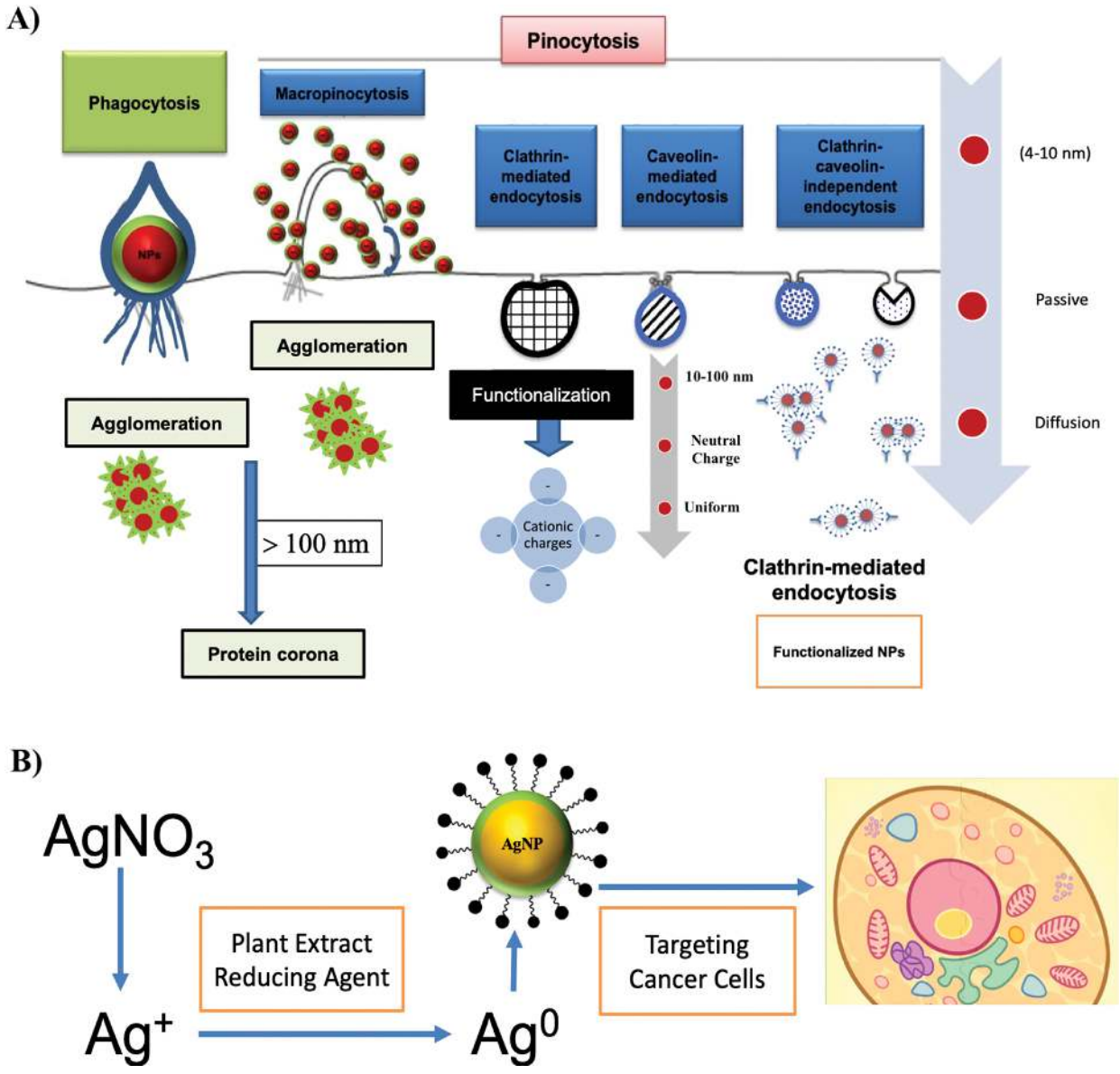

FIGURE 3.4 (a) Schematic representation of the mechanisms of NPs internalization through actively or passively receptor targeting. (b) The synthesis of AgNPs, silver nitrate reduced to AgNPs using plant extract for targeting of cancer cells. (This figure was taken from Abdellatif et al. (2024).)

3.5.2 Widespread Guide of Marketed Polypeptides

The FDA has approved the following polypeptides for sale. As biopharmaceuticals advance, novel polypeptide-based therapies may address unmet medical needs and improve global healthcare outcomes.

1. Insulin is a well-known PPT hormone that is used in the management of diabetes. There are many various marketed formulations of insulin, such as Humulin, Novolin, Lantus, and Levemir, that have been approved by the FDA (FDA 1991; Ferrer-Miralles et al., 2009).
2. Erythropoietin is a hormone that can stimulate the production of red blood cells. It has been industrialized as a recombinant PPT for the treatment of anemia accompanying chronic kidney disease, chemotherapy, and certain other conditions. Erythropoietin is approved as Epogen and Procrit by the FDA, which has impacted the management of anemia (Powe et al., 1992).
3. Growth hormone (Recombinant human growth hormone (rhGH)), such as Genotropin, Norditropin, and Humatrope, has been approved by the FDA for the treatment of growth disorders in children and hormone-related deficiencies in adults (Goldyn et al., 2011).
4. Glucagon-like peptide-1, which is a GLP-1 receptor agonist (Byetta, Victoza, Trulicity). It is a class of PPT-based drugs that are used in the treatment of type 2 diabetes. Byetta, Victoza, and

Trulicity are marketed medications that are approved by FDA-approved for glucose control and weight management in diabetic patients (Cao et al., 2023).

5. Granulocyte is a colony-stimulating factor (Neupogen, Neulasta). It is considered that factors (G-CSFs) are used to stimulate the production of white blood cells, particularly neutrophils. It is also used to reduce the risk of infection in patients undergoing certain types of cancer treatments. Neupogen and Neulasta have FDA approval, which is essential for the treatment of chemotherapy-induced neutropenia (Brave et al., 2010).
6. Interferons are considered a group of cytokines with immunomodulatory properties. They have been harnessed as PPT therapeutics for multiple sclerosis, certain types of cancers, and viral infections. The FDA approved the interferon products, such as Avonex, Betaseron, Pegasys, and Intron A, for patients facing complex medical challenges (Sondak and Kudchadkar, 2012; Summers et al., 2010).

This chapter emphasizes the significance of FDA-approved PPT as high point in biopharmaceuticals, showing their importance in modern medicine. This resource informs and inspires polypeptide therapeutic research, innovation, and clinical use, helping patients, and healthcare communities worldwide.

From a critical perspective, we examined the FDA's medication market and approvals databases, as well as other pertinent publications (FDA, Morigi et al., 2012, Drew and Aloba, n.d.; Zhong et al., 2018; Bobo et al., 2016) (Table 3.2). Despite occupying a limited fraction of the marketed products, nanomedicines have represented about 14% of the recent FDA approvals in the last two decades, which reflects rapidly growing

development if compared with other well-established dosage forms such as oral capsules, solutions, or topical formulations. LNPs were the top-ranked among FDA-approved nanomedicines (~27%), while the other types of nanomedicines represented proportions comparable to each other. LNPs have attracted attention due to their ease of preparation, scalability, and high delivery efficiency, taking into consideration their structures mimicking cellular membranes (Khalil et al., 2020). Furthermore, they are expected to attract massive attention after the recent approval of COVID-19 vaccines based on LNPs.

TABLE 3.2

List of Nanomedicines Approved by the FDA for Human Use (Younis et al., 2022b)

Product name	Description	Indication	Approval year	Manufacturer
Copaxone®	*Glatiramer* Acetate polypeptide polymer	Multiple sclerosis	1996	Teva
Curosurf®	Poractant alfa pulmonary surfactant	Respiratory distress syndrome	1999	Chiesi USA
Pegasys®	PEGylated IFN-α-2a	Viral hepatitis B, C	2002	Genentech
Neulasta®	PEGylated filgrastim	Chemotherapy-induced neutropenia	2002	Amgen
Estrasorb®	Micellar estradiol	Menopausal vasomotor symptoms	2003	Novavax
Emend®	Aprepitant nanocrystals	Antiemetic	2003	Merck

Product name	Description	Indication	Approval year	Manufacturer
Macugen®	PEGylated oligonucleotide (pegaptanib)	Neovascular macular degeneration	2004	Bausch and Lomb
Eligard®	Leuprolide acetate-loaded polymeric NPs	Prostate cancer	2004	Tolmar
Abraxane®	Nanoparticle albumin–bound paclitaxel (nab-PTX)	Non-small cell lung cancer, metastatic breast cancer, metastatic pancreatic cancer	2005	Celgene
Oncaspar®	PEGylated aspargase	Acute lymphoblastic leukemia	2006	Baxalta U.S.
Somatuline Depot®	Lanreotide acetate-loaded nanotube	Acromegaly	2007	Ipsen
PegIntron®	PEGylated IFN-α-2a	Viral hepatitis C	2008	Merck
Cimzia®	PEGylated antibody targeting TNFα	Crohn's disease	2008	UCB
Plegridy®	PEGylated IFN-β-1a	Multiple sclerosis	2014	Biogen
Ryanodex®	Nanocrystalline dantrolene sodium	skeletal muscle relaxant in malignant hyperthermia	2014	Eagle Pharmaceuticals
Adynovate®	PEGylated recombinant protein	Hemophilia	2015	Baxalta US Inc
Patisiran®	siRNA-loaded LNPs	Hereditary transthyretin-mediated amyloidosis	2018	Alnylam Pharmaceuticals

Product name	Description	Indication	Approval year	Manufacturer
Mircera®	PEGylated epoetin beta	Anemia in chronic renal diseases	2018	Vifor
Moderna COVID-19 Vaccine®	mRNA-loaded LNPs	Vaccination against COVID-19	2020	ModernaTX, Inc
Pfizer-BioNTech COVID-19 Vaccine®	mRNA-loaded LNPs	Vaccination against COVID-19	2020	Pfizer

Abbreviations: mRNA, messenger RNA; siRNA, small interfering RNA; GalNAc, N-acetylgalactosamine; IFN, interferon; TNFα, tumor necrosis factor alpha; HIV, human immunodeficiency virus. These acronyms refer to different types of biomolecules.

3.6 FUTURE PERSPECTIVES

Nanotechnology and biomaterials can be used to build next-generation delivery platforms that improve PPT therapeutic pharmacokinetics and pharmacodynamics (Ochubiojo et al., 2012). NPs-loaded PPTs are attracting biotechnology attention for drug delivery, tissue engineering, and therapies. PPTs and NPs offer a possible path to new therapeutics. Discussing the future of NP-loaded PPTs is crucial to exploring their potential (Hassan et al., 2021; Nishiyama and Kataoka, 2005).

Personalized medicine is a promising application of NP-loaded PPTs. Customizing medicine delivery systems to patient needs could improve treatment outcomes. Personalized medicine will become more available as nanotechnology and PPT behavior improve, enabling precision targeting of

diseased tissues while limiting harm to healthy cells (Singh and Sharma, 2023). Furthermore, NP-loaded PPTs' future is tied to regenerative medicine. The regulated release of growth factors and other bioactive compounds from NP-loaded PPTs can promote tissue regeneration and repair. Regulated secretion of growth factors and other bioactive compounds of NP-loaded PPTs are able to enhance repair and regeneration of tissues. The property will transform the property bone deformities, skin traumas, and neurological diseases treatment (Waris et al., 2022).

NP-capped PPTs can also include, in addition to customized medicine and regenerative medicine used to treat cancer. Transportation of therapeutic drugs to the cancerous cells without destruction of healthy cells is something that oncologists have been striving to achieve. The personalized nature and features are customizable mechanisms of delivery that are NP-capped PPTs offer a new solution to cancer treatment problems. These sophisticated techniques will be used in future research to create highly selective and powerful anticancer drugs are promising (Su et al., 2023; Liu et al., 2021).

Vaccine development and immunotherapy are part of the future of NPs-capped PPTs. A unique type of NP-based vaccine delivery systems, using immunostimulatory PPTs, is exceptional. immune-boosting approach. Such a design can enhance infectious disease vaccines and cancer vaccines. Particular delivery of antigens and adjuvants may be beneficial in vaccination and immunotherapy by adjusting immune functions (Alexander et al., 2002). NP-capped PPTs have a high potential to come up with a new generation of biotechnologies that would revolutionize healthcare and medicine.

3.7 CONCLUSIONS

To sum up, PPTs that are applied during therapeutics are efficient to be administered in a target-based manner. The peculiarity of this new method of delivery is that it can be used to treat a big variety of illnesses, which is growing with advancement in research and technology, and this promises a promising future of medical care and personalized medical care.

REFERENCES

Abd Ellah, N. H., Gad, S. F., Muhammad, K., G, E. B. & Hetta, H. F. 2020. Nanomedicine as a promising approach for diagnosis, treatment and prophylaxis against COVID-19. *Nanomedicine (Lond)*, 15, 2085–2102.

Abd Ellah, N. H., Tawfeek, H. M., John, J. & Hetta, H. F. 2019. Nanomedicine as a future therapeutic approach for Hepatitis C virus. *Nanomedicine (Lond)*, 14, 1471–1491.

Abd Elwakil, M. M., Khalil, I. A., Elewa, Y. H. A., Kusumoto, K., Sato, Y., Shobaki, N., Kon, Y. & Harashima, H. 2019. Lung-endothelium-targeted nanoparticles based on a pH-sensitive lipid and the GALA peptide enable robust Gene silencing and the regression of metastatic lung cancer. *Adv Funct Mater*, 29, 1807677.

Abdellatif, A. A. 2018. Targeting of somatostatin receptors expressed in blood cells using quantum dots coated with vapreotide. *Saudi Pharm J*, 26(8):1162–1169.

Abdellatif, A. A., El Rasoul, S. A. & Osman, S. 2015. Gold nanoparticles decorated with octreotide for somatostatin receptors targeting. *Int J Pharm Sci Res*, 7, 14–20.

Abdellatif, A. A., Zayed, G., El-Bakry, A., Zaky, A., Saleem, I. Y. & Tawfeek, H. M. 2016. Novel gold nanoparticles coated with somatostatin as a potential delivery system for targeting somatostatin receptors. *Drug Dev Ind Pharm*, 42(11), 1782–1791.

Abdellatif, A. A. H. 2019. Identification of somatostatin receptors using labeled PEGylated octreotide, as an active internalization. *Drug Dev Ind Pharm*, 45, 1707–1715.

Abdellatif, A. A. H. 2020. A plausible way for excretion of metal nanoparticles via active targeting. *Drug Dev Ind Pharm*, 46, 744–750.

Abdellatif, A. A. H., Aldalaen, S. M., Faisal, W. & Tawfeek, H. M. 2018. Somatostatin receptors as a new active targeting sites for nanoparticles. *Saudi Pharm J*, 26, 1051–1059.

Abdellatif, A. A. H., Hennig, R., Pollinger, K., Tawfeek, H. M., Bouazzaoui, A. & Goepferich, A. 2020a. Fluorescent nanoparticles coated with a somatostatin analogue target blood monocyte for efficient leukaemia treatment. *Pharm Res*, 37, 217.

Abdellatif, A. A. H., Ibrahim, M. A., Amin, M. A., Maswadeh, H., Alwehaibi, M. N., Al-Harbi, S. N., Alharbi, Z. A., Mohammed, H. A., Mehany, A. B. M. & Saleem, I. 2020b. Cetuximab conjugated with octreotide and entrapped calcium alginate-beads for targeting somatostatin receptors. *Sci Rep*, 10, 4736.

Abdellatif, A. A. H., Mohammed, H. A., Khan, R. A., Singh, V., Bouazzaoui, A., Yusuf, M., Akhtar, N., Khan, M., Al-Subaiyel, A., Mohammed, S. A. A. & Al-Omar, M. S. 2021. Nano-scale delivery: A comprehensive review of nano-structured devices, preparative techniques, site-specificity designs, biomedical applications, commercial products, and references to safety, cellular uptake, and organ toxicity. *Nanotechnol Rev*, 10, 1493–1559.

Abdellatif, A. A. H., Mostafa, M. A. H., Konno, H. & Younis, M. A. 2024. Exploring the green synthesis of silver nanoparticles using natural extracts and their potential for cancer treatment. *3 Biotech*, 14, 274.

Abdellatif, A. A. H., Tolba, N. S., Alsharidah, M., Al Rugaie, O., Bouazzaoui, A., Saleem, I., Maswadeh, H. & Ali, A. T. 2022. PEG-4000 formed polymeric nanoparticles loaded with cetuximab downregulate p21 & stathmin-1 gene expression in cancer cell lines. *Life Sci*, 295, 120403.

Abdellatif, A. A. H., Younis, M. A., Alsowinea, A. F., Abdallah, E. M., Abdel-Bakky, M. S., Al-Subaiyel, A., Hassan, Y. A. H. & Tawfeek, H. M. 2023. Lipid nanoparticles technology in vaccines: Shaping the future of prophylactic medicine. *Colloids Surf B Biointerfaces*, 222, 113111.

Adams, D., Gonzalez-Duarte, A., O'riordan, W. D., Yang, C.-C., Ueda, M., Kristen, A. V., Tournev, I., Schmidt, H. H., Coelho, T., Berk, J. L., Lin, K.-P., Vita, G., Attarian, S., Planté-Bordeneuve, V., Mezei, M. M., Campistol, J. M., Buades, J., Brannagan, T. H., Kim, B. J., Oh, J., Parman, Y.,

Sekijima, Y., Hawkins, P. N., Solomon, S. D., Polydefkis, M., Dyck, P. J., Gandhi, P. J., Goyal, S., Chen, J., Strahs, A. L., Nochur, S. V., Sweetser, M. T., Garg, P. P., Vaishnaw, A. K., Gollob, J. A. & Suhr, O. B. 2018. Patisiran, an RNAi therapeutic, for hereditary transthyretin amyloidosis. *N Engl J Med*, 379, 11–21.

Alexander, J., Oseroff, C., Dahlberg, C., Qin, M., Ishioka, G., Beebe, M., Fikes, J., Newman, M., Chesnut, R. W., Morton, P. A., Fok, K., Appella, E. & Sette, A. 2002. A decaepitope polypeptide primes for multiple CD8+ IFN-gamma and th lymphocyte responses: Evaluation of multiepitope polypeptides as a mode for vaccine delivery. *J Immunol*, 168, 6189–6198.

Alhasan, H., Terkawi, M. A., Matsumae, G., Ebata, T., Tian, Y., Shimizu, T., Nishida, Y., Yokota, S., Garcia-Martin, F., M, M. A. E., Takahashi, D., Younis, M. A., Harashima, H., Kadoya, K. & Iwasaki, N. 2022. Inhibitory role of Annexin A1 in pathological bone resorption and therapeutic implications in periprosthetic osteolysis. *Nat Commun*, 13, 3919.

Allen, T. M. & Cullis, P. R. 2013. Liposomal drug delivery systems: From concept to clinical applications. *Adv Drug Deliv Rev*, 65, 36–48.

Alley, S. C., Okeley, N. M. & Senter, P. D. 2010. Antibody-drug conjugates: Targeted drug delivery for cancer. *Curr Opin Chem Biol*, 14, 529–537.

Alshangiti, D. M., El-Damhougy, T. K., Zaher, A., Madani, M. & Mohamady Ghobashy, M. 2023. Revolutionizing biomedicine: Advancements, applications, and prospects of nanocomposite macromolecular carbohydrate-based hydrogel biomaterials: A review. *RSC Adv*, 13, 35251–35291.

Barenholz, Y. 2012. Doxil(R)–the first FDA-approved nano-drug: Lessons learned. *J Control Release*, 160, 117–34.

Beck, A., Goetsch, L., Dumontet, C. & Corvaia, N. 2017. Strategies and challenges for the next generation of antibody-drug conjugates. *Nat Rev Drug Discov*, 16, 315–337.

Betush, R. J., Urban, J. M. & Nilsson, B. L. 2018. Balancing hydrophobicity and sequence pattern to influence self-assembly of amphipathic peptides. *Biopolymers*, 110, e23099.

Birshtein, T. M., Goryunov, A. N. & Turbovich, M. L. 1974. Selection of the parameters of intramolecular interactions on the basis of analyzing potential maps and conformations of polypeptides. *Mol Biol*, 7, 560–569.

Bobo, D., Robinson, K. J., Islam, J., Thurecht, K. J. & Corrie, S. R. 2016. Nanoparticle-based medicines: A review of FDA-approved materials and clinical trials to Date. *Pharm Res*, 33, 2373–2387.

Bost, J. P., Barriga, H., Holme, M. N., Gallud, A., Maugeri, M., Gupta, D., Lehto, T., Valadi, H., Esbjörner, E. K., Stevens, M. M. & El-Andaloussi, S. 2021. Delivery of oligonucleotide therapeutics: Chemical modifications, lipid nanoparticles, and extracellular vesicles. *ACS Nano*, 15, 13993–14021.

Bouazzaoui, A. & Abdellatif, A. A. H. 2024. Vaccine delivery systems and administration routes: Advanced biotechnological techniques to improve the immunization efficacy. *Vaccine X*, 19, 100500.

Bouazzaoui, A., Abdellatif, A. A. H., Al-Allaf, F. A., Bogari, N. M., Al-Dehlawi, S. & Qari, S. H. 2021. Strategies for vaccination: Conventional vaccine approaches versus new-generation strategies in combination with adjuvants. *Pharmaceutics*, 13, 140.

Bradbury, A. R., Sidhu, S., Dubel, S. & Mccafferty, J. 2011. Beyond natural antibodies: The power of in vitro display technologies. *Nat Biotechnol*, 29, 245–254.

Brave, M., Farrell, A., Ching Lin, S., Ocheltree, T., Pope Miksinski, S., Lee, S. L., Saber, H., Fourie, J., Tornoe, C., Booth, B., Yuan, W., He, K., Justice, R. & Pazdur, R. 2010. FDA review summary: Mozobil in combination with granulocyte colony-stimulating factor to mobilize hematopoietic stem cells to the peripheral blood for collection and subsequent autologous transplantation. *Oncology*, 78, 282–288.

Brown, T. D., Whitehead, K. A. & Mitragotri, S. 2019. Materials for oral delivery of proteins and peptides. *Nat Rev Mater*, 5, 127–148.

Bruno, B. J., Miller, G. D. & Lim, C. S. 2013. Basics and recent advances in peptide and protein drug delivery. *Ther Deliv*, 4, 1443–1467.

Cao, M., Pan, C., Tian, Y., Wang, L., Zhao, Z. & Zhu, B. 2023. Glucagon-like peptide 1 receptor agonists and the potential risk of pancreatic carcinoma: A pharmacovigilance study using the FDA Adverse Event Reporting System and literature visualization analysis. *Int J Clin Pharm*, 45, 689–697.

Carpentier, J. L. 1984. Receptor-mediated endocytosis of polypeptide hormones. *Acta Histochem Suppl*, 29, 57–60.

Chan, W. C. W. 2020. Nano research for COVID-19. *ACS Nano*, 14, 3719–3720.

Chapman, A. P. 2002. PEGylated antibodies and antibody fragments for improved therapy: A review. *Adv Drug Deliv Rev*, 54, 531–545.

Chehelgerdi, M., Chehelgerdi, M., Allela, O. Q. B., Pecho, R. D. C., Jayasankar, N., Rao, D. P., Thamaraikani, T., Vasanthan, M., Viktor, P., Lakshmaiya, N., Saadh, M. J., Amajd, A., Abo-Zaid, M. A., Castillo-Acobo, R. Y., Ismail, A. H., Amin, A. H. & Akhavan-Sigari, R. 2023. Progressing nanotechnology to improve targeted cancer treatment: Overcoming hurdles in its clinical implementation. *Mol Cancer*, 22, 169.

Cheng, X., Liu, Q., Li, H., Kang, C., Liu, Y., Guo, T., Shang, K., Yan, C., Cheng, G. & Lee, R. J. 2017. Lipid nanoparticles loaded with an antisense oligonucleotide gapmer against Bcl-2 for treatment of lung cancer. *Pharm Res*, 34, 310–320.

Cheng, X., Yu, D., Cheng, G., Yung, B. C., Liu, Y., Li, H., Kang, C., Fang, X., Tian, S., Zhou, X., Liu, Q. & Lee, R. J. 2018. T7 peptide-conjugated lipid nanoparticles for dual modulation of Bcl-2 and Akt-1 in lung and cervical carcinomas. *Mol Pharm*, 15, 4722–4732.

Chithrani, B. D., Ghazani, A. A. & Chan, W. C. W. 2006. Determining the size and shape dependence of gold nanoparticle uptake into mammalian cells. *Nano Letters*, 6, 662–668.

Clauss, Z. S., Meudom, R., Su, B., Vandenberg, M. A., Saini, S. S., Webber, M. J., Chou, D. H. & Kramer, J. R. 2023. Supramolecular protein stabilization with zwitterionic polypeptide-cucurbit[7]uril conjugates. *Biomacromolecules*, 24, 481–488.

Conner, S. D. & Schmid, S. L. 2003. Regulated portals of entry into the cell. *Nature*, 422, 37–44.

Costa, S. A., Mozhdehi, D., Dzuricky, M. J., Isaacs, F. J., Brustad, E. M. & Chilkoti, A. 2019. Active targeting of cancer cells by nanobody decorated polypeptide micelle with bio-orthogonally conjugated drug. *Nano Lett*, 19, 247–254.

Darge, H. F., Andrgie, A. T., Tsai, H. C. & Lai, J. Y. 2019. Polysaccharide and polypeptide based injectable thermo-sensitive hydrogels for local biomedical applications. *Int J Biol Macromol*, 133, 545–563.

Delehanty, J. B., Mattoussi, H. & Medintz, I. L. 2009. Delivering quantum dots into cells: Strategies, progress and remaining issues. *Anal Bioanal Chem*, 393, 1091–105.

Donia, M., Osman, R., Awad, G. A. S. & Mortada, N. 2020. Polypeptide and glycosaminoglycan polysaccharide as stabilizing polymers in nanocrystals for a safe ocular hypotensive effect. *Int J Biol Macromol*, 162, 1699–1710.

Dozier, J. K. & Distefano, M. D. 2015. Site-specific PEGylation of therapeutic proteins. *Int J Mol Sci*, 16, 25831–25864.

Drew, A. & Aloba, O. n.d.. *Improving NDA Approval Odds for New Dosage Forms of Approved Products* [Online]. Available: https://camargopharma.com/resources/blog/improving-nda-approval-odds-new-dosage-forms-approved-products/ [Accessed 2021/02/03].

Emeje, M. O., Obidike, I. C., Akpabio, E. I., & Ofoefule, S. I. 2012. Nanotechnology in drug delivery. *In* Sezer, A. D. (ed.): *Recent Advances in Novel Drug Carrier Systems*. Intechopen.

Ezike, T. C., Okpala, U. S., Onoja, U. L., Nwike, C. P., Ezeako, E. C., Okpara, O. J., Okoroafor, C. C., Eze, S. C., Kalu, O. L., Odoh, E. C., Nwadike, U. G., Ogbodo, J. O., Umeh, B. U., Ossai, E. C. & Nwanguma, B. C. 2023. Advances in drug delivery systems, challenges and future directions. *Heliyon*, 9, e17488.

Ferrer-Miralles, N., Domingo-Espín, J., Corchero, J. L., et al. 2009. Microbial factories for recombinant pharmaceuticals. *Microb Cell Fact*, 8, 17. https://doi.org/10.1186/1475-2859-8-17

Food and Drug Administration. (1991). Fees for certifying insulin drugs—FDA. Interim ruleopportunity for public comment. *Fed Regist*, 56, 50248–50249.

Garbuzenko, O. B., Saad, M., Betigeri, S., Zhang, M., Vetcher, A. A., Soldatenkov, V. A., Reimer, D. C., Pozharov, V. P. & Minko, T. 2009. Intratracheal versus intravenous liposomal delivery of siRNA, antisense oligonucleotides and anticancer drug. *Pharm Res*, 26, 382–394.

Garbuzenko, O. B., Saad, M., Pozharov, V. P., Reuhl, K. R., Mainelis, G. & Minko, T. 2010. Inhibition of lung tumor growth by complex pulmonary delivery of drugs with oligonucleotides as suppressors of cellular resistance. *Proc Natl Acad Sci USA*, 107, 10737–10742.

Ge, C., Wang, Z., Wang, Y. & Wei, M. 2025. Peptide-based therapeutic and delivery strategies for inflammatory bowel disease: Challenges and future directions. *RSC Adv*, 15, 25560–25578.

Ghasemiyeh, P. & Mohammadi-Samani, S. 2018. Solid lipid nanoparticles and nanostructured lipid carriers as novel drug delivery systems: Applications, advantages and disadvantages. *Res Pharm Sci*, 13, 288–303.

Glassman, P. M. & Muzykantov, V. R. 2019. Pharmacokinetic and pharmacodynamic properties of drug delivery systems. *J Pharmacol Exp Ther*, 370, 570–580.

Goldyn, A. K., Nabhan, Z. M. & Eugster, E. A. 2011. A comparison of referral patterns to the pediatric endocrine clinic before and after FDA approval of growth hormone for idiopathic short stature. *J Pediatr Endocrinol Metab*, 24, 89–91.

Gomes Dos Reis, L., Lee, W. H., Svolos, M., Moir, L. M., Jaber, R., Engel, A., Windhab, N., Young, P. M. & Traini, D. 2020. Delivery of pDNA to lung epithelial cells using PLGA nanoparticles formulated with a cell-penetrating peptide: Understanding the intracellular fate. *Drug Dev Ind Pharm*, 46, 427–442.

Goncalves, J. & Caliceti, P. 2024. Optimizing pharmacological and immunological properties of therapeutic proteins through PEGylation: Investigating key parameters and their impact. *Drug Des Devel Ther*, 18, 5041–5062.

Gong, L., Yang, Z., Zhang, F. & Gao, W. 2022. Cytokine conjugates to elastin-like polypeptides. *Adv Drug Deliv Rev*, 190, 114541.

Hamil, A. S. & Dowdy, S. F. 2016. Synthesis and conjugation of small interfering ribonucleic neutral SiRNNs. *Methods Mol Biol*, 1364, 1–9.

Han, S. S., Li, Z. Y., Zhu, J. Y., Han, K., Zeng, Z. Y., Hong, W., Li, W. X., Jia, H. Z., Liu, Y., Zhuo, R. X. & Zhang, X. Z. 2015. Dual-pH sensitive charge-reversal polypeptide micelles for tumor-triggered targeting uptake and nuclear drug delivery. *Small*, 11, 2543–2554.

Hassan, N. U., Chaudhery, I., Ur. Rehman, A. & Ahmed, N. 2021. Polymeric nanoparticles used in tissue engineering. *In: Advances in Polymeric Nanomaterials for Biomedical Applications* (pp. 191–224). London: Elsevier.

He, Q., Wen, L., Li, Q., Xu, J. & Luo, F. 2007. Fabrication of alginate microsphere for controlled release and investigation of its release characteristics in vitro. *Sheng Wu Yi Xue Gong Cheng Xue Za Zhi*, 24, 1301–1304.

Hild, W. A., Breunig, M. & Goepferich, A. 2008. Quantum dots - Nano-sized probes for the exploration of cellular and intracellular targeting. *Eur J Pharm Biopharm*, 68, 153–168.

Hinuma, S., Fujita, K. & Kuroda, S. 2022. Specific binding and endocytosis of liposomes to HEK293T cells via myrisoylated pre-S1 peptide bound to sodium taurocholate cotransporting polypeptide. *Vaccines (Basel)*, 10, 1846.

Hoy, S. M. 2017. Nusinersen: First global approval. *Drugs*, 77, 473–479.

Huang, J., Bonduelle, C., Thevenot, J., Lecommandoux, S. & Heise, A. 2012. Biologically active polymersomes from amphiphilic glycopeptides. *J Am Chem Soc*, 134, 119–122.

Itani, R., Tobaiqy, M. & Al Faraj, A. 2020. Optimizing use of theranostic nanoparticles as a life-saving strategy for treating COVID-19 patients. *Theranostics*, 10, 5932–5942.

Jevsevar, S., Kunstelj, M. & Porekar, V. G. 2010. PEGylation of therapeutic proteins. *Biotechnol J*, 5, 113–128.

Kelf, T. A., Sreenivasan, V. K. A., Sun, J., Kim, E. J., Goldys, E. M. & Zvyagin, A. V. 2010. Non-specific cellular uptake of surface-functionalized quantum dots. *Nanotechnology*, 21, 285105.

Kempf, D. J., Sham, H. L., Marsh, K. C., Flentge, C. A., Betebenner, D., Green, B. E., Mcdonald, E., Vasavanonda, S., Saldivar, A., Wideburg, N. E., Kati, W. M., Ruiz, L., Zhao, C., Fino, L., Patterson, J., Molla, A., Plattner, J. J. & Norbeck, D. W. 1998. Discovery of ritonavir, a potent

inhibitor of HIV protease with high oral bioavailability and clinical efficacy. *J Med Chem*, 41, 602–617.

Khalil, I. A., Younis, M. A., Kimura, S. & Harashima, H. 2020. Lipid nanoparticles for cell-specific *in vivo* targeted delivery of nucleic acids. *Biol Pharm Bull*, 43, 584–595.

Kher, G., Trehan, S. & Misra, A. 2011. 7 - Antisense oligonucleotides and RNA interference. *In:* Misra, A. (ed.) *Challenges in Delivery of Therapeutic Genomics and Proteomics*. London: Elsevier.

Khvorova, A. & Watts, J. K. 2017. The chemical evolution of oligonucleotide therapies of clinical utility. *Nat Biotechnol*, 35, 238–248.

Lau, J. L. & Dunn, M. K. 2018. Therapeutic peptides: Historical perspectives, current development trends, and future directions. *Bioorg Med Chem*, 26, 2700–2707.

Li, Y., Moein Moghimi, S. & Simberg, D. 2024. Complement-dependent uptake of nanoparticles by blood phagocytes: Brief overview and perspective. *Curr Opin Biotechnol*, 85, 103044.

Lima, T., Gunnarsson, S. B., Coelho, E., Evtuguin, D. V., Correia, A., Coimbra, M. A., Cedervall, T. & Vilanova, M. 2022. Beta-glucan-functionalized nanoparticles down-modulate the proinflammatory response of mononuclear phagocytes challenged with Candida albicans. *Nanomaterials (Basel)*, 12, 3439.

Liu, C., Zhang, X., Han, X., Fang, Y., Liu, X., Wang, X., Waterhouse, G. I. N., Xu, C., Yin, H. & Gao, X. 2020. Polypeptide-templated au nanoclusters with red and blue fluorescence emissions for multimodal imaging of cell nuclei. *ACS Appl Bio Mater*, 3, 1934–1943.

Liu, G., Wang, M., He, H. & Li, J. 2021. Doxorubicin-loaded tumor-targeting peptide-decorated polypeptide nanoparticles for treating primary orthotopic colon cancer. *Front Pharmacol*, 12, 744811.

Liu, Y., Song, Z., Zheng, N., Nagasaka, K., Yin, L. & Cheng, J. 2018. Systemic siRNA delivery to tumors by cell-penetrating alpha-helical polypeptide-based metastable nanoparticles. *Nanoscale*, 10, 15339–15349.

Loira-Pastoriza, C., Vanvarenberg, K., Ucakar, B., Machado Franco, M., Staub, A., Lemaire, M., Renauld, J. C. & Vanbever, R. 2021. Encapsulation of a CpG oligonucleotide in cationic liposomes enhances its local antitumor activity following pulmonary delivery in a murine model of metastatic lung cancer. *Int J Pharm*, 600, 120504.

Lombardo, D., Kiselev, M. A. & Caccamo, M. T. 2019. Smart nanoparticles for drug delivery application: Development of versatile nanocarrier platforms in biotechnology and nanomedicine. *J Nanomater*, 2019, 1–26.

Mao, Z., Zhou, X. & Gao, C. 2013. Influence of structure and properties of colloidal biomaterials on cellular uptake and cell functions. *Biomater Sci*, 1, 896–911.

Matsumoto, R., Hara, R., Andou, T., Mie, M. & Kobatake, E. 2014. Targeting of EGF-displayed protein nanoparticles with anticancer drugs. *J Biomed Mater Res B Appl Biomater*, 102, 1792–1798.

Mattheolabakis, G., Milane, L., Singh, A. & Amiji, M. M. 2015. Hyaluronic acid targeting of CD44 for cancer therapy: From receptor biology to nanomedicine. *J Drug Target*, 23, 605–618.

Mayor, S. & Pagano, R. E. 2007. Pathways of clathrin-independent endocytosis. *Nat Rev Mol Cell Biol*, 8, 603–612.

Mccafferty, J., Griffiths, A. D., Winter, G. & Chiswell, D. J. 1990. Phage antibodies: Filamentous phage displaying antibody variable domains. *Nature*, 348, 552–554.

Meszaros, M., Phan, T. H. M., Vigh, J. P., Porkolab, G., Kocsis, A., Pali, E. K., Polgar, T. F., Walter, F. R., Bolognin, S., Schwamborn, J. C., Jan, J. S., Deli, M. A. & Veszelka, S. 2023. Targeting human endothelial cells with glutathione and alanine increases the crossing of a polypeptide nanocarrier through a blood-brain barrier model and entry to human brain organoids. *Cells*, 12, 618.

Metelev, V. G. & Oretskaya, T. S. 2021. Modified oligonucleotides: New structures, new properties, and new spheres of application. *Russ J Bioorg Chem*, 47, 339–343.

Morigi, V., Tocchio, A., Bellavite Pellegrini, C., Sakamoto, J. H., Arnone, M. & Tasciotti, E. 2012. Nanotechnology in medicine: From inception to market domination. *J Drug Deliv*, 2012, 389485.

Nafee, N., Taetz, S., Schneider, M., Schaefer, U. F. & Lehr, C. M. 2007. Chitosan-coated PLGA nanoparticles for DNA/RNA delivery: Effect of the formulation parameters on complexation and transfection of antisense oligonucleotides. *Nanomedicine*, 3, 173–183.

Nakamura, T., Sato, Y., Yamada, Y., Abd Elwakil, M. M., Kimura, S., Younis, M. A. & Harashima, H. 2022. Extrahepatic targeting of lipid nanoparticles in vivo with intracellular targeting for future nanomedicines. *Adv Drug Deliv Rev*, 188, 114417.

NIH. 2023. *Oligonucleotides Clinical Trials* [Online]. Available: https://clinicaltrials.gov/ct2/results?cond=&term=oligonucleotide&cntry=&state=&city=&dist= [Accessed 2023/02/02].

Nishiyama, N. & Kataoka, K. 2005. Medical applications of nanotechnology: Polymeric micelles for drug delivery. *Nihon Geka Gakkai Zasshi*, 106, 700–705.

Osaki, F., Kanamori, T., Sando, S., Sera, T. & Aoyama, Y. 2004. A quantum dot conjugated sugar ball and its cellular uptake on the size effects of endocytosis in the subviral region. *J Am Chem Soc*, 126, 6520–6521.

Pan, W., Zhang, L., Li, L., Cen, J., Song, R., Song, C., Zhang, G., Hu, J. & Liu, S. 2024. Engineering semicarbazide-bearing polypeptide conjugates for efficient tumor chemotherapy and imaging of tumor metastasis. *Adv Mater*, 36, e2309315.

Papadopoulos, N. & Lennartsson, J. 2018. The PDGF/PDGFR pathway as a drug target. *Mol Aspects Med*, 62, 75–88.

Peer, D., Karp, J. M., Hong, S., Farokhzad, O. C., Margalit, R. & Langer, R. 2020. Nanocarriers as an emerging platform for cancer therapy. *In: Nano-Enabled Medical Applications* (pp. 61–91). Jenny Stanford Publishing.

Perry, J. L., Tian, S., Sengottuvel, N., Harrison, E. B., Gorentla, B. K., Kapadia, C. H., Cheng, N., Luft, J. C., Ting, J. P., Desimone, J. M. & Pecot, C. V. 2020. Pulmonary delivery of nanoparticle-bound toll-like receptor 9 agonist for the treatment of metastatic lung cancer. *ACS Nano*, 14, 7200–7215.

Powe, N. R., Griffiths, R. I., De Lissovoy, G., Anderson, G. F., Watson, A. J., Greer, J. W., Herbert, R. J., Eggers, P. W., Milam, R. A. & Whelton, P. K. 1992. Access to recombinant erythropoietin by

Medicare-entitled dialysis patients in the first year after FDA approval. *JAMA*, 268, 1434–1440.

Ragelle, H., Riva, R., Vandermeulen, G., Naeye, B., Pourcelle, V., Le Duff, C. S., D'haese, C., Nysten, B., Braeckmans, K., De Smedt, S. C., Jérôme, C. & Préat, V. 2014. Chitosan nanoparticles for siRNA delivery: Optimizing formulation to increase stability and efficiency. *J Control Release*, 176, 54–63.

Ragelle, H., Vanvarenberg, K., Vandermeulen, G. & Préat, V. 2016. Chitosan Nanoparticles for SiRNA Delivery In Vitro. *In:* Shum, K. & Rossi, J. (eds.) *SiRNA Delivery Methods: Methods and Protocols*. New York: Springer New York.

Rapaka, R. S. & Urry, D. W. 1978. Coacervation of sequential polypeptide models of tropoelastin. Synthesis of H-(Val-Ala-Pro-Gly)n-Val-OMe and H-(Val-Pro-Gly-Gly)n-Val-OMe. *Int J Pept Protein Res*, 11, 97–108.

Rossino, G., Marchese, E., Galli, G., Verde, F., Finizio, M., Serra, M., Linciano, P. & Collina, S. 2023. Peptides as therapeutic agents: Challenges and opportunities in the green transition era. *Molecules*, 28, 6032.

Schmidt, B., Ribnicky, D. M., Poulev, A., Logendra, S., Cefalu, W. T. & Raskin, I. 2008. A natural history of botanical therapeutics. *Metabolism*, 57, S3–S9.

Sheikhpour, M., Barani, L. & Kasaeian, A. 2017. Biomimetics in drug delivery systems: A critical review. *J Control Release*, 253, 97–109.

Shih, T. & Lindley, C. 2006. Bevacizumab: An angiogenesis inhibitor for the treatment of solid malignancies. *Clin Ther*, 28, 1779–802.

Singh, S. & Sharma, H. 2023. Emerging applications of nanotechnology in drug delivery and medical imaging: Review. *Curr Radiopharm*, 16, 269–283.

Smith, M. R. 2003. Rituximab (monoclonal anti-CD20 antibody): Mechanisms of action and resistance. *Oncogene*, 22, 7359–7368.

Sondak, V. K. & Kudchadkar, R. 2012. Pegylated interferon for the adjuvant treatment of melanoma: FDA approved, but what is its role? *Oncologist*, 17, 1223–1224.

Song, E. Q., Zhang, Z. L., Luo, Q. Y., Lu, W., Shi, Y. B. & Pang, D. W. 2009. Tumor cell targeting using folate-conjugated fluorescent quantum dots and receptor-mediated endocytosis. *Clinical Chemistry*, 55, 955–963.

Stepanova, M., Nikiforov, A., Tennikova, T. & Korzhikova-Vlakh, E. 2023. Polypeptide-based systems: From synthesis to application in drug delivery. *Pharmaceutics*, 15, 2102.

Su, Y., Xu, W., Wei, Q., Ma, Y., Ding, J. & Chen, X. 2023. Chiral polypeptide nanoparticles as nanoadjuvants of nanovaccines for efficient cancer prevention and therapy. *Sci Bull (Beijing)*, 68, 284–294.

Summers, J., Cohen, M. H., Keegan, P. & Pazdur, R. 2010. FDA drug approval summary: Bevacizumab plus interferon for advanced renal cell carcinoma. *Oncologist*, 15, 104–111.

Sun, H., Gu, X., Zhang, Q., Xu, H., Zhong, Z. & Deng, C. 2019. Cancer nanomedicines based on synthetic polypeptides. *Biomacromolecules*, 20, 4299–4311.

Sun, M., Guo, J., Hao, H., Tong, T., Wang, K. & Gao, W. 2018. Tumour-homing chimeric polypeptide-conjugated polypyrrole nanoparticles for imaging-guided synergistic photothermal and chemical therapy of cancer. *Theranostics*, 8, 2634–2645.

Suzuki, R., Brown, G. A., Christopher, J. A., Scully, C. C. G. & Congreve, M. 2020. Recent developments in therapeutic peptides for the glucagon-like peptide 1 and 2 receptors. *J Med Chem*, 63, 905–927.

Taetz, S., Nafee, N., Beisner, J., Piotrowska, K., Baldes, C., Mürdter, T. E., Huwer, H., Schneider, M., Schaefer, U. F., Klotz, U. & Lehr, C. M. 2009. The influence of chitosan content in cationic chitosan/PLGA nanoparticles on the delivery efficiency of antisense 2′-O-methyl-RNA directed against telomerase in lung cancer cells. *Eur J Pharm Biopharm*, 72, 358–369.

Tan, L., Huan, R., Wu, L. F., Bao, Y., Ma, Y. C., Zou, Q. L. & Yong, J. 2023. Peptide-based nanomaterials: Self-assembly and applications. *Mini Rev Med Chem*, 23, 399–411.

Tewabe, A., Abate, A., Tamrie, M., Seyfu, A. & Abdela Siraj, E. 2021. Targeted drug delivery - from magic bullet to nanomedicine: Principles, challenges, and future perspectives. *J Multidiscip Healthc*, 14, 1711–1724.

Theobald, N. 2020. Emerging vaccine delivery systems for COVID-19: Functionalised silica nanoparticles offer a potentially safe and effective alternative delivery system for DNA/RNA vaccines and may be useful in the hunt for a COVID-19 vaccine. *Drug Discovery Today*, 25(9), 1556.

Thwala, L. N., Delgado, D. P., Leone, K., Marigo, I., Benetti, F., Chenlo, M., Alvarez, C. V., Tovar, S., Dieguez, C., Csaba, N. S. & Alonso, M. J. 2018. Protamine nanocapsules as carriers for oral peptide delivery. *J Control Release*, 291, 157–168.

Vargason, A. M., Anselmo, A. C. & Mitragotri, S. 2021. The evolution of commercial drug delivery technologies. *Nat Biomed Eng*, 5, 951–967.

Vázquez-Ríos, A. J., Molina-Crespo, Á., Bouzo, B. L., López-López, R., Moreno-Bueno, G. & De La Fuente, M. 2019. Exosome-mimetic nanoplatforms for targeted cancer drug delivery. *J Nanobiotechnology*, 17, 85.

Verma, A., Uzun, O., Hu, Y. H., Hu, Y., Han, H. S., Watson, N., Chen, S. L., Irvine, D. J. & Stellacci, F. 2008. Surface-structure-regulated cell-membrane penetration by monolayer-protected nanoparticles. *Nature Materials*, 7, 588–595.

Wang, X., Song, Z., Wei, S., Ji, G., Zheng, X., Fu, Z. & Cheng, J. 2021. Polypeptide-based drug delivery systems for programmed release. *Biomaterials*, 275, 120913.

Wang, Y., Zhang, L., Liu, C., Luo, Y. & Chen, D. 2024. Peptide-mediated nanocarriers for targeted drug delivery: Developments and strategies. *Pharmaceutics*, 16, 220.

Waris, A., Ali, A., Khan, A. U., Asim, M., Zamel, D., Fatima, K., Raziq, A., Khan, M. A., Akbar, N., Baset, A. & Abourehab, M. A. S. 2022. Applications of various types of nanomaterials for the treatment of neurological disorders. *Nanomaterials*, 12, 1949.

Weber, P., Dzuricky, M., Min, J., Jenkins, I. & Chilkoti, A. 2021. Concentration-independent multivalent targeting of cancer cells by genetically encoded core-crosslinked Elastin/Resilin-like polypeptide micelles. *Biomacromolecules*, 22, 4347–4356.

Wong, E. & Goldberg, T. 2014. Mipomersen (kynamro): A novel antisense oligonucleotide inhibitor for the management of homozygous familial hypercholesterolemia. *P t*, 39, 119–122.

Wu, Y. & Angelova, A. 2023. Recent uses of lipid nanoparticles, cell-penetrating and bioactive peptides for the development of brain-targeted nanomedicines against neurodegenerative disorders. *Nanomaterials (Basel)*, 13, 1066.

Xia, F., Hou, W., Zhang, C., Zhi, X., Cheng, J., De La Fuente, J. M., Song, J. & Cui, D. 2018a. pH-responsive gold nanoclusters-based nanoprobes for lung cancer targeted near-infrared fluorescence imaging and chemo-photodynamic therapy. *Acta Biomater*, 68, 308–319.

Xia, Y., Wang, C., Xu, T., Li, Y., Guo, M., Lin, Z., Zhao, M. & Zhu, B. 2018b. Targeted delivery of HES5-siRNA with novel polypeptide-modified nanoparticles for hepatocellular carcinoma therapy. *RSC Adv*, 8, 1917–1926.

Xie, J., Bi, Y., Zhang, H., Dong, S., Teng, L., Lee, R. J. & Yang, Z. 2020. Cell-penetrating peptides in diagnosis and treatment of human diseases: From preclinical research to clinical application. *Front Pharmacol*, 11, 697.

Xu, P. Y., Kankala, R. K., Wang, S. B. & Chen, A. Z. 2022. Development of highly stable ICG-polymeric nanoparticles with ultra-high entrapment efficiency using supercritical antisolvent (SAS)-combined solution casting process. *Int J Pharm*, 629, 122348.

Xue, W., Trital, A., Shen, J., Wang, L. & Chen, S. 2020. Zwitterionic polypeptide-based nanodrug augments pH-triggered tumor targeting via prolonging circulation time and accelerating cellular internalization. *ACS Appl Mater Interfaces*, 12, 46639–46652.

Yang, P., Guo, Q., Xu, S. T., Sheng, D. Y. & Zhang, Q. Z. 2021. Constructing brain abeta-targeting nanoparticles loaded with EGCG for treating Alzheimer's disease in mice. *Sichuan Da Xue Xue Bao Yi Xue Ban*, 52, 605–611.

Yang, S., Wang, M., Wang, T., Sun, M., Huang, H., Shi, X., Duan, S., Wu, Y., Zhu, J. & Liu, F. 2023. Self-assembled short peptides: Recent advances and strategies for potential pharmaceutical applications. *Mater Today Bio*, 20, 100644.

Yang, Y., Jia, Y., Xiao, Y., Hao, Y., Zhang, L., Chen, X., He, J., Zhao, Y. & Qian, Z. 2018. Tumor-targeting anti-MicroRNA-155 delivery based on biodegradable poly(ester amine) and hyaluronic acid shielding for lung cancer therapy. *Nanoscale*, 19, 2058–2069.

Younis, M. A., Khalil, I. A., Elewa, Y. H. A., Kon, Y. & Harashima, H. 2021. Ultra-small lipid nanoparticles encapsulating sorafenib and midkine-siRNA selectively-eradicate sorafenib-resistant hepatocellular carcinoma in vivo. *J Control Release*, 331, 335–349.

Younis, M. A., Khalil, I. A. & Harashima, H. 2020. Gene therapy for hepatocellular carcinoma: Highlighting the journey from theory to clinical applications. *Adv Ther*, 3, 2000087.

Younis, M. A., Sato, Y., Elewa, Y. H. A., Kon, Y. & Harashima, H. 2023. Self-homing nanocarriers for mRNA delivery to the activated hepatic stellate cells in liver fibrosis. *J Control Release*, 353, 685–698.

Younis, M. A., Tawfeek, H. M., Abdellatif, A. A. H., Abdel-Aleem, J. A. & Harashima, H. 2022a. Clinical translation of nanomedicines: Challenges, opportunities, and keys. *Adv Drug Deliv Rev*, 181, 114083.

Younis, M. A., Tawfeek, H. M., Abdellatif, A. A. H., Abdel-Aleem, J. A. & Harashima, H. 2022b. Clinical translation of nanomedicines: Challenges, opportunities, and keys. *Adv Drug Deliv Rev*, 181, 114083.

Yung, B. C., Li, J., Zhang, M., Cheng, X., Li, H., Yung, E. M., Kang, C., Cosby, L. E., Liu, Y., Teng, L. & Lee, R. J. 2016. lipid nanoparticles composed of quaternary amine-tertiary amine cationic lipid combination (QTsome) for therapeutic delivery of AntimiR-21 for lung cancer. *Mol Pharm*, 13, 653–662.

Zhang, Z., Cheng, W., Pan, Y. & Jia, L. 2020. An anticancer agent-loaded PLGA nanomedicine with glutathione-response and targeted delivery for the treatment of lung cancer. *J Mater Chem B*, 8, 655–665.

Zhong, H., Chan, G., Hu, Y., Hu, H. & Ouyang, D. 2018. A comprehensive map of FDA-approved pharmaceutical products. *Pharmaceutics*, 10, 263.

Zhu, J., Liu, H., Cai, X., Wu, W., Zhu, Z. & Yu, L. 2021. Preparation and characterization of instant casein phosphopeptide by supercritical fluid assisted atomization. *Foods*, 10, 2147.

4 Advances in Multilayer Nanoarchitectures and Activatable Protein Nanoparticles for Precision Medicine

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4.1 INTRODUCTION

4.1.1 Overview of Precision Medicine

Precision medicine is a patient-centric clinical framework that aims to tailor medical decisions, diagnostics and therapeutics to the individual biological, environmental, and lifestyle profile of each patient rather than relying on "average population" responses (Collins & Varmus, 2015). It integrates multi-layer data such as genomics, epigenomics, transcriptomics, proteomics, metabolomics, imaging features, and digital phenotypes to define patient-specific disease pathways and treatment susceptibility. The development of modern bioinformatics pipelines, AI-assisted multi-omics

analytics and large-scale biobanks are major enablers converting precision medicine from theoretical framework to clinical practice (Figure 4.1) (Shao et al., 2024).

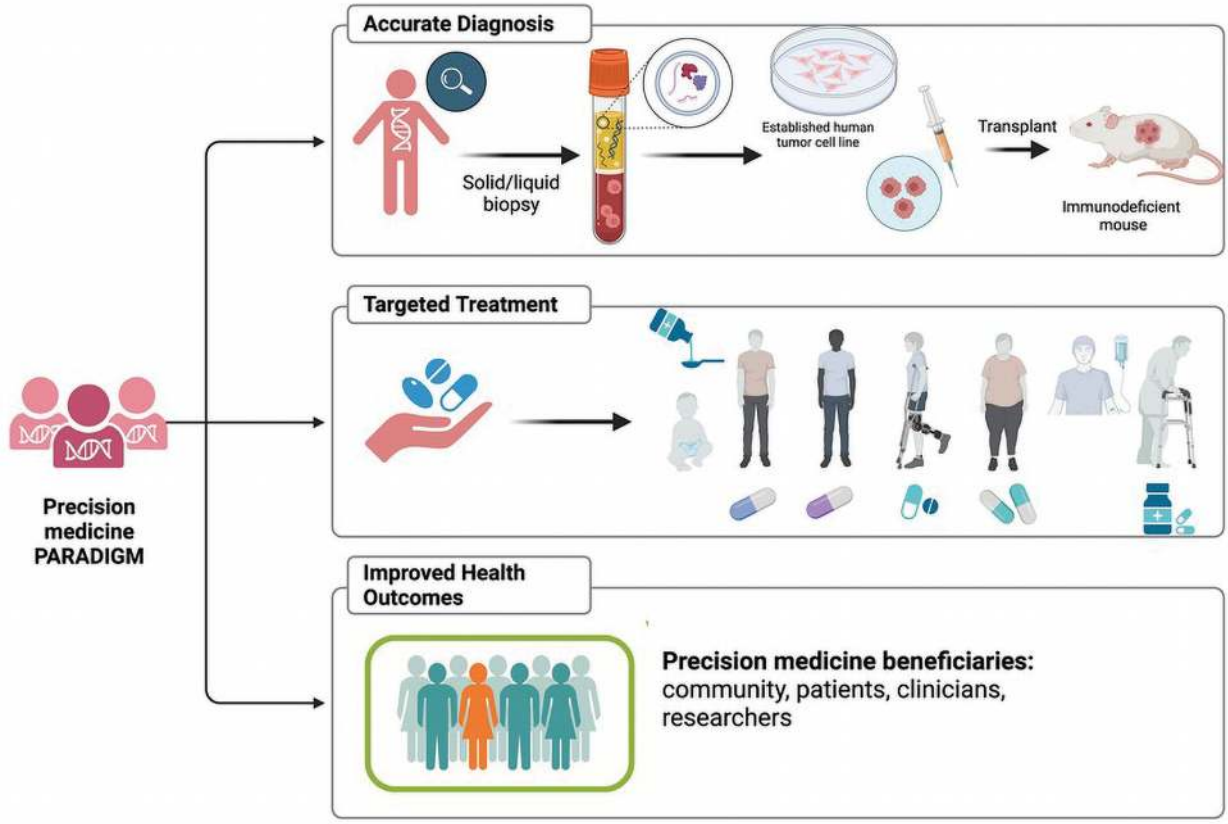

FIGURE 4.1 Precision medicine paradigm. Current approaches for precision medicine often involve assessment of various cancer drugs including chemotherapy, targeted therapies, or immunotherapy and others using patient derived tissue cancer cells or models such as spheroid or organoid, as well as orthotopic murine xenograft models. With the rise of AI-based systems and technology platforms, it is anticipated that this process can be accelerated. Created with BioRender.com. (Accessed on 18 January 2024, 2024). (Ref. Carini, C. & Seyhan, A. (2024). Tribulations and future opportunities for artificial intelligence in precision medicine. *J Transl Med*, 22. https://doi.org/10.1186/s12967-024-05067-0) ⏎

Clinical application of precision medicine is now evident in oncology, for example, genetic profiling guides selection of targeted inhibitors; in cardiology, biomarker stratification supports risk-based therapeutics, in endocrinology like precision diabetes phenotyping and in infectious diseases as pathogen genome-guided antimicrobial selection (Yi et al., 2025; Younis et al., 2022).

4.1.2 Role of Nanotechnology in Medicine

Nanotechnology has become a central enabling technology in modern medicine as nanoparticles (NPs) can be manufactured at size scales comparable to biomolecular structures such as proteins, receptors and cellular membranes allowing them to interact with biological systems with superior specificity (Abdellatif et al., 2018, 2023; Hayley Nehoff et al., 2014). The capacity to control surface chemistry, hydrophobic/hydrophilic balance, ligand presentation, and particle stiffness enables programmable interactions with diseased tissues and microenvironments, especially in solid tumors (Hayley Nehoff et al., 2014; Ramesh et al., 2022). Nanotechnology is integrated into precision diagnostics. NP-based biosensors and nano-enabled liquid biopsy platforms can detect ultra-low analyte concentrations, leveraging enhanced surface area and quantum-scale optical or electrochemical signatures. Additionally, magnetic NPs are used to improve bio-imaging and provide image-guided drug delivery and hyperthermia therapy (Abdellatif et al., 2023, 2024; Mohsin et al., 2022).

4.2 ACTIVATABLE PROTEIN NANOPARTICLES (APNPS)

4.2.1 Concept and Mechanisms

Activatable protein nanoparticles (APNPs) are protein-based supramolecular structures in which the therapeutic function is masked during systemic circulation and subsequently become functionally activated only when exposed to disease-specific biochemical triggers in the pathological microenvironment. Activation generally results in exposure of a hidden functional motif (e.g., cell-penetrating peptide, receptor-binding domain), structural transformation or disassembly, or catalytic/prodrug conversion from an inactive to an active therapeutic form (Zhao et al., 2021). APNPs are designed to leverage the intricate physiological and pathological cues within the body. By responding to specific stimuli like pH of the system, catalytic action of enzymes, or oxidation–reduction potential, these systems achieve site-specific release, enhancing desired efficacy of treatment while diminishing possible off-target effects and toxicity (Luo et al., 2024). In addition, Protease-cleavable linkers (e.g. MMP-2/9: PLGLAG, cathepsin B: GFLG) are positioned between a masking segment and the bioactive domain. In circulation, the mask blocks affinity sterically/electrostatically. Local proteolysis in tumor ECM removes the mask and exposes the binding motif. This has enabled “Pro-antibodies” and masked cytokines with 10^2–10^3 fold activity jumps only inside tumor protease pockets (Li et al., 2021). Moreover, enzyme-activated prodrug conversion is a logic in which the cytotoxic pharmacophore is chemically caged (masked) in its OFF-state by a labile promoiety that suppresses binding, solubility or electrophilicity. The nanocarrier or soluble precursor remains pharmacologically dormant during systemic circulation. Upon accumulation in the tumor microenvironment, selected disease-overexpressed enzymes (most commonly MMP-2/9, cathepsin B, legumain, β-glucuronidase, NAD(P)H quinone oxidoreductase 1 [NQO1], esterases) catalyze controlled bond cleavage—removing the prosthetic group and restoring the innate reactivity of the parent drug. The specificity arises from the fact that these enzymes are (i) overexpressed in

stromal CAF niches, necrotic cores, acidic lysosomes or hypoxic gradients, and (ii) display faster turnover locally than in blood → giving local pharmacological amplification rather than global exposure. In polymeric and dendritic nanomedicine, this principle is frequently implemented by embedding the promoiety directly in the carrier (e.g. cathepsin-B-cleavable GFLG in PAMAM/polyaspartamide) so that prodrug conversion and carrier disassembly occur coherently, which increases spatial synchrony between activation and payload release. A major advantage is that enzyme-triggered conversion can shift apparent therapeutic index by ≥10× because the rate determining step is now an endogenous kinetic gate rather than passive diffusion. However, the challenge remains OFF-state leakage—background cleavage by low basal enzyme in liver, spleen, or serum—thus current engineering focuses on sequence tuning to raise the activation energy for OFF-pathway hydrolysis while maintaining high catalytic efficiency (kcat/Km) under tumor protease micro-conditions (Abdellatif et al., 2023; Bai et al., 2022; Younis et al., 2025).

4.2.2 Design Principles

4.2.2.1 Responsive Elements

Representative elements used in APNPs mainly include pH-switchable amino-acid blocks, protease-cleavable peptides, redox cleavable bonds, hypoxia-reductive motifs, and ROS-labile linkers. The most common examples are MMP substrates such as PLGLAG, GPLGVRG, and GPLGIAGQ, which are selectively cleaved by MMP-2/MMP-9 in tumor stroma, and these sequences are widely used to “mask” RGD or apoptosis peptides and only expose them upon MMP processing inside tumors (Yu Xiao et al., 2024). pH-responsive motifs usually rely on protonation of histidine (pKa ≈6.0), and therefore, HIS-rich blocks (e.g. HHHHH) are

incorporated to undergo coil-to-α-helix shifts or to promote endosomal escape in slightly acidic tumor or endosomal pH (Chang et al., 2021). Glutamic-acid rich blocks can similarly modulate conformation at low pH. Disulfide S–S crosslinks act as redox responsive elements, which is stable in blood but rapidly cleaved in the reductive cytosol, thus used for intracellular activation (Lee et al., 2013). ROS-responsive thioketal linkers are chemical bonds that are stable under normal conditions but break in the presence of reactive oxygen species (ROS), a hallmark of certain diseases like cancer. This property makes them useful for targeted drug delivery systems, as they release drugs in a localized area by cleaving the linker and separating the therapeutic agent from a carrier, like a NP. Thioketals are generally biocompatible and biodegradable, and their cleavage is a non-toxic process that can be triggered by ROS such as hydrogen peroxide (Rinaldi et al., 2022). Schematic illustration of the chemical structure and corresponding release mechanisms of TK-based ROS-responsive nanosystems is shown in Figure 4.2.

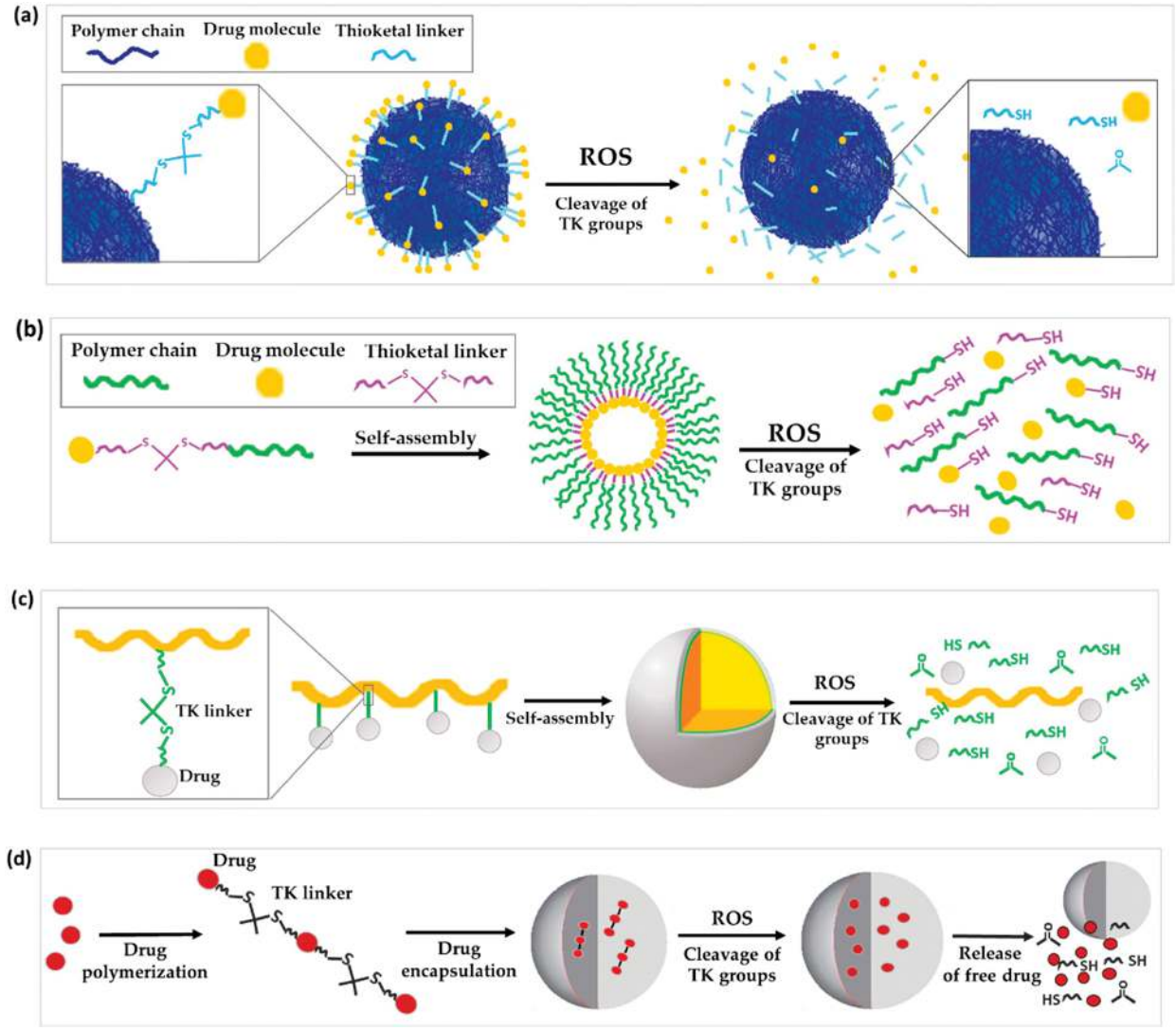

FIGURE 4.2 Schematic illustration of the chemical structure and corresponding release mechanisms of TK-based ROS-responsive nanosystems, where TK is used as (a) linker to attach drugs to the surface of nanoparticles, (b) linkage to form prodrug-based NPs, (c) linker to form polyprodrug-based NPs with drug molecules attached to the same polymer unit by TK linkages or (d) linker to form encapsulated polyprodrugs based on multiple drug molecules attached to each other via TK bridges. (Rinaldi et al., 2022) ⏎

4.2.2.2 Stability and Activation Thresholds

The main engineering challenge is to maintain structural integrity during blood circulation while ensuring activation only in the pathological microenvironment. Ideally, drug nanocarriers should not only solubilize hydrophobic anticancer drugs but also remain in circulation for a prolonged period to be introduced into tumor tissue and thus improve the efficacy of cancer therapy. Coating the surface of nanocarriers with a hydrophilic polymer constitutes the most common approach to prolong the circulation time of polymeric nanocarriers in the bloodstream. Poly(ethylene glycol) (PEG) is the polymer most commonly used to coat nanocarriers, a process known as PEGylation (Liu et al., 2014). This approach minimizes nanocarrier interaction with components of biological fluids in the bloodstream and avoids their detection by the mononuclear phagocytic system (MPS) and subsequent clearance by the kidneys (Masood, 2016). Multilayer shell engineering involves the precise deposition of alternating material layers around a NP core, primarily using the layer-by-layer (LbL) assembly technique. This method offers a high degree of control and versatility, allowing for the creation of smart, multifunctional nanomedicines with enhanced efficacy and safety for applications such as targeted drug delivery, medical imaging, and cancer therapy, where outer layers shield the activatable protein core until tumor-specific chemical gradients peel away the outer coating (Seth Rowland et al., 2024).

4.3 MULTILAYER NANOARCHITECTURES

4.3.1 Definition and Significance

The nanoarchitectures refer to the branch of combining the nanotechnology with other fields like biotechnology, organic chemistry, and supramolecular chemistry (Abdellatif et al., 2023; Ariga, 2023). These nanoarchitectures

have a complicated structure of multi-component materials compared with nanofabrication of single or few components (Ariga et al., 2019). Generally, nanoarchitectures resulted from a process called nanoarchitectonics which refers to the process of creating functional materials using nanoscale units and nanotechnology concepts. The prepared organized structure and specific design is considered the final product of nanoarchitectonics which could be in different arrangements and shapes like nanosheets, nanotubes, or complex integrated arrangement. Nanoarchitectonics based on self-assembly of atoms and molecules to develop new nanomaterials with a well-organized structure (nanoarchitectures) with specific and unique characters (Oviedo et al., 2022). They also classified according to the dimension structure of electro confinement to 0D, 1D, 2D, and 3D nanoarchitectonics (Gupta et al., 2022).

Nanoarchitectures has been proposed for different research studies and applications like materials and sensors production (Wang et al., 2024), many devices applications, energy production and catalysis, medical and biomedical fields (Nienhaus et al., 2020), and in tissue engineering (Rahmani Del Bakhshayesh et al., 2023). One of the most interesting applications is the ability of these systems to store the electrochemical energy in what is called supercapacitors. These supercapacitors can be used in hybrid electric cars, portable electronics, memory backup, and power management systems (Bonifazi et al., 2007; Chen et al., 2021; Takimiya & Nakano, 2018). Sensors based mesoporous materials with variety of applications, electrochemical, and piezoelectric sensors have been also investigated and developed (Guo et al., 2013; Zheng, 2021). In addition, the significant role of these structures in medical field regrading drug delivery, peptide, gene, and DNA-based nanoarchitectonics. It has been investigated that DNA is considered suitable and strong element for self-assembly of nanoarchitectures (Aldaye et al., 2008). In terms of environment protection against unwanted materials and other toxic components, nanoarchitecture-

based 3D-formed structures like 3D-carbon foam has a potential to protect our environment from the deleterious electromagnetic radiations, as well as the beneficial role of other 3D-based nanoarchitectures in water purification purposes (Carlberg et al., 2018; Chen et al., 2018).

The multilayer nanoarchitectures are considered an advanced tool for the production of highly engineered and functionalized smart surfaces with complex nanoarchitectures (Motta et al., 2024). A multilayer or chemically modulated structures presented by Koehler in 1970, who pointed out in his review the concept strategies for building strong solid with an enhancement of physical properties of components via sequential deposition of layers of a material (Koehler, 1970). Different tools and mechanism have been introduced for preparation of multilayers with specific characters in different industries (Koehler, 1970). For example, toughness, wear resistance, mechanical properties, and load-bearing capability, as well as their corrosion resistance have been enhanced through developing specific multilayers (Allahyarzadeh et al., 2017; Chen et al., 2020; Zhang & Kobayashi, 2020).

4.3.2 Fabrication Techniques

There are different methods used for fabrication of multilayer nanoarchitectures.

4.3.2.1 Layer-by-Layer Assembly

One of the simple and inexpensive procedure for preparation of multilayer nanoarchitectures is the layer-by layer assembly, LbL. Simply the multilayer nanoarchitectures can be obtained on a solid substrate via dipping the substrate into the solution of target components. During the process, alternative charged multilayers were produced with functional materials in relatively short time (Ariga et al., 2019; Cao et al., 2021). The driving force

for LbL assembly is generally electrostatic force, charge neutralization, and renaturation upon the adsorption of counterionic component materials onto a charged surface result in charge inversion, which enables the alternate adsorption of cationic and anionic species as nanofilms. Finally, the number and sequences of the produced layers can be controlled by changing the sequence of the adsorption processes. Another used forces for LbL production include hydrogen bonding (Han et al., 2022), metal coordination (Rubinstein & Vaskevich, 2010), charge transfer (Rezvan, 2025), supramolecular inclusion (Wang et al., 2018), stereocomplex formation (Im et al., 2021), and sol/gel reactions (Malucelli, 2020). In addition, fully automated process has been also adapted for LbL assembly production (Mets et al., 2013). Spin-coating LbL has been also developed to prepare LbL in relatively very short period of time ranging from sub-second to second time interval per layer instead of traditionally used immersion adsorption steps which take several minutes (Kriaa et al., 2025). The LbL approach generates quite flexible and non-crystalline multi-component assemblies. LbL assembled materials often contain structural and morphological variations or even defects that hinder the formation of grain boundaries, which in turn are favorable for the facile diffusion of small molecules and open possibilities for dynamic functions. These imperfect features are highly advantageous for sequential nanoreactors, protective coatings, and drug delivery systems (Zyuzin et al., 2019). An example on the LbL assembly and its role in DNA transfection pathway is illustrated in Figure 4.3.

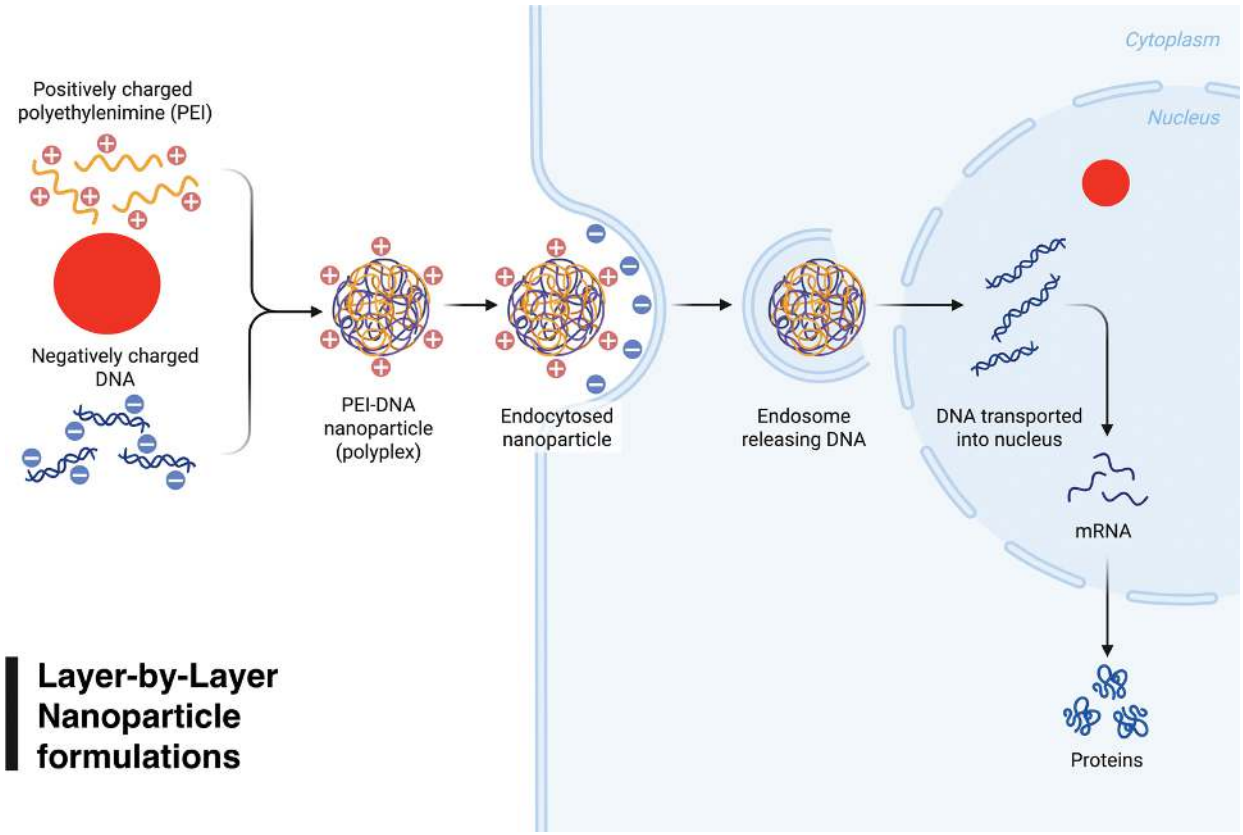

FIGURE 4.3 This figure illustrates the polyethylenimine (PEI)-mediated DNA transfection pathway. Positively charged PEI binds to negatively charged DNA to form PEI-DNA NPs (polyplexes). These NPs are endocytosed into cells and escape the endosome to release DNA. The released DNA enters the nucleus, where it is transcribed into mRNA and subsequently translated into proteins, demonstrating the process of gene expression following PEI-based DNA delivery. ⏎

4.3.2.2 Electrodeposition Methods

Another effective tool in preparation of multilayer nanoarchitectures is the electrodeposition technique. During this process, multilayers are formed and deposited onto a surface via application of an electric field (Dorozhkin, 2015). Different categories of materials can be deposited involves metals, metal alloys, metal oxides, nanomaterials, semiconductors, and conductive polymers (Sebastián et al., 2018). The final shape and morphology of the

produced nanoarchitectures will depend on the used substrate and the composition of the electrolytes. The most commonly used deposition techniques are physical vapor deposition (PVD) and chemical vapor deposition (CVD), chemical and electrochemical techniques, physical coating spraying, and roll-to-roll coating methods (Khadem et al., 2017). Electrodeposition and vapor phase deposition have been widely employed in the manufacturing of multilayer films. However, electrodeposition has always been facile for the production of alloyed multilayer coatings of various metals (Aliofkhazraei et al., 2021).

4.4 APPLICATIONS IN PRECISION MEDICINE

Precision medicine aims to tailor therapeutic interventions for individual patients by considering their unique biological characteristics, disease phenotypes, and environmental factors, thus optimizing treatment efficacy and minimizing side effects. Also known as "the right drug, at the right dose, at the right time," precision medicine requires much information to be collected from patients and preferably analyzed before the choice of a specific therapeutic intervention. Several basic concepts must be gradually considered during such process, and important terminologies have emerged in the literature and practicing (Ezike et al., 2023; Mitchell et al., 2021; Zhang et al., 2022).

Therapeutic targeting is a general and significant approach in precision medicine. It aims to deliver drug or therapeutic agents only to diseased cells/tissues/organs that is targeted and avoid healthy ones that will also significantly reduce possible side effects. Various types of diseases such as cancers, genetic disorders and infectious diseases or viruses such as COVID-19 viruses and SARS can be good candidates of therapeutic targeting application. Biomarkers can be defined as measurable indicators of disease

etiology, disease burden or therapeutic efficacy. Biomarkers can be identified during early stage of the same disease or at the beginning of one new infectious viruses. Patient stratification combines disease identification and sub-classification. Individualized precise medicine can be prescribed based on these classifications. It is also important to note that a same type of cancer or diseases may exhibit very different features within different populations or even individuals. Personalized dosing focuses on providing patients with precise dosing strategy, such as dosing routes, formulations, frequencies, durations, and co-administrations (Ahmad et al., 2023).

4.4.1 Cancer Therapy

Tumor heterogeneity, an inherent characteristic of all tumors, is fundamentally connected to tumorigenesis, progression, and therapeutic response. Tumors evolve through time and space that alters their microbiological features which renders difficulties in penetrating and in delivering anticancer agents uniformly to the entire mass simultaneously. Tumor biology affects the design principles of cancer nanomedicine for selective delivery, deep penetration, homogeneous distribution, and controllable release of payloads now and at later stages when needed. Cancer remains a major global disease and morbidity. In 2020, about 19.3 million new cases, and 10 million deaths distributed worldwide. Cancer types account for 47% of the cases and 55% of the deaths. About 40% of the individuals risk developing cancer. One in five men and one in six women can die from cancer. Targeted drug delivery, imaging and diagnostics, treatment of genetic disorders, antiviral, and antibacterial applications are diverse fields in cancer therapy. In addition to cancer therapy, the similar strategy can be extended to other therapeutic areas nowadays. Growing interest and increased funding provide motivation to establish, evaluate, and

standardize platform technology that can lower the research, development, and evaluation (Chakraborty et al., 2022; Guo et al., 2023).

4.4.1.1 Targeted Drug Delivery

Drug delivery systems are versatile formulations that allow the controlled delivery of active ingredients at specific rates over defined periods of time. Various types of materials can be used as drug carriers, such as nanomaterials, hydrogels, polymers, lipids, proteins, and microcrystals. Multifunctional drug delivery systems designed to fulfill multiplex functions are highly desirable in anticancer therapy. To precisely kill tumor cells and avoid side effects to normal tissues, drug delivery systems must meet several requirements: (1) selective targeting to tumor cells, (2) penetration of the tumor stroma after reaching the target cells, (3) intracellular delivery to bypass endosomal trapping, and (4) controlled release of payloads able to induce apoptosis or necrosis to enact cell killing in response to intracellular stimulating signals as presented in Figure 4.4. Targeted drug delivery aims to improve the drug delivery efficiency and reduce side effects of drugs. Targeting moieties can be engineered onto particles to bond to specific receptors on diseased tissues. Various targeting approaches toward different receptors have been embarked, including the delivery of chemotherapeutics (e.g., paclitaxel, doxorubicin, cisplatin, gemcitabine) to tumor cells, delivering anti-HIV drugs toward CD4+ T lymphocytes infected with HIV, and the targeting of antibiotics toward bacteria for effective antibacterial treatment. By selecting specific targeting moieties, a wide variety of drugs, including small molecules, peptides, proteins, nucleic acids, siRNAs, and anti-miRs, can be incorporated (Korsmeyer, 2016; Mitchell et al., 2021).

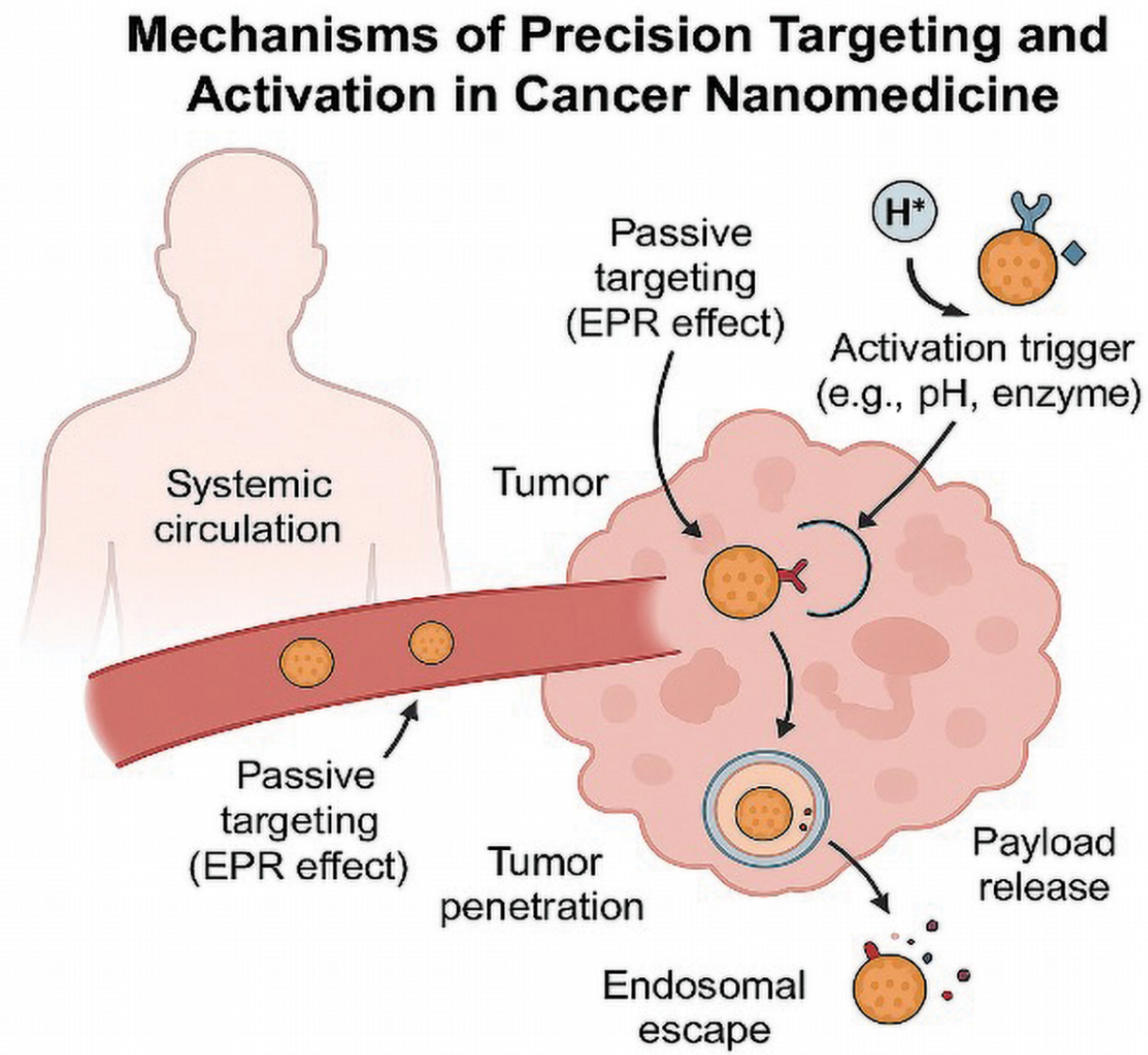

FIGURE 4.4 Illustration of the key mechanisms of precision targeting and activation used in cancer nanomedicine, highlighting NP delivery from systemic circulation to controlled release at the tumor site. ⏎

4.4.1.2 Imaging and Diagnostics

Imaging and diagnostics play critical roles in identifying diseases and monitoring treatment progress. X-ray, computed tomography (CT), magnetic resonance imaging (MRI), ultrasound, and optical imaging are widely used

imaging techniques. Contrast agents such as iron oxide, bismuth, iodine, lanthanide, and organic dyes are added to establish contrast in various imaging modalities. Unlike conventional contrast agents, activatable protein NPs improve imaging quality and diagnostic accuracy by enabling imaging only after reaching the target tissue. They reduce background noise during circulation and provide signals after achieving target tissue accumulation, such as in tumors, thereby enhancing contrast-to-noise ratio and time-to-signal increase. Activatable protein NPs can carry diverse imaging agents to combine imaging modalities and establish materials that respond to diverse spatiotemporal cues, such as pH, redox, enzymes, and external stimuli (Key & Leary, 2014; Sridhar et al., 2017).

Molecular imaging, a noninvasive, quantitative, and highly sensitive imaging technique captures cellular and molecular changes at the whole organism level. It covers physiological and pathological processes, including cancer, cardiovascular diseases, and neurodegenerative diseases. Molecular imaging technologies track disease evolution and therapeutic responses by visualizing biomarker expression. NPs establish a platform for molecular imaging in cells and organisms. Magnetic resonance imaging (MRI), due to its high spatial resolution and deep tissue penetration, monitors brain metabolism, tumor focus, and drug metabolism. NPs that deliver targeted imaging and therapeutic reagents to specific cellular substructures enable multimodal imaging and therapy evaluation (Rahman, 2023).

4.4.2 Treatment of Genetic Disorders

Treatment of genetic disorders encompasses numerous strategies such as the delivery of nucleic acids to control gene expression, small molecules to replenish deficient enzymes, and proteins to correct misfolding. Multilayer nanoarchitectures enable a variety of these approaches by providing

functionalization and triggering mechanisms tailored to specific targets, thereby overcoming barriers imposed by plasma proteins, blood vessels, and biological membranes. For all three genetic disorder targets, multilayer configurations offer advantages in circulation, specificity, and release compared to alternative platforms like single-component lipid- or polymer-based systems as well as activatable protein NPs (Borges et al., 2024; Hasan et al., 2025).

Gene expression modulation can be achieved using small interfering (si)RNA and messenger (m)RNA, which regulate a range of diseases from cancer and neurodegenerative disorders to metabolic syndromes and fibrosis. Therapeutic siRNA design relies on extensive in silico simulation to identify optimal configurations; bioactivity can then be confirmed using multilayer nanoarchitectures that protect the payload from degradation and promote endosomal escape, targeting mechanisms based on consensus promoter motifs, and additional features that enhance cellular uptake and cytoplasmic release. Nucleic acids constituting loss-of-function disease genes offer similar potential but necessitate different delivery strategies because safety considerations for excess protein differ from those for siRNA combinations of preferably highly delivered mRNA encoding the missing protein remain eligible. MRNA-based vaccines against infectious diseases exemplify extensive research to identify the most appropriate constructs (Lin et al., 2015).

4.4.3 Antiviral and Antibacterial Applications

Among the myriad applications pursued in contemporary research, antiviral and antibacterial treatments stand to benefit significantly from multilayer nanoarchitectures and activatable protein NPs. Nanotechnology offers distinct avenues for improvement over conventional antiviral regimes,

including enhanced bioavailability and controlled release, while also facilitating novel intracellular delivery strategies for previously inaccessible biomolecular cargo. For instance, multiple polymeric and lipid-based NP formulations have demonstrated capacity to formulate specific antiviral agents such as acyclovir or ganciclovir within a protecting matrix to stabilize the payload and govern pharmacokinetics.

Core–shell–protein hybrid platforms enable penetration of cytosolic membranes for delivery of antisense oligonucleotides. Further, metallic NPs such as silver or copper sulfide can directly bind biomolecules or disrupt viral replication. Efficient strategies are additionally required to reach target tissues and mitigate the development of resistance. Surface functionalization to exploit pathogen-specific targets, along with tailored properties or disassembly routes to deliver selective agents, have emerged as relevant paradigms in this regard (Lin et al., 2015; Milovanovic et al., 2017). The comparative summary of multilayer nanoarchitecture and activatable protein NP applications in precision medicine is presented in Table 4.1.

TABLE 4.1

Comparative Summary of Applications of Multilayer Nanoarchitectures and Activatable Protein Nanoparticles in Precision Medicine

Application Area	Role of Multilayer Nanoarchitectures	Role of Activatable Protein Nanoparticles	Key Challenges
Cancer Therapy	Targeted drug delivery, deep penetration, endosomal escape	Activatable imaging agents, real-time monitoring, theranostics	Tumor heterogeneity, delivery to dense stroma

Application Area	Role of Multilayer Nanoarchitectures	Role of Activatable Protein Nanoparticles	Key Challenges
Genetic Disorders	Protection of siRNA/mRNA, controlled expression, multilayer protection	Protein-level delivery, responsive to cellular triggers	Delivery across barriers, protein folding fidelity
Antiviral/Antibacterial	Controlled release, penetration into infected cells, broad targeting	Specific activation upon pathogen interaction, reduced off-target effects	Viral mutation, resistance development
Imaging & Diagnostics	Carrier for contrast agents, multimodal functionalization	Activatable only at target tissue, reduced noise, improved specificity	Tissue penetration, real-time response

4.5 CHALLENGES AND LIMITATIONS

The development of multilayer nanoarchitectures and activatable protein NPs offers great potential for precision medicine; however, significant challenges remain before these strategies achieve widespread clinical translation. Balancing biocompatibility, drug release kinetics, and targeting efficiency is a prerequisite that has not yet been fully met. For multilayer nanoarchitectures, obstructions to effective biodistribution and accumulation of signal-enhancing therapeutics still exist. Surface modifications that optimize blood circulation can diminish interactions with cellular membranes and prevent extracellular biological barriers from being crossed. Citrate-capped gold NPs, for example, remain predominantly in the blood pool and experience minimal penetration into tumor tissue even after prolonged circulation times. Strategies such as core–shell architectures that prolong blood circulation while facilitating tumor accumulation and penetration have been explored, but a comprehensive, unified design framework remains elusive (Korsmeyer, 2016; Mitchell et al., 2021).

APNPs initially appear more advanced; multiple NP architectures can be delivered to various tissue types, and triggering mechanisms compatible with biological systems have been developed. Nevertheless, a critical need persists. Detailed understanding of how different triggering cues influence the biodistribution, accumulation, and efficacy of APNPs remains limited. Other factors, including particle formulations, delivery route, and external variables, also modulate these properties. For example, tunable redox-responsive APNPs loaded with tumor-targeting DNA dox have been applied, but the optimal design for solid tumors remains unknown, illustrating the critical need for systematic exploration in this area (Nguyen et al., 2024; Shi et al., 2024).

Both multilayer nanoarchitectures and APNPs represent promising directions for precision medicine and extensive ongoing research is dedicated to advancing their experimental and theoretical development. Nevertheless, comprehensive design frameworks that guide the optimal selection of materials, formats, triggering mechanisms, and other parameters in relation to predetermined targets are not yet established. These frameworks remain pivotal for the effective application of multilayer nanoarchitectures and APNPs in precision medicine.

4.5.1 Biocompatibility and Toxicity

Biocompatibility is crucial for any bioengineered tool or medical device intended for human use. NPs can trigger an immune response, and modifying surface chemistry and compositions affects their interaction with cells and immune systems. Biocompatibility continues to be assessed mainly using polymeric nanocarriers, lipid-based NPs, and inorganic materials, and a clear consensus has not emerged. Analysis of APNPs indicates that their stabilization against proteolysis and aggregation is not routinely

characterized during biocompatibility evaluation, despite being vital for therapeutic efficacy and safety. This property is examined alongside surface charge and hydrophilicity, and criterial thresholds for mammalian cells are proposed. Risk factors also tie to biodegradation, aggregation, and lipopolysaccharide contamination of APNPs (Naahidi, 2013).

4.5.2 Scalability and Manufacturing

Reproducibility and cost-effectiveness of APNP fabrication remain major hurdles, along with the establishment of appropriate quality control protocols and characterization techniques to ensure that materials produced on different time scales are equivalent. As manufacturing processes scale up, solutions such as miniaturization and batch microfluidics may offer an improved pathway (Chan et al., 2016; Korsmeyer, 2016).

The length and complexity of preclinical and clinical studies mandated for regulatory approval also increase substantially with the progression from the laboratory to clinical testing. In an influential analysis, the FDA identified five specific preclinical investigations as necessary to support new drug applications.

4.5.3 Regulatory Considerations

With the rapid development of advanced materials for drug delivery and robust research on biomaterials, pharmaceuticals, and biologics formulations, biopharmaceutics and pharmaceutical nanotechnology have made significant strides in recent years. It was reported that integrated approaches involving these materials focus on the designing, engineering, and/generation of nanocarriers that permit encapsulation of drugs and rejuvenate their half-lives while retaining effectiveness. Various active and passive targeting strategies have been employed according to the biology

properties of the major organ systems such as liver, kidney, lung, and circulating vessels to direct the nanocarriers. Intracellular, cytoplasmic, and/or organelle targeting approaches are further studied to enhance efficacy or safety profiles. Recent developments further enhance these targeting concepts by allowing extensive and real-time monitoring of the preclinical and clinical material distribution pathways. Such passive, active, or mechanism-driven physicochemical targeting can provide more versatility and flexibility for formulations, hands—on bioassays and *in vitro* options to comprehensively probe these directional targeting hypotheses. These new materials offer innovative platforms to decipher the Polysaccharide drug delivery website, and drug-disease combinations at the molecular and cellular level to improve the chance of success against diseases (Gao et al., 2024; Thakur et al., 2025).

4.6 FUTURE DIRECTIONS

Multilayer nanoarchitectures and APNPs can transform precision medicine by enabling the targeted delivery of therapeutics, imaging agents, or biologics to diseased tissues. The emerging design frameworks that underpin these technologies are informed by contemporary notions of precision medicine and the mechanisms through which particle properties dictate circulation and distribution patterns *in vivo*. Nevertheless, substantial hurdles remain to translating these concepts into effective clinical applications and ensuring that the underlying technologies are sufficiently versatile and well defined.

In contemporary precision medicine, patient stratification based on molecular features or timely biological information ensures that only patients who are likely to benefit from a therapeutic receive the agent. Underlying these approaches is targeted delivery using a system that

selectively transports a drug, imaging agent, or genetic material to the designated site. When such strategies are directly linked to prevailing design principles, certain constraints emerge that help guide material selection and engineering strategies, as detailed further for multilayer nanoarchitectures and APNPs (Mitchell et al., 2021).

4.6.1 Innovations in Nanoengineering

Nanomedicine has gained prominence in science and medicine, targeting interactions at molecular scales to develop sophisticated tools for timely, precise diagnosis and therapeutic intervention. Advances in nanoengineering address multiple pressing global health needs. With growing incidence and mortality of diverse diseases, sturdy platforms capable of rapid yet patient-adapted development are called for. Contemporary therapies routinely rely on poorly targeted pharmaceuticals after disease establishes footholds. NPs are engineered materials with precise physicochemical properties spanning the 1–100 nm scale; they enhance biodistribution and intracellular access of covalently or non-covalently loaded agents to amplify treatment efficacy. The capacity to deliver cargoes selectively to concerned cells enables innovative treatment paradigms (Mitchell et al., 2021).

Multilayer nanoarchitectures represent design concepts promoting the organized construction of multiple component layers on nanoparticles, facilitating patient-tailored NP platforms capable of addressing diverse diseases and spatio-temporal control. These multilayer nanoarchitectures are amenable to bioorthogonal functionalization strategies permitting combination of varying layers composed of different materials to optimize circulation, targeting, and cargo release profiles, thus accommodating a wider range of pharmaceutical agents than single-component nanoparticles. Furthermore, diverse triggers can activate individual layers to control release

in response to external or physiological stimuli such as temperature, pH, redox conditions, enzymes, and exogenous factors, thus permitting investigation of different therapeutic strategies (Korsmeyer, 2016).

4.6.2 Integration with Other Therapeutic Modalities

Multilayer nanoarchitectures and APNPs offer avenues for combination therapies—multiple concurrent or sequential treatments that improve clinical outcomes beyond monotherapies. Ideally, a separate regimen can act synergistically and be administered simultaneously with an activatable nanoparticle. Alternately, a self-contained regimen can favorably influence effector cells, administer less persistently, or trigger corresponding outputs. Such maneuvers can extend transit times, facilitate deeper penetrating second therapies, and curtail multiple-drug resistance (Chakraborty et al., 2022). Platforms enabling these combination approaches will fulfill the broader promise of precision medicine (Chakraborty et al., 2022).

Multidrug combination remains an attractive approach in cancer therapy for improving efficacy, reducing toxicity, and overcoming drug resistance. However, existing strategies often result in compromised therapeutic indices owing to systemic side effects. In parallel, delivery approaches for targeting biomacromolecules against genetic disorders remain constrained by biocompatibility, inactivity at target sites, and impeded cell entrance. Generating activatable protein nanocarriers driven by pH and other endogenous signals proficiently addresses drug resistance in cancer therapy and genetic disorder therapy (Shrestha, 2012).

4.6.3 Personalized Medicine Approaches

The application of multilayer nanoarchitectures and APNPs for precision medicine addresses patient heterogeneity by enabling tailored therapies.

Precision medicine refers to targeted therapeutic strategies based on individual patient characteristics. Nanomaterials and nanoparticles can augment the precision of cancer diagnosis and therapy, infectious disease management, genetic disorder treatment, and antimicrobial applications. By engineering multilayer nanoarchitectures with stimuli-responsive components or APNPs with specific triggering cues, these approaches modulate pharmacokinetics, biodistribution, and drug release to optimize therapeutic effectiveness and safety for specific patient populations. The overarching goal of multilayer nanoarchitectures remains to decouple circulation or targeting from drug release, whereas APNPs provide a distinctive strategy to accomplish the same objective (Mitchell et al., 2021).

4.7 CONCLUSIONS

Precision medicine identifies biomarkers correlated with clinical outcomes to monitor disease progression and treatment efficacy. Personalized medicine selects therapies based on specific biomarkers, while patient-oriented medicine optimizes treatment designs for individual patients. In non-targeted therapy, all patients receive the same regimen, assuming uniform responses and side effects. Multilayer nanoarchitectures address therapeutic targeting principles, selecting nanoparticle sizes based on biomarker profiles rather than universal constraints. The design considers the relationship between selectivity and competitive treatments, with APNPs featuring adaptive designs. Therapy controlled by multiple biocues equates to multi-dimensional precision medicine, as control dimensions correspond to different identifiers. Enzymes activate therapeutics released spatially, targeting tissues affected by metabolic disorders like hereditary tyrosinemia through the release of specific genes. Controlling metabolite release at various tissues can distinguish between viral and bacterial infections,

addressing virus killing and super-infection resistance. Challenges remain in broad clinical adoption of multilayer nanoarchitectures and APNPs.

REFERENCES

Abdellatif, A. A. H., Ahmed, F., Mohammed, A. M., Alsharidah, M., Al-Subaiyel, A., Samman, W. A., Alhaddad, A. A., Al-Mijalli, S. H., Amin, M. A., Barakat, H., & Osman, S. K. (2023). Recent advances in the pharmaceutical and biomedical applications of cyclodextrin-capped gold nanoparticles. *Int J Nanomedicine*, *18*, 3247–3281. https://doi.org/10.2147/IJN.S405964⏎

Abdellatif, A. A. H., Aldalaen, S. M., Faisal, W., & Tawfeek, H. M. (2018). Somatostatin receptors as a new active targeting sites for nanoparticles. *Saudi Pharm J*, *26*(7), 1051–1059. https://doi.org/10.1016/j.jsps.2018.05.014⏎

Abdellatif, A. A. H., Alshubrumi, A. S., & Younis, M. A. (2024). Targeted nanoparticles: the smart way for the treatment of colorectal cancer. *AAPS PharmSciTech*, *25*(1), 23. https://doi.org/10.1208/s12249-024-02734-9⏎

Abdellatif, A. A. H., Scagnetti, G., Younis, M. A., Bouazzaoui, A., Tawfeek, H. M., Aldosari, B. N., Almurshedi, A. S., Alsharidah, M., Rugaie, O. A., Davies, M. P. A., Liloglou, T., Ross, K., & Saleem, I. (2023). Non-coding RNA-directed therapeutics in lung cancer: Delivery technologies and clinical applications. *Colloids Surf B Biointerfaces*, *229*, 113466. https://doi.org/10.1016/j.colsurfb.2023.113466

Abdellatif, A. A. H., Younis, M. A., Alsowinea, A. F., Abdallah, E. M., Abdel-Bakky, M. S., Al-Subaiyel, A., Hassan, Y. A. H., & Tawfeek, H. M. (2023). Lipid nanoparticles technology in vaccines: Shaping the future of prophylactic medicine. *Colloids Surf B Biointerfaces*, *222*, 113111. https://doi.org/10.1016/j.colsurfb.2022.113111

Ahmad, A., Imran, M., & Ahsan, H. (2023). Biomarkers as biomedical bioindicators: Approaches and techniques for the detection, analysis, and validation of novel biomarkers of diseases. *Pharmaceutics*, *15*(6), 1630.⏎

Aldaye, F. A., Palmer, A. L., & Sleiman, H. F. (2008). Assembling materials with DNA as the guide. *Science*, *321*(5897), 1795–1799. https://doi.org/10.1126/science.1154533⏎

Aliofkhazraei, M., Walsh, F. C., Zangari, G., Köçkar, H., Alper, M., Rizal, C., Magagnin, L., Protsenko, V., Arunachalam, R., Rezvanian, A., Moein, A., Assareh, S., & Allahyarzadeh, M. H. (2021). Development of electrodeposited multilayer coatings: A review of fabrication, microstructure, properties and applications. *Appl Surf Sci Adv*, *6*, 100141. https://doi.org/10.1016/j.apsadv.2021.100141⏎

Allahyarzadeh, M. H., Aliofkhazraei, M., Rouhaghdam, A. S., Torabinejad, V., Alimadadi, H., & Ashrafi, A. (2017). Electrodeposition mechanism and corrosion behavior of multilayer nanocrystalline nickel-tungsten alloy. *Electrochim Acta*, *258*, 883–899. https://doi.org/10.1016/j.electacta.2017.11.139⏎

Ariga, K. (2023). Materials nanoarchitectonics: Collaboration between chem, nano and mat. *ChemNanoMat*, *9*(7), e202300120. https://doi.org/10.1002/cnma.202300120⏎

Ariga, K., Ahn, E., Park, M., & Kim, B.-S. (2019). Layer-by-layer assembly: Recent progress from layered assemblies to layered nanoarchitectonics. *Chem Asian J*, *14*(15), 2553–2566. https://doi.org/10.1002/asia.201900627⏎

Ariga, K., Jia, X., & Shrestha, L. K. (2019). Soft material nanoarchitectonics at interfaces: Molecular assembly, nanomaterial synthesis, and life control. *Mol Syst Des Eng*, *4*(1), 49–64. https://doi.org/10.1039/C8ME00094H

Ariga, K., Nishikawa, M., Mori, T., Takeya, J., Shrestha, L. K., & Hill, J. P. (2019). Self-assembly as a key player for materials nanoarchitectonics. *Sci Technol Adv Mater*, 20(1), 51–95. (1468-6996 (Print)).

Bai, X., Smith, Z. L., Wang, Y., Butterworth, S., & Tirella, A. (2022). Sustained drug release from smart nanoparticles in cancer therapy: A comprehensive review. *Micromachines*, *13*(10), 1623. https://www.mdpi.com/2072-666X/13/10/1623⏎

Bonifazi, D., Enger, O., & Diederich, F. (2007). Supramolecular [60]fullerene chemistry on surfaces. *Chem Soc Rev*, *36*(2), 390–414. https://doi.org/10.1039/B604308A⏎

Borges, J., Zeng, J., Liu, X. Q., Chang, H., Monge, C., Garot, C., Ren, K. f., Machillot, P., Vrana, N. E., & Lavalle, P. (2024). Recent developments in layer-by-layer assembly for drug delivery and tissue engineering applications. *Adv Healthcare Mater*, *13*(8), 2302713.

Cao, L. A.-O. X., Huang, Y., Parakhonskiy, B. A.-O., & Skirtach, A. A.-O. (2021). Nanoarchitectonics beyond perfect order – Not quite perfect but quite useful. *Nanoscale Horiz*, 6(12), 877–897. (2040-3372 (Electronic)).

Carlberg, M., Koppel, T., Ahonen, M., & Hardell, L. (2018). Case-control study on occupational exposure to extremely low-frequency electromagnetic fields and the association with meningioma. *BioMed Res Int*, *2018*(1), 5912394. https://doi.org/10.1155/2018/5912394

Chakraborty, K., Tripathi, A., Mishra, S., Mallick, A. M., & Roy, R. S. (2022). Emerging concepts in designing next-generation multifunctional nanomedicine for cancer treatment. *Biosci Rep*, *42*(7), BSR20212051.

Chan, H. F., Ma, S., & Leong, K. W. (2016). Can microfluidics address biomanufacturing challenges in drug/gene/cell therapies? *Regener Biomater*, *3*(2), 87–98.

Chang, L., Bai, H., Yao, J., Liu, H., Gou, S., Zhong, C., Zhang, Y., & Ni, J. (2021). New designed pH-responsive histidine-rich peptides with antitumor activity. *J Drug Target*, *29*(6), 651–659. https://doi.org/10.1080/1061186X.2021.1873351

Chen, G., Shrestha, L. K., & Ariga, K. (2021). Zero-to-two nanoarchitectonics: Fabrication of two-dimensional materials from zero-dimensional fullerene. *Molecules*, *26*(15), 4636.

Chen, W. Y., Chen, H. W., Li, W. P., Huang, J. C., Yu, H. S., Duh, J. G., Lan, S., & Feng, T. (2020). Compositionally modulated microstructure in nano-layered Ni-P metallic glass composite coating prepared by electrodeposition. *Surf Coat Technol*, *389*, 125636. https://doi.org/10.1016/j.surfcoat.2020.125636

Chen, Y., Song, X., Zhao, T., Xiao, Y., Wang, Y., & Chen, X. (2018). A phosphorylethanolamine-functionalized super-hydrophilic 3D graphene-based foam filter for water purification. *J Hazard Mater*, *343*, 298–303. https://doi.org/10.1016/j.jhazmat.2017.09.045

Dorozhkin, S. V. (2015). 7 - Surface modification of magnesium and its biodegradable alloys by calcium orthophosphate coatings to improve corrosion resistance and biocompatibility. In T. S. N. S. Narayanan, I.-S. Park, & M.-H. Lee (Eds.), *Surface Modification of Magnesium and Its Alloys for Biomedical Applications* (pp. 151–191). Woodhead Publishing. https://doi.org/10.1016/B978-1-78242-078-1.00007-4

Ezike, T. C., Okpala, U. S., Onoja, U. L., Nwike, C. P., Ezeako, E. C., Okpara, O. J., Okoroafor, C. C., Eze, S. C., Kalu, O. L., & Odoh, E. C. (2023). Advances in drug delivery systems, challenges and future directions. *Heliyon*, *9*(6), e16945.

Gao, X. J., Ciura, K., Ma, Y., Mikolajczyk, A., Jagiello, K., Wan, Y., Gao, Y., Zheng, J., Zhong, S., & Puzyn, T. (2024). Toward the integration of machine learning and molecular modeling for designing drug delivery nanocarriers. *Adv Mater*, *36*(45), 2407793.

Guo, L., Yang, J., Wang, H., & Yi, Y. (2023). Multistage self-assembled nanomaterials for cancer immunotherapy. *Molecules*, *28*(23), 7750.

Guo, W., Duan, X., Shen, Y., Qi, K., Wei, C., & Zheng, W. (2013). Ionothermal synthesis of mesoporous SnO2 nanomaterials and their gas sensitivity depending on the reducing ability of toxic gases. *Phys Chem Chem Phys*, *15*(27), 11221–11225. https://doi.org/10.1039/C3CP51663F

Gupta, D., Varghese, B. S., Suresh, M., Panwar, C., & Gupta, T. K. (2022). Nanoarchitectonics: Functional nanomaterials and nanostructures—a review. *J Nanopart Res*, *24*(10), 196. https://doi.org/10.1007/s11051-022-05577-2

Collins, F. S., & Varmus, H. (2015). A new initiative on precision medicine. NEJM. https://doi.org/10.1056/NEJMp1500523

Han, S. Y., Yun, G., Nguyen, D. T., Kang, E. K., Lee, H., Kim, S., Kim, B. J., Park, J. H., & Choi, I. S. (2022). Hydrogen bonding-based layer-by-layer assembly of nature-derived eggshell membrane hydrolysates and coffee melanoidins in single-cell nanoencapsulation. *ChemNanoMat*, *8*(5), e202100535. https://doi.org/10.1002/cnma.202100535

Hasan, E., Lewis, C. J., Hernandez, J. G., Gentile, P., & Ferreira, A. M. (2025). Layer-by-layer assembly: Advancing skin repair, one layer at a time. *RSC Adv*, *15*(18), 13908–13923.

Hayley Nehoff, N., Domanovitch, L., Taurin, S., & Greish, K. (2014). Nanomedicine for drug targeting: Strategies beyond the enhanced permeability and retention effect. *Int J Nanomed*, *9* 2539–2555. https://doi.org/http://dx.doi.org/10.2147/IJN.S47129

Im, S. H., Im, D. H., Park, S. J., Chung, J. J., Jung, Y., & Kim, S. H. (2021). Stereocomplex polylactide for drug delivery and biomedical applications: A review. *Molecules*, *26*(10), 3051.

Liu, J., Huang, Y., Kumar, A., Tan, A., Jin, S., Mozhi, A., & Liang, X. J. (2014). pH-sensitive nano-systems for drug delivery in cancer therapy. *Biotechnol Adv*, *32*(4), 693–710. https://doi.org/10.1016/j.biotechadv.2013.11.009

Key, J., & Leary, J. F. (2014). Nanoparticles for multimodal in vivo imaging in nanomedicine. *Int J Nanomed*, *9*, 711–726.

Khadem, M., Penkov, O. V., Yang, H.-K., & Kim, D.-E. (2017). Tribology of multilayer coatings for wear reduction: A review. *Friction*, *5*(3), 248–262. https://doi.org/10.1007/s40544-017-0181-7

Koehler, J. S. (1970). Attempt to design a strong solid. *Phys Rev B*, *2*(2), 547–551. https://doi.org/10.1103/PhysRevB.2.547

Korsmeyer, R. (2016). Critical questions in development of targeted nanoparticle therapeutics. *Regener Biomater*, *3*(2), 143–147.

Kriaa, K., Fadhillah, F., Alghamdi, A. M., Ali, F. A. A., & Aljlil, S. (2025). Spin-spray assisted layer-by-layer assembly of polyelectrolyte multilayer nanofiltration membranes with competitive organic fouling resistance. *Desal Water Treat*, *323*, 101306. https://doi.org/10.1016/j.dwt.2025.101306

Lee, M. H., Yang, Z., Lim, C. W., Lee, Y. H., Dongbang, S., Kang, C., & Kim, J. S. (2013). Disulfide-cleavage-triggered chemosensors and their biological applications. *Chem Rev*, *113*(7), 5071–5109. https://doi.org/10.1021/cr300358b

Li, Y., Z, C., Li, G., Deng, G., Zhang, H., Sun, Y., & An, F. (2021). Protease-triggered bioresponsive drug delivery for the targeted theranostics of malignancy. *Acta Pharm Sin B*, *11*(*8*), 2220–2242. https://doi.org/10.1016/j.apsb.2021.01.017

Lin, G., Zhang, H., & Huang, L. (2015). Smart polymeric nanoparticles for cancer gene delivery. *Mol Pharm*, *12*(2), 314–321.

Luo, Y., Zhang, Z., Fang, D., Peng, Y., Li, J., Zhang, H., Chen, Z., Liu, M., Zhang, C., Liu, L., Qian, X., Rao, Q., & Gu, K. (2024). Dendritic polymer-based nanomedicines remodel the tumor stroma: Improve drug penetration and enhance antitumor immune response. *Advanced Materials*, *36*(15), 2401304. https://doi.org/10.1002/adma.202401304

Malucelli, G. (2020). Sol-gel and layer-by-layer coatings for flame-retardant cotton fabrics: Recent advances. *Coatings*, *10*(4), 339.

Masood, F. (2016). Polymeric nanoparticles for targeted drug delivery system for cancer therapy. *Mater Sci Eng C*, *60*, 569–578. https://doi.org/10.1016/j.msec.2015.11.067

Mets, J. M., Wilson, J. T., Cui, W., & Chaikof, E. L. (2013). An automated process for layer-by-layer assembly of polyelectrolyte multilayer thin films on viable cell aggregates. *Adv Healthcare Mater*, 2(2), 229–234. (2192-2640 (Print)).

Milovanovic, M., Arsenijevic, A., Milovanovic, J., Kanjevac, T., & Arsenijevic, N. (2017). Nanoparticles in antiviral therapy. In *Antimicrobial Nanoarchitectonics* (pp. 383–410). Elsevier.

Mitchell, M. J., Billingsley, M. M., Haley, R. M., Wechsler, M. E., Peppas, N. A., & Langer, R. (2021). Engineering precision nanoparticles for drug delivery. *Nat Rev Drug Discov*, *20*(2), 101–124.

Mohsin, A., Hussain, M. H., Mohsin, M. Z., Zaman, W. Q., Aslam, M. S., Shan, A., Dai, Y., Khan, I. M., Niazi, S., Zhuang, Y., & Guo, M. (2022). Recent advances of magnetic nanomaterials for bioimaging, drug delivery, and cell therapy. *ACS Appl Nano Mater*, *5*(8), 10118–10136. https://doi.org/10.1021/acsanm.2c02014

Motta, M. A., Mulko, L., Marin, E., Larrañaga, A., & Calderón, M. (2024). Polypeptide-based multilayer nanoarchitectures: Controlled assembly on planar and colloidal substrates for biomedical applications. *Adv Colloid Interface Sci*, *331*, 103248. https://doi.org/10.1016/j.cis.2024.103248

Naahidi, S. (2013). *Biocompatibility Evaluation of Engineered Amino Acid Pairing Peptides for Drug Delivery* (Doctoral dissertation, University of Waterloo).

Nguyen, L. N., Ngo, W., Lin, Z. P., Sindhwani, S., MacMillan, P., Mladjenovic, S. M., & Chan, W. C. (2024). The mechanisms of nanoparticle delivery to solid tumours. *Nat Rev Bioeng*, *2*(3), 201–213.

Nienhaus, K., Wang, H., & Nienhaus, G. U. (2020). Nanoparticles for biomedical applications: Exploring and exploiting molecular interactions at the nano-bio interface. *Mater Today Adv*, *5*, 100036. https://doi.org/10.1016/j.mtadv.2019.100036

Oviedo, L. R., Oviedo, V. R., Martins, M. O., Fagan, S. B., & da Silva, W. L. (2022). Nanoarchitectonics: The role of artificial intelligence in the design and application of nanoarchitectures. *J Nanopart Res*, *24*(8), 157. https://doi.org/10.1007/s11051-022-05535-y

Rahman, M. (2023). Magnetic resonance imaging and iron-oxide nanoparticles in the era of personalized medicine. *Nanotheranostics*, *7*(4), 424.

Rahmani Del Bakhshayesh, A., Saghebasl, S., Asadi, N., Kashani, E., Mehdipour, A., Nezami Asl, A., & Akbarzadeh, A. (2023). Recent advances in nano-scaffolds for tissue engineering applications: Toward natural therapeutics. *WIREs Nanomed Nanobiotechnol*, *15*(6), e1882. https://doi.org/10.1002/wnan.1882

Ramesh, M., Janani, R., Deepa, C., & Rajeshkumar, L. (2022). Nanotechnology-enabled biosensors: A review of fundamentals, design principles, materials, and applications. *Biosensors (Basel)*, *13*(1). https://doi.org/10.3390/bios13010040

Rezvan, V. H. (2025). Charge transfer complexes: A review survey. *Results Chem*, *17*, 102600. https://doi.org/10.1016/j.rechem.2025.102600

Rinaldi, A., Caraffi, R., Grazioli, M. V., Oddone, N., Giardino, L., Tosi, G., Vandelli, M. A., Calzà, L., Ruozi, B., & Duskey, J. T. (2022). Applications of the ROS-responsive thioketal linker for the production of smart nanomedicines. *Polymers (Basel)*, *14*(4), 687. https://doi.org/10.3390/polym14040687

Rubinstein, I., & Vaskevich, A. (2010). Self-assembly of nanostructures on surfaces using metal–organic coordination. *Isr J Chem*, *50*(3), 333–346. https://doi.org/10.1002/ijch.201000024

Sebastián, P., Climent, V., Feliu, J. M., & Gómez, E. (2018). Ionic liquids in the field of metal electrodeposition. In Wandelt, K. (Ed.), *Encyclopedia of Interfacial Chemistry* (pp. 690–700). Elsevier. https://doi.org/10.1016/B978-0-12-409547-2.13379-7

Seth Rowland, A. A., Whalley, R. D., Ferreira, A. M., Kotov, N., & Gentile, P. (2024). Layer-by-layer nanoparticle assembly for biomedicine: Mechanisms, technologies, and advancement via acoustofluidics. *ACS Appl Nano Mater*, *7*(14), 15874–15902. https://doi.org/10.1021/acsanm.4c02463

Shao, Y., Li, X., Sun, Y., & Qin, G. (2024). Artificial intelligence-driven precision medicine: multi-omics and spatial multi-omics approaches in diffuse large b-cell lymphoma (DLBCL). *Front Biosci*, *29*(12), 404. https://doi.org/10.31083/j.fbl2912404

Shi, S., Ou, X., & Cheng, D. (2024). Nanoparticle-facilitated therapy: Advancing tools in peripheral nerve regeneration. *Int J Nanomed*, *19*, 19–34.

Shrestha, R. (2012). *Multi-Functional Bio-Synthetic Hybrid Nanostructures for Enhanced Cellular Uptake, Endosomal Escape and Targeted Delivery Toward Diagnostics and Therapeutics* (Doctoral dissertation, University of Alberta).

Sridhar, S., Mishra, S., Gulyás, M., Padmanabhan, P., & Gulyás, B. (2017). An overview of multimodal neuroimaging using nanoprobes. *Int J Mol Sci*, *18*(2), 311.

Takimiya, K., & Nakano, M. (2018). Thiophene-fused naphthalene diimides: New building blocks for electron deficient π-functional materials. *Bull Chem Soc Jpn*, *91*(1), 121–140. https://doi.org/10.1246/bcsj.20170298

Thakur, S., Godela, R., Mandava, K., & Kolure, R. (2025). Advances in nanocarrier technology for drug encapsulation: A comprehensive overview. *Discov Mater*, *5*(1), 124.

Wang, S., Yang, L., Wang, Q., Fan, Y., Shang, J., Qiu, S., Li, J., Zhang, W., & Wu, X. (2018). Supramolecular self-assembly of layer-by-layer graphene film driven by the synergism of π–π and

hydrogen bonding interaction. *J Photochem Photobiol A Chem*, *355*, 249–255. https://doi.org/10.1016/j.jphotochem.2017.09.023⏎

Wang, W. A.-O., Yang, R., & Liu, M. A.-O. (2024). Design and fabrication of micro/nano sensors and actuators. *Micromachines*, 15(6), 674. https://doi.org/10.3390/mi15060674⏎

Yi, M., Chen, T. H. H., Lin, C. H., & Fann, Y. C. (2025). Unlocking precision medicine: Clinical applications of integrating health records, genetics, and immunology through artificial intelligence. *J Biomed Sci*, *32*, 16. https://doi.org/10.1186/s12929-024-01110-w⏎

Younis, M. A., Alsogaihi, M. A., Abdellatif, A. A. H., & Saleem, I. (2025). Nanoformulations in the treatment of lung cancer: Current status and clinical potential. *Drug Dev Ind Pharm*, *51*(9), 1088–1104. https://doi.org/10.1080/03639045.2024.2437562⏎

Younis, M. A., Tawfeek, H. M., Abdellatif, A. A. H., Abdel-Aleem, J. A., & Harashima, H. (2022). Clinical translation of nanomedicines: Challenges, opportunities, and keys. *Adv Drug Deliv Rev*, *181*, 114083. https://doi.org/10.1016/j.addr.2021.114083⏎

Yu Xiao, J. L., Wang, R., Fan, Y., Han, X., Fu, Y., Alepuz, P., Wang, W., & Liang, A. (2024). eIF5A promotes +1 programmed ribosomal frameshifting in Euplotes octocarinatus. *Int J Biol Macromol*, *254*(1). https://doi.org/10.1016/j.ijbiomac.2023.127743⏎

Zhang, S., & Kobayashi, S. (2020). Multilayering process of electrodeposited nanocrystalline iron–nickel alloys for further strengthening. *J Mater Sci*, *55*(13), 5627–5638. https://doi.org/10.1007/s10853-020-04378-z⏎

Zhang, Y., Yang, H., Yu, Y., & Zhang, Y. (2022). Application of nanomaterials in proteomics-driven precision medicine. *Theranostics*, *12*(6), 2674.⏎

Zhao, X., Bai, J., & Yang, W. (2021). Stimuli-responsive nanocarriers for therapeutic applications in cancer. *Cancer Biol Med*, *18*(2), 319–335.⏎

Zheng, Y. (2021, 10–12 Dec. 2021). Applications of electrochemical biosensors for glucose detection. In *2021 3rd International Academic Exchange Conference on Science and Technology Innovation (IAECST)*. IEEE.⏎

Zyuzin, M. V., Timin, A. S., & Sukhorukov, G. B. (2019). Multilayer capsules inside biological systems: State-of-the-art and open challenges. *Langmuir*, *35*(13), 4747–4762. https://doi.org/10.1021/acs.langmuir.8b04280⏎

5 Nanocarrier-Based Approaches for Intracellular Protein Transport in Therapeutic Applications

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5.1 INTRODUCTION

Intracellular protein delivery is widely recognized as a vital advancement in medicine. The advancement of microinjection and electro-transfection technologies has been made possible by continuous research and innovation, enabling the effective delivery of substances such as antibodies and active proteins in vitro (Bouazzaoui et al., 2025). While microinjection technology enables direct injection of biological agents into the cytoplasm, its processing efficiency is relatively low, and each operation is constrained to a single cell. To enhance processing efficiency, high-throughput protein delivery methodologies that can create transient pores in the cell membrane have been developed (Bouazzaoui et al., 2023). These methods are primarily based on electroporation technology, hyperosmotic solutions, and

mechanical force. However, these methods destroy the cell membrane integrity, thereby enabling the exchange of substances between the cytoplasm and the external medium. This may result in the toxic effects on the cells and interference with the normal cell functions. Furthermore, these technologies necessitate the use of costly equipment and intricate operational conditions, which constrains their applicability in medical research and treatment, both *in vivo* and *in vitro* (Abdellatif et al., 2025, Bouazzaoui et al., 2025).

The advent of gene editing technology that relies on electroporation technology for protein delivery has facilitated the approval of the first gene editing therapeutic drug, Casgevy, which can be used to treat transfusion-dependent beta-thalassemia (TDT) and sickle cell disease (SCD). This therapeutic strategy first requires obtaining CD34+ hematopoietic stem and progenitor cells from healthy donors (Frangoul et al., 2025, 2021). These cells are genetically modified by CRISPR-Cas9 gene-editing system that targets the BCL11A enhancer. CRISPR-Cas9 is delivered inside the cells by electroporation. This approach has shown strong efficacy, but it remains limited by its complexity, and the exceptionally high cost (approximately $2.2 million), and the risk of adverse effects related to infections and chemotherapy (Frangoul et al., 2021).

With the new technologies of controlled gene editing and intracellular antibody, therapeutics require more delivery platforms that enable precise protein targeting with enhanced tissue and cellular specificity (Abdellatif et al., 2022). Therefore, novel methods are needed to ensure efficient interaction between the loaded protein and intracellular targets without cytotoxicity. Many new nanomaterial carriers have been developed, including polymers, lipid nanoparticles, and protein nanoparticles. Nanomaterial-based delivery systems can efficiently deliver proteins of different sizes and isoelectric points to other cells while maintaining their

biological activity. To achieve cytoplasmic delivery, cell-penetrating peptides (CPPs) and phase-separated peptides can be used to traverse the cell membrane, improving cellular entry efficiency directly. Biomimetic nanocarriers can protect biological macromolecules from degradation within endosomes and lysosomes, thereby improving the bioavailability of imaging agents or therapeutic agents. Nanomaterial-mediated cytoplasmic protein delivery has significant advantages, including protecting proteins from degradation, extending the half-life of protein drugs, responsive release, targeted delivery, and improving drug efficacy (Abdellatif et al., 2022, Bouazzaoui and Abdellatif, 2024).

These advantages make that nanomaterials have broad application prospects in the field of drug delivery. Compared to traditional delivery methods such as electroporation, nanomaterial-mediated cytoplasmic protein delivery offers a safe, efficient, and high-uptake alternative, driving the development of intracellular target-related drugs in research. See different intracellular targets and different types of functionalized drugs in (Figure 5.1).

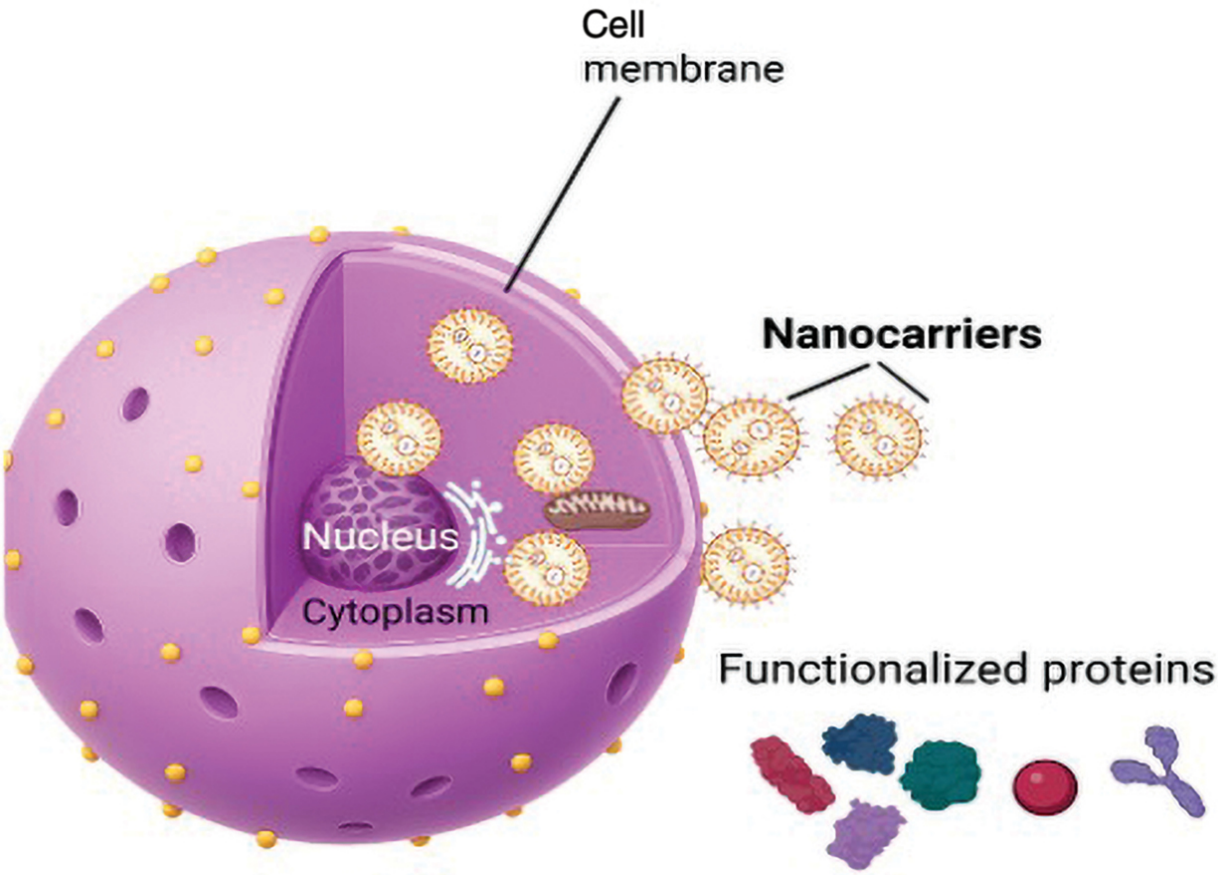

FIGURE 5.1 Nanocarrier-mediated delivery of functionalized proteins into a mammalian cell. (Schematic illustration showing nanocarriers interacting with the cell membrane and releasing functionalized proteins into the cytoplasm. The cross-section highlights key intracellular structures, including the nucleus and cytoplasm, and demonstrates how engineered nanocarriers facilitate the transport of proteins across the cell membrane for intracellular therapeutic or diagnostic applications.)

We aim to provide a comprehensive overview of recent discoveries and technologies in nanodelivery systems for protein drugs. Polymers, liposomes, and protein nanoparticles are examples of emerging nanocarriers designed to meet the needs of various applications. These continuous technological innovations in the field of protein nanocarriers have become a focal point of attention due to their potential to deliver advantages and broad

application prospects. We also focused on discussing the applications of liposome-based protein delivery systems in preclinical studies, especially in antitumor treatment and fluorescent imaging. In this chapter, we will take a closer look at the essential principles for designing nanocarriers to meet the practical applications of protein nanoparticle and polymer nanoparticle delivery systems (Figure 5.2). Finally, we have summarized the crucial parameters that control protein delivery efficiency and provided a future view for the development of cytoplasmic delivery systems. The field of protein nanocarriers will continue to inspire researchers to develop new technologies for precise, personalized cytoplasmic protein delivery, thereby continuously driving the development of novel protein drugs. See different types of protein nanocarriers, as shown in Figure 5.2.

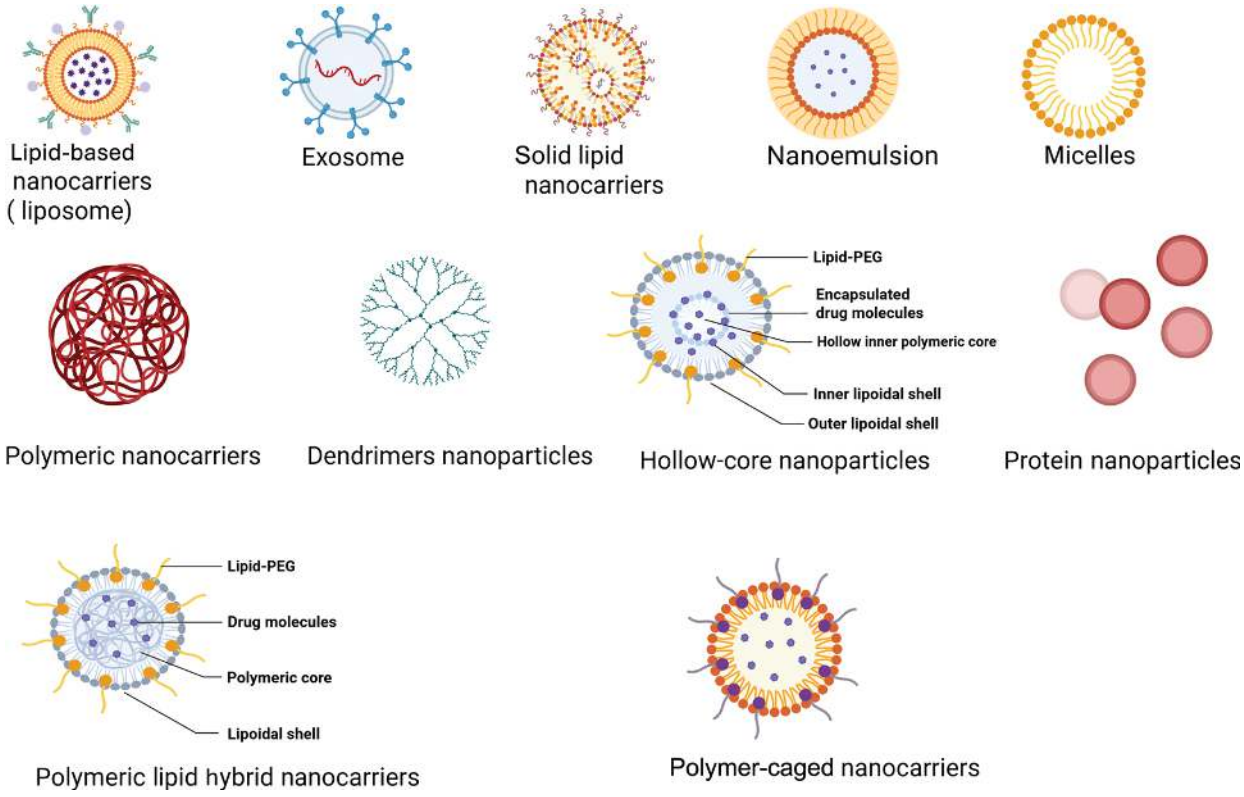

FIGURE 5.2 Nanocarrier systems for protein transport. (Schematic illustration showing structures and formulas for protein transport.) ⏎

5.2 NANOCARRIER SYSTEMS FOR PROTEIN TRANSPORT

Organic nanocarriers are solid particles formulated from organic compounds, lipids, and polymers, ranging in size from 10nm to 1 μm. Utilizing biopolymers in manufacturing these carriers extends their benefits and applications. Their biopolymer-based systems can be simpler to develop, which can lead to increased productivity and streamlined manufacturing processes. In addition, organic nanocarriers have tunable surface properties that can be designed for particular biomedical applications such as imaging or targeted drug delivery. A notable advantage of biopolymer-based carriers is that organic nanocarriers are biocompatible which mitigates negative responses from a host and is safe in living systems. Biopolymer-based carriers have high stability, preserving the integrity of encapsulated drugs or imaging agents during storage and transport which is critical for preserving therapeutic or diagnostic efficacy. Another important advantage is that these systems are biocompatible and after drug delivery, these carriers are eliminated or metabolized in the body, which mitigates long-term toxic accumulation. A wide range of biopolymers have been used to create organic nanocarriers which include lipid-based organic systems, dendrimers, and nanomicelles and others as illustrated in. Each of these has unique properties (Younis et al., 2025, Bouazzaoui and Abdellatif, 2024, Abdellatif et al., 2023).

The addition of polymers in the engineering of nanocarriers has significantly enhanced their therapeutic efficacy by site-specific targeted delivery of those drugs and minimizing their side effects. Polymers have been employed, either alone or in combination with inorganic nanomaterials, to fabricate multifunctional DDSs (Kim et al., 2024). Block copolymers, when employed in the preparation of drug-loaded nanocarriers or for surface

modification, augment the interaction between NPs and target tissue, resulting in improved biodistribution, prolonged circulation time, and enhanced uptake (Islam et al., 2025). Polymeric nanocarriers are amphiphilic block copolymers with varying hydrophobicity that are natural or synthetic (De et al., 2022). They may be formulated through nanoprecipitation or double-emulsion method. One advantage of their core–shell characteristics is that they can encapsulate hydrophobic drugs for controlled-release delivery, thereby increasing drug half-life in the body (Vosoughi et al., 2025). Not only that, but also, the polymeric nanocarriers can be equipped with ligands for targeted drug delivery, making them highly effective carriers with improved biological activity and bioavailability (Yun et al., 2025, Ly et al., 2024).

The intracellular delivery efficiency of nanocarrier systems is highly dependent on physicochemical properties, of the nanocarriers, including surface charge, particle size, and material composition, which collectively determine uptake mechanisms and endosomal escape behavior. For instance, using positively charged carriers, such as cationic liposomes, PEI-derived polymers, fluorinated polymers, and guanidinium-functionalized systems, improved binding to the negatively charged plasma membrane, thereby promoting rapid internalization via clathrin-mediated, caveolin-mediated, or macropinocytic endocytosis. It has been shown that particle size is also an important factor. Nanocarriers between 30 and 150 nm generally achieve optimal endocytic uptake, whereas larger particles may shift toward macropinocytosis or exhibit reduced intracellular transport. Another important player is the composition of the nanocarrier which highly affects its cytosolic delivery; for example, lipid-based carriers can undergo membrane fusion or pH-dependent destabilization polymeric systems frequently exploit the proton-sponge effect or dynamic covalent interactions to rupture endosomes and CPP-modified carriers enable direct translocation

or transient pore formation. Adjusting these parameters, nanocarriers can minimize lysosomal degradation, enhance cytosolic release, and significantly improve the intracellular bioavailability of therapeutic proteins (Abdellatif et al., 2022, Younis et al., 2022, Singh et al., 2021).

5.2.1 Lipid-Based Nanocarriers

5.2.1.1 Liposomes

Liposomal systems integrated with protein-based therapeutics are an exciting new approach for biotechnology that can enhance the stability and delivery performance of protein drugs. Liposomes resemble biological membranes, are highly biocompatible and adaptable, and can interact closely with proteins for targeting, transport, and prolonged release. Liposomes can enter cells by several different pathways: by fusing with the plasma membrane, by internalizing through endocytosis, or by exchanging lipids. All these mechanisms require physical interactions and electrostatic complementarity between the lipid bilayers and the cellular membrane (Nsairat et al., 2022, Tenchov et al., 2021, Trucillo et al., 2020). Protein and liposome interaction can be divided broadly into two approaches: encapsulation and surface modification (Park et al., 2024a). Encapsulation approaches include methods in which a protein is entrapped within the lipid bilayer to produce a stable liposome–protein complex (Burger et al., 2025). This strategy has particular potential for targeting and delivering therapeutic proteins, such as glucose oxidase, catalase, horseradish peroxidase, and superoxide dismutase, as enzymes in the therapeutic context (Anwar et al., 2025, Liao et al., 2025, Sen et al., 2024, Melo et al., 2020, Kim et al., 2019). Proteins are susceptible to environmental conditions, such as temperature, pH, and exposure to denaturing conditions, which can lead to loss of conformation and bioactivity. In forming a structure with liposomes, the protein is encased by a

thermo-stable vessel and protected from conditions that may lead to denaturation or inactivation via the reestablishment of its conformation (Masson and Lushchekina, 2022). In addition, the liposomal carrier may offer a microenvironment where release kinetics can be tailored to prolong the protein's pharmacologically active action (Chen et al., 2024b). In the case of modifications, using chemical or physical means to modify proteins within liposomal systems makes use of "spontaneous liposomal fusion," creating a relocated protein. In Table 5.1, we compared between different nanocarriers for protein delivery.

TABLE 5.1

Comparison of Nanocarriers for Protein Delivery

Category	Type	Advantages	Limitations	References
A. Lipid-based	Liposomes	Biocompatible; protect proteins; tunable surface modification; strong clinical translation precedent	Physical/storage instability; opsonization and rapid clearance; scale-up and sterility challenges	(Singh et al., 2017, Akbarzadeh et al., 2013, Torchilin, 2007)
A. Lipid-based	Nanoemulsions	High solubilization capacity for hydrophobic payloads; well-established, scalable formulation routes	Require surfactants/co-surfactants that may destabilize proteins; limited control over protein release kinetics	(Binauld and Stenzel, 2013, Wu et al., 2010, Kim et al., 2010)

Category	Type	Advantages	Limitations	References
A. Lipid-based	Micelles/Polymeric micelles	Simple preparation; suitable for amphiphilic constructs; stimuli-responsive designs (e.g., pH, redox) are possible	Low *in vivo* stability due to critical micelle concentration (CMC) dissociation; premature cargo release; potential renal clearance	(Palmerston Mendes et al., 2017, Kim et al., 2010)
B. Polymeric	Fluorinated polymers	Enhance protein stability and shelf-life; tune surface interactions (stealth properties); improve formulation robustness	Complex multi-step synthesis; potential for biopersistence and toxicity depending on fluorination pattern and polymer length	(Du et al., 2019, Zhang et al., 2008)
B. Polymeric	Boronated polymers/PBA-based	Reversible covalent binding to cis-diols (e.g., on glycoproteins); enable glucose/pH-responsive release	Physiological instability of boronate esters (hydrolysis); risk of off-target glycan binding; slow release kinetics	(Shi et al., 2011a, Sengupta et al., 2005)
B. Polymeric	Guanidinium-functionalized polymers	Promote strong membrane interaction and efficient cytosolic delivery via bidentate H-bonding; effective in pre-clinical studies	Strong binding can retard cargo release and reduce efficacy; potential charge-related cytotoxicity and non-specific interactions	(Torchilin, 2008)

Category	Type	Advantages	Limitations	References
B. Polymeric	Heterocyclic polymers (biomedical)	Diverse chemical functionality for stimuli-responsiveness and tunable degradation; e.g., pyridine for pH-sensitivity	Often require multi-step syntheses; reactive functional groups may perturb protein structure if not carefully designed and controlled	(Sahu et al., 2024, Mcgrath et al., 1995)
B. Polymeric	Dendrimers	High loading capacity; multivalency for enhanced targeting; controlled architecture; strong endosomal buffering (e.g., PAMAM)	Cytotoxicity concerns (especially with cationic generations); costly and complex scale-up; potential immunogenicity	(Karadurmus and Erturk, 2025, Wang et al., 2022)
C. Polymeric-Lipid Hybrid	Polymer core-lipid shell hybrid carriers (LPHNPs)	Combine polymeric control over release with lipid biocompatibility and stealth coatings; improved payload protection	Complex multi-step formulation and difficult scale-up; risk of phase separation and batch-to-batch heterogeneity	(Hassan et al., 2025, Sivadasan et al., 2021)
C. Polymeric-Lipid Hybrid	Polymer-Caged Nanobins (PCNs)	Stimuli-responsive polymer shell around a liposomal core; allows modular surface modification and triggered release	Complex fabrication; many constructs remain in pre-clinical stages; long-term stability can be a challenge	(Lee et al., 2009)

Category	Type	Advantages	Limitations	References
C. Polymeric-Lipid Hybrid	Monolithic LPNPs / Mixed lipid-polymer systems	Flexible composition for co-delivery of multiple drugs; enables tailored release profiles	Significant heterogeneity; challenges in reproducibility and complex characterization required	(Hassan et al., 2025)
C. Polymeric-Lipid Hybrid	Lipid bilayer-coated nanocarriers (core–shell)	Biomimetic shell improves stealth and biocompatibility; polymeric core provides mechanical strength and stability	Difficulty in achieving a uniform, stable coating; limited payload capacity for some constructs; manufacturing complexity	(Giram et al., 2024, Lee et al., 2022)
C. Polymeric-Lipid Hybrid	Hollow-core / Lipid–Polymer–Lipid (L-P-L) nanocarriers	High cargo capacity; potential for sequential or multi-layer release control	Complex synthesis and characterization; concerns regarding stability under physiological stress	(Arbaz et al., 2025, Ma, 2019)
D. Protein-Based and Peptide-Modified	Self-assembling protein nanoparticles (SAPNs, nanocages)	Genetically programmable; precise multivalent display; intrinsic biocompatibility (ideal for vaccines, targeted delivery)	Risk of aggregation; significant manufacturing, sterility, and scale-up challenges; limited to specific cargo types without engineering	(Sahab-Negah et al., 2024, Zhang et al., 2023, Park and Champion, 2023)

Category	Type	Advantages	Limitations	References
D. Protein-Based and Peptide-Modified	Nanocarriers modified with cell-penetrating peptides (CPPs)	Enhance cellular uptake and endosomal escape; modular conjugation strategy applicable across many carrier types	Non-specific uptake; potential for toxicity and immunogenicity; stability of peptide conjugates in serum can be low	(Jankowski et al., 2025, Gessner and Neundorf, 2020)

5.2.1.1.1 Solid Lipid Nanocarriers

To overcome certain limitations associated with polymeric nanocarriers, lipid systems have emerged as another attractive option and have seen widespread use as delivery systems for lipophilic drugs. Specifically, solid lipid nanoparticles (SLNs) have garnered significant attention in the previous decade (Bukke et al., 2024, Akanda et al., 2023, Viegas et al., 2023). SLNs are representative of a new generation of lipid emulsions ranging from submicrometers to nanoparticles wherein the lipid phase has taken on a solid lipid in place of a liquid lipid. Switching to a solid lipid has several benefits: small particle size, large surface area, high drug loading, and enhanced interfacial interactions, all of which make SLNs ideal candidates to improve their performance for pharmaceutical, nutraceutical, and bioactive compound delivery. SLNs can be made up of natural or synthetic lipids, with a lipid core that is solid at room and body temperature (Safta et al., 2024).

Along with their safer profile compared to cationic liposomes, SLNs are still considered safe for use in human systems. This submicron colloidal carrier system enables lipids to be dispersed in water or an aqueous surfactant, offering good biocompatibility and improved drug delivery systems compared to lipid-based systems (Ghasemiyeh and Mohammadi-

Samani, 2018). The versatility SLNs have created in their potential as a dynamic drug carrier system for intravenous applications has led researchers to consider novel drug carriers. For example, curcumin-loaded SLNs have an added value as an anti-amyloid, anti-inflammatory, and neuroprotective effects compared to conventional curcumin formulations in mouse models of Alzheimer's disease (Genchi et al., 2024, Campisi et al., 2022, Maiti et al., 2018).

5.2.1.2 Nanoemulsions (NEs)

Nanoemulsions (NEs) are colloidal dispersions of two immiscible liquids with surfactants used to stabilize a mixture. NEs are typically made with water, oil, and an emulsifier in a defined ratio. NEs have a lot of advantages, including good biocompatibility, kinetic stability, ability to facilitate cellular transport via paracellular and transcellular transport, and insulation of encapsulated cargo from hydrolysis and enzymatic degradation (Jacob et al., 2024). NEs can be used to carry a broad range of compounds, including both hydrophilic and hydrophobic compounds. Oil-in-water (O/W) NEs are ideal for hydrophobic molecules, while water-in-oil (W/O) systems are suitable for hydrophilic payloads. NEs can be administered through many routes: nasal, ocular, oral, and intravenous (Manzoor et al., 2023). NEs have great functioning ability along with a significant opportunity for therapeutic effects. Chitosan-coated rosmarinic acid NEs have been previously shown to protect astrocytes under neuroinflammation conditions, when the astrocytes were exposed to lipopolysaccharide that induces neuroinflammation, on cell death, and associated redox state (Fachel et al., 2020). Similarly, siRNA-loaded NEs demonstrated the therapeutic potential of this formulation in reducing TNF-α levels, indicating the potential of NEs as treatments for neuroinflammatory scenarios (Yadav et al., 2016). In a different study on

microparticle delivery, ropinirole, a dopamine agonist that has been additionally prescribed along with levodopa, is complicated by low bioavailability and a short half-life (Silva et al., 2021). Using a NE based on transdermal gels, which delivered improved drug absorption properties, demonstrated better physical properties (less skin irritation and lower systemic toxicity) compared with traditional formulations (Choudhury et al., 2017).

5.2.1.3 Micelles

The presence of micelles challenges our understanding when considering drug formulations in aqueous environments. Micelles are self-assembling nanosized colloidal particles with a hydrophobic core and hydrophilic shell that can be used as drug carriers for poorly soluble drugs (Biswas et al., 2013). Amphiphilic copolymers containing hydrophobic and hydrophilic blocks have been recognized as new materials that can form stable micelles (Kataoka et al., 2012). Polymeric micelles, including lipid-core micelles created by conjugating soluble copolymers to lipids, have shown excellent stability *in vitro* and *in vivo* and positive biocompatibility (Ghezzi et al., 2021). This allows for the solubilization of numerous poorly soluble pharmaceuticals, many of which are in preclinical and clinical research (Hwang et al., 2020). Poly(ethylene glycol) (PEG), with a molecular weight of 2–15 kDa, is the most frequent hydrophilic segment in micellar drug delivery systems and offers several advantages for applications in micellar systems (Shi et al., 2022). PEG is highly soluble, nontoxic, and has no charge, so it can form a hydrophilic corona surrounding micelles to inhibit nonspecific interactions with blood components and extend the time a drug circulates in systemic circulation (Shi et al., 2022). Other hydrophilic polymers, including poly(N-vinyl pyrrolidone) (PVP) and poly(N-isopropyl

acrylamide), have also been used in the consideration of their ability to stabilize micelles. Examples of lipid-based micelles, such as PEG-diacyllipid conjugate systems devised by Torchilin and coworkers, not only utilize the hydrophobic lipid core to improve micelle stability (Jhaveri and Torchilin, 2014).

Another example of innovative micellar systems is the PEG–cholic acid conjugates that offer high drug loading capacities as shown through a variety of therapeutic applications (including paclitaxel) (Ghezzi et al., 2021). These micelles were shown to have better anticancer actions and lesser toxicity than standard formulations in both animal models and clinical trials. PEG–cholic acid micelles (including PEG2K–cholic acid) also show extended release and extensive drug loading, and they offer essential opportunities for clinically relevant drug delivery systems (Xiao et al., 2011). Yokoyama et al. also elucidated their own results and the manner in which adriamycin-conjugated polyethylene glycol–poly (aspartic acid) block copolymers (PEG–P[Asp(ADR)]) could micelle in aqueous solution and have in vivo antitumor activity (Yokoyama et al., 1991). Clearly, the report did not separate immobilized and chemically or physically separated adriamycin since both chemistries exist in the proposed micelle. Additionally, some of the adriamycin derivatives may be impurities, while others could lead to side effects.

5.2.2 Cell Derivate

Extracellular vesicles (EVs) as protein carriers are nano- to microsized bilayer phospholipid vesicles that are secreted by almost all cell types to facilitate intercellular communication (Ragni, 2025). In addition to their diagnostic uses, EVs have been recognized as drug delivery vehicles due to their relatively efficient cellular uptake, as well as their reduced immune

clearance compared to typical liposome formulations (Du et al., 2023a). EVs typically contain the proteins from their source cell, which is in common with the potential to use EVs as protein delivery vehicles. Normally, EVs are loaded with specific proteins by engineering cells to overexpress the desired protein, followed by the isolation of EVs from the culture medium using ultracentrifugation (Wiklander et al., 2019). Successful examples of protein-loaded EVs include CD47@exosomes (Wen et al., 2025) and arrestin domain containing protein 1 ([ARRDC1]-mediated microvesicles) (Wang et al., 2018). Although there has been success with these examples, the translation of EVs to the clinic has been constrained by low yields and variation in quality (Klyachko et al., 2020). To address the issues associated with EVs, cellular vesicles (CVs) are designed as alternative EVs. CVs are nano-sized (15–300 nm) vesicles prepared via the sonication and extrusion of plasma membranes from cancer cells that can retain functional proteins and protein surface markers (Li et al., 2025). Research teams have fabricated CVs that express high levels of PD-1 (Kiełbowski et al., 2025) and dual proteins (SIRPα and PD-1) using similar gene-editing strategies (Lin et al., 2025).

5.2.2.1 Polymeric Nanocarriers

Functional polymer-mediated biomacromolecular delivery occupies an essential position in the field of protein delivery and has long been a focus of attention. Natural polymers such as chitosan (Desai et al., 2023), alginic acid (Masoumi Shahrbabak et al., 2025), cyclodextrin (Payamifar et al., 2025), and chondroitin sulfate (Mneimneh and Mehanna, 2024), as well as polymeric materials such as PLGA (Alsaab et al., 2022) and PEI (Beach et al., 2024), can be used for protein delivery due to their good biocompatibility and biodegradability. Polymer–protein complexes formed

through intermolecular interactions often exhibit instability and are prone to decomposition in buffered solutions, posing a critical challenge that needs to be addressed in this field (Pritzlaff et al., 2023). Compared with nucleic acids such as DNA and RNA, proteins have a relatively low net charge, which undoubtedly increases the difficulty of forming stable complexes with polymer carriers. To successfully prepare protein complexes with good stability, we urgently need to explore the practical application of cationic polymers. Mixing different functional ligands onto cationic polymers can make a better binding affinity between polymers and proteins, effectively reducing charge repulsion between cationic polymers during the formation of polymer–protein complexes (Lv et al., 2019). We will explore the progress in polymers modified with various functional ligands for protein cytosolic delivery, aiming to provide valuable references and inspiration for future developments in this field.

5.2.2.2 Fluorinated Polymers

Because of their unique ability to interact ionically with various biomolecules and bind to the anionic regions of proteins, fluorinated polymers have drawn much interest (Xin et al., 2024). Through the fluorophilic effect, they form highly stable nanoformulations via synergistic assembly and thus hold immense promise in biomedical engineering (Jeon et al., 2025). Such formulations stabilize the proteins against environmental degradation and target their delivery, therefore opening a whole horizon of therapeutic opportunities. The fluorinated chains impart dual hydrophobic and lipophobic characters to the polymer, which helps encode proteins with precisely controlled encapsulation and preserve biologic activity.

Cheng Yiyun's research group made a scientific breakthrough when they revealed the "fluoroamphiphiles" phenomenon of fluorinated polymers in

protein cytosolic delivery (Zhang et al., 2018). They grafted an assortment of fluorinated small-molecule compounds onto polyethylenimine, thereby creating an extensive library of fluorinated polymer materials. This discovery gave birth to an entirely new route for the application of fluorinated polymers in biomedicine. The group further confirmed the usefulness of their approach by using model proteins with different moieties (Akbarian and Chen, 2022).

5.2.2.3 Phenylboronic Acid (PBA) in Boronated Polymers

Phenylboronic acid (PBA), a typical electron-deficient group in boronated polymers, is responsible for their biomedical applications (Ryu et al., 2019). PBA can form stable nitrogen–boronic acid coordination with primary amines, as well as imidazole groups rich in lone pair electrons, enabling them to bind efficiently to proteins (Martínez-Aguirre et al., 2021). In addition, the guanidinium groups of arginine residues in proteins may interact strongly with PBA through guanidinium–π–π interactions, which can further enhance the affinity between boronated polymers and proteins (Zhu et al., 2024).

When high-density grafted PBA is used on cationic polymers, these polymers can achieve deep binding with cationic and anionic regions on protein surfaces through multiple modes of interaction (Zhang et al., 2021). This not only strengthens drug–cell interactions but also drug targeting and therapeutic efficacy. However, polymers with relatively low PBA grafting ratios tend to bind weakly with proteins (Hwang et al., 2020). To overcome this limitation, researchers introduced natural polyphenols. Because they contain a high density of phenolic hydroxyl groups and aromatic rings, polyphenols are strong hydrogen bond formers (Kerr-Phillips et al., 2022). They can engage in hydrophobic interactions with biomolecules such as

proteins and nucleic acids. This compensates for the low PBA density, improving the performance of boronic acid-modified polymers.

Furthermore, boronate-modified polymers can specifically bind to catechin groups on protein surfaces via catechin–boronate ester bonds (Ren et al., 2023). These dynamic covalent bonds are physiologically stable but cleavable in acidic microenvironments at the target site, allowing for controlled release (Nazli et al., 2024), and those techniques improve therapeutic specificity with reduced off-target effects. It has been demonstrated that the incorporation of natural polyphenols can improve the efficacy of boronate-modified dendrimer polymers by at least fivefold (Ozuna-Valencia et al., 2024). These findings suggest a promising approach for drug delivery and tumor treatment, providing a pathway to developing safer and more efficient medical treatments.

5.2.2.4 Guanidinium-Functionalized Polymers

Guanidinium-functionalized polymers have attracted tremendous attention among biomedical researchers because of their favorable chemical properties and high intracellular delivery capacity. Guanidinium-functionalized polymers show high protein binding affinities and effective facilitation of cell membrane interactions, thereby providing enhanced cellular uptake (Chen et al., 2024a, Lv et al., 2020). Such properties render guanidinium-functionalized polymers as favorable candidates for applications in protein delivery, drug delivery, and cellular imaging.

One of the most versatile aspects of guanidinium is its higher alkalinity. The guanidinium group has a relatively high pKa of 13.6, which renders it even more basic than the arginine side chains (pKa 12.5) and lysine (pKa 10.5). Because of their strong basicity, guanidinium groups can form very stable interactions, such as salt bridges and hydrogen bonds, with the

carboxyl groups of acidic amino acids, such as glutamic acid and aspartic acid (Negi et al., 2023). These interactions also significantly enhance polymer–protein binding, facilitating the correct intracellular localization and distribution of guanidinium-functionalized carriers.

Guanidinium-rich peptides, polymers, and nanoparticles exhibit exceptional, reliable membrane-penetrating ability. They have a high affinity for the cell membrane, ensuring rapid binding and efficient internalization via endocytic pathways, thereby making them effective in protein and drug delivery. A good representative example is provided by Wang et al., who conjugated poly (β-amino ester) (PAE) with phenylguanidine groups to enhance its performance of cytosolic protein delivery. The guanidine-achieved PAE possessed excellent protein-binding ability and high internalization efficiency, efficiently delivering CRISPR-Cas9 RNP complexes into GFP-expressing HeLa cells and inducing over 80% knockout of GFP expression (Wang et al., 2023c).

Later studies with phenylbiguanide-conjugated polymers revealed even more enhanced results. Compared with monoguanidine-functionalized analogs, the biguanide-derived polymers were more active with respect to protein binding and amplified facilitation of cell membrane interaction and cellular uptake (Yu et al., 2019).

Interestingly, biguanidines exhibit lower cytotoxicity than monoguanidines despite their higher charge density. The explanation of this is not fully understood, but it offers valuable insight into the optimal design of future protein delivery vehicles of high efficacy and low toxicity. This result identifies guanidinium-functionalized polymers as likely candidates for efficient and safe intracellular protein delivery (Zhu et al., 2024, Wang et al., 2023c, Yu et al., 2019).

5.2.2.5 Heterocyclic Polymers

Heterocyclic polymers form a prominent group of materials that find application in the construction of delivery systems for biological macromolecules, particularly cytosolic proteins. With their unique structural and electronic properties, they contain regulatory ability, which plays a pivotal role in maintaining the stability and biological function of proteins upon delivery. The regulatory ability guarantees effective delivery of therapeutic proteins into the cell while maintaining their functional integrity (He et al., 2024, Wang et al., 2024a).

Heterocyclic organic compounds are of prime interest in this regard due to the presence of heteroatoms such as nitrogen (N), oxygen (O), and sulfur (S) in their ring systems. These heteroatoms with high electronegativity induce strong inductive effects on the heterocyclic chain. This, in turn, modifies the charge distribution along the polymer chain, directly affecting the polymer's ability to interact with proteins and other biomacromolecules. This inherent characteristic makes heterocyclic polymers highly effective in maximizing both binding affinity and drug delivery efficiency of therapeutics (Song et al., 2024, Tao et al., 2023).

Functionalized polyethylenimines (PEIs) and PEGs are examples of the heterocyclic polymer's ability to be employed in delivery. Through modulation of coordination and hydrogen bonding, these polymers can be designed to regulate their charge characteristics and cargo-binding capacity tightly. For instance, PEIs bearing heterocyclic substituents can enhance electrostatic interactions with proteins and reduce nonspecific toxicity. At the same time, PEG derivatives functionalized with heterocycles can facilitate solubility, stability, and circulation times *in vivo* (Postupalenko et al., 2014).

The promise of heterocyclic polymers to combine tunable charge architecture with diversified modes of molecular interaction—ranging from ionic and hydrogen bonding through coordination chemistry—positions

them as prime candidates for the next generation of macromolecule and protein delivery vehicles. Their adaptability in structure enables them to provide the platform on which to construct multifunctional delivery platforms that can simultaneously match stability, efficacy, and biocompatibility (Benjaminsen et al., 2013).

5.2.2.6 Heterocyclic Polymers for Biomedical Applications

PEG is currently one of the most commonly used polymers in biomedical fields with multiple applications in protein conjugation, drug delivery, surface engineering, and tissue engineering (Zhao et al., 2024). PEGylation, which is well studied methods, relies on the covalent attachment of PEG chains to peptides or proteins. The modification considerably enhance pharmacokinetic features, i.e., enhanced aqueous solubility, prolonged systemic half-life, improved conformational stability, and reduced immunogenicity (Wasyłeczko et al., 2024). Thus, PEGylation has become a cornerstone strategy in the enhancement of the therapeutic utility of biologics in clinical applications. In addition to PEG, heterocyclic polymer derivatives have shown great promise for protein delivery in the cytosol.

For instance, pyridine thiourea-substituted polyethylenimines (PEIs) possess high protein-binding capacity (Postupalenko et al., 2014). The polymers can be linked into target proteins, as green fluorescent protein (GFP), by strong ionic forces and hydrophobic interactions, thereby preserving proteins intact and active during delivery. Strong binding not only improves delivery stability but also prevents premature release or protein drug degradation. Apart from single polymers, heterocyclic units can be incorporated into hybrid delivery systems. When combined with biocompatible delivery platforms such as liposomes, polymeric nanoparticles, or lipid–polymer hybrids, heterocyclic polymers deliver

multifunctional platforms that can be tailored. Hybrid systems allow for targeted drug delivery of protein therapeutic proteins to the target tissues, along with the improvement of their bioavailability and in vivo stability. Such adaptability underscores the potential of heterocyclic polymers in filling the gap between research innovation and medical use.

Nevertheless, several challenges remain. The decision to use the material in the formulation of heterocyclic polymers relies in part on its ability to exert certain synthetic control and to undergo chemically reproducible modification. Preservation of biocompatibility, cytotoxicity reduction, and preservation of long-term stability in the elaborate biological environment require advanced synthetic methods and valid characterization techniques (Eltaib, 2025). Malhotra et al. formulated nanoparticles that were surface-coated with phosphonate-based biocompatible polymers and reached long-term stability without defeating delivery performance (Malhotra et al., 2022). This highlights the importance of innovative polymer design and the careful selection of materials to overcome significant limitations. Proper utilization of heterocyclic polymer-based delivery system design and formulation will be crucial in the near future to increase efficiency as well as specificity.

Other advanced functionalization methods, in conjunction with future-generation analytical instruments such as high-throughput screening and real-time microscopy, will drive the development of safer, more effective delivery systems.

5.2.2.7 Dendrimers

Dendrimers are branched, organized macromolecules that can be prepared through repetitive chemical reactions originating from a core structure. They have been well-studied as carriers for drugs, genes, and proteins and

function through covalent conjugation, ionic interactions, or adsorption (Fadaei et al., 2025). Dendrimers offer several notable advantages, including highly controllable biodistribution and pharmacokinetics, structural and chemical uniformity for reproducibility, and multifunctionality through numerous surface groups that can be utilized to enhance solubility, targeting specificity, and binding with various biomolecules (Chis et al., 2020). Many dendrimers encapsulate drugs in 1%–5% range, which allows for sufficient amounts of drugs or drug derivatives to be encapsulated while also retaining dendrimer structural stability and dendrimer-drug stability (Menjoge et al., 2010, Milhem et al., 2000). Certain dendrimers can cause elevations in liver enzymes or leukocyte infiltration in the liver from brief administration in animal models (Porterfield et al., 2023), Yet, considerable evidence exists across the literature showing the success of dendrimers in health applications (Alamos-Musre et al., 2025). In studies using dendrimers, they promoted antioxidant levels and inhibition of neuroinflammatory responses that alleviated the effects of neuroinflammation, contributing to positive outcomes in developing zebrafish with cerebral palsy (Kannan et al., 2012, Wang et al., 2009). Notably, dendritic polyglycerol sulfates (dPGS) are significant inhibitors of both anti-inflammatory and complement activities (Dernedde et al., 2010). Studies indicate that dPGS can alter amyloid-beta fibril formation, downregulate neuroinflammatory mediators such as lipocalin-2, and positively affect decreased neuroglial cell function, suggesting applications in neurodegenerative disorders such as Alzheimer's disease (Tianyi et al., 2023, Maysinger et al., 2018, 2015).

Poly(amidoamine) (PAMAM) dendrimers are commonly used in applications as nanocarriers because of the tertiary amine groups on the branch points of the dendrimers (Yang et al., 2015). The amines can complex with metal ions when in aqueous solution and then be reduced to produce nanocarriers encapsulated within the dendrimers (Scott et al., 2005). Various

formulations using dendrimers have been developed as nanodrugs for many therapeutic purposes, including antiviral polylysine dendrimers marked with sulfonated naphthyl groups, antibacterial PPL dendrimers composed of tertiary alkylammonium groups, and chitosan-dendrimer hybrids to enhance antibacterial action (Sharma et al., 2014). Most notably, when used with anti-inflammatory or antioxidant agents, advanced PAMAM dendrimers have shown promise as anti-inflammatory agents to inhibit inflammation in traumatic brain injury by blocking proinflammatory cytokines and preventing NF-κB activation (Sharma et al., 2020), and similar results have been found for dendrimer–N-acetyl-L-cysteine to increase antioxidant levels intracellularly and protect microglia, as well as astrocytes, against excitotoxicity (Sharma et al., 2018). Dendrimers entangle in a hydrogel form, crosslinked networks that can swell in an aqueous solution (Kaga et al., 2016). Hydrogels generally modified with PEG will still swell in aqueous solution and are very useful and desirable in prolonged applications related to cartilage tissue engineering, ocular injury repair, and targeted drug delivery (Peppas et al., 1999). Dendrimers have also been helpful for transdermal drug delivery because they aid in drug delivery by solubilizing components, allowing drugs to be more broadly distributed in circulation in plasma (Kirkby et al., 2024).

5.2.3 Polymeric Lipid Hybrid Nanocarriers

The formation of stable hybrid vesicles in polymer–lipid hybrid nanocarriers is mainly influenced by differences in chemical composition and the size of the hydrophobic segments of polymers and lipids (Chountoulesi et al., 2024). These hybrid systems have the key features of both components into a unified structure enabling versatile combinations that overcome the individual limitations of polymers or lipids (Lok et al., 2025). Polymers offer

the advantage of controlled drug release and improved stability, while lipids, due to their biomimetic nature, enhance drug-loading capacity. Compared with carriers made solely of polymers or lipids, hybrid particles exhibit greater stability and prolonged therapeutic efficacy *invivo* (Parveen et al., 2023).

The vesicular structures formed in polymer–lipid hybrid systems are controlled by factors such as the polymer-to-lipid molar ratio and the thermotropic phase state of phospholipids (Chountoulesi et al., 2024). These assemblies can range from homogeneous polymer–lipid mixtures to microdomains enriched in either component, changing the polymer/lipid molar ratio alters vesicle architecture: higher polymer content generally yields thicker and more stable membranes, whereas higher lipid content produces more flexible structures with distinct biological functions (Parveen et al., 2023).

The lipid part controls the phase state through its main-chain transition temperature (Tm), which is no less important in determining membrane properties. Below Tm, lipids adopt a gel-like phase, producing more rigid, ordered membranes, whereas above Tm they transition to a liquid-crystalline phase, generating flexible, dynamic bilayers. In hybrid vesicles, this phase behavior parallels that of mixed liposomes prepared from lipids with different melting temperatures (Tm values) (Bhojoo et al., 2018, Caffrey, 2000)

Polymer—the size mismatch between polymer chains and lipid tails—also shapes lipid interactions in these hybrids. Liposomal membranes typically range from 3–5nm in thickness, whereas polymersomes vary between 5 and 50nm (Rideau et al., 2018). A pronounced mismatch introduces high line tension at polymer-rich and lipid-rich interfaces. This arises from thickness disparities that can expose hydrophobic polymer segments to the aqueous phase, thereby increasing the overall system energy

(Brodszkij and Städler, 2024). The system can either choose between conformational adaptation and coalescing into larger domains. In conformational adaptation, the polymer chains at the lipid–polymer interface undergo elastic deformation to decrease line tension, but sacrificing entropy as chain conformations are restricted (Wang et al., 2023a, Paul and Weikl, 2016). Similarly, the deformation of lipid membranes with coexisting phases bends in order to accommodate differences in composition (Baumgart et al., 2005). Specifically, hybrid vesicles formed from a rigid polymer (polystyrene) and a fluid lipid (dioleoylphosphatidylcholine (DOPC)) tend to deform polymer chains at the boundary to minimize exposure to water and stabilize the lipid–polymer interface (Bochicchio and Monticelli, 2016). In circumstances where conformational adaptation is not possible, the system may resort to simply growing larger lipid or polymer domains to have less interface and lower the line tension present (Wongsirojkul et al., 2022). In systems where polymer chains are too bulky or rigid to deform (such as hybrid vesicles made from polyethylene glycol–polylactic acid (PEG–PLA) and dipalmitoylphosphatidylcholine (DPPC)), the structure is usually observable as conglomerated lipid domains with an isolated polymer structure to reduce boundary exposure (Fauquignon et al., 2022).

The adaptable properties of hybrid vesicles are primarily determined by the properties of the pure polymer, including molar mass, chain length, and the rigidity of its hydrophobic backbone. Too much rigidity or substantial size mismatch may cause stable domains not to be formed. Instead, no domains are formed, resulting in the distribution of components being uniformly distributed throughout the vesicle (Reimhult and Virk, 2021). The study of line tension in polymer–lipid hybrid vesicles produced values more similar to those observed in multiphase lipid vesicles, indicating that line tension measurements can help expose the structure and properties of these systems (Fauquignon et al., 2022). Hybrid vesicles are unique in that they

can combine polymers and lipids into the same system/covalent structure, which makes them very promising drug delivery carriers (Lok et al., 2025). With proper engineering and careful formulation, hybrid vesicles can be designed to optimize drug delivery for a range of unique therapeutics.

5.2.3.1 Polymer Core–Lipid Shell Hybrid Carriers

Polymer core–lipid shell nanocarriers have made significant strides in drug delivery and have been researched extensively for their therapeutic applications. These systems combine the favorable properties of their respective components: the polymeric core and lipid shell (Lok et al., 2025, Chountoulesi et al., 2024, Sivadasan et al., 2021). They were developed by combining a liposome and a polymeric-based nanocarrier to form a hybrid system containing a polymer backbone with a lipid backbone or backbone (Chountoulesi et al., 2024). They can be thought of as composed of some composite structure with the polymer backbone designed and enclosed by the lipid layer(s) with interfacial regions occupied by various aqueous or buffer phases (Wu, 2016). The lipid shell, wherein the traditional liposomes are made of cationic or anionic phospholipids, allows for various electrostatic interactions between oppositely charged forms. Generally, three structural layers are typically identified: (1) a biodegradable hydrophobic polymeric core (e.g., PLGA); (2) an intermediate lipid region that acts as a barrier against leakage and penetration from water; (3) an outer lipid–PEG conjugate shell, which can load the outer hydrophilic drugs (Troutier et al., 2005). This structural setup provides structural stability and maintains the formulation integrity. While this method of loading water-soluble ionic drugs is quite limited, packaging hydrophobic drugs allows for easier encapsulation in polymer core–lipid shell hybrid carriers (Sivadasan et al., 2021). At the same time, the use of ionic drugs with counterion polymers

improves the loading efficiencies, and the lipid shell can enhance stability overall (Parveen et al., 2023). The adaptability of the polymer–lipid hybrids has been well established, as they are even able to modify solid lipid nanoparticles (SLNs) to deliver positively charged hydrophilic drugs (Gajbhiye et al., 2023).

5.2.3.2 Polymer-Caged Nanobins

Polymer-caged nanobins are novel systems in the drug delivery systems (DDS) arena, specifically designed to improve drug release, stability, and leakage control. Polymer-caged nanobins can be customized through their outer polymer layers to give specific surface properties. For example, polyacrylic acid has some properties due to the carboxyl groups present on its surface, which serve as active attachment points for linkers that confer pH-responsive properties (Lee et al., 2010). The basic process for making polymer-caged nanobins involves starting with liposomes, followed by some form of surface modification, such as coating with a polymer like cholesterol-functionalized polyacrylic acid. This modification can create carboxylate groups directly onto the liposome surface, thereby enhancing functionality (Cao et al., 2022). Drug-release profiles can be significantly tuned through these types of modifications. Another possible way to provide polymer shielding to the liposomal core is the use of pH-sensitive bridge linkers, thereby generating a pH-sensitive cross-linked polymer shell around the liposome (Sobol et al., 2025). These polymer coatings provide several advantages, including protection of liposomes, enhancing structural stability, decreasing premature drug leakage, and limiting release at site-targeted induced areas (Sriwidodo et al., 2022).

5.2.3.3 Monolithic LPNPs–Mixed Lipid–Polymer Systems

Monolithic lipid–polymer nanoparticles (LPNPs)/mixed lipid–polymer systems offer a novel paradigm for nanocarrier design that combines lipid and polymer components in novel drug delivery systems (Rahat et al., 2024). These carriers may form colloidal particles in which lipid particles are dispersed in a polymer matrix, thereby optimizing drug delivery. In general, these polymers are composed of a copolymer with hydrophilic and hydrophobic segments and lipids, which explains their amphiphilic properties that facilitate drug encapsulation and delivery (Xu et al., 2013). These lipid–polymer hybrid nanoparticles also combine the mechanistic aspects of liposomes, which are vesicular particles formed from phospholipids that resemble the cell membrane in structure and properties, with the self-assembly properties of phospholipids. In this respect, monolithic LPNPs or mixed lipid–polymer nanoparticles differ from traditional liposomes in terms of structure and properties, providing distinct advantages and challenges (Persano et al., 2021). For instance, the incorporation of PEG–lipid conjugates can improve circulation times and stability; however, excess incorporation of PEG–lipid conjugates can favorably enhance micelle formation, whereas phospholipid complicated aggregated vesicular particles. Nevertheless, monolithic LPNPs—mixed lipid–polymer systems—hold great potential for drug delivery (Liu et al., 2025).

Lipids bring together the advantages of biocompatibility and membrane-mimicking properties, in addition to stability and sustained-release properties attributed to polymers. These systems offer a versatile platform for the targeted and prolonged delivery of therapeutics (Shah et al., 2025).

5.2.3.4 Lipid Bilayer-Coated Nanocarriers

Coating self-assembled nanocarriers with lipid bilayers represents a promising approach to enhancing the stability and efficacy of drug delivery systems (DDS) in conditions where PEG fails to stabilize them *in vivo* (Plaza-Oliver et al., 2021). The lipid bilayer in lipid bilayer–coated nanoparticles is derived from RBC membranes, which offers additional advantages, like biocompatibility and controlled release. The lipid bilayer-encapsulated nanocarriers are generally made by heating RBCs to break them apart into membrane-derived fragments and encapsulating polymeric nanocarriers in the erythrocytes during the formation (Le et al., 2021). Lipid bilayer coating of nanocarriers offers enhanced biological surface strength while maximizing *in vivo* stability by mimicking the complex surface chemistry of RBCs (Parveen et al., 2023). Lipid bilayer-encapsulated nanocarrier membranes replicate a biomimetic structure that facilitates and supports drug delivery. The erythrocyte-derived vesicles surround the drug payload, while the stiff lipid barrier can promote slower release kinetics and robust sustained therapeutic action. Yet again, a limitation of lipid bilayer coating nanocarriers is the presence of blood group antigens in the RBC membrane, which may complicate crossmatching (Liao et al., 2024, Lei et al., 2022, Li et al., 2021a). However, lipid bilayer-encapsulated nanocarriers are still appealing as biocompatible DDS, with stability and promising therapeutic potential for controlled release therapies (Mehta et al., 2023).

5.2.3.5 Hollow-Core Nanoparticles/Lipid–Polymer–Lipid Nanocarriers

This special type of nanocarrier is composed of a concentric architecture comprising an innermost hollow lipid core, a middle polymer layer, with an outer lipid–PEG shell. They are formulated by using a double-emulsification solvent evaporation method (Shi et al., 2011b). In this process, lipids and polymers are emulsified to form concentric layers, producing polymer–lipid

complexes with properties similar to poly (lactic-co-glycolic acid) (PLGA) nanocarriers (Olshefsky et al., 2022). Each layer plays a role: the outer lipid–PEG layer improves *in vivo* stability, while the inner cationic lipid layer enhances anionic drugs encapsulation (Lu et al., 2023, Sivadasan et al., 2021). This structure ensures the efficient combined delivery of several drugs, making these nanocarriers particularly suitable for applications such as multidrug-resistant cancer therapy.

5.2.4 Protein Nanoparticles

The delivery of protein drugs to the cytosol (cytosolic delivery) via protein nanoparticles has recently progressed and emerged as a new frontier in biomedicine (Eweje et al., 2025). Protein nanoparticles can be characterized as nanocarriers formed of self-assembling single or multiple proteins that transport biomolecules intracellularly. New design strategies for protein nanoparticles have been to use (i) phase separation into condensates to modulate protein interactions and parameters such as temperature, pH, or ionic strength (used as either nanocarriers or imaging devices) (Zhu et al., 2024); (ii) computer-designed nanoparticles where design is performed using modeling of the structure, dimensions, and surface characteristics enabling targeted sites and distributions improving delivery efficiencies and reducing side effects; or (iii) combining the protein with short peptides termed CPP nanocarriers based on the ability of these peptides to cross membranes enabling improved intracellular transport (Herpoldt et al., 2024). In summary, these approaches illustrate the potential for further development of protein nanoparticles to enable cytosolic drug delivery.

5.2.4.1 Protein Self-Assembly Condensates with Liquid Phase Separation

Phase-separated protein self-assembly condensates capitalize on proteins' ability to undergo liquid–liquid phase separation under certain conditions to form condensates that facilitate controlled delivery of proteins (Zhu et al., 2024). As previously described, they create areas that are highly concentrated locally yet relatively permeable, allowing for efficient intermolecular interactions and controlled diffusion. Proteins contained within condensates at a localized area can translocate to their target sites via direct membrane translocation or typical endocytosis such as clathrin-mediated, caveolin-mediated, or macropinocytosis-mediated endocytosis (Zhu et al., 2024) (e.g., transferrin-functionalized Flutax-2 peptide condensates observed to translocate via clathrin-mediated endocytosis) (Zhu et al., 2024). YBX1 condensates are capable of recruiting miRNAs and encapsulating them into exosomes (Liu et al., 2021).

Condensates offer the added advantages of rapid formation relative to liposomes or polymeric nanoparticles, as well as minimal cytotoxicity. Additionally, they can be produced without the use of organic solvents, providing a safe and objective means of delivering proteins to cells (Liu et al., 2021, Paul and Weikl, 2016). Recent studies highlight the flexible nature of protein self-assembly condensates. For instance, glucose-responsive peptide–protein complexes exhibit characteristic droplet formation under metabolic stimuli, and pH-sensitive HBpep coacervates can disassemble in reducing environments to release their protein cargo (Zhu et al., 2024, Yuan et al., 2024). Remember that condensation can facilitate the efficient packaging of biomolecules into condensates, thereby bypassing endosomal degradation and enhancing tissue targeting and therapeutic delivery.

Despite advancements in drug delivery, gene therapy, and regenerative medicine, there are still limitations. Protein condensates lack the stability afforded by solid nanoparticles; this is important as their disassembly before acting at the target site may compromise the effectiveness. Research is

needed to increase stability and control release kinetics, site specificity of delivery, and safety profiles for *in vivo* studies (Zhu et al., 2024, Dragwidge et al., 2024).

5.2.4.2 Self-assembling Protein Nanoparticles

The field of self-assembling protein nanoparticles, in its present form, emerged during the early 2000s, and when the principle of computational design was introduced for understanding protein folding process, intermolecular interactions and assembly (López-Sagaseta et al., 2016). Engineered protein sequences for self-assembling nanoparticles are synthesized by genetic engineering and induced to assemble into diverse nanoscale structural morphologies (spherical, rod, and tube) for the desired function (López-Sagaseta et al., 2016, Kobayashi et al., 2015). These self-assembling protein nanoparticles offer potential applications in the fields of biomedicine and nanotechnology, including drug delivery, biosensors, tissue engineering, and nanoelectronics (Hughes et al., 2024). About drug delivery, self-assembling protein nanoparticles could potentially achieve targeted drug delivery and address efficacy and minimizing side effect challenges (Hughes et al., 2024, Olshefsky et al., 2022).

David Baker has been a major contributor to the field of self-assembling protein nanoparticles, advancing the field of protein design through the application of "top-down" design principles and reinforcement learning (Lutz et al., 2023). The Baker group has also used Monte Carlo Tree Search to develop new architectures—disk-shaped nanopores, ultra-compact icosahedra with enhanced antigen display, and bioactive fusion-protein icosahedra carrying multiple domains such as angiopoietin-1. The biologically active protein nanoparticles produced by Baker's lab have demonstrated superior immunogenicity, angiogenesis induction, and cell

signaling activation, and have strong potential in vaccine design and development and regenerative medicine. The top-down design method, unlike conventional methods, can create structural motifs of proteins that are not found in nature, which chemically and structurally expand the design space of programmable protein nanomaterials.

Traditional physics-based tools such as Rosetta required heavy computation and expert manual refinement for protein–protein interface design. To overcome these limitations, de Haas et al. applied the deep learning model ProteinMPNN to engineer two-component tetrahedral nanomaterials ("T33") from distinct trimeric subunits (De Haas et al., 2024). These nanomaterials were assembled *in vitro* from pure individual components, which is an essential milestone for scalable biomanufacturing. The Protein MPNN interfaces were designed with increased polarity, helping components fit together more smoothly and reducing hurdles to extend the application of protein interface design. (De Haas et al., 2024).

In addition to AI-based design, Huddy et al. constructed a geometry-based method using standardized protein building blocks (Huddy et al., 2024). This approach simplifies the construction of assemblies ranging from small oligomers to large nanocages and linear "train track" structures, with tunable size and geometry. Such scalable strategies address challenges in achieving structural regularity in complex assemblies (Huddy et al., 2023).

Overall, computational modeling and AI are contributing to the field of protein nanocarriers through the design of self-assembling protein nanoparticles and protein engineering to generate nanostructures with precise shapes and functions. A promising application of protein nanocarriers is in nanomedicine and biotechnology. However, there are still challenges in production efficiency, stability, and biocompatibility; ongoing efforts aim to improve their use in these fields (De Haas et al., 2024).

In conclusion, the fast progress of computationally designed self-assembling protein nanoparticles is considered a platform for the next generation of biomedical applications (Huddy et al., 2023).

5.2.5 Nanocarriers Modified with Cell-Penetrating Peptides (CPPs)

CPPs are small peptides with the ability to shuttle some macromolecules through the cytoplasmic membranes and, as such, are of great interest for drug delivery as well as other biomedical uses (Kardani and Bolhassani, 2021). Currently, more than 1,800 peptides are cataloged in the CPPsite 2.0 database and are generally categorized as amphipathic, cationic, or hydrophobic peptides (Zhu et al., 2024).

Nanocarriers are often modified with CPPs to enhance the nanocarrier's ability to gain entry into cells. Such modification is typically achieved through electrostatic adsorption or covalent bonding at the nanoparticle surface. Such modification leads to increased cellular uptake and increased delivery of therapeutic agents within the cell (Kardani and Bolhassani, 2021).

The transport of CPP-modified nanocarriers occurs via two major pathways: direct membrane translocation and endocytosis. In the first mode, amphipathic CPPs insert into lipid bilayers and cross the membrane directly (Herce et al., 2009), or transiently disrupt the bilayer to form pores that allow passive cargo diffusion (Park et al., 2024b). In the second mode, CPP–cargo complexes enter cells through energy-dependent endocytic pathways, including pinocytosis, clathrin-mediated, and caveolin-mediated endocytosis (Ruseska and Zimmer, 2020, Guo et al., 2016).

In mucosal tissues, CPP modification enhances interaction with mucus and epithelium, prolonging drug residence. Rehmani et al. conducted a promising study by engineering glycosaminoglycan-binding-enhanced

transduction (GET) nanocarriers for the oral delivery of insulin, achieving over 22-fold higher concentration in intestinal models (Rehmani et al., 2023). Neurotherapeutics also benefit from this technology. For example, CPP-modified nanocarriers enhance the penetration of the blood–brain barrier. Barra et al. utilized liposomes equipped with viral fusion peptides to deliver neuroprotective agents in an *in vitro* brain barrier model (Barra et al., 2022).

CPPs also suffer from serious obstacles such as serum instability, endosomal degradation, and cytotoxicity. To overcome those obstacles, incorporating lysosomal escape sequences and optimizing peptide design can enhance the efficiency and safety of the nanocarriers.

5.2.6 Enzymes Delivered Using Liposomes

Enzyme-based therapeutics have emerged as a significant class of drugs for the treatment of various diseases, specifically those caused by congenital enzyme deficiencies. Enzyme replacement therapy (ERT) allows for the restoration of absent or deficient enzymatic activity through the provision of functional enzymes to patients. As an example, infantile neurofibromatosis, a lysosomal storage disorder, causes the accumulation of undegraded metabolites not because of enzyme inactivity or degradation, but because of the absence of the enzyme palmitoyl-protein thioesterase 1 (PPT1). The loss of PPT1 activity causes granular deposits in lysosomes (Mukherjee et al., 2019, Geraets et al., 2016). Enzyme replacement therapy allows correcting such metabolic defects by restoring enzyme activity to the tissues and cells that depend on that enzyme activity. Despite the promise of enzyme replacement therapy as a therapeutic option, the clinical implementation of ERT is limited by the stability of the enzyme *in vivo*, the circulation half-life of the enzyme, and immunogenicity (Geraets et al., 2016). Encapsulating

enzymes into liposomes offers the opportunity to address many of the challenges outlined above. Liposomal encapsulation can stabilize enzymes from degradation, stabilize the enzyme, and reduce immunogenicity. For example, Santi et al. delivered lysosomal delivery of PPT1 using liposomes for the treatment of infantile neurofibromatosis (Wang et al., 2023b). The liposomes encapsulated PPT1, which showed restored cellular enzymatic effect on fibroblasts of CLN1 patients and facilitated the protein delivery to the central nervous system, altered intracellular pathways that provided therapeutic impact.

Besides enzyme replacement, delivery systems composed of liposomes have been utilized to deliver catalytic enzymes that ultimately may be therapeutic or cytotoxic. An example to consider is glucose oxidase (GOx), which has been used for studies surrounding the death of tumor cells. By encapsulating GOx within liposomes, the delivery of catalytically active GOx to tumor cells becomes possible, where glucose can be oxidized to form hydrogen peroxide (Li et al., 2023). More sophisticated systems have been devised wherein the delivery of the enzyme is coupled to a multifunctional therapeutic platform. One of the examples is the Mn-based nanoprobe NanoMn–GOx–PTX, which combines manganese, which acts as a core with a phospholipid bilayer, in order to co-deliver glucose oxidase, paclitaxel and a fluorescent dye(Wang et al., 2023b). It was designed in a manner where the manganese ions and therapeutic payloads are released from liposomes in response to tumor cell-acidic microenvironments. Manganese ions also catalyze the hydrogen peroxide formed by GOx into reactive oxygen species (ROS), while paclitaxel delivered its chemotherapeutic effects, which resulted in a synergistic effect and exhibited antitumor effects. Another novel approach toward liposome-based enzyme nanoreactors has been reported. Wang et al. designed a GOx and horseradish peroxidase (HRP) co-encapsulated liposome delivery system (Gao et al.,

2023). The system encompasses two functioning enzymes capable of catalyzing, under enzyme-like confined conditions.

Because of their distinctive catalytic properties, enzymes can not only produce characteristic colorimetric reactions with substrates but also serve as valuable tools for monitoring enzyme activity (Lee et al., 2025b). For this reason, they are often employed as model proteins to assess the efficiency of delivery systems. Such evaluation is typically carried out by detecting intracellular enzyme activity or by observing fluorescence signals.

A clear example is the important antioxidant enzyme that converts harmful superoxide radicals into oxygen and hydrogen peroxide, called superoxide dismutase (SOD). Clinical applications of SOD are typically in conditions with high levels of reactive oxygen species (ROS), such as rheumatoid arthritis, inflammatory diseases, and ischemia–reperfusion injury (Mccord and Fridovich, 1969). But this application is facing many challenges. It shows a very short half-life in circulation, rapid renal clearance, and poor tissue accumulation where it is needed (Zelko et al., 2002).

Using PEG (PEGylation) is an effective strategy to change the surface of liposomes with PEG and hence solve the issue (Allen and Cullis, 2013). This process reduces recognition and clearance by the mononuclear phagocyte system, allowing SOD-loaded liposomes to have a longer half-life and achieve higher concentrations in target tissues.

Liposomes offer many advantages. However, using protein nanocarriers, such as liposomes, also poses many challenges (Lombardo and Kiselev, 2022, Colletier et al., 2002). One of these challenges is that the protein may lose its activity during formulation. Liposome preparation involves several steps, which may affect the protein's structure. pH and temperature control are other factors that affect both the assembly of phospholipids and the stability of the protein. pH affects how lipids self-assemble and determines

how efficiently the protein can be encapsulated, while precise temperature management is essential to maintain both liposome structure and protein function.

Liposomes have also been explored for delivering ribonucleoprotein (RNP) complexes such as CRISPR-Cas9, a powerful genome-editing tool with significant potential for treating genetic diseases.

5.2.7 Liposomes for the Delivery of Ribonucleoproteins (RNP)

Clustered regularly interspaced short palindromic repeats (CRISPR)-associated protein 9 (Cas9) represents a powerful genome-editing tool that has clearly indicated a potential for treating diseases at the genetic level (Khoshandam et al., 2024). However, the safe and efficient delivery of CRISPR-Cas9 components into host cells is a major obstacle that hampers its ability to be translated into the clinic (Rauf et al., 2025). Unlike gene delivery that involves the introduction of DNA into cells, RNP complexes can have better advantages because they can provide immediate gene-editing activity without relying on cellular expression of the protein, with the benefits of minimizing intracellular off-target activity and duration of exposure (Ramakrishnan et al., 2025, Kaupbayeva et al., 2024, Du et al., 2023b).

However, another barrier to cellular introduction is that Cas9 has a relatively high molecular weight (size) of ~160 kDa. Liposome encapsulation offers a viable solution for direct delivery of Cas9 RNP into the target cell while protecting it from degradation by cellular nucleases. Two applications of liposomal technology are to protect and transport the Cas9 RNP protein complex; protection allows for the RNP to remain intact and functional, while transport ensures the complex reaches the target cell. Liposome-mediated delivery represents a safe and efficient platform for new

non-toxic and targeted gene-editing therapies(Akbaba et al., 2023, Yip, 2020).

Uses for Gene Editing Complexes Delivered Using Liposomes. The liposome delivery of gene-editing protein complexes has been utilized in a broad range of biomedical disciplines, such as ophthalmology, otology, and dermatology (Yin et al., 2023). In corneal disease models, Mirjalili Mohanna et al. developed a novel lipid nanoparticle (LNP) system that successfully combined pre-formed RNPs with template DNA and delivered it to cultured mouse cortical neurons, leading to in vitro genome editing (Mirjalili Mohanna et al., 2022). In follow-up *in vivo* experiments, they injected RNPs and DNA templates encapsulated within LNPs into mouse corneas, which served as proof of concept for the efficient gene editing of corneal tissues for the first time (Mirjalili Mohanna et al., 2022). In hearing disorders, Tao et al. demonstrated that liposome-mediated *in vivo* delivery of CRISPR-Cas9 RNP complexes can affect the gene editing of Otoab alleles, resulting in improved survival of outer hair cells and hearing restoration (Tao et al., 2023). In a similar vein, a combination of lipid and AAV-mediated delivery of Streptococcus pyogenes Cas9 (SpCas9) RNP complexes was used in allele-specific editing of the transmembrane channel-like 1 (TMC1) gene, and in mouse models of dominant hearing loss with hair cell defects, auditory function was restored (György et al., 2019).

In their research on dermatology, Bolsoni et al. have shown that LNPs deliver editing tools inside the living epidermis of human skin, allowing for in situ genome editing capabilities. This work demonstrates the ability to do so in living tissue methods that could be used here to treat rare, monogenic skin disorders (Bolsoni et al., 2023). Together, these studies confirm the range of applicable disease contexts of the liposome-mediated delivery platforms for therapeutic genome editing. Advanced liposome systems based on CRISPR-Cas9 for controlled release. To move liposome-based genome

editing into clinical practice, new generations of liposomal systems will transform how CRISPR-Cas9 RNP complexes are delivered (Yin et al., 2023). Some of these systems have the capability of precise, stimulus-responsive delivery (Dubey and Mostafavi, 2023). The next-generation light-triggered liposomes, for example, create spatiotemporally controlled Cas9 activity. By adding photosensitive molecules, such as verteporfin (VP), to the lipid bilayer, scientists have developed liposomes that undergo destabilization upon exposure to light of a predetermined wavelength. Upon irradiation with 690 nm light, VP produces singlet oxygen that oxidizes the unsaturated lipids, affecting the integrity of the membrane, resulting in the release of the encapsulated RNP (Liu et al., 2021). This means that Cas9 activity can be confined to illuminated tissue and provide enhanced specificity to the target, reducing off-target activity (Guo et al., 2023).

5.2.8 Advanced Liposome Systems for Controlled CRISPR-CAS9 Delivery

To advance the clinical applications of liposomal approaches for genome editing, next-generation liposomal systems have been designed to fine-tune stimulus-responsive release of CRISPR-Cas9 RNP complexes with high specificity (Li et al., 2024, Xie et al., 2021, Aksoy et al., 2020). Recently, the spatiotemporal regulation of Cas9 activity has become possible by embedding photosensitive molecules, such as verteporfin (VP), into the lipid bilayer, with liposomes designed to be destabilized upon exposure to light of a defined wavelength. Under 690 nm irradiation, VP generates singlet oxygen, which oxidizes the unsaturated lipids in the membrane, causing the membrane integrity to decrease, hence releasing encapsulated RNPs (Chen et al., 2017). This is an exciting mechanism as it allows for Cas9 activity to be limited to illuminated tissues, increasing target specificity and decreasing off-target effects.

One of the interesting studies was done by Yan and colleagues. They developed a liposome that carries phosphorylated DNA, aiding stimulus-responsive, cell-specific delivery (Kanazhevskaya et al., 2025). These liposomes are engineered to fuse with the cell membrane once exposed to pH or UV stimuli to release proteins into the cytoplasm, mimicking virus fusion. This platform has also proven useful to efficiently deliver proteins of various sizes and charges into the target cells (Lee et al., 2025a). Together, these emerging liposome systems will provide considerable capabilities to achieve temporal, spatial, and cell-type–specific control of CRISPR-Cas9 activity (Aksoy et al., 2020). These advancements represent significant future value for improvement in specificity, safety, and accessibility.

5.2.9 Liposomes for Fluorescence Imaging

Liposome–protein complexes have become very effective and accurate labeling and imaging reagents for use in cell labeling and imaging applications (Shahsavari et al., 2024, Petersen et al., 2012). Liposomes in these complexes rely on superior membrane-fusion capacity to facilitate cellular uptake. At the same time, the proteins provide valuable specificity due to their ability to recognize molecular patterns and targets. As a result, the combination of these properties enables accurate labeling and detailed imaging of cells, which can subsequently be observed by microscopy.

Liposomal formulations for imaging exhibit strong targeting capabilities, enabling precise localization within specific cell types, subcellular compartments, or organelles.

Not only that but also the high-quality imaging properties enable visualization of morphology, cellular structure, and functional states. This property of liposome–protein complexes elucidates cellular dynamics, including cell proliferation, metabolism, signaling, and differentiation, and

has tremendously contributed to our understanding of these complex biological processes. These new nanocarrier inventions provide deeper insights into more sophisticated biology principles and enable more effective theoretical and practical approaches for disease diagnosis and various therapeutic modalities. For example, iRGD-ICG-10-HCPT-PFP-NPs, which is a multifunctional ultrasound molecular probe, composed of a series of CPPs and phase-transformation lipid nanoparticles containing 10-hydroxycamptothecin (Li et al., 2021b). It was demonstrated, with the use of low-intensity focused ultrasound, that this liposome–protein probe could precisely provide diagnosis and therapy of hepatocellular carcinoma and be visualized with ultrasound/photoacoustic (PA) dual-mode imaging. The strong targeting and tumor-penetration capabilities of the drug delivery system improved therapeutic efficacy and opened a new window of therapeutic options for liver cancer diagnosis and therapy.

Fluorescent proteins have been widely used along with liposome-based delivery systems to follow target proteins to gain a better view of nanoparticle uptake quality, efficiency, and subcellular visualization. This has created essential tools and perspectives for the growth of nanomedicine and delivery research (Arul et al., 2023). Hamliton et al. undertook a high-throughput liposome screening strategy and utilized this cationic liposome to attain intracellular delivery of OspF (Hamilton et al., 2024). This research demonstrates the ability to selectively inhibit the MAPK signaling pathway, alleviate tumor growth in cancer cells, and manipulate the immune response of macrophages. To further increase the encapsulation efficiency of lipid nanoparticles, the researchers produced a genetic fusion of OspF with a negatively charged green fluorescent protein (GFP). This confusion aided the self-assembly of the cationic lipid nanoparticles through electrostatic interactions to improve delivery performance and provide a strong method of exploring protein transport dynamics at the cellular level. To sum up, it can

be stated that liposome–protein complexes not only contribute to a mechanistic understanding of liposome-mediated delivery but also expand the realm of potential applications of drug delivery strategies and therapeutic development in the future.

5.3 CHALLENGES

Due to fast progress in biotechnology and nanotechnology, the field of nanocarrier-mediated protein delivery has undergone significant expansion. Yet, some barriers still exist, despite substantial achievements being made. These comprise the constraints of targeting specificity, cell penetration peptide efficiency, lysosomal escape efficiency, reversible release capability, and maintenance of protein activity. Addressing these issues warrants further improvement of the nanocarrier design as well as the exploration of novel delivery strategies and technologies to achieve a more accurate and efficient mode of protein delivery.

There are many hurdles to the clinical translation of nanocarrier-mediated protein delivery, such as manufacturing scalability, batch-to-batch consistency, and protein conformation preservation. Yet, several strategic approaches can address these limitations and support commercial readiness. One of the approaches to overcome those hurdles is establishing large-scale production platforms, including microfluidic mixers, continuous flow nanoprecipitation, and high-pressure homogenization, which provide precise control over nanocarrier size, homogeneity, and cargo encapsulation while remaining compatible with GMP workflows. Also, in order to overcome issues with protein stability during formulation and storage, cryoprotectant-assisted lyophilization, incorporation of PEG or zwitterionic surface chemistries, and pH and ionic environment optimization are implemented to minimize denaturation. We also need to establish rigorous analytical

protocol, such as real-time monitoring of physicochemical properties, tests that confirm protein structure remains intact, and routine checks for endotoxins and other impurities. In addition, validated *in vitro–in vivo* correlation models are essential to show that nanoparticle behavior in the lab reliably predicts pharmacokinetics and biodistribution in living systems. By considering quality by design (QbD) principles into the process from the very beginning and clearly defining the key quality features that need to be controlled, we can make it easier to meet regulatory expectations, reduce variability between batches, and ultimately streamline the path of protein nanomedicines from early research to clinical testing and commercial production.

Compared with electroporation, nanocarrier-based protein delivery tends to be both more effective and much gentler on cells. While electroporation can push proteins into cells efficiently, it does so by forcing open temporary pores in the membrane, which often harms the cells, lowers their survival, and makes the method difficult to use with delicate or primary cells. Nanocarriers work very differently—they rely on natural cellular pathways like endocytosis or membrane fusion, allowing proteins to enter without damaging the membrane. They also protect proteins from breaking down, help them circulate longer in the body, and are designed to release their cargo inside the cytosol more efficiently. One more advantage of nanocarriers is that it can be designed to target specific tissues or cell types. This approach could result in reducing unwanted side effects and enabling applications that electroporation simply cannot achieve safely. For these reasons, nanocarriers offer a more biocompatible, flexible, and clinically practical approach to delivering proteins into cells.

5.3.1 Targeting Ability

One of the major hurdles in protein delivery to cells with nanocarriers is achieving efficient targeting. Therapeutically, proteins must be delivered in high purity to a specific organ, tissue, or cell type (Zhu et al., 2024). However, the elaborate biological setup presents a multitude of challenges, including hindrance in distribution of nanocarriers *in vivo* by blood circulation, intercellular space, or cellular membranes.

The RGD peptide is well studied and is known to bind specifically to the integrin αvβ3 receptor, an important receptor in cellular adhesion processes (Yamada et al., 2023, Yu et al., 2014). The cyclic RGD (cRGD) peptide is more stable in structure than the linear form and therefore has a stronger binding affinity and selectivity for integrin receptors (Li et al., 2021c, Bellis, 2011). Since αvβ3 integrin is expressed much higher degrees in a number of tumor cells, cRGD has emerged as a widely pursued targeting ligand in the diagnosis and therapeutic treatment of cancer (Gu et al., 2023). With the help of cRGD, the developed liposomes can actively bind to tumor cells so that the delivery of therapeutic agents can be done in a targeted way (Wu et al., 2024).

Liposomes have some disadvantages; for instance, their physicochemical properties render them vulnerable to removal from the body via the mononuclear phagocyte system, reducing their general effectiveness in drug delivery (Liu et al., 2022a). To counter this, scientists are creating new methods of enabling liposome functionality. For instance, Li et al. created a biomimetic hybrid nanoparticle, miR497/TP-HENP, by merging liposomes and exosomes. This system effectively encapsulated the TP and miR497 chemotherapeutic agents and exploited the homologous targetability property of tumor cell–derived exosomes and the cRGD peptide. Consequently, the hybrid system achieved both longer circulation within the tumor region and greater tumor specificity (Li et al., 2022). In the same manner, in the field of recombinant protein research, RGD derivatives are

also vital. A good example is the two-disulfide-bridged cyclic polypeptide RGD4C, which exhibits reasonable binding specificity toward tumor neovascular endothelial cells (Huang et al., 2021). All such findings are evidence of the potential role RGD-based approaches may have toward increasing the selectivity of targeting of nanocarrier systems.

5.3.2 Efficiency of CPPs

CPPs are essential reagents for protein delivery systems, as they can deliver significant biomolecules—e.g., proteins, DNA, and RNA—into cells with high specificity. This property renders them of monumental significance in drug discovery, disease treatment, and numerous biomedical applications (Moreno-Vargas and Prada-Gracia, 2025, Munyendo et al., 2012). Although CPPs have great potential, efficiency of delivery is a formidable barrier for their practical application (Ghorai et al., 2023).

Several parameters control CPP-mediated transport. The sequence, size, and post-translational modification of CPPs significantly impact their ability to penetrate membranes (Liu et al., 2022b). The intracellular environment is another controlling factor since cells are heterogeneous with respect to the composition and structure of membranes and exhibit differential sensitivity to the uptake of CPPs. The multifaceted nature renders it challenging to achieve reproducible and reliable delivery outcomes in heterogeneous biological systems.

5.3.3 Lysosomal escape Efficiency

Nanoparticles, when they are relatively large (>100 nm), typically cannot penetrate the cell membrane directly. In this case, endocytosis is considered the primary method of cell entry (Sahay et al., 2010). In this case, the cell membrane surrounds the nanoparticle to form a vesicle that then buds off

into the cytoplasm. Following cellular uptake, nanoparticles are commonly transported to lysosomes, acidic organelles enriched with hydrolytic enzymes. These enzymes can degrade both the nanoparticle carrier and its associated payload, including therapeutic proteins, enzymes, or antibodies, thereby reducing the efficiency of intracellular cargo delivery. Because of this, factors must be engineered into the nanoparticle to liberate the nanoparticles and any cargo from lysosomes to maintain structural integrity and biological activity. The successful release of nanoparticles into the cytoplasm is critical once the developers have designed strategies to access intracellular targets and create a therapeutic response.

Nanoparticles can also enhance cellular uptake through surface functionalization and the addition of ligands, such as transferrin, antibodies, or RGD peptides, to promote receptor-mediated endocytosis. When ligands bind to the membrane and specific receptors for the ligands, the nanoparticle can pass through the membrane and form vesicles. It is also worth mentioning that endocytic patterns vary by cell type; for example, macrophages exhibit a strong propensity to engulf foreign particles. The lysosomal escape efficiency is closely associated with the pH of the intracellular microenvironment. The low pH in endo/lysosomal organelles is a valuable property that can be used to activate nanocarriers in a controlled manner. For example, cationic polymer-based nanoparticles utilize the pH of the endosomes or lysosomes by using the proton sponge effect to disrupt lysosomal membranes (Pei and Buyanova, 2019). By sequestering protons, these polymer-based nanoparticles will cause an increase in the pH of the endosome/lysosome, which leads to membrane instability, rupture, and release of the protein cargo into the cytoplasm (Wojnilowicz et al., 2019).

In addition to pH-responsiveness, some specific cells, in particular cancer cells, have the benefit of having an enzymatic profile that could allow for selective nanocarrier activation. Cancer cells have increasingly proteases

localized in the lysosomal compartment, such as cathepsin B (CTSB) that can be used as a specific nanocarrier activation strategy (Karimi et al., 2016). Chen et al., utilizing the aforementioned properties, developed an antibody delivery system that was responsive to the enzymatic activity of endo-/lysosomes (Chen et al., 2024c). They utilized anti-nucleopore complex antibodies (anti-NPC) as a model protein and modified them with a PEG-pAsp(DET) polymer backbone, which allowed for selective delivery of the antibodies to intracellular complementary target sites through the use of a CTSB cleavable val-cit linker. The established strategy ensured selective intracellular release of antibodies in cancer cells (Chen et al., 2024c). In addition to the respected pH response, the 1,2-diaminoethane groups in the PEG-pAsp(DET) polymer backbone elicited experimentally memorialized membrane destabilization in endo/lysosomal compartments, addressing a mechanistic gap for efficient endosome escape (Hall et al., 2017, Uchida et al., 2014).

5.3.4 Limited Reversible Release Capabilities

One of the biggest challenges with protein delivery through nanocarriers is the ability to achieve controlled release, due to the overly strong affinity between the protein and the carrier materials (Zhu et al., 2024). In response, researchers have developed a number of functional polymers, including guanidine-rich and boronate-containing systems, designed to facilitate the delivery of proteins into the cytoplasm. However, the problem remains that even these polymers have overly strong affinities for proteins, resulting in delayed release after the protein has been taken up into the intracellular milieu (Chen et al., 2023, Yu et al., 2019). This means the protein cargo can remain sequestered within the carrier for too long to be bioavailable or to retain biological activity.

Such obstacles pose major limitations for the expanded use of such systems for delivery of protein therapeutics. For nanocarriers to be effective, these systems need to have precise, reversible release mechanisms. Ideally, the system would allow proteins to be released from the carrier in a controlled way due to intracellular cues such as pH, redox potential, or enzymatic activation that allows for spatiotemporally controlled release of a protein. Being responsive in this way would allow the active protein to be released in a timely manner, at the proper subcellular location, in order to provide improved therapeutic activity and to be able to more reliably invoke specific functions in cells.

5.3.5 Maintenance of Protein Activity

A key hurdle in the development of protein-based therapies is maintaining protein activity during the preparation of nanocarriers (Yin et al., 2017). The encapsulation process can subject proteins to very harsh physical and chemical conditions, including high temperatures, rapid shear forces, extreme pH conditions, and reactive chemical reagents. Such conditions can destabilize the delicate tertiary and quaternary structure of proteins, or proteins like enzymes, leading to denaturation, aggregation, or irreversible loss of function (Çalbaş et al., 2024).

The complexity of multi-step synthesis routes also does not alleviate these issues, as each step of the preparation process increases both the risk of degradation or modification of protein stability, while being counterproductive to manufacturing effort and efficiency (Shi et al., 2025). Covalent or non-covalent modification of protein side chains during formulation, such as Portmanteau methods including but not limited to fluorination, heterocyclic substitutions, or exposure to organic solvents, can change the protein's conformational structure, charge distributions, or

surface chemistry. The alteration of these characteristics can mask or expose critical binding sites, inhibiting or abnormally activating biological function (Newberry and Raines, 2017).

5.3.6 Scalability and Manufacturability Challenges of Protein Nanocarriers

The mass production of protein nanocarriers is considered a significant challenge in advancing laboratory research to the clinical and commercial stages. Proteins are highly susceptible to physical and chemical stresses during formulation due to their structure. This poses unique challenges to maintaining their therapeutic activity while formulating them into advanced nanocarrier platforms. The process of manufacturing the nanoparticle should be under strict quality controls to meet regulatory, safety, and efficacy requirements. The nanocarriers should have a uniform nanoparticle size, high-yield encapsulation, batch-to-batch reproducibility, and scalability from production lines to small-scale laboratory experiments.

5.3.6.1 Scalability Concerns Encapsulation Efficiency

Another issue that is usually seen is the loss of proteins in the formulation process in emulsification, solvent evaporation and spray-drying methods. Loss in encapsulation efficiency affects dose delivery of the treatment while also reducing cost efficiency overall because proteins are relatively expensive to manufacture. This is particularly a concern in large-scale manufacturing, as loss equals loss of money.

5.3.6.2 Controlling Particle Size

Particles should be monodispersing to allow reproducibility of pharmacokinetics, biodistribution, and drug action. Heterogeneity in size distribution can come from factors as inconsequential as changes in mixing

rates, solvent ratios, or temperature during scaling up. Large particles may be cleared quickly by the immune system, while small particles could cause unwanted early release of the protein payload. Reproducibility of size across large lots will require sophisticated equipment and optimization of the process (Ernsting et al., 2013).

5.3.7 Process Understanding and Yield

If a process is to be scalable to produce nanocarriers, then process speed, cost, and quality of product must have adequate compromises. Commercialization will need high throughput, but most nanoparticle fabrication processes available are also slow and expensive. Obtaining satisfactory yields without a reduction in quality is one of the most challenging aspects of scale-up manufacture (Bi et al., 2025).

5.3.8 Manufacturing Issues

5.3.8.1 Protein Stability and Loss

During nanocarrier preparation, proteins can be denatured, aggregated, or degraded by enzymes. Each step in the nanocarrier preparation (such as homogenization, shear stress, organic solvent exposure, or even exposure to heat stress) can affect the structure of the proteins. Proteins can typically only be stabilized by appropriately optimizing the conditions or relying on stabilizers and/or protective coatings, which makes the production process inconveniently complicated (Kaltbeitzel and Wich, 2023).

5.3.8.2 Sterile Manufacturing

Meeting sterility is a requirement for clinical nanomedicine. Sterile environments may require high-grade clean room suites and sterile filtration,

which can increase costs tremendously. Sterilization procedures should also be monitored rigorously, as heat or gamma irradiation may destroy protein-based nanocarriers, creating a trade-off between excessive sterilization and excessive instability.

5.3.8.3 Reproducibility and Uniformity

A significant concern in nanocarrier production is batch-to-batch inconsistency, as slight differences in mixing rates, pH, or excipient ratios can lead to substantial discrepancies in dosing and nanocarrier activity. There will need to be development of highly sophisticated measurement and control approaches that provide the reproducibility required for commercial-scale production.

5.3.8.3.1 Downstream Processing

The purification of nanocarriers from residual proteins, solvents, or by-products is a lengthy and costly downstream matter that requires a multitude of downstream processes. It can also lead to the secondary loss of proteins during purification steps that require filtration, centrifugation, or chromatography. Standardized and simplified purification of protein nanocarriers is essential to ensure consistent production on a large scale.

5.3.8.3.2 Lack of a Standardized Approach

The lack of standard production and analysis methods for protein nanocarriers is a significant impediment to industrialization. Without using standardized methods of characterization (such as encapsulation efficiency calculations, release kinetics, and stability), comparisons between studies and regulatory compliance become problematic.

5.3.8.4 Issues Pending Resolution

5.3.8.4.1 New Processing Methods

As new methods of manufacturing, such as microfluidics, electrospraying, and self-assembly methods, become more precise and scalable, they should provide greater reproducibility compared to conventional emulsification-based methods. The application of microfluidics, electrospraying, and self-assembly can enhance encapsulation efficiency, allowing for higher protein loadings to be achieved.

5.3.8.5 Organic Solvent-Free Synthesis

By removing organic solvents, not only is there better protein stability, but downstream purification is also easier. Nanoprecipitation in aqueous systems and supercritical fluid technology has the potential to be viable, scalable, and safer alternatives.

5.3.8.6 Real-Time Imaging and AI Integration

New process analytical technologies (PAT), meshed with artificial intelligence, will be investigated to monitor and control nanoparticle synthesis in real-time. These technologies will enable predictive modeling, facilitate rapid troubleshooting and optimization, and ensure reasonable assurance of batch-to-batch consistency in bulk production.

5.3.8.7 Biocompatibility and FDA Approval

The use of clinically tested materials (e.g., poly(lactic-co-glycolic acid) (PLGA), chitosan, lipid-based carriers) is beneficial for regulatory processes. It improves biocompatibility through established and understood degradation mechanisms and safety profiles, facilitating long-term therapy.

5.3.8.7.1 Standardization and Guidelines

Joint efforts from academia, industry and regulation will facilitate the development of standard procedures and regulatory documents. These will include harmonized approaches for characterizing nanoparticles, stability testing, and quality, leading to fewer bureaucratic impediments for clinical translation.

5.4 PRECLINICAL AND CLINICAL ADVANCEMENTS

The process of integrating a nanocarrier-based therapeutic from a conceptual design to a clinically proven treatment involves multiple stages. In this section, we describe the correct path for transition from laboratory-scale studies to human clinical trials, focusing on the milestones and challenges affecting clinical translation. We will first discuss how in vitro and animal models provide preliminary data on a nanocarrier's efficacy, safety, and biodistribution. We will then move to how to transition to the clinical trial landscape, discussing the current status of human studies, the significant role of regulatory bodies, and providing specific, verified examples of both finished and ongoing trials to illustrate the immense potential of this field (Figure 5.3).

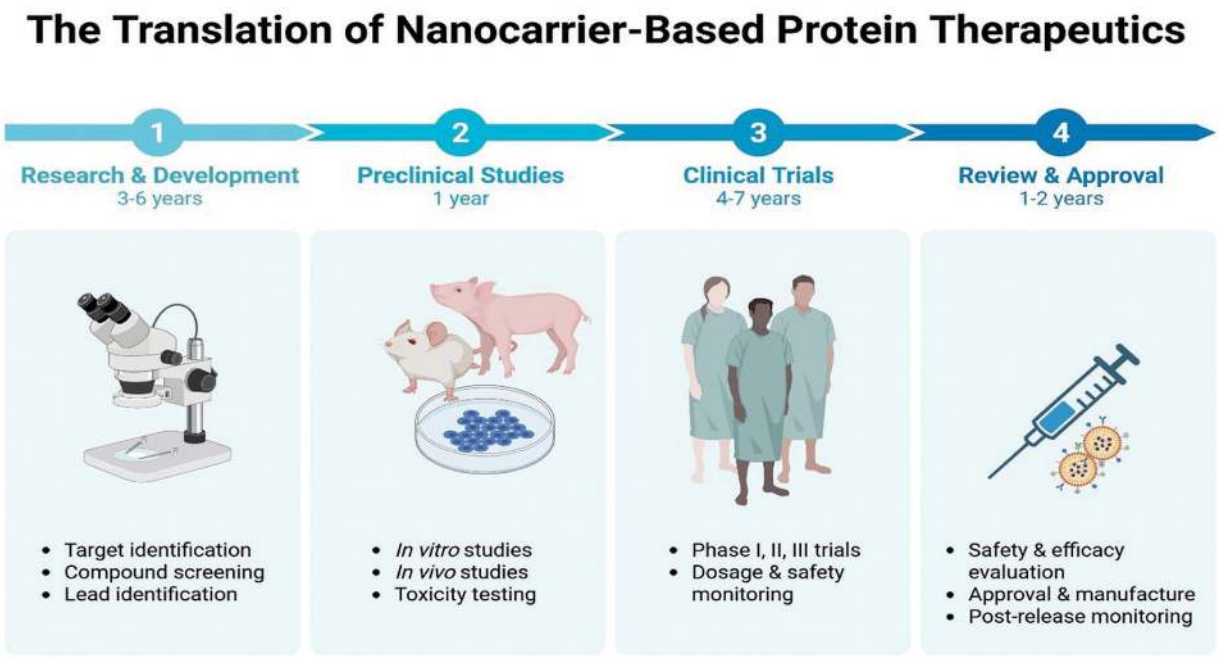

FIGURE 5.3 This flowchart maps the multi-stage path from laboratory design to clinical approval. *In vitro* studies define the mechanism of action, confirming endocytic uptake kinetics and successful endosomal escape (using models like HeLa and A549 cells) and initial safety (RAW 264.7 cells). *In vivo* validation begins with Rodent Models to establish PK/PD and the EPR effect, progressing to large animal models to predict immunogenicity. Final clinical translation requires navigating regulatory hurdles and proving efficacy in human trials, building on the success of approved systems like PEGylated proteins (Oncaspar) and albumin-bound nanoparticles (Abraxane). ⏎

5.4.1 *IN VITRO* AND ANIMAL STUDIES

Preclinical investigations are the foundation upon which nanocarrier-based protein therapies are built. These studies move beyond simple cargo encapsulation to carefully probe the complex synergy between the nanocarrier and the host's biological systems (Muraleedharan et al., 2024). The primary objective is to demonstrate therapeutic efficacy and a favorable toxicological profile *in vivo* and *in vitro*. A key determinant of success is the nanocarrier's ability to facilitate endosomal escape, a process that liberates

the therapeutic protein from the endo-lysosomal pathway to reach its cytosolic or nuclear target (Desai et al., 2024). Most nanocarriers are internalized by cells via endocytosis, a process that can be mediated by clathrin or caveolin, or through macropinocytosis (Sahay et al., 2010). Once inside the cell, the nanocarrier is sequestered within an endosome, a membrane-bound organelle that progressively acidifies from a neutral pH (pH ~7.4) to a more acidic environment (pH ~5.5–6.0 in early endosomes, and pH ~4.5–5.0 in late endosomes/lysosomes) (Stewart et al., 2016). The acidic pH inside the endosome triggers what is known as the "proton sponge effect" in many polymer-based nanocarriers. Initially, secondary and tertiary amines become protonated, and chloride ions and water rush in, causing the endosome to swell and eventually rupture, releasing the protein cargo into the cytoplasm (Almulathanon, 2018). In LNPs and lipid-based systems, acidic pH protonates ionizable lipids, altering their structure. This change makes it easier for the LNPs to fuse with the endosomal membrane and deliver their cargo (Gyanani and Goswami, 2023). Diverse cell lines from well-characterized panels are essential for testing how different nanocarriers behave inside cells, enabling high-throughput and tightly controlled in vitro mechanistic studies. HeLa cells (human cervical cancer cells) are highly utilized epithelial cells, which serve as a standard model for quantitatively investigating cellular internalization dynamics, tracking the kinetics of endocytic uptake, and meticulously verifying the efficacy of the nanocarrier to execute endosomal escape for successful cytoplasmic or nuclear delivery of the therapeutic agent (Renero-Lecuna et al., 2019). A549 cells (human lung adenocarcinoma cells) are representative models of solid tumor pathology. A549 cells are crucial for accurately determining the cytotoxicity of the released drug and calculating the half-maximal inhibitory concentration (IC 50), which validates the payload's specific anti-proliferative or pro-apoptotic effect in a malignancy-relevant context (Fan

and Li, 2021). RAW 264.7 cells (murine macrophages) are phagocytic cell line is essential for assessing the nanocarrier's immunological inertness. Studies here quantify the extent of macrophage recognition and phagocytosis and measure the release of inflammatory mediators to predict potential acute inflammatory reactions *in vivo* (Zheng et al., 2019). Table 5.2 comprehensively demonstrates the distinct roles and key outcomes evaluated by various in vitro cell lines when used for preclinical assessment of nanocarrier-based therapeutics.

TABLE 5.2

Various In Vitro Cell Lines Used for Preclinical Assessment of Nanocarrier-Based Therapeutics

Cell Line (Source)	Type of Cell/Tissue Origin	Primary Scientific Application Focus	Key Mechanism/Outcome Evaluated (Scientific Language)
HeLa Cells (Human Cervical Cancer)	Epithelial/Non-phagocytic	Cellular Fate and Uptake Mechanism	Quantifying endocytic uptake kinetics (CME, CVME) and verifying successful endosomal escape to liberate the therapeutic payload.
A549 Cells (Human Lung Adenocarcinoma)	Epithelial/Non-phagocytic	Targeted Therapeutic Efficacy	Determining the half-maximal inhibitory concentration (IC50) to validate the payload's specific cytotoxicity or anti-proliferative effect.

Cell Line (Source)	Type of Cell/Tissue Origin	Primary Scientific Application Focus	Key Mechanism/Outcome Evaluated (Scientific Language)
HepG2 Cells (Human Liver Cancer)	Epithelial/Non-phagocytic	Uptake, Toxicity, and Liver Targeting	Studying drug response in a liver-relevant context and assessing general nanocarrier toxicity and uptake mechanisms.
Caco-2 Cells (Human Colorectal Adenocarcinoma)	Epithelial/Intestinal Barrier Model	Oral Absorption and Permeability	Modeling the intestinal epithelial barrier to test nanocarrier integrity, mucoadhesiveness, and oral drug permeability.
RAW 264.7 Cells (Murine Macrophage)	Professional Phagocyte	Immunological Recognition and Clearance	Quantifying macrophage recognition/phagocytosis and measuring inflammatory mediator release to predict clearance by the Mononuclear Phagocyte System (MPS).
THP-1 Cells (Human Monocyte/Macrophage-like)	Professional Phagocyte	Human Immune Response	Assessing the potential for the nanocarrier material to trigger an inflammatory cascade in a human-relevant immune cell model.

One of the critical evaluation steps for nanocarrier proteins is conducting preclinical biosafety and efficacy studies in animal models. There are two essential types of analyses: pharmacokinetics (PK) and pharmacodynamics (PD), which must be performed in *in vivo* studies using mice, rats, or non-human primates. PK studies estimate the half-life, clearance rate, and

biodistribution of the nanocarrier for proteins. A clear example is nanoparticles with a size of 10–100 nm, which tend to accumulate in tumors via the enhanced permeability and retention (EPR) effect, which may explain why leaky tumor neovasculature and poor lymphatic drainage lead to localized drug delivery (Ravindran et al., 2018). In PD studies, we measure the specific protein of interest and its potential efficacy at the cellular and tissue levels, for example, its ability to reduce expression of an oncogenic protein or its ability to activate specific cell signaling pathways (Zhu et al., 2024). So, in general, rodent animal models are considered as an initial toxicity profile, allowing researchers to determine the maximum tolerated dose (MTD) and identify acute organ-specific toxicity associated with the nanocarrier material or its encapsulated payload. Researchers view safety assessments as multidimensional, with monitoring both acute and chronic toxicity critical. For instance, the immunogenicity of the protein nanocarriers is a significant concern. Another crucial problem is that our body's reticuloendothelial system (RES), including macrophages in the liver (Kupffer cells) and the spleen, may encounter nanoparticles as foreign antigens, resulting in systemic inflammation and reduced therapeutic efficacy (Colino et al., 2020). To help overcome this obstacle, strategies as PEGylation (covalent attachment of PEG) are used. This strategy helps create a hydrophilic "stealth" coating to reduce protein opsonization and subsequent phagocytic clearance. Unfortunately, PEGylation itself can elicit an immune response, and the formation of anti-PEG antibodies can be a limiting factor during repeat administrations (Tamura et al., 2011). In later steps of the preclinical evaluation, larger animal models, such as rabbits, pigs, and non-human primates (NHPs), are required to bridge the physiological gap to human patients (Milane, 2017). These models are chosen for their closer alignment in organ size, dermal thickness, and immunological complexity compared to rodents. Their utility is primarily

focused on biodistribution and drug targeting, and their immunogenicity risk assessment. These models are used to confirm the intended tissue tropism and evaluate the efficiency of passive targeting (such as the accumulation via the EPR effect in tumor vasculature) or active targeting (via surface-conjugated ligands) in a system more relevant to human physiological compartmentalization (Dilliard and Siegwart, 2023). Due to their more complex immune systems, larger animals are the gold standard for predicting adverse immune reactions, specifically evaluating the potential for the nanocarrier material to elicit a significant humoral immune response (anti-drug antibody production) or complement activation, which are critical safety concerns for clinical translation (Celis-Giraldo et al., 2021).

5.4.2 Clinical Trials: Current Status

The transition of nanocarrier-based protein therapeutics from lab research to clinical applications has been a slow but steady process, marked by breakthroughs such as mRNA vaccines (Agrawal and Kulkarni, 2024). These vaccines have shown that this slow rate of progress in complex delivery systems can be turned into rapid, massive production of vaccines encapsulated in lipid nanoparticles (LNPs), which are safe and effective in millions of people. This step has made the LNP platform accredited and is now being explored for a variety of other therapeutic proteins, from monoclonal antibodies to gene-editing enzymes (Wang et al., 2024b). The regulatory pathway for accrediting nanomedicines is not similar to conventional small-molecule drugs. The FDA's Office of Pharmaceutical Quality (OPQ) and the Center for Devices and Radiological Health (CDRH) are increasingly involved in the review process due to the complex physical and chemical properties of nanoparticles (Bobo et al., 2016) (Table 5.3).

TABLE 5.3

Nanocarrier-Based Protein Therapeutics in Clinical Development

Therapeutic/Product Name	Nanocarrier Type	Protein Co
Abraxane (Paclitaxel)	Albumin-Bound Nanoparticle (Nab-Technology)	Human Serum Albun
Oncaspar (Pegaspargase)	Polymer-Protein Conjugate (PEGylation)	L-Asparaginase—Ca
Adagen (Pegademase)	Polymer-Protein Conjugate (PEGylation)	Adenosine Deaminas
Neulasta (Pegfilgrastim)	Polymer-Protein Conjugate (PEGylation)	Granulocyte Colony-
Cimzia (Certolizumab pegol)	PEGylated Antibody Fragment	Certolizumab (Anti-T
Krystexxa (Pegloticase)	Polymer-Protein Conjugate (PEGylation)	Uricase—Cargo
SB-485232 (IL-18 with PLD)	PEGylated Liposome	Interleukin 18 (IL-18
Olaratumab (with PLD)	PEGylated Liposome (Co-delivery)	Olaratumab (Monocl

[a]Completed; [b]Active

5.4.3 Case Studies

The theoretical principles and preclinical successes of nanocarriers for intracellular protein transport have been validated by several clinical achievements. Here, we highlight two key case studies. Demonstrating how these nanocarrier platforms have revolutionized modern medicine, the most compelling and globally impactful example of nanocarrier-based therapy is the rapid development and deployment of mRNA vaccines for COVID-19. Two main vaccines, BNT162b2 (Pfizer-BioNTech) and mRNA-1273 (Moderna), rely on the delivery of a synthetic, modified messenger RNA molecule (Schoenmaker et al., 2021). The mRNA payload encodes the

prefusion-stabilized spike (S). The SARS-CoV-2 virus's spike protein is the primary antigen the immune system recognizes.

Studies have shown that after intramuscular injection, LNPs are taken up by antigen-presenting cells, such as dendritic cells and macrophages, as well as by nearby muscle cells (Anselmo et al., 2021). Once internalized through endocytosis, the LNPs become enclosed within an endosome. As the endosome acidifies, the ionizable lipids in the LNPs become protonated, prompting the particles to shift into a non-lamellar hexagonal phase structure. This structural shift allows the LNPs to fuse with the endosomal membrane, releasing the mRNA directly into the cytoplasm. Importantly, the mRNA never enters the nucleus, eliminating any possibility of integration into the host genome (Ilyichev et al., 2020). Once in the cytoplasm, the cell's ribosomes translate the mRNA into the spike protein. These spike proteins are then displayed on the cell surface through both MHC class I and class II pathways, activating $CD8^+$ cytotoxic T cells—which eliminate infected cells—and $CD4^+$ helper T cells, which stimulate B cells to produce neutralizing antibodies) (Ilyichev et al., 2020). The strong, multifaceted immune response triggered by this nanocarrier-based delivery approach underscores its effectiveness. Clinical data from trials such as Pfizer–BioNTech's NCT04368728 and Moderna's NCT04283461 showed more than 94% efficacy in preventing symptomatic COVID-19, paving the way for rapid regulatory approval and marking a major shift in vaccine development (Polack et al., 2020).

Nanocarriers have played an important role in cancer therapy for decades, with some of the first approved nanomedicines being liposomal drug formulations. These systems were originally developed to improve the therapeutic index of highly toxic chemotherapies by reducing their systemic exposure and concentrating the drug within the tumor microenvironment. Doxorubicin, for example, is a powerful chemotherapy drug but has dose-

limiting cardiotoxicity. To address this, it was encapsulated in a PEGylated liposome, creating Doxil. The PEGylation adds a protective "stealth" coating that dramatically extends the drug's circulation time—from minutes to several hours (Islam et al., 2022). With this longer circulation, the liposomes preferentially accumulate in tumors through the enhanced permeability and retention (EPR) effect. Once there, they slowly release doxorubicin into the tumor interstitium, where cancer cells can take it up. By reducing exposure to heart tissue, Doxil significantly lowers cardiotoxicity while maintaining its anti-tumor activity, making it a key treatment for Kaposi's sarcoma and ovarian cancer (Sohail et al., 2021).

Paclitaxel is another essential chemotherapeutic agent, but its conventional formulation requires Cremophor EL, a solvent known to cause severe hypersensitivity reactions (Fraguas-Sánchez et al., 2022). To overcome this limitation, Abraxane was developed. It uses a nanocarrier system that replaces the solvent with human serum albumin (HSA), offering a safer and more effective delivery approach.. The paclitaxel is bound to albumin in the form of nanoparticles (approximately 130 nm in diameter). The mechanism of delivery is two-fold. During passive targeting, the nanoparticles accumulate in tumors via the EPR effect. Tumor cells have a high metabolic demand and tend to overexpress SPARC, an albumin-binding protein. This makes them good targets for albumin-based nanocarriers. When paclitaxel is formulated in albumin-bound nanoparticles, the particles can be actively taken up by tumor cells through the SPARC-mediated pathway, leading to much higher drug accumulation inside the tumor (Yardley, 2013). This approach greatly improves both the safety profile and the therapeutic efficacy of paclitaxel, and it is now approved for treating metastatic breast cancer, non–small cell lung cancer, and pancreatic cancer. Gene delivery using CRISPR-Cas9 RNPs is still relatively new, but the field is advancing quickly. Although viral vectors were traditionally used, their

immunogenicity and the risk of unintended genomic integration have encouraged the move toward non-viral nanocarriers. Lipid and polymeric nanoparticles, in particular, can encapsulate the Cas9 protein and its guide RNA, protecting them from degradation and helping them reach the cytoplasm. From there, the RNP complex can enter the nucleus to perform the intended edit (Zhang et al., 2021). Several of these systems are now being evaluated in clinical trials, such as those targeting retinal degeneration, where localized injection allows precise correction of disease-causing mutations with minimal systemic exposure.

5.5 LIMITATIONS AND SAFETY CONSIDERATIONS

Translating nanocarrier-based protein therapies into the clinic comes with several substantial challenges, most of which relate to safety and manufacturing. Because nanoparticles have unique physical and chemical properties, they can interact with the body in ways that are not always predictable. Some inorganic nanoparticles, for instance, generate reactive oxygen species (ROS), leading to oxidative stress, cellular damage, and inflammation. The immune system can also recognize many nanocarriers as foreign. Macrophages of the mononuclear phagocyte system—especially those in the liver and spleen—tend to clear them quickly from the bloodstream, which reduces therapeutic efficacy and can sometimes trigger systemic immune responses (Colino et al., 2020). Approaches such as PEGylation can help reduce this immune recognition, but repeated administration may lead to the development of anti-PEG antibodies. This results in faster clearance and a diminished therapeutic effect over time. Manufacturing adds another layer of difficulty. Protein therapeutics are highly sensitive molecules and can easily denature or aggregate during encapsulation or storage, which often necessitates strict cold-chain

conditions. These requirements complicate distribution, particularly in settings with limited infrastructure. Regulatory and ethical considerations also play a major role. The structural complexity of nanocarriers makes it challenging to maintain consistent, batch-to-batch quality—an essential requirement for regulatory agencies such as the FDA. Ethical concerns, especially those related to genome editing applications and the possibility of off-target effects, further underscore the need for robust oversight. Table 5.4 summarizes these central limitations, covering intrinsic toxicity, immune clearance, cold-chain logistics, and the key regulatory and ethical barriers that still need to be addressed.

TABLE 5.4

Key Translational Hurdles: Toxicity, Immunogenicity, and Logistical Challenges Limitin

Category	Limitation	
Toxicity (Intrinsic)	**Cytotoxicity from Components**	High concentrations of materials (C
	Oxidative Stress Induction	Certain materials (inorganic nanop
Immunogenicity	**Rapid Clearance by MPS**	Phagocytic cells (Kupffer cells in l
	ABC Phenomenon (Anti-PEG)	Repeated dosing of PEGylated nan
	Complement Activation	Nanocarriers can trigger the innate
Stability & Logistics	**Protein Denaturation/Aggregation**	Proteins are fragile and can lose na
	“Cold Chain” Requirement	Many formulations (especially mR
Regulatory and Commercial	**Inconsistent Quality (QbD)**	The complexity of nanocarriers ma
	High Manufacturing Cost	Complex fabrication processes, spe
Ethical Implications	**Off-Target Gene Editing Risk**	Nanocarriers for gene editing (CRI
	Germline Editing Concern	The potential to edit germline cells

5.5.1 Toxicity and Immunogenicity Risks

The unique physical and chemical properties of nanocarriers, such as their small size and high surface-area-to-volume ratio, and their novel compositions, can lead to unintended, and often detrimental, interactions with biological systems. The most crucial factor is cytotoxicity, induced by the nanocarrier itself or by the released cargo. For instance, some cationic polymers, such as polyethylenimine PEI, though an effective agent for endosomal escape, are capable of disturbing the cell membrane and mitochondrial function. It leads to apoptosis or necrosis at high concentrations (Colino et al., 2020). This is often associated with the polymer's positive charge, which exerts an interfering influence on the negatively charged cell membrane. Further, some inorganic nanoparticles, such as those prepared from gold or iron oxide, might cause oxidative stress and result in the generation of reactive oxygen species ROS, whose overproduction leads to a cascade in cellular function. Damage, including lipid peroxidation, denaturation of proteins, and DNA damage, finally compromises the viability of the cells (Mohammadinejad et al., 2019). Another important problem is presented by immunogenicity. Nanoparticles may be recognized by the immune system as invasive foreign bodies, thus promoting a wide variety of immune responses. The innate immune system generally reacts first. The phagocytic cells of the MPS are mostly Kupffer cells and liver and macrophages in the spleen that rapidly eliminate nanoparticles from the circulation—a process termed opsonization, where serum proteins adhere to the nanoparticle surface, thus marking it for phagocytic uptake. In this case, such clearance not only diminishes therapeutic efficacy but can also cause a systemic inflammatory response (Ernst et al., 2021). Other than that, nanoparticles may also activate the complement system of the innate immune system, the result of which is the

formation of the membrane attack complex and rapid clearance (Aljabali et al., 2023).

The adaptive immune system can also be invoked with subsequent production of nanocarrier antibodies, including against PEGylated nanocarriers intended to be "stealthy." While the first dose may be stealthy, repeated dosing has been shown to induce formation of anti-PEG IgM, which accelerates the clearance of subsequent doses and in effect erases the treatment through a phenomenon called the ABC phenomenon (Pan et al., 2025; Ko et al., 2022).

5.5.2 Stability and Storage Challenges

The stability of nanocarrier systems is one of the major technical barriers that influences both the shelf life and the therapeutic efficacy of the final product. Proteins are inherently fragile molecules susceptible to degradation and denaturation by factors such as temperature fluctuations, pH changes, and mechanical stress. The encapsulation process itself can involve organic solvents or high shear forces and can compromise protein integrity. Even after successful encapsulation, maintaining the protein's native three-dimensional structure and biological activity over time is challenging. The physical stability of the nanocarrier is also an issue. Nanoparticles may aggregate or fuse together during storage, which affects their size, surface properties, and overall performance. Aggregation can lead to rapid clearance by the MPS and may provoke an immune response.

Furthermore, many nanocarrier formulation types—including mRNA vaccines—rely upon a "cold chain" for storage at very low temperatures, for example, −80°C or −20°C; this presents significant logistical and financial challenges for distribution, especially in low-resource settings with limited refrigeration infrastructure. Efforts are still being made to develop

thermostable nanocarriers that can be stored at room temperature, which would greatly enhance their global accessibility and provide significant cost reductions (Alshawwa et al., 2022).

5.5.3 Regulatory and Commercial Hurdles

The way to get nanomedicines through the regulatory process is long and complicated. Regulatory Agencies like the FDA and the EMA have to grapple with certain unique challenges in evaluating these complex systems. Unlike a simple small molecule, a nanocarrier is a complex drug product consisting of multiple components, even slight variations in one single component might modify its efficacy and safety profile (Soares et al., 2018). The QbD approach, which ensures that manufacturing processes are well-controlled to produce consistent batch-to-batch product, is crucial but difficult to implement. Regulators require extensive data on the nanocarrier's size, shape, surface charge, polydispersity index (PDI), and encapsulation efficiency, in addition to the standard drug substance and product release criteria.

The lack of standardized procedures for the characterization of nanomedicines can further complicate the approval process 311. From a commercial perspective, the One of the major barriers is the high cost of research, development, and manufacturing of nanocarriers (Liu et al., 2020). The complex fabrication processes, the need for special equipment, and expensive starting Materials contribute to high production costs, hence making such advanced therapies prohibitively expensive, limiting accessibility for patients, especially in treatments that involve chronic or widespread diseases. These treatments' cost-effectiveness needs to be weighed against their therapeutic benefits (Foulkes et al., 2020).

5.5.4 Ethical Implications

The use of nanocarriers, especially for advanced applications involving gene editing, raises profound ethical questions. The ability to deliver CRISPR-Cas9 and other gene-editing tools with high precision into cells opens the door to editing the human genome. While this holds immense promise for treating human genetic disorders, concerns remain regarding off-target effects. Besides, the Cas9 protein may induce unintended DNA cleavage at non-target sites, potentially resulting in deleterious mutations elsewhere in the genome (Wasti et al., 2023). Further, the possibility of germline editing, which involves modifications in sperm, egg, or early embryos and hence heritable, represents a main ethical flashpoint. The prospect of "designer babies" Exacerbation of social inequalities also raises serious questions for the society and regulatory bodies (Zingg et al., 2019). Moreover, there are dual-use potentials of nanocarrier technology. The very same systems designed for therapeutic delivery could be misused for non-therapeutic purposes or as biological weapons. These ethical considerations demand careful deliberation, public discourse, and a robust, transparent regulatory framework to ensure responsible innovation and prevent misuse (Domingues et al., 2022).

5.6 FUTURE DIRECTIONS AND PERSONALIZED MEDICINE

The future of nanocarrier technology is intrinsically tied to the integration of leading-edge computational and biological advancements. The next generation of nanomedicines will progress from a one-size-fits-all approach toward a highly personalized and adaptable system, driven by innovations in design, data analysis, and manufacturing.

5.6.1 Innovations in Nanocarrier Technology: AI-Driven Design and 3D-Printed Nanocarriers

Traditional designing of nanocarriers is a time-consuming and trial-and-error process. This work process has completely turned around by applying artificial intelligence and machine learning. AI algorithms can analyze huge data regarding material properties, biological interactions, and clinical outcomes to predict optimal nanocarrier formulations for specific therapeutic applications. The in silico approach dramatically accelerates the research and development pipeline by screening millions of possible combinations and identifying promising candidates for every given protein, disease target, and route of administration. For instance, with AI, it is possible to predict how the size of a nanocarrier, its surface charge according to zeta potential, and the material it is made of will influence its stability in the bloodstream, its uptake by target cells, and its immune response profile, reducing the need for extensive experimental screening (Omidian and Polymers, 2025).

In addition to design, advanced manufacturing techniques like 3D printing and microfluidics are enabling the creation of nanocarriers with unprecedented precision and reproducibility. Microfluidic devices allow for the rapid and controlled self-assembly of nanoparticles with a narrow size distribution, which is critical for ensuring consistent performance and meeting regulatory standards. 3D printing is being explored to create nanostructured materials with tunable release profiles (Akhtar et al., 2025). For example, a 3D-printed implant could contain a depot of nanocarriers that slowly release a therapeutic protein over an extended period, or a stimuli-responsive system that releases its payload only when triggered by a specific condition like a change in pH or temperature in the tumor microenvironment (Kutlehria et al., 2022).

5.6.2 Integration with Omics Technologies: Tailoring Delivery Systems Using Genomic/Proteomic Data

The true promise of nanocarriers lies in their potential to enable personalized medicine, which requires a deep understanding of a patient's individual biology. This is where "omics" technologies come in. By integrating data from genomics, proteomics, and transcriptomics, clinicians and researchers can create a molecular portrait of a patient's disease (Vitorino, 2024).

By sequencing a patient's tumor genome, a clinician can identify unique driver mutations or gene amplifications. A nanocarrier can then be designed to deliver a therapeutic protein that specifically targets this mutated protein or gene, minimizing damage to healthy cells (Kim et al., 2021). The analysis of a cell's protein expression profile can reveal unique surface markers that are overexpressed on diseased cells, such as specific receptors on cancer cells. A nanocarrier can be functionalized with a corresponding ligand (e.g., an antibody fragment or an aptamer) that binds with high affinity to this protein, ensuring precise and targeted delivery (Da Costa Marques et al., 2023). Analyzing the messenger RNA (mRNA) expression patterns can provide a real-time snapshot of the biological state of a cell. This information can be used to design nanocarriers that deliver proteins to correct dysregulated molecular pathways (Qi et al., 2024). This convergence of data allows for the design of nanocarriers that are not just for a disease, but for a specific patient's disease, ushering in the era of precision nanomedicine.

5.6.3 Role in Personalized Therapeutics: Patient-Specific Nanocarriers for Precision Medicine

The ultimate future vision for nanocarrier technology is to integrate it into a personalized medicine framework, moving beyond the current "one-size-fits-

all" model to create highly customized treatments; this approach involves a closed-loop system that would first identify a unique molecular target specific to the patient's individual pathology (such as a unique tumor protein profile in cancer), then design a nanocarrier with optimal properties (including material, size, and charge) specifically tailored to navigate that patient's unique biological environment, and finally, rapidly fabricate the personalized nanocarrier using advanced methods like microfluidics or 3D printing, thereby maximizing therapeutic efficacy while minimizing systemic side effects by sparing healthy tissue (Dey et al., 2024).

5.6.4 Global Health Implications: Accessibility and Cost-Effectiveness in Low-Resource Settings

While nanocarrier technology holds immense promise, its high cost and logistical complexity currently limit its accessibility. However, this technology has the potential to address global health disparities if key challenges are overcome. Research is focused on developing low-cost, scalable manufacturing processes to reduce the final price of nanocarrier-based therapies (Bosetti and Jones, 2019). Many current nanomedicines require a cold chain for storage and distribution, which is not feasible in many low-resource settings. Innovations in thermostable formulations that can be stored at room temperature are critical for making these treatments globally accessible, as demonstrated by the potential for cold-chain-free mRNA vaccines (Wu et al., 2020).

The development of nanocarrier-based diagnostic tools, such as paper-based biosensors, and portable nanomedicines for point-of-care applications, could enable rapid and accurate diagnosis and treatment in remote areas, reducing the reliance on centralized medical infrastructure. By focusing on these areas, nanocarrier technology can transition from an advanced

technology for a privileged few to a powerful tool for improving health outcomes worldwide (Lemmerman et al., 2020).

5.7 CONCLUSION

This chapter has provided a comprehensive overview of nanocarrier-based approaches for intracellular protein transport, a field at the nexus of materials science, biology, and medicine. We began by establishing the critical need for this technology in addressing diseases where proteins play a central role, from cancer to genetic disorders. We then delved into the major barriers to protein delivery, highlighting the instability of proteins and the difficulty of cellular uptake.

The core of our discussion was a comprehensive overview of the diverse nanocarrier systems available, including liposomes, polymeric nanoparticles, and advanced hybrid systems. We detailed the strategies for their design, fabrication, and functionalization, emphasizing the importance of material selection, encapsulation techniques, and targeted delivery mechanisms. The success stories of mRNA vaccines and the progress of protein-based cancer therapies in preclinical and clinical settings serve as powerful testaments to the efficacy and growing maturity of this field.

However, we also acknowledged the significant hurdles that remain. Toxicity, immunogenicity, stability, and regulatory complexities are not trivial challenges but areas of active research and development. The ethical implications, particularly concerning gene editing, require careful and ongoing consideration.

Looking ahead, the future is bright. The convergence of nanocarrier technology with AI-driven design, 3D printing, and omics technologies promises to usher in an era of truly personalized and precision medicine. As we continue to innovate, it is imperative that we also work to ensure that

these life-saving therapies are accessible and affordable for a global population. The journey of nanocarrier-based intracellular protein transport is a testament to the power of interdisciplinary science, promising a new chapter in the fight against disease.

5.7.1 Conclusions and Perspectives

Nanocarrier-mediated intracellular protein delivery methods have come a long way in recent years. Recent advances in cytoplasmic protein targeting, utilizing liposome-derived nanocarriers, protein self-assembly, and polymers, are documented in this review. Precise control of nanocarrier size, shape, and surface chemistry has enabled a significant enhancement in protein stability, biological activity, and targeted delivery efficacy. For example, size control introduces stability to intra- and extracellular conditions (Islam et al., 2025), whereas shape engineering increases intracellular mobility and enhances transport to target locations. Furthermore, the efficiency of nanocarrier delivery largely depends on the electronegativity of functional ligands—typically featuring fluorine, boronate, or guanidine-rich polymers (Jacob et al., 2024)—with further enhanced protein delivery performance achieved through targeted ligand modifications. Stimuli-sensitive nanocarriers are a further key advance, as structural change or controlled release of proteins can be achieved based on environmental stimuli like temperature, pH, or light and thus enable spatiotemporal control of drug delivery (Islam et al., 2025, Jacob et al., 2024).

No less groundbreaking is the development of protein design technologies, as these hold potentially multidimensional roles in drug design, synthetic biology, and enzyme engineering. Most proteins, such as MYC and KRAS, have been considered “undruggable” for a long time as

they do not possess sites for small-molecule **binding** (Kaltbeitzel & Wich, 2023). Reason-based design of proteins allows the build-up of proteins with structure and function optimized for a specific task and thereby broadens the scope of potential drug targets (De Haas et al., 2024). Apart from drug discovery, protein design improves enzyme activity, optimizes production of delivery platforms, and lowers production cost—widespread availability in the clinic of new protein therapies (Kaltbeitzel & Wich, 2023). In targeted medicine, the designer design of tumor-associated receptor-binding proteins or antibodies offers routes for particular anticancer therapies and effective gene-editing treatments (Eweje et al., 2025). Together, these advances place the technology of protein design at the center of next-generation protein delivery systems.

Clinical development of protein-loaded nanocarriers is still in its early stages despite these advancements. For the technology to advance more quickly, next-generation work needs to focus on: 1. Design and assembly of new vectors with enhanced encapsulation efficiency, biocompatibility, and targeting, inspired by natural transport systems, bacterial extracellular contractile injection machineries, and virus-like particles. 2. Optimization of protein modification approach by chemical and genetic means to enhance the stability, bioactivity, and trans-barrier transportability of proteins. 3. Increased delivery system controllability through higher-order release mechanisms like light- or chemically inducible activation to achieve high-precision temporal as well as spatial control. 4. Fostering interdisciplinarity between materials science, chemistry, biology, and medicine to support innovation in delivery technology.

Conclusion Protein delivery via nanocarrier-delivery systems holds immense potential for therapeutics and has outstanding promise for translational applications to the clinic over the coming years. As science

advances and technology improves, this is an area that will catalyze historic breakthroughs in the treatment of human disease.

REFERENCES

Abdellatif, A. A. H., Aljutayli, A. & Mohammed, A. M. 2025. Thermosensitive chitosan hydrogel loaded cetuximab: A novel approach for parenteral controlled delivery. *Drug Dev Ind Pharm*, 51, 597–605.

Abdellatif, A. A. H., Scagnetti, G., Younis, M. A., Bouazzaoui, A., Tawfeek, H. M., Aldosari, B. N., Almurshedi, A. S., Alsharidah, M., Rugaie, O. A., Davies, M. P. A., Liloglou, T., Ross, K. & Saleem, I. 2023. Non-coding RNA-directed therapeutics in lung cancer: Delivery technologies and clinical applications. *Colloids Surf B Biointerfaces*, 229, 113466.

Abdellatif, A. A. H., Younis, M. A., Alsharidah, M., Al Rugaie, O. & Tawfeek, H. M. 2022. Biomedical applications of quantum dots: Overview, challenges, and clinical potential. *Int J Nanomed*, 17, 1951–1970.

Agrawal, M. & Kulkarni, V. 2024. Current Clinical Advancements of Nanocarriers for Brain Targeting and Limitations in Clinical Translation. *Application of Nanocarriers in Brain Delivery of Therapeutics*. Springer.

Akanda, M., Mithu, M. D. S. H. & Douroumis, D. 2023. Solid lipid nanoparticles: An effective lipid-based technology for cancer treatment. *J Drug Deliv Sci Technol*, 86, 104709.

Akbaba, H., Erel-Akbaba, G., Başpinar, Y. & Şentürk, Ş. 2023. Design of liposome formulations for CRISPR/Cas9 enzyme immobilization: Evaluation of 5-alpha-reductase enzyme knockout for androgenic disorders. *ACS Omega*, 8, 46101–46112.

Akbarian, M. & Chen, S. H. 2022. Instability challenges and stabilization strategies of pharmaceutical proteins. *Pharmaceutics*, 14(11), Article 2533.

Akbarzadeh, A., Rezaei-Sadabady, R., Davaran, S., Joo, S. W., Zarghami, N., Hanifehpour, Y., Samiei, M., Kouhi, M. & Nejati-Koshki, K. 2013. Liposome: Classification, preparation, and applications. *Nanoscale Res Lett*, 8, 102.

Akhtar, M., Nehal, N., Gull, A., Parveen, R., Khan, S., Khan, S. & Ali, J. 2025. Explicating the transformative role of artificial intelligence in designing targeted nanomedicine. *Expert Opin Drug Deliv*, 22(7), 971–991.

Aksoy, Y. A., Yang, B., Chen, W., Hung, T., Kuchel, R. P., Zammit, N. W., Grey, S. T., Goldys, E. M. & Deng, W. 2020. Spatial and temporal control of CRISPR-Cas9-mediated gene editing delivered via a light-triggered liposome system. *ACS Appl Mater Interfaces*, 12, 52433–52444.

Alamos-Musre, S., Beltrán-Chacana, D., Moyano, J., Márquez-Miranda, V., Duarte, Y., Miranda-Rojas, S., Olguín, Y., Fuentes, J. A., González-Nilo, D. & Otero, M. C. 2025. From structure to function: The promise of PAMAM dendrimers in biomedical applications. *Pharmaceutics*, 17, 927.

Aljabali, A. A., Obeid, M. A., Bashatwah, R. M., Serrano-Aroca, Á., Mishra, V., Mishra, Y., El-Tanani, M., Hromić-Jahjefendić, A., Kapoor, D. N., Goyal, R., Naikoo, G. A. & Tambuwala, M. M. 2023. Nanomaterials and their impact on the immune system. *Int J Mol Sci*, 24(3), 2008.

Allen, T. M. & Cullis, P. R. 2013. Liposomal drug delivery systems: From concept to clinical applications. *Adv Drug Deliv Rev*, 65, 36–48.

Almulathanon, A. 2018. *Efficacy and Toxicity of Linear Polyamidoamine Polymers Used in Gene Delivery*. University of Nottingham.

Alsaab, H. O., Alharbi, F. D., Alhibs, A. S., Alanazi, N. B., Alshehri, B. Y., Saleh, M. A., Alshehri, F. S., Algarni, M. A., Almugaiteeb, T., Uddin, M. N. & Alzhrani, R. M. 2022. PLGA-based nanomedicine: History of advancement and development in clinical applications of multiple diseases. *Pharmaceutics*, 14(12), Article 2728.

Alshawwa, S. Z., Kassem, A. A., Farid, R. M., Mostafa, S. K. & Labib, G. S. J. P. 2022. Nanocarrier drug delivery systems: characterization, limitations, future perspectives and implementation of artificial intelligence. *Pharmaceutics*, 14, 883.

Anselmo, A. C., Mitragotri, S. J. B. & Medicine, T. 2021. Nanoparticles in the clinic: An update post COVID-19 vaccines. *Bioeng Transl Med*, 6, e10246.

Anwar, S., Sarwar, T., Khan, A. A. & Rahmani, A. H. 2025. Therapeutic applications and mechanisms of superoxide dismutase (SOD) in different pathogenesis. *Biomolecules*, 15(8), Article 1130.

Arbaz, S. J., Ramulu, B. & Yu, J. S. 2025. Rational design of hierarchical hollow-core dual-shell amorphous 3D nanospheres as an effective electrode material for hybrid supercapacitors. *J Mater Chem A*, 13, 9514–9527.

Arul, M. R., Alahmadi, I., Turro, D. G., Ruikar, A., Abdulmalik, S., Williams, J. T., Sanganahalli, B. G., Liang, B. T., Verma, R. & Kumbar, S. G. 2023. Fluorescent liposomal nanocarriers for targeted drug delivery in ischemic stroke therapy. *Biomater Sci*, 11, 7856–7866.

Barra, T., Falanga, A., Bellavita, R., Laforgia, V., Prisco, M., Galdiero, S. & Valiante, S. 2022. gH625-liposomes deliver PACAP through a dynamic in vitro model of the blood-brain barrier. *Front Physiol*, 13, 932099.

Baumgart, T., Das, S., Webb, W. W. & Jenkins, J. T. 2005. Membrane elasticity in giant vesicles with fluid phase coexistence. *Biophys J*, 89, 1067–80.

Beach, M. A., Nayanathara, U., Gao, Y., Zhang, C., Xiong, Y., Wang, Y. & Such, G. K. 2024. Polymeric nanoparticles for drug delivery. *Chem Rev*, 124, 5505–5616.

Bellis, S. L. 2011. Advantages of RGD peptides for directing cell association with biomaterials. *Biomaterials*, 32, 4205–4210.

Benjaminsen, R. V., Mattebjerg, M. A., Henriksen, J. R., Moghimi, S. M. & Andresen, T. L. 2013. The possible “proton sponge” effect of polyethylenimine (PEI) does not include change in lysosomal pH. *Mol Ther*, 21, 149–157.

Bhojoo, U., Chen, M. & Zou, S. 2018. Temperature induced lipid membrane restructuring and changes in nanomechanics. *Biochim Biophys Acta Biomembr*, 1860, 700–709.

Bi, Y., Xie, S., Li, Z., Dong, S. & Teng, L. 2025. Precise nanoscale fabrication technologies, the “last mile” of medicinal development. *Acta Pharm Sin B*, 15, 2372–2401.

Binauld, S. & Stenzel, M. H. 2013. Acid-degradable polymers for drug delivery: A decade of innovation. *Chem Commun*, 49, 2082–2102.

Biswas, S., Vaze, O. S., Movassaghian, S. & Torchilin, V. P. 2013. Polymeric Micelles for the Delivery of Poorly Soluble Drugs. *Drug Delivery Strategies for Poorly Water-Soluble Drugs*. Wiley.

Bobo, D., Robinson, K. J., Islam, J., Thurecht, K. J. & Corrie, S. R. J. P. R. 2016. Nanoparticle-based medicines: A review of FDA-approved materials and clinical trials to date. *Pharm Res*, 33, 2373–2387.

Bochicchio, D. & Monticelli, L. 2016. Chapter Five - The Membrane Bending Modulus in Experiments and Simulations: A Puzzling Picture. *In:* Iglič, A., Kulkarni, C. V. & Rappolt, M. (eds.) *Advances in Biomembranes and Lipid Self-Assembly*. Academic Press.

Bolsoni, J., Liu, D., Mohabatpour, F., Ebner, R., Sadhnani, G., Tafech, B., Leung, J., Shanta, S., An, K., Morin, T., Chen, Y., Arguello, A., Choate, K., Jan, E., Ross, C. J. D., Brambilla, D., Witzigmann, D., Kulkarni, J., Cullis, P. R. & Hedtrich, S. 2023. Lipid nanoparticle-mediated hit-and-run approaches Yield efficient and safe in situ gene editing in human skin. *ACS Nano*, 17, 22046–22059.

Bosetti, R. & Jones, S. L. J. N. 2019. Cost–effectiveness of nanomedicine: Estimating the real size of nano-costs. *Nanomedicine*, 14, 1367–1370.

Bouazzaoui, A. & Abdellatif, A. A. H. 2024. Vaccine delivery systems and administration routes: Advanced biotechnological techniques to improve the immunization efficacy. *Vaccine X*, 19, 100500.

Bouazzaoui, A., Bogari, N. M., Al-Allaf, F. A., Ekram, S. N., Athar, M., Dannoun, A., Schubert, T., Syed, S. N., Youssef, A. R., Alqahtani, M. & Abdellatif, A. A. H. 2023. Anti-E. coli Immunoglobulin Yolk (IgY): Reduction of pathogen receptors and inflammation factors could be caused by decrease in E. coli load. *Heliyon*, 9, e13876.

Bouazzaoui, A., Keedwa, K. M., Sahl, S. M., Al-Hashmi, M. S., Alzabeedi, K. H., Albshri, M. H., Bukhari, M. A., Qabani, F. H., Mula, I. A., Badr, O. I., Abdellatif, A. A. H. & Bogari, N. M. 2025. Lymphocyte subsets in Covid-19 Saudi patients: Infection caused significant reduction in peripheral blood lymphocyte subset. *Clin Lab*, 71(5), 851–857.

Brodszkij, E. & Städler, B. 2024. Advances in block copolymer-phospholipid hybrid vesicles: From physical–chemical properties to applications. *Chem Sci*, 15, 10724–10744.

Bukke, S. P. N., Venkatesh, C., Bandenahalli Rajanna, S., Saraswathi, T. S., Kusuma, P. K., Goruntla, N., Balasuramanyam, N. & Munishamireddy, S. 2024. Solid lipid nanocarriers for drug delivery: Design innovations and characterization strategies—a comprehensive review. *Discov Appl Sci*, 6, 279.

Burger, M., Brigger, F., Mantella, V. & Leroux, J.-C. 2025. Encapsulation of protein/DNA complexes into unilamellar liposomes via annexin-mediated membrane recruitment and sonication. *Cell Rep Methods*, 5, 101073.

Caffrey, M. 2000. A lipid's eye view of membrane protein crystallization in mesophases. *Curr Opin Struct Biol*, 10, 486–497.

Çalbaş, B., Keobounnam, A. N., Korban, C., Doratan, A. J., Jean, T., Sharma, A. Y. & Wright, T. A. 2024. Protein–polymer bioconjugation, immobilization, and encapsulation: A comparative review towards applicability, functionality, activity, and stability. *Biomater Sci*, 12, 2841–2864.

Campisi, A., Sposito, G., Pellitteri, R., Santonocito, D., Bisicchia, J., Raciti, G., Russo, C., Nardiello, P., Pignatello, R., Casamenti, F. & Puglia, C. 2022. Effect of unloaded and curcumin-loaded solid lipid nanoparticles on tissue transglutaminase isoforms expression levels in an experimental model of Alzheimer's disease. *Antioxidants (Basel)*, 11(6), Article 1119.

Cao, Y., Dong, X. & Chen, X. 2022. Polymer-modified liposomes for drug delivery: From fundamentals to applications. *Pharmaceutics*, 14(8), Article 1688.

Celis-Giraldo, C. T., López-Abán, J., Muro, A., Patarroyo, M. A. & Manzano-Román, R. J. V. 2021. Nanovaccines against animal pathogens: The latest findings. *Vaccines*, 9, 988.

Chen, C., Gao, P., Wang, H., Cheng, Y. & Lv, J. 2023. Histidine-based coordinative polymers for efficient intracellular protein delivery via enhanced protein binding, cellular uptake, and endosomal escape. *Biomater Sci*, 11, 1765–1775.

Chen, C.-T., Weng, C.-C., Fan, K.-P., Barman, S. R., Pal, A., Liu, C.-B., Li, Y.-K., Lin, Z.-H. & Chang, C.-C. 2024a. Guanidinium-functionalized polymer dielectrics for triboelectric bacterial

detection. *ACS Appl Mater Interfaces*, 16, 1502–1510.

Chen, J., Hu, S., Sun, M., Shi, J., Zhang, H., Yu, H. & Yang, Z. 2024b. Recent advances and clinical translation of liposomal delivery systems in cancer therapy. *Eur J Pharm Sci*, 193, 106688.

Chen, P., Yang, W., Mochida, Y., Li, S., Hong, T., Kinoh, H., Kataoka, K. & Cabral, H. 2024c. Selective intracellular delivery of antibodies in cancer cells with nanocarriers sensing endo/lysosomal enzymatic activity. *Angew Chem Int Ed*, 63, e202317817.

Chen, W., Deng, W. & Goldys, E. M. 2017. Light-triggerable liposomes for enhanced endolysosomal escape and gene silencing in PC12 cells. *Mol Ther Nucleic Acids*, 7, 366–377.

Chis, A. A., Dobrea, C., Morgovan, C., Arseniu, A. M., Rus, L. L., Butuca, A., Juncan, A. M., Totan, M., Vonica-Tincu, A. L., Cormos, G., Muntean, A. C., Muresan, M. L., Gligor, F. G. & Frum, A. 2020. Applications and limitations of dendrimers in biomedicine. *Molecules*, 25, 3982.

Choudhury, H., Gorain, B., Pandey, M., Chatterjee, L. A., Sengupta, P., Das, A., Molugulu, N. & Kesharwani, P. 2017. Recent update on nanoemulgel as topical drug delivery system. *J Pharm Sci*, 106, 1736–1751.

Chountoulesi, M., Pippa, N., Forys, A., Trzebicka, B. & Pispas, S. 2024. Structure-based evaluation of hybrid lipid–polymer nanoparticles: The role of the polymeric guest. *Polymers*, 16, 290.

Colino, C. I., Lanao, J. M. & Gutierrez-Millan, C. J. F. I. I. 2020. Targeting of hepatic macrophages by therapeutic nanoparticles. *Front Immunol*, 11, 218.

Colletier, J. P., Chaize B., Winterhalter, M. & Fournier, D. 2002. Protein encapsulation in liposomes: Efficiency depends on interactions between protein and phospholipid bilayer. *BMC Biotechnol*, 2, Article 9.

Da Costa Marques, R., Hüppe, N., Speth, K. R., Oberländer, J., Lieberwirth, I., Landfester, K. & Mailänder, V. J. A. B. 2023. Proteomics reveals time-dependent protein corona changes in the intracellular pathway. *Acta Biomater*, 172, 355–368.

De, R., Mahata, M. K. & Kim, K. T. 2022. Structure-based varieties of polymeric nanocarriers and influences of their physicochemical properties on drug delivery profiles. *Adv Sci (Weinh)*, 9, e2105373.

De Haas, R. J., Brunette, N., Goodson, A., Dauparas, J., Yi, S. Y., Yang, E. C., Dowling, Q., Nguyen, H., Kang, A., Bera, A. K., Sankaran, B., De Vries, R., Baker, D. & King, N. P. 2024. Rapid and automated design of two-component protein nanomaterials using ProteinMPNN. *Proc Natl Acad Sci*, 121, e2314646121.

Dernedde, J., Rausch, A., Weinhart, M., Enders, S., Tauber, R., Licha, K., Schirner, M., Zügel, U., Von Bonin, A. & Haag, R. 2010. Dendritic polyglycerol sulfates as multivalent inhibitors of inflammation. *Proc Natl Acad Sci U S A*, 107, 19679–84.

Desai, N., Rana, D., Salave, S., Gupta, R., Patel, P., Karunakaran, B., Sharma, A., Giri, J., Benival, D. & Kommineni, N. 2023. Chitosan: A potential biopolymer in drug delivery and biomedical applications. *Pharmaceutics*, 15(4), Article 1313.

Desai, N., Rana, D., Salave, S., Benival, D., Khunt, D. & Prajapati, B. G. J. M. 2024. Achieving endo/lysosomal escape using smart nanosystems for efficient cellular delivery. *Molecules*, 29, 3131.

Dey, S., Hassan, S. & Pandey, R. K. 2024. Nanomedicine in Targeted Drug Delivery: Precision Therapeutics for Personalized Medicine. *Nanomedicine: Innovations, Applications, and Breakthroughs in the Quest for Health and Medicine's Future*. Springer.

Dilliard, S. A. & Siegwart, D. J. J. N. R. M. 2023. Passive, active and endogenous organ-targeted lipid and polymer nanoparticles for delivery of genetic drugs. *Nat Rev Mater*, 8, 282–300.

Domingues, C., Santos, A., Alvarez-Lorenzo, C., Concheiro, A., Jarak, I., Veiga, F., Barbosa, I., Dourado, M. & Figueiras, A. J. A. N. 2022. Where is nano today and where is it headed? A review of nanomedicine and the dilemma of nanotoxicology. *ACS Nano*, 16, 9994–10041.

Dragwidge, J. M., Wang, Y., Brocard, L., De Meyer, A., Hudeček, R., Eeckhout, D., Grones, P., Buridan, M., Chambaud, C., Pejchar, P., Potocký, M., Winkler, J., Vandorpe, M., Serre, N., Fendrych, M., Bernard, A., De Jaeger, G., Pleskot, R., Fang, X. & Van Damme, D. 2024. Biomolecular condensation orchestrates clathrin-mediated endocytosis in plants. *Nat Cell Biol*, 26, 438–449.

Du, M., Ouyang, Y., Meng, F., Zhang, X., Ma, Q., Zhuang, Y., Liu, H., Pang, M., Cai, T. & Cai, Y. 2019. Polymer-lipid hybrid nanoparticles: A novel drug delivery system for enhancing the activity of Psoralen against breast cancer. *Int J Pharm*, 561, 274–282.

Du, S., Guan, Y., Xie, A., Yan, Z., Gao, S., Li, W., Rao, L., Chen, X. & Chen, T. 2023a. Extracellular vesicles: A rising star for therapeutics and drug delivery. *J Nanobiotechnol*, 21, 231.

Du, Y., Liu, Y., Hu, J., Peng, X. & Liu, Z. 2023b. CRISPR/Cas9 systems: Delivery technologies and biomedical applications. *Asian J Pharm Sci*, 18, 100854.

Dubey, A. K. & Mostafavi, E. 2023. Biomaterials-mediated CRISPR/Cas9 delivery: Recent challenges and opportunities in gene therapy. *Front Chem*, 11, 1259435.

Eltaib, L. 2025. Polymeric nanoparticles in targeted drug delivery: Unveiling the impact of polymer characterization and fabrication. *Polymers (Basel)*, 17(7), Article 833.

Ernst, L. M., Casals, E., Italiani, P., Boraschi, D. & Puntes, V. J. N. 2021. The interactions between nanoparticles and the innate immune system from a nanotechnologist perspective. *Nanomaterials*, 11, 2991.

Ernsting, M. J., Murakami, M., Roy, A. & Li, S. D. 2013. Factors controlling the pharmacokinetics, biodistribution and intratumoral penetration of nanoparticles. *J Control Release*, 172, 782–794.

Eweje, F., Ibrahim, V., Shajii, A., Walsh, M. L., Ahmad, K., Alrefai, A., Miyasato, D., Davis, J. R., Ham, H., Li, K., Roehrl, M., Haller, C. A., Liu, D. R., Chen, J. & Chaikof, E. L. 2025. Self-assembling protein nanoparticles for cytosolic delivery of nucleic acids and proteins. *Nat Biotechnol*, 44(4), 602–615.

Fachel, F. N. S., Dal Prá, M., Azambuja, J. H., Endres, M., Bassani, V. L., Koester, L. S., Henriques, A. T., Barschak, A. G., Teixeira, H. F. & Braganhol, E. 2020. Glioprotective effect of chitosan-coated rosmarinic acid nanoemulsions against lipopolysaccharide-induced inflammation and oxidative stress in rat astrocyte primary cultures. *Cell Mol Neurobiol*, 40, 123–139.

Fadaei, M. R., Fadaei, M. S., Kheirieh, A. E., Hatami, H., Rahmanian-Devin, P., Tayebi-Khorrami, V., Fathabadi, M. F., Baradaran Rahimi, V. & Askari, V. R. 2025. Overview of dendrimers as

promising drug delivery systems with insight into anticancer and anti-microbial applications. *Int J Pharm X*, 10, 100390.

Fan, M., & Li, J. (2021). A novel combinational nanodrug delivery system induces synergistic inhibition of lung adenocarcinoma cells in vitro. *Lett Drug Des Discov*, 18(1). https://doi.org/10.2174/1570180817999200719152426

Fauquignon, M., Ibarboure, E. & Le Meins, J. F. 2022. Hybrid polymer/lipid vesicles: Influence of polymer architecture and molar mass on line tension. *Biophys J*, 121, 61–67.

Foulkes, R., Man, E., Thind, J., Yeung, S., Joy, A. & Hoskins, C. 2020. The regulation of nanomaterials and nanomedicines for clinical application: Current and future perspectives. *Biomater Sci*, 8, 4653–4664.

Fraguas-Sánchez, A. I., Lozza, I. & Torres-Suárez, A. I. J. C. 2022. Actively targeted nanomedicines in breast cancer: From pre-clinal investigation to clinic. *Cancers*, 14, 1198.

Frangoul, H., Altshuler, D., Cappellini, M. D., Chen, Y.-S., Domm, J., Eustace Brenda, K., Foell, J., De La Fuente, J., Grupp, S., Handgretinger, R., Ho Tony, W., Kattamis, A., Kernytsky, A., Lekstrom-Himes, J., Li Amanda, M., Locatelli, F., Mapara Markus, Y., De Montalembert, M., Rondelli, D., Sharma, A., Sheth, S., Soni, S., Steinberg Martin, H., Wall, D., Yen, A. & Corbacioglu, S. 2021. CRISPR-Cas9 gene editing for sickle cell disease and β-thalassemia. *N Engl J Med*, 384, 252–260.

Frangoul, H., Stults, A., Bruce, K., Domm, J., Carroll, C., Aide, S., Duckworth, M., Evans, M. & Mcmanus, M. 2025. Best practices in gene therapy for sickle cell disease and transfusion-dependent β-Thalassemia. *Transplant Cell Ther*, 31, 352.e1–352.e10.

Gajbhiye, K. R., Salve, R., Narwade, M., Sheikh, A., Kesharwani, P. & Gajbhiye, V. 2023. Lipid polymer hybrid nanoparticles: A custom-tailored next-generation approach for cancer therapeutics. *Mol Cancer*, 22, 160.

Gao, Y., Wang, K., Zhang, J., Duan, X., Sun, Q. & Men, K. 2023. Multifunctional nanoparticle for cancer therapy. *MedComm (2020)*, 4, e187.

Genchi, G., Lauria, G., Catalano, A., Carocci, A. & Sinicropi, M. S. 2024. Neuroprotective effects of curcumin in neurodegenerative diseases. *Foods*, 13(2), Article 292.

Geraets, R. D., Koh, S. Y., Hastings, M. L., Kielian, T., Pearce, D. A. & Weimer, J. M. 2016. Moving towards effective therapeutic strategies for neuronal ceroid lipofuscinosis. *Orphanet J Rare Dis*, 11, 40.

Gessner, I. & Neundorf, I. 2020. Nanoparticles modified with cell-penetrating peptides: Conjugation mechanisms, physicochemical properties, and application in cancer diagnosis and therapy. *Int J Mol Sci*, 21(17), Article 6372.

Ghasemiyeh, P. & Mohammadi-Samani, S. 2018. Solid lipid nanoparticles and nanostructured lipid carriers as novel drug delivery systems: Applications, advantages and disadvantages. *Res Pharm Sci*, 13, 288–303.

Ghezzi, M., Pescina, S., Padula, C., Santi, P., Del Favero, E., Cantù, L. & Nicoli, S. 2021. Polymeric micelles in drug delivery: An insight of the techniques for their characterization and assessment in biorelevant conditions. *J Control Release*, 332, 312–336.

Ghorai, S. M., Deep, A., Magoo, D., Gupta, C. & Gupta, N. 2023. Cell-penetrating and targeted peptides delivery systems as potential pharmaceutical carriers for enhanced delivery across the blood-brain barrier (BBB). *Pharmaceutics*, 15(3), Article 842.

Giram, P. S., Nimma, R., Bulbule, A., Yadav, A. S., Gorain, M., Venkata Radharani, N. N., Kundu, G. C. & Garnaik, B. 2024. Engineered PLGA core–lipid shell hybrid nanocarriers improve the efficacy and safety of irinotecan to combat colon cancer. *ACS Biomater Sci Eng*, 10, 6661–6676.

Gu, Y., Dong, B., He, X., Qiu, Z., Zhang, J., Zhang, M., Liu, H., Pang, X. & Cui, Y. 2023. The challenges and opportunities of αvβ3-based therapeutics in cancer: From bench to clinical trials. *Pharmacol Res*, 189, 106694.

Guo, C., Ma, X., Gao, F. & Guo, Y. 2023. Off-target effects in CRISPR/Cas9 gene editing. *Front Bioeng Biotechnol*, 11, 1143157.

Guo, Z., Peng, H., Kang, J. & Sun, D. 2016. Cell-penetrating peptides: Possible transduction mechanisms and therapeutic applications. *Biomed Rep*, 4, 528–534.

Gyanani, V. & Goswami, R. J. P. 2023. Key design features of lipid nanoparticles and electrostatic charge-based lipid nanoparticle targeting. *Pharmaceutics*, 15, 1184.

György, B., Nist-Lund, C., Pan, B., Asai, Y., Karavitaki, K. D., Kleinstiver, B. P., Garcia, S. P., Zaborowski, M. P., Solanes, P., Spataro, S., Schneider, B. L., Joung, J. K., Géléoc, G. S. G., Holt, J. R. & Corey, D. P. 2019. Allele-specific gene editing prevents deafness in a model of dominant progressive hearing loss. *Nat Med*, 25, 1123–1130.

Hall, A., Lächelt, U., Bartek, J., Wagner, E. & Moghimi, S. M. 2017. Polyplex evolution: Understanding biology, optimizing performance. *Mol Ther*, 25, 1476–1490.

Hamilton, A. G., Swingle, K. L., Thatte, A. S., Mukalel, A. J., Safford, H. C., Billingsley, M. M., El-Mayta, R. D., Han, X., Nachod, B. E., Joseph, R. A., Metzloff, A. E. & Mitchell, M. J. 2024. High-throughput in vivo screening identifies differential influences on mRNA lipid nanoparticle immune cell delivery by administration route. *ACS Nano*, 18, 16151–16165.

Hassan, A. A. A., Ramadan, E., Kristó, K., Regdon, G., Jr. & Sovány, T. 2025. Lipid-polymer hybrid nanoparticles as a smart drug delivery system for peptide/protein delivery. *Pharmaceutics*, 17(2), Article 321.

He, X., Wang, J., Liu, X., Niu, Q., Li, Z., Chen, B. & Xiong, Q. 2024. Hypoxia-responsive hydrogen-bonded organic framework-mediated protein delivery for cancer therapy. *Adv Healthcare Mater*, 13, 2400747.

Herce, H. D., Garcia, A. E., Litt, J., Kane, R. S., Martin, P., Enrique, N., Rebolledo, A. & Milesi, V. 2009. Arginine-rich peptides destabilize the plasma membrane, consistent with a pore formation translocation mechanism of cell-penetrating peptides. *Biophys J*, 97, 1917–1925.

Herpoldt, K.-L., López, C. L., Sappington, I., Pham, M. N., Srinivasan, S., Netland, J., Montgomery, K. S., Roy, D., Prossnitz, A. N., Ellis, D., Wargacki, A. J., Pepper, M., Convertine, A. J., Stayton, P. S. & King, N. P. 2024. Macromolecular cargo encapsulation via in vitro assembly of two-component protein nanoparticles. *Adv Healthcare Mater*, 13, 2303910.

Huang, C.-C., Liu, F.-R., Feng, Q., Pan, X.-Y., Song, S.-L. & Yang, J.-L. 2021. RGD4C peptide mediates anti-p21Ras scFv entry into tumor cells and produces an inhibitory effect on the human

colon cancer cell line SW480. *BMC Cancer*, 21, 321.

Huddy, T. F., Hsia, Y., Kibler, R. D., Xu, J., Bethel, N., Nagarajan, D., Redler, R., Leung, P. J. Y., Courbet, A., Yang, E. C., Bera, A. K., Coudray, N., Calise, S. J., Davila-Hernandez, F. A., Weidle, C., Han, H. L., Li, Z., Mchugh, R., Reggiano, G., Kang, A., Sankaran, B., Dickinson, M. S., Coventry, B., Brunette, T. J., Liu, Y., Dauparas, J., Borst, A. J., Ekiert, D., Kollman, J. M., Bhabha, G. & Baker, D. 2023. Blueprinting expandable nanomaterials with standardized protein building blocks. *bioRxiv,* 2023-06.

Huddy, T. F., Hsia, Y., Kibler, R. D., Xu, J., Bethel, N., Nagarajan, D., Redler, R., Leung, P. J., Weidle, C. & Courbet, A. 2024. Blueprinting extendable nanomaterials with standardized protein blocks. *Nature*, 627, 898–904.

Hughes, K. J., Cheng, J., Iyer, K. A., Ralhan, K., Ganesan, M., Hsu, C.-W., Zhan, Y., Wang, X., Zhu, B., Gao, M., Wang, H., Zhang, Y., Huang, J. & Zhou, Q. A. 2024. Unveiling trends: Nanoscale materials shaping emerging biomedical applications. *ACS Nano*, 18, 16325–16342.

Hwang, D., Ramsey, J. D. & Kabanov, A. V. 2020. Polymeric micelles for the delivery of poorly soluble drugs: From nanoformulation to clinical approval. *Adv Drug Deliv Rev*, 156, 80–118.

Ilyichev, A., Orlova, L., Sharabrin, S., Karpenko, L. I. & Dzharullaeva, S. 2020. mRNA technology as one of the promising platforms for the SARS-CoV-2 vaccine development. *Vaccines*, 24, 802.

Islam, M. R., Patel, J., Back, P. I., Shmeeda, H., Adamsky, K., Yang, H., Alvarez, C., Gabizon, A. A. & La-Beck, N. M. J. N. 2022. Comparative effects of free doxorubicin, liposome encapsulated doxorubicin and liposome co-encapsulated alendronate and doxorubicin (PLAD) on the tumor immunologic milieu in a mouse fibrosarcoma model. *J Immunother Cancer*, 6, 451.

Islam, S., Ahmed, M. M. S., Islam, M. A., Hossain, N. & Chowdhury, M. A. 2025. Advances in nanoparticles in targeted drug delivery–A review. *Results Surf Interfaces*, 19, 100529.

Jacob, S., Kather, F. S., Boddu, S. H. S., Shah, J. & Nair, A. B. 2024. Innovations in nanoemulsion technology: Enhancing drug delivery for oral, parenteral, and ophthalmic applications. *Pharmaceutics*, 16(3), Article 377.

Jankowski, A. M., Ensign, M. A. & Maisel, K. 2025. Cell-penetrating peptides as facilitators of cargo-specific nanocarrier-based drug delivery. *Nanoscale*, 17(35), 20006–20019.

Jeon, S., Jeon, Y., Lim, J.-Y., Kim, Y., Cha, B. & Kim, W. 2025. Emerging regulatory mechanisms and functions of biomolecular condensates: Implications for therapeutic targets. *Signal Transduct Target Ther*, 10, 4.

Jhaveri, A. M. & Torchilin, V. P. 2014. Multifunctional polymeric micelles for delivery of drugs and siRNA. *Front Pharmacol*, 5, Article 77.

Kaga, S., Arslan, M., Sanyal, R. & Sanyal, A. 2016. Dendrimers and dendrons as versatile building blocks for the fabrication of functional hydrogels. *Molecules*, 21, 497.

Kaltbeitzel, J. & Wich, P. R. 2023. Protein-based nanoparticles: From drug delivery to imaging, nanocatalysis and protein therapy. *Angew Chem Int Ed*, 62, e202216097.

Kanazhevskaya, L. Y., Zhdanova, P. V., Chernonosov, A. A. & Koval, V. V. 2025. Off-target interactions in the CRISPR-Cas9 machinery: Mechanisms and outcomes. *Biochem Biophys Rep*, 43, 102134.

Kannan, S., Dai, H., Navath, R. S., Balakrishnan, B., Jyoti, A., Janisse, J., Romero, R. & Kannan, R. M. 2012. Dendrimer-based postnatal therapy for neuroinflammation and cerebral palsy in a rabbit model. *Sci Transl Med*, 4, 130ra46.

Karadurmus, L. & Erturk, A. S. 2025. Recent emerging trends in dendrimer research: Electrochemical sensors and their multifaceted applications in biomedical fields or healthcare. *Biosens Bioelectron*, 273, 117172.

Kardani, K. & Bolhassani, A. 2021. Cppsite 2.0: An available database of experimentally validated cell-penetrating peptides predicting their secondary and tertiary structures. *J Mol Biol*, 433, 166703.

Karimi, M., Eslami, M., Sahandi-Zangabad, P., Mirab, F., Farajisafiloo, N., Shafaei, Z., Ghosh, D., Bozorgomid, M., Dashkhaneh, F. & Hamblin, M. R. 2016. pH-Sensitive stimulus-responsive nanocarriers for targeted delivery of therapeutic agents. *Wiley Interdiscip Rev Nanomed Nanobiotechnol*, 8, 696–716.

Kataoka, K., Harada, A. & Nagasaki, Y. 2012. Block copolymer micelles for drug delivery: Design, characterization and biological significance. *Adv Drug Deliv Rev*, 64, 37–48.

Kaupbayeva, B., Tsoy, A., Safarova Yantsen, Y., Nurmagambetova, A., Murata, H., Matyjaszewski, K. & Askarova, S. 2024. Unlocking genome editing: Advances and obstacles in CRISPR/Cas delivery technologies. *J Funct Biomater*, 15(2), Article 28.

Kerr-Phillips, T., Damavandi, M., Pilkington, L. I., Whitehead, K. A., Travas-Sejdic, J. & Barker, D. 2022. Effects of neutral, anionic and cationic polymer brushes grafted from poly(para-phenylene vinylene) and poly(para-phenylene ethynylene) on the polymer's photoluminescent properties. *Polymers*, 14, 2767.

Khoshandam, M., Soltaninejad, H., Mousazadeh, M., Hamidieh, A. A. & Hosseinkhani, S. 2024. Clinical applications of the CRISPR/Cas9 genome-editing system: Delivery options and challenges in precision medicine. *Genes Dis*, 11, 268–282.

Kiełbowski, K., Plewa, P., Szulc, J., Ćmil, M., Bakinowska, E. & Pawlik, A. 2025. Extracellular vesicles and PD-L1—A review of complex immunoregulatory properties and clinical importance. *Biomedicines*, 13, 1356.

Kim, M., Shin, M., Zhao, Y., Ghosh, M. & Son, Y.-O. 2024. Transformative impact of nanocarrier-mediated drug delivery: Overcoming biological barriers and expanding therapeutic horizons. *Small Sci*, 4, 2400280.

Kim, S., Kim, M., Jung, S., Kwon, K., Park, J., Kim, S., Kwon, I. & Tae, G. 2019. Co-delivery of therapeutic protein and catalase-mimic nanoparticle using a biocompatible nanocarrier for enhanced therapeutic effect. *J Control Release*, 309, 181–189.

Kim, S., Shi, Y., Kim, J. Y., Park, K. & Cheng, J.-X. 2010. Overcoming the barriers in micellar drug delivery: Loading efficiency, in vivo stability, and micelle–cell interaction. *Expert Opin Drug Deliv*, 7, 49–62.

Kim, T., Nam, K., Kim, Y. M., Yang, K. & Roh, Y. H. J. A. N. 2021. DNA-assisted smart nanocarriers: Progress, challenges, and opportunities. *ACS Nano*, 15, 1942–1951.

Kirkby, M., Sabri, A. H. B., Holmes, A., Moss, G. P. J. & Scurr, D. 2024. PAMAM dendrimers as mediators of dermal and transdermal drug delivery: A review. *J Pharm Pharmacol*, 76, 1284–1300.

Klyachko, N. L., Arzt, C. J., Li, S. M., Gololobova, O. A. & Batrakova, E. V. 2020. Extracellular vesicle-based therapeutics: Preclinical and clinical investigations. *Pharmaceutics*, 12(11), Article 1111.

Ko, C.-N., Zang, S., Zhou, Y., Zhong, Z. & Yang, C. J. J. O. N. 2022. Nanocarriers for effective delivery: Modulation of innate immunity for the management of infections and the associated complications. *J Nanobiotechnol*, 20, 380.

Kobayashi, N., Yanase, K., Sato, T., Unzai, S., Hecht, M. H. & Arai, R. 2015. Self-assembling nano-architectures created from a protein nano-building block using an intermolecularly folded dimeric de novo protein. *J Am Chem Soc*, 137, 11285–11293.

Kutlehria, S., D'souza, A., Bleier, B. S. & Amiji, M. M. J. M. P. 2022. Role of 3D printing in the development of biodegradable implants for central nervous system drug delivery. *Mol Pharm*, 19, 4411–4427.

Le, Q. V., Lee, J., Lee, H., Shim, G. & Oh, Y. K. 2021. Cell membrane-derived vesicles for delivery of therapeutic agents. *Acta Pharm Sin B*, 11, 2096–2113.

Lee, H. W., Kharel, S. & Loo, S. C. J. 2022. Lipid-coated hybrid nanoparticles for enhanced bacterial biofilm penetration and antibiofilm efficacy. *ACS Omega*, 7, 35814–35824.

Lee, S., Nyamzaya, K., Han, J., Song, Y., Lee, J., Jo, J. H., Bang, S., Lim, E.-K., Haam, S. & Kim, E. 2025a. Membrane fusion-inspired nanomaterials: Emerging strategies for infectious disease and cancer diagnostics. *Adv Healthcare Mater*, n/a, 2500485.

Lee, S. M., Song, Y., Hong, B. J., Macrenaris, K. W., Mastarone, D. J., O'halloran, T. V., Meade, T. J. & Nguyen, S. T. 2010. Modular polymer-caged nanobins as a theranostic platform with enhanced magnetic resonance relaxivity and pH-responsive drug release. *Angew Chem Int Ed Engl*, 49, 9960–9964.

Lee, S.-M., Chen, H., O'halloran, T. V. & Nguyen, S. T. 2009. "Clickable" polymer-caged nanobins as a modular drug delivery platform. *J Am Chem Soc*, 131, 9311–9320.

Lee, Y., Haizan, I., Sim, S. B. & Choi, J.-H. 2025b. Colorimetric biosensors: Advancements in nanomaterials and cutting-edge detection strategies. *Biosensors*, 15, 362.

Lei, W., Yang, C., Wu, Y., Ru, G., He, X., Tong, X. & Wang, S. 2022. Nanocarriers surface engineered with cell membranes for cancer targeted chemotherapy. *J Nanobiotechnol*, 20, 45.

Lemmerman, L. R., Das, D., Higuita-Castro, N., Mirmira, R. G. & Gallego-Perez, D. 2020. Nanomedicine-based strategies for diabetes: Diagnostics, monitoring, and treatment. *Trends Endocrinol Metab*, 31, 448–458.

Li, A., Zhao, Y., Li, Y., Jiang, L., Gu, Y. & Liu, J. 2021a. Cell-derived biomimetic nanocarriers for targeted cancer therapy: Cell membranes and extracellular vesicles. *Drug Deliv*, 28, 1237–1255.

Li, H., Shi, S., Wu, M., Shen, W., Ren, J., Mei, Z., Ran, H., Wang, Z., Tian, Y., Gao, J. & Zhao, H. 2021b. iRGD peptide-mediated liposomal nanoparticles with photoacoustic/ultrasound dual-modality imaging for precision theranostics against hepatocellular carcinoma. *Int J Nanomed*, 16, 6455–6475.

Li, L., He, D., Guo, Q., Zhang, Z., Ru, D., Wang, L., Gong, K., Liu, F., Duan, Y. & Li, H. 2022. Exosome-liposome hybrid nanoparticle codelivery of TP and miR497 conspicuously overcomes chemoresistant ovarian cancer. *J Nanobiotechnol*, 20, 50.

Li, M., Chen, F., Yang, Q., Tang, Q., Xiao, Z., Tong, X., Zhang, Y., Lei, L. & Li, S. 2024. Biomaterial-based CRISPR/Cas9 delivery systems for tumor treatment. *Biomater Res*, 28, 0023.

Li, N., Qiu, S., Fang, Y., Wu, J. & Li, Q. 2021c. Comparison of linear vs. cyclic RGD pentapeptide interactions with integrin α(v)β(3) by molecular dynamics simulations. *Biology (Basel)*, 10(10), Article 1024.

Li, S., Wang, Q., Jia, Z., Da, M., Zhao, J., Yang, R. & Chen, D. 2023. Recent advances in glucose oxidase-based nanocarriers for tumor targeting therapy. *Heliyon*, 9, e20407.

Li, W., Zhang, H., Chen, L., Huang, C., Jiang, Z., Zhou, H., Zhu, X., Liu, X., Zheng, Z., Yu, Q., He, Y., Gao, Y., Ma, J. & Yang, L. 2025. Cell membrane-derived nanovesicles as extracellular vesicle-

mimetics in wound healing. *Mater Today Bio*, 31, 101595.

Liao, J., Gong, L., Xu, Q., Wang, J., Yang, Y., Zhang, S., Dong, J., Lin, K., Liang, Z., Sun, Y., Mu, Y., Chen, Z., Lu, Y., Zhang, Q. & Lin, Z. 2024. Revolutionizing neurocare: Biomimetic nanodelivery via cell membranes. *Adv Mater*, 36, 2402445.

Liao, Y., Zhang, Z., Zhao, Y., Zhang, S., Zha, K., Ouyang, L., Hu, W., Zhou, W., Sun, Y. & Liu, G. 2025. Glucose oxidase: An emerging multidimensional treatment option for diabetic wound healing. *Bioactive Mater*, 44, 131–151.

Lin, X., Yue, L., Cheng, K. & Rao, L. 2025. Engineering cellular vesicles for immunotherapy. *Acc Mater Res*, 6, 327–339.

Liu, P., Chen, G. & Zhang, J. 2022a. A review of liposomes as a drug delivery system: Current status of approved products, regulatory environments, and future perspectives. *Molecules*, 27(13), Article 4523.

Liu, W., Zhang, M., Lv, H. & Yang, C. 2025. Formulation-driven optimization of PEG-lipid content in lipid nanoparticles for enhanced mRNA delivery in vitro and in vivo. *Pharmaceutics*, 17(1), Article 112.

Liu, X., Tang, I., Wainberg, Z. A. & Meng, H. J. S. 2020. Safety considerations of cancer nanomedicine—a key step toward translation. *Sci Adv*, 16, 2000673.

Liu, X. M., Ma, L. & Schekman, R. 2021. Selective sorting of microRNAs into exosomes by phase-separated YBX1 condensates. *Elife*, 10, e71982.

Liu, Y., Zhao, Z. & Li, M. 2022b. Overcoming the cellular barriers and beyond: Recent progress on cell penetrating peptide modified nanomedicine in combating physiological and pathological barriers. *Asian J Pharm Sci*, 17, 523–543.

Lok, K.-H., Loo, H. L. & Chuah, L.-H. 2025. Topical and transdermal lipid-polymer hybrid nanoparticles (LPN): An integration in advancing dermatological treatments. *Drug Deliv Transl Res*, 15(11), 4277–4313.

Lombardo, D. & Kiselev, M. A. 2022. Methods of liposomes preparation: Formation and control factors of versatile nanocarriers for biomedical and nanomedicine application. *Pharmaceutics*,

14(3), 543.

López-Sagaseta, J., Malito, E., Rappuoli, R. & Bottomley, M. J. 2016. Self-assembling protein nanoparticles in the design of vaccines. *Comput Struct Biotechnol J*, 14, 58–68.

Lu, Y., Cheng, D., Niu, B., Wang, X., Wu, X. & Wang, A. 2023. Properties of poly (lactic-co-glycolic acid) and progress of poly (lactic-co-glycolic acid)-based biodegradable materials in biomedical research. *Pharmaceuticals*, 16, 454.

Lutz, I. D., Wang, S., Norn, C., Courbet, A., Borst, A. J., Zhao, Y. T., Dosey, A., Cao, L., Xu, J., Leaf, E. M., Treichel, C., Litvicov, P., Li, Z., Goodson, A. D., Rivera-Sánchez, P., Bratovianu, A. M., Baek, M., King, N. P., Ruohola-Baker, H. & Baker, D. 2023. Top-down design of protein architectures with reinforcement learning. *Science*, 380, 266–273.

Lv, J., Fan, Q., Wang, H. & Cheng, Y. 2019. Polymers for cytosolic protein delivery. *Biomaterials*, 218, 119358.

Lv, J., Tan, E., Wang, Y., Fan, Q., Yu, J. & Cheng, Y. 2020. Tailoring guanidyl-rich polymers for efficient cytosolic protein delivery. *J Control Release*, 320, 412–420.

Ly, P.-D., Ly, K.-N., Phan, H.-L., Nguyen, H. H. T., Duong, V.-A. & Nguyen, H. V. 2024. Recent advances in surface decoration of nanoparticles in drug delivery. *Front Nanotechnol*, 6, Article 1345678.

Ma, D. 2019. Chapter 1 - Hybrid Nanoparticles: An Introduction. *In:* Mohapatra, S., Nguyen, T. A. & Nguyen-Tri, P. (eds.) *Noble Metal-Metal Oxide Hybrid Nanoparticles*. Woodhead Publishing.

Maiti, P., Paladugu, L. & Dunbar, G. L. 2018. Solid lipid curcumin particles provide greater anti-amyloid, anti-inflammatory and neuroprotective effects than curcumin in the 5xFAD mouse model of Alzheimer's disease. *BMC Neurosci*, 19, 7.

Malhotra, K., Fuku, R., Kumar, B., Hrovat, D., Van Houten, J., Piunno, P. A. E., Gunning, P. T. & Krull, U. J. 2022. Unlocking long-term stability of upconversion nanoparticles with biocompatible phosphonate-based polymer coatings. *Nano Lett*, 22, 7285–7293.

Manzoor, M., Sharma, P., Murtaza, M., Jaiswal, A. K. & Jaglan, S. 2023. Fabrication, characterization, and interventions of protein, polysaccharide and lipid-based nanoemulsions in food and

nutraceutical delivery applications: A review. *Int J Biol Macromol*, 241, 124485.

Martínez-Aguirre, M. A., Flores Alamo, M., Elisa Trejo-Huizar, K. & Yatsimirsky, A. K. 2021. Boronic acid complexes with amino phenolic N,O-ligands and their use for non-covalent protein fluorescence labeling. *Bioorg Chem*, 113, 104993.

Masoumi Shahrbabak, S., Jalali, S. M., Fathabadi, M. F., Tayebi-Khorrami, V., Amirinejad, M., Forootan, S., Saberifar, M., Fadaei, M. R., Najafi, Z. & Askari, V. R. 2025. Modified alginates for precision drug delivery: Advances in controlled-release and targeting systems. *Int J Pharm X*, 10, 100381.

Masson, P. & Lushchekina, S. 2022. Conformational stability and denaturation processes of proteins investigated by electrophoresis under extreme conditions. *Molecules*, 27(15), Article 4932.

Maysinger, D., Gröger, D., Lake, A., Licha, K., Weinhart, M., Chang, P. K. Y., Mulvey, R., Haag, R. & Mckinney, R. A. 2015. Dendritic polyglycerol sulfate inhibits microglial activation and reduces hippocampal CA1 dendritic spine morphology deficits. *Biomacromolecules*, 16, 3073–3082.

Maysinger, D., Ji, J., Moquin, A., Hossain, S., Hancock, M. A., Zhang, I., Chang, P. K. Y., Rigby, M., Anthonisen, M., Grütter, P., Breitner, J., Mckinney, R. A., Reimann, S., Haag, R. & Multhaup, G. 2018. Dendritic polyglycerol sulfates in the prevention of synaptic loss and mechanism of action on glia. *ACS Chem Neurosci*, 9, 260–271.

Mccord, J. M. & Fridovich, I. 1969. Superoxide dismutase. An enzymic function for erythrocuprein (hemocuprein). *J Biol Chem*, 244, 6049–6055.

Mcgrath, M. P., Sall, E. D. & Tremont, S. J. 1995. Functionalization of polymers by metal-mediated processes. *Chem Rev*, 95, 381–398.

Mehta, M., Bui, T. A., Yang, X., Aksoy, Y., Goldys, E. M. & Deng, W. 2023. Lipid-based nanoparticles for drug/gene delivery: An overview of the production techniques and difficulties encountered in their industrial development. *ACS Mater Au*, 3, 600–619.

Melo, M. N., Pereira, F. M., Rocha, M. A., Ribeiro, J. G., Diz, F. M., Monteiro, W. F., Ligabue, R. A., Severino, P. & Fricks, A. T. 2020. Immobilization and characterization of horseradish peroxidase

into chitosan and chitosan/PEG nanoparticles: A comparative study. *Process Biochem*, 98, 160–171.

Menjoge, A. R., Kannan, R. M. & Tomalia, D. A. 2010. Dendrimer-based drug and imaging conjugates: Design considerations for nanomedical applications. *Drug Discov today*, 15, 171–185.

Milane, L. S. 2017. Translational Nanomedicine. *Nanomedicine for Inflammatory Diseases*. CRC Press.

Milhem, O., Myles, C., Mckeown, N., Attwood, D. & D'emanuele, A. 2000. Polyamidoamine Starburst® dendrimers as solubility enhancers. *Int J Pharm*, 197, 239–241.

Mirjalili Mohanna, S. Z., Djaksigulova, D., Hill, A. M., Wagner, P. K., Simpson, E. M. & Leavitt, B. R. 2022. LNP-mediated delivery of CRISPR RNP for wide-spread in vivo genome editing in mouse cornea. *J Control Release*, 350, 401–413.

Mneimneh, A. T. & Mehanna, M. M. 2024. Chondroitin sulphate: An emerging therapeutic multidimensional proteoglycan in colon cancer. *Int J Biol Macromol*, 254, 127672.

Mohammadinejad, R., Moosavi, M. A., Tavakol, S., Vardar, D., Hosseini, A., Rahmati, M., Dini, L., Hussain, S., Mandegary, A. & Klionsky, D. J. J. A. 2019. Necrotic, apoptotic and autophagic cell fates triggered by nanoparticles. *Autophagy*, 15(1), 4–33.

Moreno-Vargas, L. M. & Prada-Gracia, D. 2025. Exploring the chemical features and biomedical relevance of cell-penetrating peptides. *Int J Mol Sci*, 26, 59.

Mukherjee, A. B., Appu, A. P., Sadhukhan, T., Casey, S., Mondal, A., Zhang, Z. & Bagh, M. B. 2019. Emerging new roles of the lysosome and neuronal ceroid lipofuscinoses. *Mol Neurodegener*, 14, 4.

Munyendo, W. L., Lv, H., Benza-Ingoula, H., Baraza, L. D. & Zhou, J. 2012. Cell penetrating peptides in the delivery of biopharmaceuticals. *Biomolecules*, 2, 187–202.

Muraleedharan, A., Acharya, S. & Kumar, R. J. A. O. 2024. Recent updates on diverse nanoparticles and nanostructures in therapeutic and diagnostic applications with special focus on smart protein nanoparticles: A review. *Appl Sci*, 9, 42613–42629.

Nazli, A., Irshad Khan, M. Z., Rácz, Á. & Béni, S. 2024. Acid-sensitive prodrugs; a promising approach for site-specific and targeted drug release. *Eur J Med Chem*, 276, 116699.

Negi, I., Jangra, R., Gharu, A., Trant, J. F. & Sharma, P. 2023. Guanidinium–amino acid hydrogen-bonding interactions in protein crystal structures: Implications for guanidinium-induced protein denaturation. *Phys Chem Chem Phys*, 25, 857–869.

Newberry, R. W. & Raines, R. T. 2017. 4-fluoroprolines: Conformational analysis and effects on the stability and folding of peptides and proteins. *Top Heterocycl Chem*, 48, 1–25.

Nsairat, H., Khater, D., Sayed, U., Odeh, F., Al Bawab, A. & Alshaer, W. 2022. Liposomes: Structure, composition, types, and clinical applications. *Heliyon*, 8, e09394.

Olshefsky, A., Richardson, C., Pun, S. H. & King, N. P. 2022. Engineering self-assembling protein nanoparticles for therapeutic delivery. *Bioconjugate Chem*, 33, 2018–2034.

Omidian, H. J. J. O. B. & Polymers, C. 2025. AI-powered breakthroughs in material science and biomedical polymers. *J Biomater Sci Polym Ed*, 40, 161–174.

Ozuna-Valencia, K. H., Rodríguez-Félix, F., Márquez-Ríos, E., Moreno-Vásquez, M. J., Graciano-Verdugo, A. Z., Robles-García, M. Á., Aubourg-Martínez, S. P., Quintero-Reyes, I. E., López-Corona, B. E. & Tapia-Hernández, J. A. 2024. Improving the properties of polysaccharide-based films by incorporation of polyphenols through free radical grafting: A review. *Polysaccharides*, 5, 672–697.

Palmerston Mendes, L., Pan, J. & Torchilin, V. P. 2017. Dendrimers as nanocarriers for nucleic acid and drug delivery in cancer therapy. *Molecules*, 22(9), Article 1401.

Pan, J., Wang, Y., Chen, Y., Zhang, C., Deng, H., Lu, J. & Chen, W. J. J. O. N. 2025. Emerging strategies against accelerated blood clearance phenomenon of nanocarrier drug delivery systems. *J Nanobiotechnol*, 23, 138.

Park, I., Uchida, D., Jeon, S., Aikawa, K., Hiroyoshi, N. & Ito, M. 2024a. Microencapsulation-based surface modification for improving flotation separation of Cu-Zn mixed sulfide minerals. *Minerals Eng*, 213, 108756.

Park, J. & Champion, J. A. 2023. Development of self-assembled protein nanocage spatially functionalized with HA stalk as a broadly cross-reactive influenza vaccine platform. *ACS Nano*, 17, 25045–25060.

Park, S., Kim, J., Oh, S. S. & Choi, S. Q. 2024b. Arginine-rich cell-penetrating peptides induce lipid rearrangements for their active translocation across laterally heterogeneous membranes. *Adv Sci*, 11, 2404563.

Parveen, S., Gupta, P., Kumar, S. & Banerjee, M. 2023. Lipid polymer hybrid nanoparticles as potent vehicles for drug delivery in cancer therapeutics. *Med Drug Discov*, 20, 100165.

Paul, F. & Weikl, T. R. 2016. How to distinguish conformational selection and induced fit based on chemical relaxation rates. *PLoS Comput Biol*, 12, e1005067.

Payamifar, S., Foroozandeh, A., Pourmadadi, M., Abdouss, M. & Hasanzadeh, M. 2025. Cyclodextrin-based nanocarriers as promising scaffolds for overcoming challenges of doxorubicin delivery in cancer chemotherapy. *Carbohydr Polym Technol Appl*, 9, 100677.

Pei, D. & Buyanova, M. 2019. Overcoming endosomal entrapment in drug delivery. *Bioconjug Chem*, 30, 273–283.

Peppas, N. A., Keys, K. B., Torres-Lugo, M. & Lowman, A. M. 1999. Poly(ethylene glycol)-containing hydrogels in drug delivery. *J Control Release*, 62, 81–87.

Persano, F., Gigli, G. & Leporatti, S. 2021. Lipid-polymer hybrid nanoparticles in cancer therapy: Current overview and future directions. *Nano Express*, 2, 012006.

Petersen, A. L., Hansen, A. E., Gabizon, A. & Andresen, T. L. 2012. Liposome imaging agents in personalized medicine. *Adv Drug Deliv Rev*, 64, 1417–1435.

Plaza-Oliver, M., Santander-Ortega, M. J. & Lozano, M. V. 2021. Current approaches in lipid-based nanocarriers for oral drug delivery. *Drug Deliv Transl Res*, 11, 471–497.

Polack, F. P., Thomas, S. J., Kitchin, N., Absalon, J., Gurtman, A., Lockhart, S., Perez, J. L., Pérez Marc, G., Moreira, E. D. & Zerbini, C. J. N. E. J. O. M. 2020. Safety and efficacy of the BNT162b2 mRNA Covid-19 vaccine. *N Engl J Med*, 383, 2603–2615.

Porterfield, J. E., Sharma, R., Jimenez, A. S., Sah, N., Mccracken, S., Zhang, L., An, H. T., Lee, S., Kannan, S., Sharma, A. & Kannan, R. M. 2023. Galactosylated hydroxyl-polyamidoamine dendrimer targets hepatocytes and improves therapeutic outcomes in a severe model of acetaminophen poisoning-induced liver failure. *Bioeng Transl Med*, 8, e10486.

Postupalenko, V., Sibler, A.-P., Desplancq, D., Nominé, Y., Spehner, D., Schultz, P., Weiss, E. & Zuber, G. 2014. Intracellular delivery of functionally active proteins using self-assembling pyridylthiourea-polyethylenimine. *J Control Release*, 178, 86–94.

Pritzlaff, A., Ferré, G., Dargassies, E., Williams, C. O., Gonzalez, D. D. & Eddy, M. T. 2023. Conserved protein–polymer interactions across structurally diverse polymers underlie alterations to protein thermal unfolding. *ACS Central Sci*, 9, 685–695.

Qi, L., Li, Z., Liu, J. & Chen, X. J. A. M. 2024. Omics-enhanced nanomedicine for cancer therapy. *Adv Mater*, 36, 2409102.

Ragni, E. 2025. Extracellular vesicles: Recent advances and perspectives. *FBL*, 30, 45.

Rahat, I., Yadav, P., Singhal, A., Fareed, M., Purushothaman, J. R., Aslam, M., Balaji, R., Patil-Shinde, S. & Rizwanullah, M. 2024. Polymer lipid hybrid nanoparticles for phytochemical delivery: Challenges, progress, and future prospects. *Beilstein J Nanotechnol*, 15, 1473–1497.

Ramakrishnan, M., Kaul, R., Sharma, A., Ahmad, Z., Vijayakanth, V., Keerthana, K., Gao, Z., Zhou, M. & Wei, Q. 2025. CRISPR RNP-mediated transgene-free genome editing in plants: Advances, challenges and future directions for tree species. *Plant Cell Environ*, https://doi.org/10.1111/pce.70176.

Rauf, M. A., Rao, A., Sivasoorian, S. S. & Iyer, A. K. 2025. Nanotechnology-based delivery of CRISPR/Cas9 for cancer treatment: A comprehensive review. *Cells*, 14, Article 112.

Ravindran S, Suthar JK, Rokade R, Deshpande P, et al. Pharmacokinetics, Metabolism, Distribution and Permeability of Nanomedicine. *Current Drug Metabolism*. 2018;19(4):327–344. doi: 10.2174/1389200219666180305154119.

Rehmani, S., Mclaughlin, C. M., Eltaher, H. M., Moffett, R. C., Flatt, P. R. & Dixon, J. E. 2023. Orally-delivered insulin-peptide nanocomplexes enhance transcytosis from cellular depots and

improve diabetic blood glucose control. *J Control Release*, 360, 93–109.

Reimhult, E. & Virk, M. M. 2021. Hybrid lipopolymer vesicle drug delivery and release systems. *J Biomed Res*, 35, 301–309.

Ren, Q., Cheng, Y. & Lv, J. 2023. Boronate building blocks for intracellular protein delivery. *Adv Healthc Mater*, 12, e2202049.

Renero-Lecuna, C., Iturrioz-Rodríguez, N., González-Lavado, E., Padín-González, E., Navarro-Palomares, E., Valdivia-Fernández, L., García-Hevia, L., Fanarraga, M. L. & González-Legarreta, L. J. J. O. N. 2019. Effect of size, shape, and composition on the interaction of different nanomaterials with HeLa cells. *J Nanobiotechnol*, 17, 7518482.

Rideau, E., Dimova, R., Schwille, P., Wurm, F. R. & Landfester, K. 2018. Liposomes and polymersomes: A comparative review towards cell mimicking. *Chem Soc Rev*, 47, 8572–8610.

Ruseska, I. & Zimmer, A. 2020. Internalization mechanisms of cell-penetrating peptides. *Beilstein J Nanotechnol*, 11, 101–123.

Ryu, J. H., Lee, G. J., Shih, Y. V., Kim, T. I. & Varghese, S. 2019. Phenylboronic acid-polymers for biomedical applications. *Curr Med Chem*, 26, 6797–6816.

Safta, D. A., Bogdan, C. & Moldovan, M. L. 2024. SLNs and NLCs for skin applications: Enhancing the bioavailability of natural bioactives. *Pharmaceutics*, 16(4), Article 458.

Sahab-Negah, S., Khawaja, A. M., Kendall, L. & Heiss, J. D. 2024. Peptide- and Protein-Based Nanoparticles. *In:* Kateb, B., Heiss, J. D., Yu, J. S. & Hsieh, M. (eds.) *The Textbook of Nanoneuroscience and Nanoneurosurgery*. Cham: Springer Nature Switzerland.

Sahay, G., Alakhova, D. Y. & Kabanov, A. V. 2010. Endocytosis of nanomedicines. *J Control Release*, 145, 182–195.

Sahu, D., Sreekanth, P. S. R., Behera, P. K., Pradhan, M. K., Patnaik, A., Salunkhe, S. & Cep, R. 2024. Advances in synthesis, medicinal properties and biomedical applications of pyridine derivatives: A comprehensive review. *Eur J Med Chem Reports*, 12, 100210.

Schoenmaker, L., Witzigmann, D., Kulkarni, J. A., Verbeke, R., Kersten, G., Jiskoot, W. & Crommelin, D. J. J. I. J. O. P. 2021. mRNA-lipid nanoparticle COVID-19 vaccines: Structure and

stability. *Int J Pharm*, 601, 120586.

Scott, R. W. J., Wilson, O. M. & Crooks, R. M. 2005. Synthesis, characterization, and applications of dendrimer-encapsulated nanoparticles. *J Phys Chem B*, 109, 692–704.

Sen, A., Oswalia, J., Yadav, S., Vachher, M. & Nigam, A. 2024. Recent trends in nanozyme research and their potential therapeutic applications. *Curr Res Biotechnol*, 7, 100205.

Sengupta, S., Eavarone, D., Capila, I., Zhao, G., Watson, N., Kiziltepe, T. & Sasisekharan, R. 2005. Temporal targeting of tumour cells and neovasculature with a nanoscale delivery system. *Nature*, 436, 568–572.

Shah, R. M., Jadhav, S. R., Bryant, G., Kaur, I. P. & Harding, I. H. 2025. On the formation and stability mechanisms of diverse lipid-based nanostructures for drug delivery. *Adv Colloid Interface Sci*, 338, 103402.

Shahsavari, S., Rad, M. B., Hajiaghajani, A., Rostami, M., Hakimian, F., Jafarzadeh, S., Hasany, M., Collingwood, J. F., Aliakbari, F., Fouladiha, H., Bardania, H., Otzen, D. E. & Morshedi, D. 2024. Magnetoresponsive liposomes applications in nanomedicine: A comprehensive review. *Biomed Pharmacother*, 181, 117665.

Sharma, A., Mejía, D., Maysinger, D. & Kakkar, A. 2014. Design and synthesis of multifunctional traceable dendrimers for visualizing drug delivery. *RSC Adv*, 4, 19242–19245.

Sharma, R., Kambhampati, S. P., Zhang, Z., Sharma, A., Chen, S., Duh, E. I., Kannan, S., Tso, M. O. M. & Kannan, R. M. 2020. Dendrimer mediated targeted delivery of sinomenine for the treatment of acute neuroinflammation in traumatic brain injury. *J Control Release*, 323, 361–375.

Sharma, R., Sharma, A., Kambhampati, S. P., Reddy, R. R., Zhang, Z., Cleland, J. L., Kannan, S. & Kannan, R. M. 2018. Scalable synthesis and validation of PAMAM dendrimer-N-acetyl cysteine conjugate for potential translation. *Bioeng Transl Med*, 3, 87–101.

Shi, D., Beasock, D., Fessler, A., Szebeni, J., Ljubimova, J. Y., Afonin, K. A. & Dobrovolskaia, M. A. 2022. To PEGylate or not to PEGylate: Immunological properties of nanomedicine's most popular component, polyethylene glycol and its alternatives. *Adv Drug Deliv Rev*, 180, 114079.

Shi, J., Xiao, Z., Kamaly, N. & Farokhzad, O. C. 2011a. Self-assembled targeted nanoparticles: Evolution of technologies and bench to bedside translation. *Accounts Chem Res*, 44, 1123–1134.

Shi, J., Xiao, Z., Votruba, A. R., Vilos, C. & Farokhzad, O. C. 2011b. Differentially charged hollow core/shell lipid-polymer-lipid hybrid nanoparticles for small interfering RNA delivery. *Angew Chem Int Ed Engl*, 50, 7027–31.

Shi, J., Yuan, B., Yang, H. & Sun, Z. 2025. Recent advances on protein engineering for improved stability. *BioDesign Res*, 7, 100005.

Silva, S., Almeida, A. J. & Vale, N. 2021. Importance of nanoparticles for the delivery of antiparkinsonian drugs. *Pharmaceutics*, 13, 508.

Singh, S., Sharma, N., Behl, T., Sarkar, B. C., Saha, H. R., Garg, K., Singh, S. K., Arora, S., Amran, M. S., Abdellatif, A. A. H., Bilgrami, A. L., Ashraf, G. M. & Rahman, M. S. 2021. Promising strategies of colloidal drug delivery-based approaches in psoriasis management. *Pharmaceutics*, 13(7), Article 1005.

Singh, Y., Meher, J. G., Raval, K., Khan, F. A., Chaurasia, M., Jain, N. K. & Chourasia, M. K. 2017. Nanoemulsion: Concepts, development and applications in drug delivery. *J Control Release*, 252, 28–49.

Sivadasan, D., Sultan, M. H., Madkhali, O., Almoshari, Y. & Thangavel, N. 2021. Polymeric lipid hybrid nanoparticles (PLNs) as emerging drug delivery platform-a comprehensive review of their properties, preparation methods, and therapeutic applications. *Pharmaceutics*, 13, 1291.

Soares, S., Sousa, J., Pais, A. & Vitorino, C. J. F. I. C. 2018. Nanomedicine: principles, properties, and regulatory issues. *Front Chem*, 6, 360.

Sobol, Ż., Chiczewski, R. & Wątróbska-Świetlikowska, D. 2025. Advances in liposomal drug delivery: Multidirectional perspectives on overcoming biological barriers. *Pharmaceutics*, 17(1), Article 88.

Sohail, M., Sun, Z., Li, Y., Gu, X. & Xu, H. 2021. Research progress in strategies to improve the efficacy and safety of doxorubicin for cancer chemotherapy. *Expert Rev Anticancer Ther*, 21, 1385–1398.

Song, Q., Wang, D., Li, H., Wang, Z., Sun, S., Wang, Z., Liu, Y., Lin, S., Li, G. & Zhang, S. 2024. Dual-response of multi-functional microsphere system to ultrasound and microenvironment for enhanced bone defect treatment. *Bioactive Mater*, 32, 304–318.

Sriwidodo, Umar, A. K., Wathoni, N., Zothantluanga, J. H., Das, S. & Luckanagul, J. A. 2022. Liposome-polymer complex for drug delivery system and vaccine stabilization. *Heliyon*, 8, e08934.

Stewart, M. P., Lorenz, A., Dahlman, J., & Sahay, G. 2016. Challenges in carrier-mediated intracellular delivery: Moving beyond endosomal barriers. *WIREs Nanomed Nanobiotechnol*, 8(3), 465–478. https://doi.org/10.1002/wnan.1369

Tamura, M., Ichinohe, S., Tamura, A., Ikeda, Y. & Nagasaki Y. 2011. In vitro and in vivo characteristics of core-shell type nanogel particles: Optimization of core cross-linking density and surface poly(ethylene glycol) density in PEGylated nanogels. *Acta Biomaterialia*, 7(9), 3354–3361.

Tao, Y., Lamas, V., Du, W., Zhu, W., Li, Y., Whittaker, M. N., Zuris, J. A., Thompson, D. B., Rameshbabu, A. P., Shu, Y., Gao, X., Hu, J. H., Pei, C., Kong, W. J., Liu, X., Wu, H., Kleinstiver, B. P., Liu, D. R. & Chen, Z. Y. 2023. Treatment of monogenic and digenic dominant genetic hearing loss by CRISPR-Cas9 ribonucleoprotein delivery in vivo. *Nat Commun*, 14, 4928.

Tenchov, R., Bird, R., Curtze, A. E. & Zhou, Q. 2021. Lipid nanoparticles─from liposomes to mRNA vaccine delivery, a landscape of research diversity and advancement. *ACS Nano*, 15, 16982–17015.

Tianyi, C., Xiang, L., Dan, L. & Youqi, T. 2023. Development of small molecules for disrupting pathological amyloid aggregation in neurodegenerative diseases. *Ageing Neurodegener Dis*, 3, 18.

Torchilin, V. P. 2007. Micellar nanocarriers: pharmaceutical perspectives. *Pharm Res*, 24, 1–16.

Torchilin, V. P. 2008. Tat peptide-mediated intracellular delivery of pharmaceutical nanocarriers. *Adv Drug Deliv Rev*, 60, 548–558.

Troutier, A.-L., Delair, T., Pichot, C. & Ladavière, C. 2005. Physicochemical and interfacial investigation of lipid/polymer particle assemblies. *Langmuir*, 21, 1305–1313.

Trucillo, P., Campardelli, R. & Reverchon, E. 2020. Liposomes: From bangham to supercritical fluids. *Processes*, 8, 1022.

Uchida, H., Itaka, K., Nomoto, T., Ishii, T., Suma, T., Ikegami, M., Miyata, K., Oba, M., Nishiyama, N. & Kataoka, K. 2014. Modulated protonation of side chain aminoethylene repeats in N-substituted polyaspartamides promotes mRNA transfection. *J Am Chem Soc*, 136, 12396–12405.

Viegas, C., Patrício, A. B., Prata, J. M., Nadhman, A., Chintamaneni, P. K. & Fonte, P. 2023. Solid lipid nanoparticles vs. nanostructured lipid carriers: A comparative review. *Pharmaceutics*, 15(3), Article 758.

Vitorino, R. J. P. 2024. Transforming clinical research: The power of high-throughput omics integration. *Pharmaceuticals*, 12, 25.

Vosoughi, P., Naghib, S. M., Jafari, T. & Kangarshahi, B. M. 2025. Chitosan-encapsulated lipid-based nanovesicles for therapeutic applications and tissue engineering: A review. *Carbohydr Polym Technol Appl*, 10, 100805.

Wang, B., Navath, R. S., Romero, R., Kannan, S. & Kannan, R. 2009. Anti-inflammatory and anti-oxidant activity of anionic dendrimer-N-acetyl cysteine conjugates in activated microglial cells. *Int J Pharm*, 377, 159–168.

Wang, J., Li, B., Qiu, L., Qiao, X. & Yang, H. 2022. Dendrimer-based drug delivery systems: History, challenges, and latest developments. *J Biol Eng*, 16, 18.

Wang, J., Sundaravadivelu Devarajan, D., Nikoubashman, A. & Mittal, J. 2023a. Conformational properties of polymers at droplet interfaces as model systems for disordered proteins. *ACS Macro Lett*, 12(11), 1472–1478.

Wang, Q., Yu, J., Kadungure, T., Beyene, J., Zhang, H. & Lu, Q. 2018. ARMMs as a versatile platform for intracellular delivery of macromolecules. *Nat Commun*, 9, 960.

Wang, R., Yu, Y., Gai, M., Mateos-Maroto, A., Morsbach, S., Xia, X., He, M., Fan, J., Peng, X., Landfester, K., Jiang, S. & Sun, W. 2023b. Liposomal enzyme nanoreactors based on

nanoconfinement for efficient antitumor therapy. *Angew Chem Int Ed*, 62, e202308761.

Wang, S., Ding, P., Shen, L., Fan, D., Cheng, H., Huo, J., Wei, X., He, H. & Zhang, G. 2024a. Inhalable hybrid nanovaccines with virus-biomimetic structure boost protective immune responses against SARS-CoV-2 variants. *J Nanobiotechnol*, 22, 76.

Wang, T., Yu, T., Li, W., Liu, Q., Sung, T.-C. & Higuchi, A. J. I. J. O. P. 2024b. Design and lyophilization of mRNA-encapsulating lipid nanoparticles. *Int J Pharm*, 662, 124514.

Wang, X., Li, Y., Wang, X., Sandoval, D. M., He, Z., A, S., Saez, I. L. & Wang, W. 2023c. Guanidyl-rich poly (β amino ester) s for universal functional cytosolic protein delivery and clustered regularly interspaced short palindromic repeats (CRISPR) Cas9 ribonucleoprotein based gene editing. *ACS Nano*, 17, 17799–17810.

Wasti, S., Lee, I. H., Kim, S., Lee, J.-H. & Kim, H. J. F. I. G. 2023. Ethical and legal challenges in nanomedical innovations: A scoping review. *Front Genet*, 14, 1163392.

Wasyłeczko, M., Wojciechowski, C. & Chwojnowski, A. 2024. Polyethersulfone polymer for biomedical applications and biotechnology. *Int J Mol Sci*, 25, 4233.

Wen, X., Hao, Z., Yin, H., Min, J., Wang, X., Sun, S. & Ruan, G. 2025. Engineered extracellular vesicles as a new class of nanomedicine. *Chem Bio Eng*, 2, 3–22.

Wiklander, O. P. B., Brennan, M., Lötvall, J., Breakefield, X. O. & El Andaloussi, S. 2019. Advances in therapeutic applications of extracellular vesicles. *Sci Transl Med*, 11(492), eaav8523.

Wojnilowicz, M., Glab, A., Bertucci, A., Caruso, F. & Cavalieri, F. 2019. Super-resolution imaging of proton sponge-triggered rupture of endosomes and cytosolic release of small interfering RNA. *ACS Nano*, 13, 187–202.

Wongsirojkul, N., Masuta, A., Shimokawa, N. & Takagi, M. 2022. Control of line tension at phase-separated lipid domain boundaries: Monounsaturated fatty acids with different chain lengths and osmotic pressure. *Membranes*, 12, 781.

Wu, L.-P., Wang, D., Li, Z. J. M. S. & C, E. 2020. Grand challenges in nanomedicine. *Mater Sci Eng C*, 106, 110302.

Wu, W., Aiello, M., Zhou, T., Berliner, A., Banerjee, P. & Zhou, S. 2010. In-situ immobilization of quantum dots in polysaccharide-based nanogels for integration of optical pH-sensing, tumor cell imaging, and drug delivery. *Biomaterials,* 31, 3023–31.

Wu, X. Y. 2016. Strategies for optimizing polymer-lipid hybrid nanoparticle-mediated drug delivery. *Expert Opin Drug Deliv*, 13, 609–612.

Wu, Y., Zeng, C., Lv, J., Li, H., Gao, J., Liu, Z. & Yuan, Z. 2024. Tumor-targeted cRGD-coated liposomes encapsulating optimized synergistic cepharanthine and IR783 for chemotherapy and photothermal therapy. *Int J Nanomed*, 19, 6145–6160.

Xiao, K., Luo, J., Li, Y., Lee, J. S., Fung, G. & Lam, K. S. 2011. PEG-oligocholic acid telodendrimer micelles for the targeted delivery of doxorubicin to B-cell lymphoma. *J Control Release*, 155, 272–81.

Xie, R., Wang, Y. & Gong, S. 2021. External stimuli-responsive nanoparticles for spatially and temporally controlled delivery of CRISPR-Cas genome editors. *Biomater Sci*, 9, 6012–6022.

Xin, J., Lu, X., Cao, J., Wu, W., Liu, Q., Wang, D., Zhou, X. & Ding, D. 2024. Fluorinated organic polymers for cancer drug delivery. *Adv Mater*, 36, 2404645.

Xu, W., Ling, P. & Zhang, T. 2013. Polymeric micelles, a promising drug delivery system to enhance bioavailability of poorly water-soluble drugs. *J Drug Deliv*, 2013, 340315.

Yadav, S., Gandham, S. K., Panicucci, R. & Amiji, M. M. 2016. Intranasal brain delivery of cationic nanoemulsion-encapsulated TNFα siRNA in prevention of experimental neuroinflammation. *Nanomedicine*, 12, 987–1002.

Yamada, Y., Onda, T., Wada, Y., Hamada, K., Kikkawa, Y. & Nomizu, M. 2023. Structure–activity relationships of RGD-containing peptides in integrin αvβ5-mediated cell adhesion. *ACS Omega*, 8, 4687–4693.

Yang, J., Zhang, Q., Chang, H. & Cheng, Y. 2015. Surface-engineered dendrimers in gene delivery. *Chem Rev*, 115, 5274–5300.

Yardley, D. A. 2013. nab-Paclitaxel mechanisms of action and delivery. *J Control Release*, 170, 365–372.

Yin, L., Yuvienco, C. & Montclare, J. K. 2017. Protein based therapeutic delivery agents: Contemporary developments and challenges. *Biomaterials*, 134, 91–116.

Yin, X., Harmancey, R., Mcpherson, D. D., Kim, H. & Huang, S. L. 2023. Liposome-based carriers for CRISPR genome editing. *Int J Mol Sci*, 24(5), 4621.

Yip, B. H. 2020. Recent advances in CRISPR/Cas9 delivery strategies. *Biomolecules*, 10(8), 1168.

Yokoyama, M., Okano, T., Sakurai, Y., Ekimoto, H., Shibazaki, C. & Kataoka, K. 1991. Toxicity and antitumor activity against solid tumors of micelle-forming polymeric anticancer drug and its extremely long circulation in blood. *Cancer Res*, 51, 3229–3236.

Younis, M. A., Alsogaihi, M. A., Abdellatif, A. A. H. & Saleem, I. 2025. Nanoformulations in the treatment of lung cancer: current status and clinical potential. *Drug Dev Ind Pharm*, 51, 1088–1104.

Younis, M. A., Tawfeek, H. M., Abdellatif, A. A. H., Abdel-Aleem, J. A. & Harashima, H. 2022. Clinical translation of nanomedicines: Challenges, opportunities, and keys. *Adv Drug Deliv Rev*, 181, 114083.

Yu, C., Tan, E., Xu, Y., Lv, J. & Cheng, Y. 2019. A guanidinium-rich polymer for efficient cytosolic delivery of native proteins. *Bioconjug Chem*, 30, 413–417.

Yu, Y.-P., Wang, Q., Liu, Y.-C. & Xie, Y. 2014. Molecular basis for the targeted binding of RGD-containing peptide to integrin αVβ3. *Biomaterials*, 35, 1667–1675.

Yuan, F., Gollapudi, S., Day, K. J., Ashby, G., Sangani, A., Malady, B. T., Wang, L., Lafer, E. M., Huibregtse, J. M. & Stachowiak, J. C. 2024. Ubiquitin-driven protein condensation stabilizes clathrin-mediated endocytosis. *PNAS Nexus*, 3, 342.

Yun, Y., An, J., Kim, H. J., Choi, H. K. & Cho, H.-Y. 2025. Recent advances in functional lipid-based nanomedicines as drug carriers for organ-specific delivery. *Nanoscale*, 17, 7617–7638.

Zelko, I. N., Mariani, T. J. & Folz, R. J. 2002. Superoxide dismutase multigene family: A comparison of the CuZn-SOD (SOD1), Mn-SOD (SOD2), and EC-SOD (SOD3) gene structures, evolution, and expression. *Free Radic Biol Med*, 33, 337–349.

Zhang, L., Chan, J. M., Gu, F. X., Rhee, J.-W., Wang, A. Z., Radovic-Moreno, A. F., Alexis, F., Langer, R. & Farokhzad, O. C. 2008. Self-assembled lipid−polymer hybrid nanoparticles: A robust drug delivery platform. *ACS Nano*, 2, 1696–1702.

Zhang, L., Yao, L., Zhao, F., Yu, A., Zhou, Y., Wen, Q., Wang, J., Zheng, T. & Chen, P. 2023. Protein and peptide-based nanotechnology for enhancing stability, bioactivity, and delivery of anthocyanins. *Adv Healthc Mater*, 12, e2300473.

Zhang, S., Shen, J., Li, D. & Cheng, Y. 2021. Strategies in the delivery of Cas9 ribonucleoprotein for CRISPR/Cas9 genome editing. *Theranostics*, 11, 614–648.

Zhang, Z., Shen, W., Ling, J., Yan, Y., Hu, J. & Cheng, Y. 2018. The fluorination effect of fluoroamphiphiles in cytosolic protein delivery. *Nat Commun*, 9, 1377.

Zhao, J., Liu, L., Wang, Y., Huang, Z., Zhang, Y., Qiao, R., Davis, T. P., Tang, Z., Song, W. & Chen, X. 2024. Heterocyclic molecules tethered branched polymers with innate immune stimulating activity. *CCS Chemistry*, 6, 1278–1288.

Zheng, H., Li, J., Luo, X., Li, C., Hu, L., Qiu, Q., Ding, J., Song, Y. & Deng, Y. 2019. Murine RAW264. 7 cells as cellular drug delivery carriers for tumor therapy: A good idea? *Cancer Chemother Pharmacol*, 83(2), 361–374.

Zhu, C., Mu, J. & Liang, L. 2024. Nanocarriers for intracellular delivery of proteins in biomedical applications: Strategies and recent advances. *J Nanobiotechnol*, 22, 688.

Zingg, R. & Fischer, M. J. 2019. The consolidation of nanomedicine. *Wiley Interdiscip Rev Nanomed Nanobiotechnol*, 11, e1569.

6 Novel Approaches and Advances in Therapeutic Glycoprotein Delivery and Applications

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6.1 INTRODUCTION

Among the various biomolecules explored for therapeutic purposes, glycoproteins stand out for their complexity and range of biological roles [1, 2]. However, the peptide chains alone do not define their roles. Often, the specific pattern of glycan attachment reshapes folding and alters receptor interactions, which together shape biological outcomes [3, 4]. These carbohydrate additions are far from being decorative. They govern essential parameters, such as half-life in circulation or even immunogenic masking [5].

Erythropoietin, which is vital for red blood cell production, has much of its pharmacological stability toward glycans that protect it from proteases and help it circulate longer [6]. Similarly, the SARS-CoV-2 spike protein

exploits glycosylation to facilitate ACE2 binding and partially evade immune surveillance [7–10]. This makes glycoproteins central players in physiology and disease, and not merely bystanders of these processes.

Clinically, these findings are transformative. For instance, monoclonal antibodies have redefined their role in the treatment of cancer and autoimmune diseases. They are glycoproteins engineered with extraordinary specificity: trastuzumab targets HER2-positive breast cancer, whereas rituximab binds CD20 in lymphomas; both rely on carefully tuned peptide and glycan profiles [11–13]. In metabolic diseases like Gaucher and Fabry disease, recombinant glycoprotein enzymes compensate for missing lysosomal functions. Their effectiveness still depends on the precise management of glycosylation to ensure correct trafficking [14, 15].

This promise is not without substantial complications. Ironically, the glycosylation that enables these sophisticated functions also complicates their production and makes them fragile, challenging small-molecule drugs. Even small shifts in cell culture or purification can alter glycan patterns. This, in turn, alters pharmacokinetics and may trigger immune responses [16–18]. Scaling up the manufacturing of such compounds is not a minor undertaking. Ensuring consistent batches with delicate structures adds further complexity. Recent technological advances have begun to mitigate these challenges. Lipid nanoparticles protect fragile proteins, whereas polymeric and stimuli-sensitive systems aim for site-specific release [19–22]. Simultaneously, refined glycoengineering in non-human systems yields more human-like glycans, thereby curbing unexpected immune reactions [23, 24].

This chapter examines glycoprotein structures, functions, and therapeutic potential, with an emphasis on formidable delivery and stabilization challenges. It surveys advanced formulation strategies and how they aim to protect structures, guide glycoproteins, and adjust their pharmacokinetics.

Examples from oncology, enzyme therapies, and vaccines demonstrate how glycoprotein engineering is increasingly dovetailing with precision medicine. It also highlights enduring problems, immunogenicity, regulatory barriers, and difficulties of scaling up, which still steer the translation of these biomolecules into clinical use.

6.2 BIOLOGICAL SIGNIFICANCE OF GLYCOPROTEINS

Multicellular organisms rely on glycoproteins for various biological processes This may appear dramatic, but once we trace how these molecules mediate communication and immune defense, it becomes difficult to argue otherwise [25]. They are vital not only because of their polypeptide backbone but also because of the attached glycans that tune everything from folding to receptor affinity and serum persistence [26] (Figure 6.1).

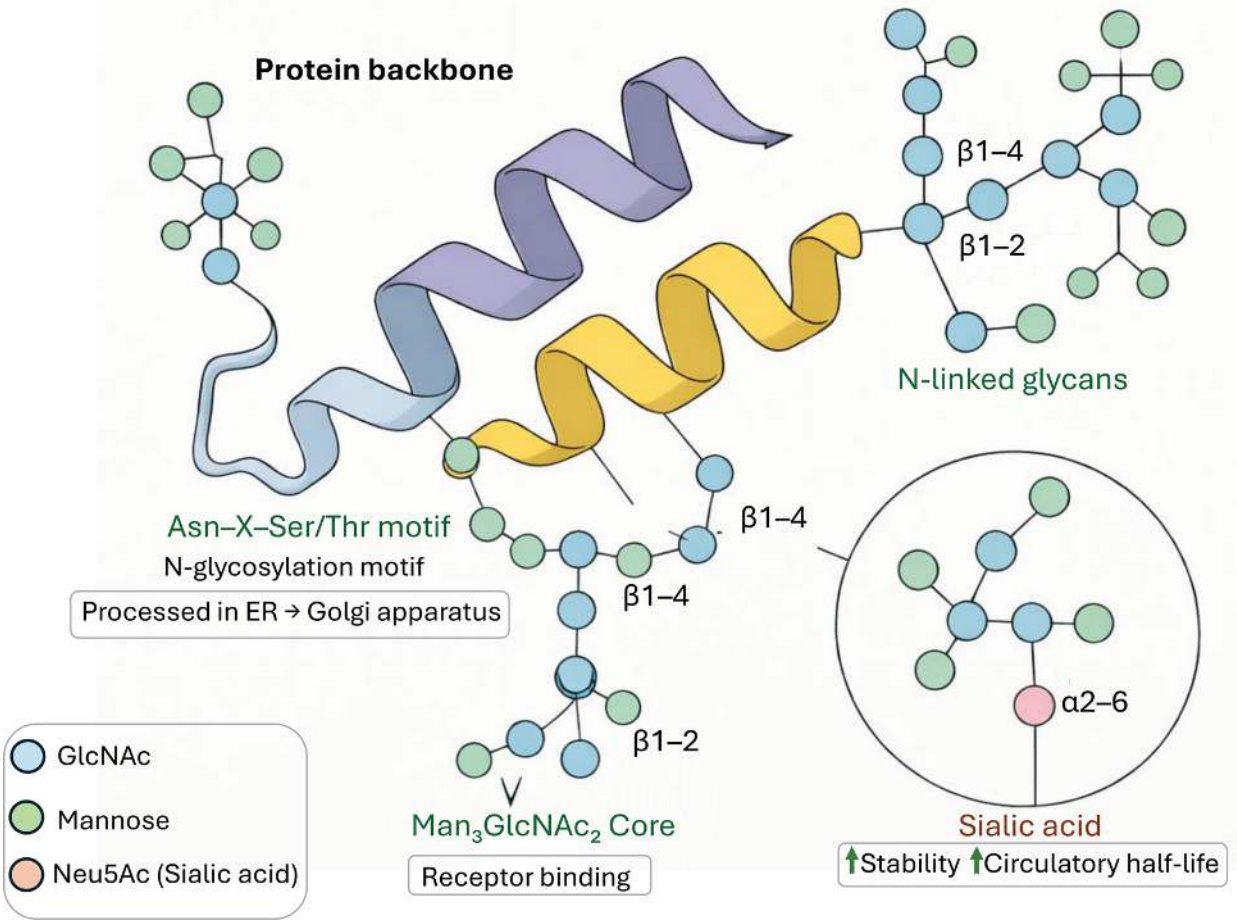

FIGURE 6.1 Structural organization and functional consequences of N-linked glycosylation in therapeutic glycoproteins. (Schematic representation of the canonical Asn–X–Ser/Thr sequon and the conserved $Man_3GlcNAc_2$ core structure of N-linked glycans, illustrating enzymatic processing from the endoplasmic reticulum to the Golgi apparatus. Terminal glycan modifications, including β1–2/β1–4 branching and α2–6 sialylation (Neu5Ac), are depicted in relation to their effects on conformational stability, receptor recognition, and circulatory half-life. The figure emphasizes how site-specific glycan architecture modulates pharmacokinetic behavior and receptor-mediated interactions of biotherapeutics.) ⏎

For instance, in signaling, glycoproteins operate as both ligands and receptors, orchestrating intricate signaling cascades. Erythropoietin is a classic example of this; its glycan branches are not peripheral decorations

but essential stabilizers that extend its half-life and directly underpin its effectiveness in stimulating erythropoiesis [8]. The same principle applies to other hormones, such as follicle-stimulating hormone, whose biological potency is significantly diminished when glycosylation is disrupted.

The glycoprotein architecture of the immune system is arguably even more complex. Immunoglobulins are glycoproteins at their core; their glycan structures influence not only solubility and stability but also how they engage Fc receptors and activate complement pathways [16, 27]. Cytokines, such as interleukins, follow a similar mechanism, with glycosylation often dictating receptor-binding kinetics or shielding key epitopes from proteolytic clipping. This intricate sugar-coding mechanism is particularly evident in pathogens that exploit glycoproteins. The glycan shield of the SARS-CoV-2 spike protein is not accidental; it helps mask viral surfaces from neutralizing antibodies while still allowing tight ACE2 engagement [7, 8, 28]. Glycoproteins are rarely involved in signaling or immunity. They also lend themselves to structural integrity issues. Considering laminin or collagen, heavily glycosylated extracellular matrix proteins maintain the tissue architecture and modulate cell adhesion [29, 30]. Disruption of glycosylation results in compromised basement membranes or defective connective tissue.

On the enzymatic side, glycoproteins populate lysosomes, where they break down the complex substrates. Glycosylation is not just about trafficking these enzymes correctly (although mannose-6-phosphate tagging is famous for directing lysosomal delivery [14]), it also stabilizes proteins against harsh intracellular conditions. In diseases such as Gaucher or Fabry disease, the breakdown of this glycoprotein machinery leads to substrate accumulation and multisystem pathology, underscoring the importance of these structures [31].

What is perhaps most striking is how broadly these roles span: from cell surface receptors modulating immunity to enzymes ensuring metabolic

clean-up, glycoproteins underwrite numerous aspects of biological homeostasis [16, 17, 32]. This functional breadth is precisely why they have become high-value therapeutic targets, but also why their delicate architecture poses several pharmaceutical challenges. Interfering with their glycosylation often alters more than superficial features or attempts to replicate it outside the body; you are often tampering with more than a superficial feature; you are altering core bioactivity [16, 17, 33, 34].

6.3 THERAPEUTIC POTENTIAL AND CHALLENGES

If there is a single thread running through the modern history of biopharmaceuticals, it is the growing appreciation for glycoproteins, not merely as passive scaffolds or messengers, but as actively engineered therapeutics. Their structural sophistication enables functions beyond the reach of small molecules, such as ultra-specific receptor binding, immune modulation, and enzyme replacement deep inside the lysosomes. Yet, the very complexity that makes these molecules so powerful also renders them unusually challenging to develop and deploy [35–37].

Take monoclonal antibodies. These are arguably the poster children for glycoprotein therapeutics. They have reshaped oncology, allowing clinicians to target tumor-associated antigens with precision once confined to theoretical speculation. Trastuzumab zeroes in on HER2-positive breast cancer, whereas rituximab targets CD20 on B cells, each benefiting from glycosylation patterns that stabilize the molecule and influence Fc-mediated immune effects [11, 12]. Without carefully tuned glycans, their half-lives drop and their ability to recruit immune effector functions falters.

Consequently, enzyme replacement therapy (ERT) is available. Disorders such as Gaucher’s and Fabry’s are not just textbook curiosities; they are examples of how a missing or malfunctioning glycoprotein can cascade into

a systemic disease. The recombinant enzymes administered are glycoproteins engineered to display mannose-6-phosphate groups that facilitate lysosomal uptake [14, 15]. Strip away these sugar motifs and break down cellular trafficking, both literally and therapeutically.

Yet, these advances coexist with significant hurdles. The complexity that makes glycoproteins clinically useful also makes them delicate and unpredictable. Their three-dimensional architecture, stabilized by glycans, is vulnerable to shifts in pH, oxidation, and even mechanical stress. Aggregation can expose hydrophobic domains, potentially triggering immune responses or hastening clearance from the bloodstream [38, 39].

Manufacturing can address these problems. Even subtle variations in cell culture conditions, including oxygen levels, nutrient supply, and minor pH drifts, can alter glycosylation patterns, producing batches with different pharmacokinetic or immunogenic profiles [17, 18]. Regulatory bodies should pay close attention to this issue, requiring exhaustive characterization to confirm that each production lot is functionally equivalent.

Clearance also remains a significant challenge. Glycoproteins often encounter receptor-mediated pathways designed to whisk them out of the circulation. Asialoglycoprotein receptors in the liver rapidly bind glycoproteins missing terminal sialic acids, whereas mannose receptors on macrophages mop up high-mannose variants, both natural housekeeping mechanisms that unfortunately work against extended therapeutic presence [40, 41].

Thus, advances in delivery systems have begun to matter profoundly. From lipid nanoparticles and polymer carriers to emerging metal-based platforms, these technologies aim to prolong, shield labile structures, and release cargo precisely when required [19, 21, 42]. Simultaneously, glycoengineering pushes us closer to human-like glycan profiles, reducing the risks of anti-drug antibodies and erratic pharmacodynamics [23, 24].

Therefore, its therapeutic potential is enormous and may be unmatched by other biological classes. Still, there is a need for careful design and scientific humility. Every advance highlights both our progress and the persistent need for further refinement before these intricate systems can be reliably applied. These applications span oncology, infectious diseases, and metabolic disorders (Figure 6.2).

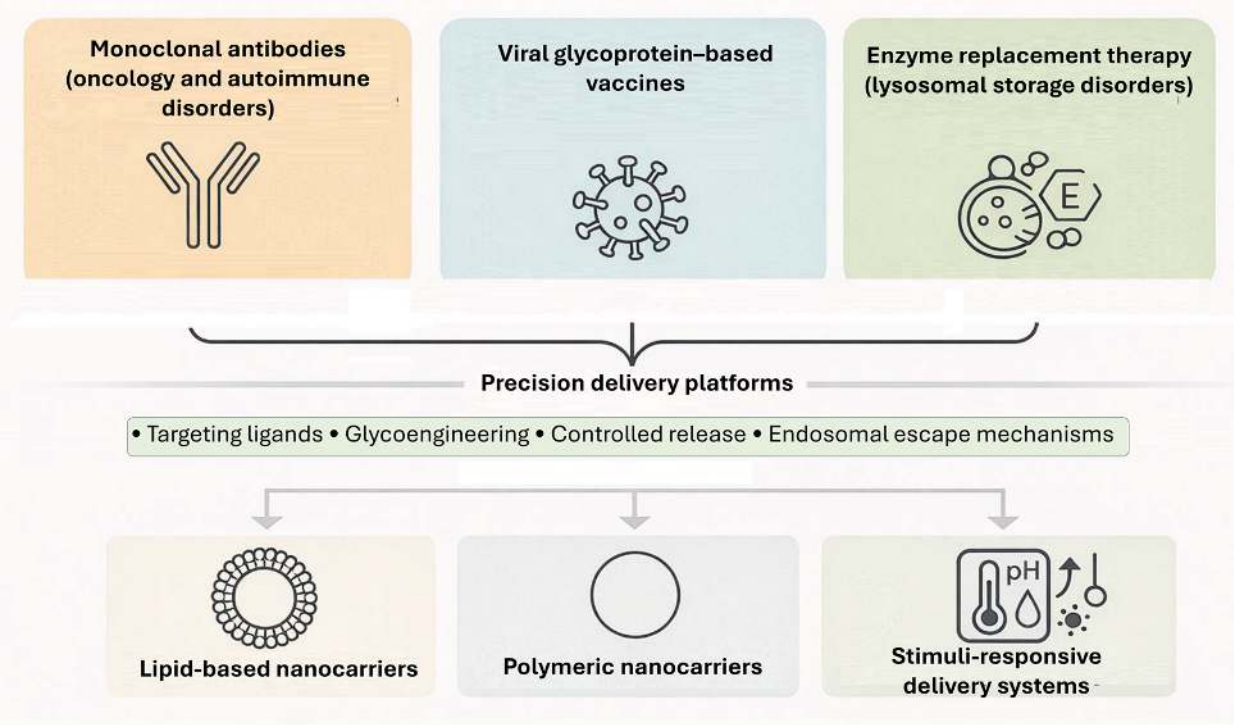

FIGURE 6.2 Precision glycoprotein delivery platforms across major therapeutic modalities. (Conceptual overview linking glycoengineering strategies and nanocarrier systems—including lipid-based, polymeric, and stimulus-responsive platforms—to three principal therapeutic classes: monoclonal antibodies, viral glycoprotein–based vaccines, and enzyme replacement therapies for lysosomal storage disorders. The schematic integrates targeting ligands, controlled-release systems, and endosomal escape mechanisms, highlighting the convergence of glycan design and nanotechnology in precision biotherapeutic delivery.) ⏎

6.3.1 Stability and Degradation

One of the more persistent challenges in deploying glycoproteins therapeutically is to keep them intact long enough to perform their functions. Unlike small molecules, which generally withstand environmental stressors, glycoproteins have intricate three-dimensional architectures and delicate glycan branches, which are surprisingly vulnerable to a wide spectrum of destabilizing forces [16, 43].

Chemically, they face a constant barrage. Oxidative environments, whether from reactive oxygen species *in vivo* or trace contaminants during processing, can modify residues such as methionine, tryptophan, and cysteine. These subtle changes ripple out, sometimes compromising local folding or creating new hydrophobic patches that encourage aggregation [44]. Deamidation is another culprit; when asparagine or glutamine side chains are hydrolyzed into aspartic acids, they introduce charge alterations that can perturb tertiary structures, subtly at first, but enough to undermine receptor binding or accelerate clearance [45].

Hydrolysis occurs under harsh pH conditions. Glycoproteins can fragment outright, severing peptide bonds, and delicate glycosidic linkages. This can strip away glycans that normally stabilize the protein core or shield key epitopes from immune detection. In addition, non-enzymatic glycation reactions with reducing sugars produce advanced glycation end products, muddying solubility and stability in ways that often escape straightforward prediction [43, 46].

Maintaining these conformations under physical stress is challenging. Glycoproteins rely on an intricate balance of non-covalent forces to maintain their conformation. Even modest shifts in temperature, ionic strength, or mechanical agitation (e.g., during vial shaking or infusion pumping) can tip the balance, leading to local unfolding or global denaturation [47]. Once hydrophobic regions are exposed, aggregation often follows, and aggregated

glycoproteins do not just lose bioactivity; they also become prime targets for immune recognition [16, 48, 49].

Therefore, adsorption is an important consideration. Glycoproteins can stick to container walls or delivery devices, which might sound trivial, but actually result in measurable losses, particularly at low concentrations, typical of many biologics [16]. Precipitation is another concern, whether it is driven by extremes in pH or by reaching solubility thresholds.

Overlaying this with the reality of enzymatic degradation. Even when administered intravenously, glycoproteins navigate the bloodstream, which is rich in proteases and glycosidases. Endopeptidases, such as trypsin or chymotrypsin, can cleave peptide backbones, whereas glycosidases systematically nibble away at glycans, dismantling features that prolong half-life and reduce immunogenic exposure [50, 51]. In lysosomes, where many therapeutics ultimately aim to deliver their payloads, the combined activity of proteases and glycosidases can dramatically accelerate glycoprotein degradation [52]. Given these vulnerabilities, it is evident that no single factor solely dictates glycoprotein stability. Instead, chemical modifications, physical stressors, and continuous enzymatic assaults converge to compromise structural integrity and reduce therapeutic efficacy [53].

For this reason, scientists have put much effort into stabilizing buffers, protective excipients, and site-specific mutations that replace labile residues, all just to buy these remarkable molecules more time to work [54–56]. Without these defenses, the odds are unfortunately stacked against glycoproteins, maintaining their functional form long enough to achieve the clinical goals. The combined influence of oxidation, deamidation, hydrolysis, and other destabilizing forces creates a formidable challenge in maintaining glycoprotein efficacy. These threats, along with their

pharmacological effects and current mitigation approaches, are summarized in Table 6.1.

TABLE 6.1

Stability Challenges in Glycoprotein Therapeutics and Mitigation Strategies

Challenge Type	Specific Factor	Mechanism/Examples	Impact on Therapeutic Performance	Mitigation Strategies/Ref.
Chemical Modifications	Oxidation	Reactive oxygen species (ROS) and dissolved O_2 oxidize methionine, tryptophan, cysteine in erythropoietin and mAbs.	Alters folding, reduces receptor binding, promotes aggregation.	Antioxidants, methionine analogs, oxygen-controlled environments [36, 44]
	Deamidation	Asparagine hydrolyzes to Asp or isoAsp, notably in IgG Fc domains.	Changes charge, distorts local conformation, exposes new epitopes.	Stabilizing excipients, site-directed mutagenesis at hotspots [45]
	Maillard Reaction (Glycation)	Non-enzymatic reaction with reducing sugars, especially lysosomal enzymes.	Forms AGEs, lowers solubility and stability, risks immunogenicity.	Avoid reducing sugars, add stabilizers (e.g., trehalose) [43]

Challenge Type	Specific Factor	Mechanism/Examples	Impact on Therapeutic Performance	Mitigation Strategies/Ref.
	Isomerization	Asp → isoAsp conversion in cytokines and Fc-fusions.	Subtle backbone reshaping, lowers receptor affinity, potency loss.	Strategic amino acid substitutions to eliminate sensitive residues [45]
	Disulfide Scrambling	Redox or mechanical stress reshuffles disulfide bonds (e.g. mAbs).	Misfolding, aggregation, loss of bioactivity.	Optimized redox buffers, gentle processing [36].
Physical and Mechanical	Aggregation	Partial unfolding from oxidation or stress exposes hydrophobics.	Aggregates lower bioactivity, raise immunogenic risk.	Surfactants (polysorbates), controlled agitation, stabilizers [36, 44]
	Adsorption	Low-dose glycoproteins adhere to glass/plastic surfaces.	Measurable drug loss, potential fragment formation.	Tween 20, HSA, siliconized containers [16]
	Shear Stress	Pumping, mixing, infusion induce partial unfolding.	Aggregation, reduced stability, increased clearance.	Low-shear tubing, gentle agitation, surfactants [16]

Challenge Type	Specific Factor	Mechanism/Examples	Impact on Therapeutic Performance	Mitigation Strategies/Ref.
	Precipitation	Extremes in pH, temp, or protein concentration.	Precipitates reduce bioavailability, complicate formulation.	Buffer near neutral pH, lyophilization as needed [47]
Enzymatic Degradation	Proteolysis	Endo-/exopeptidases (trypsin, chymotrypsin, carboxy-/aminopeptidase).	Cleaves backbone, undermines integrity, accelerates clearance.	Encapsulation, protease inhibitors, glycoengineering [50, 51]
	Glycosidase Trimming	Exo-/endoglycosidases (β-galactosidase, α-mannosidase, endo-β-N-acetylglucosaminidase).	Removes glycans, shortening half-life, altering targeting.	Glycoengineering to reinforce glycoforms, nanoparticle shielding [50, 51]
	Dual Lysosomal Action	Cathepsins + glycosidases in lysosomes.	Simultaneous peptide and glycan degradation, fast loss of function.	Mannose-6-phosphate tagging, protective carriers [14, 15]

6.3.2 Immunogenicity

Because of their elegance and functional promise, glycoproteins carry an unavoidable reality: the immune system notices them, sometimes more readily than we would like. Immunogenicity has long been a major issue in the development of glycoprotein-based therapeutics, partly because it is

closely related to the structural identity. These elaborate glycans that help ensure stability and extend circulation can also mark glycoproteins as "foreign" under incorrect circumstances [57, 58]. Starting with the protein backbone. Even with fully humanized sequences, minor misfolding or local aggregation can expose cryptic epitopes, regions normally buried in the native conformation, and suddenly ripe for B-cell recognition. Aggregates, in particular, appear to be immunological red flags; they cluster identical epitopes in ways that effectively supercharge immune recognition [16]. This is one reason why manufacturers obsess the minimization of aggregation beyond preserving the bioactivity.

Thus, the glycan component becomes crucial. Non-human expression systems, such as yeast, plants, and certain insect lines, often decorate proteins with sugar residues, such as α-Gal or Neu5Gc, motifs that are not typically found in humans. These are highly immunogenic and capable of provoking robust antibody responses, which can either neutralize the therapeutic agent outright or form immune complexes that complicate clearance and safety profiles [58]. Even in mammalian systems, slight shifts in glycosylation patterns (due to stress, feed variation, or bioreactor conditions) can lead to glycoforms that are subtly different from those of the patient, potentially tipping the immune scales. These pathways significantly influence the therapeutic half-life and immune recognition (Figure 6.3).

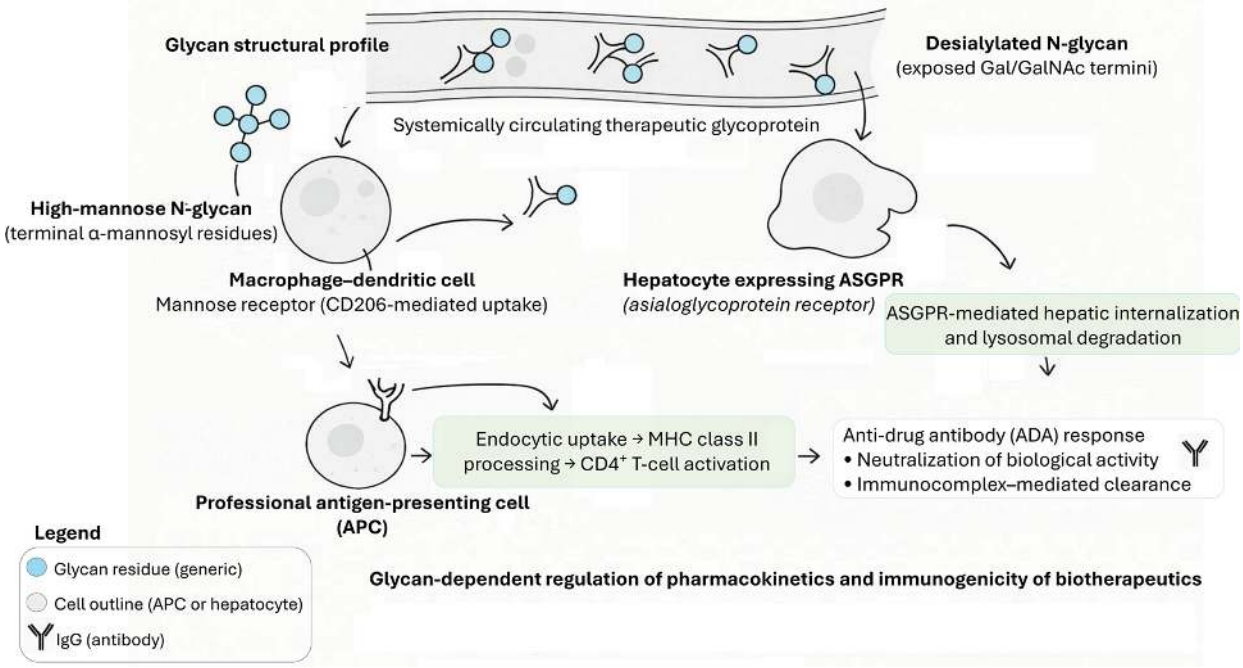

FIGURE 6.3 Glycan-dependent regulation of pharmacokinetics and immunogenicity of circulating biotherapeutics. (Diagrammatic representation of how distinct N-glycan profiles influence receptor-mediated uptake and immune recognition. High-mannose glycans promote CD206-dependent internalization by macrophage–dendritic cells, facilitating MHC class II processing and CD4+ T-cell activation, whereas desialylated glycans with exposed Gal/GalNAc residues trigger ASGPR-mediated hepatic clearance. Downstream consequences include anti-drug antibody formation, neutralization of biological activity, and immune-complex–mediated elimination. The figure underscores glycan composition as a critical determinant of systemic exposure and immunogenic risk.) ⏎

Importantly, glycans themselves do not merely decorate these proteins; they actively participate in immune recognition. Mannose-rich structures, for example, are recognized by innate immune receptors like the mannose receptors on macrophages. This is not necessarily malicious; it is an evolved system to help clear pathogens, but it means glycoproteins with exposed mannose can be flagged for rapid uptake and disposal, undermining

therapeutic goals while simultaneously presenting antigens to immune cells [41, 58].

Further, muddying the water are impurities. It is possible for accidental adjuvants to be produced by trace amounts of host cell proteins, DNA, or even materials that have leached from the production equipment. These adjuvants can increase the immune system's vigilance against the treatment itself. It is difficult to reverse the formation of anti-drug antibodies (ADAs) after they have been created; they may continue to exist, undermine the effectiveness of the treatment, or cause hypersensitivity reactions in certain circumstances [59]. The route of administration is another key variable. Subcutaneous or intramuscular injections linger longer in local tissues, providing antigen-presenting cells ample opportunities to process and present fragments to the immune system. In contrast, intravenous infusions disperse rapidly, sometimes reducing the local concentration gradients that drive robust immune priming, although this is hardly foolproof [58].

Several countermeasures have been developed in this regard. PEGylation is often used to sterically shield epitopes, dampen immune recognition, and prolong circulation [60–63]. Meanwhile, advanced cell line engineering has pushed glycosylation profiles closer to those of a fully human, trimming away xenoantigen risks [23, 24]. In some cases, controlled immunosuppression is accompanied by therapy, particularly enzyme replacement treatments, to minimize neutralizing antibody formation [64].

Despite these innovations, it remains difficult to predict immunogenicity with certainty. The vigilance of the immune system, honed over evolutionary time, often means that we cannot anticipate every response. Therefore, continuous monitoring, patient-specific assessments, and, perhaps most importantly, humility in our expectations, remain essential when navigating the complex terrain of glycoprotein therapeutics [65].

6.3.3 Bioavailability

Here, bioavailability refers simply to the fraction of the administered dose that reaches intact and active systemic circulation [66–68]. That said, this is rarely straightforward for the glycoproteins. They are large, complex, and structurally fragile, which means that they face a gauntlet of biological and chemical hurdles almost immediately after their administration [69]. The route of administration is the primary determinant. Intravenous injection offers 100% bioavailability, bypassing digestive enzymes and absorption barriers. Therefore, many glycoprotein drugs, such as monoclonal antibodies, recombinant enzymes, and even some glycoprotein-based vaccines, are administered in this manner [40, 69]. This is not merely an IV route that comes with trade-offs in terms of patient convenience, the risk of infusion reactions, and healthcare delivery costs.

Oral delivery introduces multiple additional challenges. The gastrointestinal tract is rich in proteases that cleave peptide backbones, not to mention glycosidases that systematically chop away carbohydrate branches [69]. Even if the fragments survive, their sheer size and hydrophilicity render passive diffusion across the gut epithelium unlikely. This means that oral glycoprotein formulations, outside of some experimental encapsulation efforts, have largely remained inaccessible [70–72].

Subcutaneous or intramuscular routes are a compromise, offering easier administration but introducing variability in absorption rates. Local enzymatic degradation at the injection site can reduce the fraction that enters the circulation, and these depot environments may also amplify immunogenicity by prolonging exposure to antigen-presenting cells [58]. Beyond the delivery route, systemic clearance mechanisms are large. Even when glycoproteins enter circulation, they are often rapidly targeted for removal. For instance, the liver's asialoglycoprotein receptor recognizes

desialylated glycoproteins and rapidly clears them from the circulation [40, 73]. Mannose receptors on macrophages play a similar role to high-mannose glycoforms, a mechanism that evolved to clear pathogens but unfortunately does not discriminate against our carefully engineered therapeutics [41].

Glycosylation is a double-edged process. Properly configured terminal sialylation can hide galactose residues, thereby prolonging the circulatory half-life. Aberrant or incomplete glycosylation, due to production quirks or inherent instability, can dramatically accelerate clearance [23]. This is one reason why manufacturers devote much effort to tightly controlling culture conditions and post-translational processing.

Aggregation also accelerates clearance and can induce an immune response. Aggregated glycoproteins tend to be cleared more rapidly; worse, they can trigger immune recognition, resulting in anti-drug antibodies that neutralize the therapeutic and may accelerate their clearance at subsequent doses [16, 44].

Researchers have explored multiple strategies for mitigating these challenges. Encapsulation in nanoparticles, whether lipid or polymeric, can shield glycoproteins from proteases and delay clearance [21, 74]. PEGylation sterically hinders receptor interactions and proteolysis, often doubling or tripling the serum half-life of the drug [60, 61]. Every modification introduces new complexities, such as altered tissue distribution, potential new immunogenic epitopes, or unpredictable pharmacodynamics [75].

Thus, the bioavailability of glycoproteins is not only a matter of dose versus drug release. It involves an intricate balance between absorption barriers, metabolic stability, molecular architecture, and host immune vigilance. Getting it right often means iterative design and sometimes uncomfortable trade-offs; favoring stability here might mean slower receptor

engagement [76]. The nature of working with such sophisticated biological tools is enormous, and so is the care needed to bring this promise to fruition.

6.3.4 Production and Scalability

If designing glycoproteins that work in principle is challenging, producing them reliably, economically, and consistently is another level of difficulty. This is one of the underappreciated realities of biopharmaceutical development: no matter how elegant a molecule might be in the laboratory, it is ultimately the manufacturing hurdles that often determine whether it ever reaches patients [17, 18].

A large part of this complexity stems from the glycosylation process. Unlike the polypeptide backbone, which is dictated by the genetic sequence and is thus relatively predictable, glycan attachment arises from a web of post-translational enzymatic steps inside the host cell. These processes are sensitive to various variables, such as nutrient availability, oxygen tension, pH shifts, and even subtle differences in bioreactor shear forces [17, 77]. As a result, you can end up with a heterogeneous mix of glycoforms, some of which may have dramatically different pharmacokinetics or immunogenicity.

Mammalian expression systems, particularly in Chinese hamster ovary (CHO) cells, are widely used because they approximate human-like glycosylation better than yeast or insect cells [23]. However, this approach is imperfect. They can still introduce minor structural motifs, such as certain fucosylations or sialic acid linkages, that differ subtly from native human patterns. Even slight discrepancies can affect receptor recognition or trigger unwanted immune surveillance, underscoring why regulatory agencies demand exhaustive glycoform profiling before approving a production process [78].

Scaling up to industrial volumes magnifies these issues only. Maintaining consistent oxygenation, nutrient gradients, and waste removal in massive bioreactors is non-trivial, and slight local fluctuations can skew the glycosylation machinery toward alternative branching patterns [18, 77]. As the process moves downstream, purification must not only eliminate DNA and proteins from the host cell, but it must also separate gynoprotein that are closely related while maintaining the delicate tertiary structure of the therapy. For the purpose of achieving reproducible glycan profiles, chromatography techniques have become extremely fine-tuned and frequently proprietary [79]. There is also an economic dimension to this issue. The production of glycoproteins in mammalian systems is inherently costly and involves slow-growing cells, expensive media, and extensive quality control at each step. This is partly why glycoprotein therapeutics remain among the most expensive drugs in the market, straining insurance systems and often limiting accessibility in lower-resource settings [80].

Researchers are not idle in facing these challenges. Advances in glycoengineering have enabled the modification of modified modification systems to yield more uniform, human-compatible glycans, trimming down heterogeneity, and reducing immunogenic surprises [23, 24]. New bioreactor designs manage local microenvironments and smooth gradients that could disrupt enzyme activity. Even continuous manufacturing, a departure from batch procedures, is being investigated to stabilize production parameters over time. Biological systems are still variable despite these developments. Chemical synthesis can replicate small-molecule medications, but glycoproteins are live cell products, making them complex and unpredictable. Ensure each batch is functionally and physically identical is a regulatory need for patient safety, not just a technical challenge [81]. Therefore, while it is tempting to focus exclusively on molecular design or clinical results, the truth is that for glycoprotein drugs, robust, scalable, and

carefully monitored production is every bit as critical to therapeutic success. The hidden infrastructure underpins the clinical promise of these molecules [82].

6.4 ADVANCED DELIVERY PLATFORMS

The inherent fragility and complex clearance profiles of glycoproteins require more than those of passive transport. Over the past two decades, a diverse array of delivery platforms has emerged, each designed to address specific biological challenges of drug delivery. Some mimic cell membranes, while others use synthetic polymers, inorganic features, or respond to disease cues. They are not interchangeable. Each system has unique advantages and trade-offs for stability, targeting, controlled release, or manufacturability. The following subsections discuss these advanced delivery modalities, setting the stage for how they reshape therapeutic possibilities. Each has its own strengths and practical limitations, as is often the case in drug delivery (Figure 6.4).

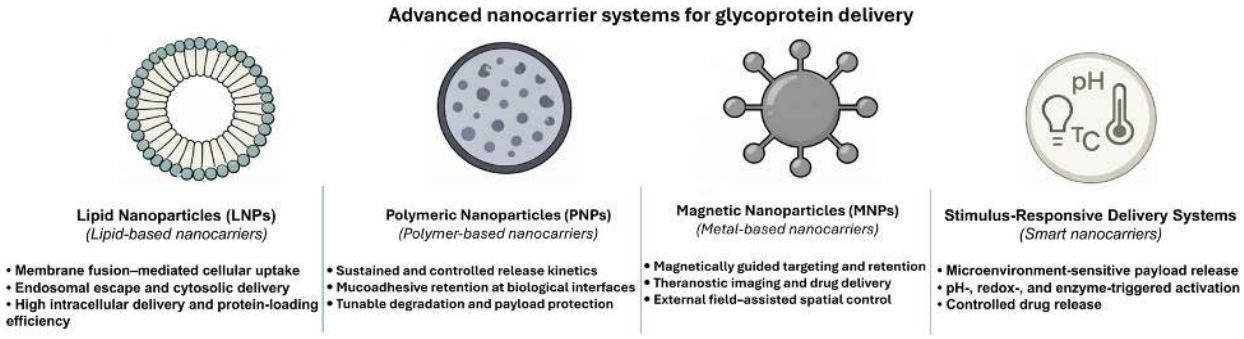

FIGURE 6.4 Advanced nanocarrier systems for glycoprotein delivery. (Comparative overview of lipid nanoparticles (LNPs), polymeric nanoparticles (PNPs), magnetic nanoparticles (MNPs), and stimulus-responsive systems. Mechanistic attributes include membrane fusion and cytosolic delivery (LNPs), tunable degradation and mucoadhesion (PNPs), magnetically guided targeting (MNPs), and microenvironment-triggered activation (pH, redox, enzyme-sensitive platforms). The schematic

highlights design variables governing intracellular trafficking, payload protection, and spatial targeting.) ⏎

6.4.1 Lipid Nanoparticles

When researchers first began experimenting with lipid-based carriers, the primary allure was straightforward: these structures with their bilayer architecture bear an uncanny resemblance to biological membranes. This similarity is greater than superficial. This allows lipid nanoparticles (LNPs) to merge with cell membranes, facilitating direct payload delivery into the cytosol [19, 20, 83]. This characteristic is particularly advantageous for glycoproteins, which often struggle with proteolytic degradation and have poor membrane permeability.

Liposomes, the best-known class of LNPs, have evolved dramatically since early days. They are no longer just passive bubbles; modern liposomes can be functionalized with targeting ligands or even antibodies themselves, creating constructs that actively home to specific tissues or cell types [84, 85]. In oncology, liposomes conjugated with monoclonal antibodies have been used to directly direct cytotoxic drugs to tumor beds, thereby minimizing collateral damage to healthy tissues [84]. Researchers have now applied similar principles to glycoproteins, where local concentrations often dictate their therapeutic success. Therefore, they play a role in the development of vaccines. The COVID-19 pandemic has brought lipid nanoparticles into the global spotlight, most notably through mRNA vaccines encoding glycoprotein antigens, such as the SARS-CoV-2 spike protein. LNPs protect fragile mRNA and serve as adjuvants. They enhance uptake and drive strong immune responses [19, 86]. Similar concepts have been extended to the direct delivery of glycoprotein subunits. Encapsulation

shields these antigens and enables a more controlled presentation to the immune system [87, 88].

In the context of enzyme replacement, LNPs have also shown promise. Delivering glycoprotein enzymes to lysosome-deficient tissues or across the blood–brain barrier in certain experimental designs remains a formidable challenge. Lipid-based carriers offer a route to protect these enzymes in transit and potentially exploit receptor-mediated endocytosis for targeted uptake [89].

Like many delivery approaches, these approaches also have drawbacks. Liposomes are prone to physical instability, with the risk of leakage or fusion during storage. Pharmacokinetics can also be unpredictable and influenced by factors such as particle size, surface charge, and precise lipid composition. Moreover, while PEGylation of liposomal surfaces can extend circulation time by evading mononuclear phagocyte clearance, it can also sometimes provoke anti-PEG antibodies upon repeated administration, a trade-off still being worked out [23, 90, 91].

Despite these limitations, lipid nanoparticles have undeniably altered the landscape of glycoprotein delivery. These materials are biocompatible and structurally versatile. Researchers can tailor them by modifying lipids, adding ligands, or adjusting the encapsulation methods to fit the unique challenges presented by each glycoprotein. In doing so, they moved from experimental curiosities to frontline solutions, a shift unlikely to reverse anytime soon [71].

Although lipid nanoparticles have largely set the pace for clinical translation, thanks in no small part to their success in mRNA and vaccine delivery, they are hardly the only promising avenue. In fact, the push to engineer even more precise release profiles, longer circulation times, and better mechanical stability has turned the spotlight toward polymeric nanoparticles. These systems have their own set of advantages (and, of

course, their own quirks), expanding the toolkit available to tackle the multifaceted delivery challenges posed by glycoproteins [91].

6.4.2 Polymeric Nanoparticles

If lipid nanoparticles rely on their biomimetic qualities, polymeric nanoparticles adopt a different approach: harnessing synthetic versatility to fine-tune almost every aspect of delivery, from release kinetics to tissue targeting. This flexibility is a big part of why they have become an attractive option for transporting sensitive macromolecules such as glycoproteins [92–94]. At their core, polymeric nanoparticles (PNPs) are constructed from biodegradable and biocompatible materials such as poly(lactic-co-glycolic acid) (PLGA), poly(lactic acid) (PLA), and chitosan, which are typical suspects [92]. Each polymer has distinct degradation profiles and loading characteristics, allowing researchers to tailor the systems to the half-life or release requirements of specific glycoproteins. Whether a rapid burst is required to initiate an immune response. A slow and sustained drip may be required to maintain therapeutic enzyme levels. With PNPs, the polymer ratios or crosslinking densities can be adjusted to match those reported in pharmaceutical studies [91, 95].

Encapsulation within PNPs does more than simply ferry glycoproteins from point A to point B. It protects them en route, shielding delicate peptide and glycan bonds from proteases and glycosidases that might otherwise degrade the cargo before it reaches the target tissues [92, 93]. Moreover, encapsulation can mitigate aggregation risks and maintain the glycoprotein in a more stable microenvironment throughout transport and initial release.

Functionalization adds an additional control layer. By grafting antibodies, peptides, or even sugar ligands onto nanoparticle surfaces, PNPs can actively target cell populations, a feature that is particularly appealing

for applications such as tumor-specific delivery or directing glycoproteins to macrophages in ERTs [42, 96]. Chitosan-based formulations have inherent mucoadhesive properties that can enhance absorption across mucosal barriers, opening doors for nasal or oral routes that might otherwise be off-limits to glycoprotein therapeutics [92, 97].

Polymeric systems can also serve stealth functions. PEGylation of the nanoparticle surface reduces recognition by the mononuclear phagocyte system, prolonging circulation time, much like in lipid nanoparticles, but with tunable polymer chains that allow finer control over hydrodynamic diameter and surface charge [23]. This translates into more time in circulation and more opportunities to find and interact with the intended targets.

However, these advantages do not imply that polymeric systems are trouble-free. Batch-to-batch consistency can pose challenges, particularly for more elaborate multicomponent formulations. There is also the issue of long-term biocompatibility of certain polymers, although materials such as PLGA have developed extensive safety profiles over decades of clinical use [92, 96].

However, the appeal of polymeric nanoparticles lies in their expansive customizability. For glycoproteins that demand delicate handling, precise release, or targeted delivery, which, truthfully, is most of them, PNPs offer a robust platform that can be engineered to meet highly specific therapeutic objectives. In many ways, they complement more biologically inspired approaches, such as lipid carriers, broadening the design palette for tackling what remains one of the most challenging classes of therapeutics.

6.4.3 Metal-Based Nanoparticles

Although lipid and polymeric systems often exhibit biocompatibility and organic resemblance, metal-based nanoparticles (MNPs) offer unique physicochemical properties that can be exploited for both delivery and auxiliary functions. Gold, silver, and iron oxide each bring more than just a structural scaffold; they offer optical, magnetic, or catalytic features that open entirely new therapeutic possibilities [98–100].

Gold nanoparticles (AuNPs) are best known in this category. Their surfaces are easily functionalized through thiol linkages, allowing stable attachment of glycoproteins or targeting ligands [99, 101, 102]. However, beyond simple delivery, their plasmonic characteristics make them responsive to light, enabling local heating or photothermal release when irradiated at specific wavelengths. This has been explored in oncology, where AuNPs carrying monoclonal antibodies or cytokines can be directed to tumors and selectively activated by near-infrared light to enhance their therapeutic impact [103].

Iron oxide nanoparticles, particularly in their superparamagnetic form, provide different types of external control. These particles can be guided by magnetic fields to concentrate in the desired anatomical regions to help steer glycoprotein payloads through an otherwise indiscriminate circulatory system [89, 104]. Moreover, because they also serve as contrast agents for magnetic resonance imaging (MRI), they straddle the line between therapeutic and diagnostic, what's often called "theranostics." For example, anti-CD3 monoclonal antibodies conjugated to iron oxide cores have been investigated for simultaneous T-cell targeting and tumor imaging [105, 106]. Although their direct role in glycoprotein delivery remains more exploratory, coupling glycoprotein therapeutics with AgNPs has been proposed as a dual-action approach in contexts such as viral infections, leveraging both targeted protein activity and broad-spectrum antimicrobial effects [98, 106].

These advantages, however, come with important caveats. Metal cores must be carefully engineered and coated to avoid cytotoxicity or unintended immune activation. Surface chemistry plays a significant role; poorly stabilized nanoparticles can aggregate or release metal ions that disrupt cellular homeostasis [22, 107]. Therefore, many formulations incorporate dextran, PEG, or other polymers as protective shells, striking a balance between exploiting the unique attributes of the metal and mitigating its disadvantages.

However, the payoff is substantial. Metal-based nanoparticles expand the toolkit for glycoprotein delivery well beyond passive carriers, integrating externally controllable features, such as magnetism and photoreactivity. This not only opens doors for precision targeting but also enables combination strategies, imagining directing a glycoprotein therapeutic to a tumor, verifying its accumulation through MRI, and then triggering localized release via heat or light. It is an approach that continues to evolve and is promising to reshape how we think about the site-specific delivery of these structurally complex biomolecules.

6.5 STIMULI-RESPONSIVE NANOPARTICLES

If conventional delivery systems mainly rely on passive circulation and hope for some degree of preferential uptake, stimuli-responsive nanoparticles flip the paradigm. They are engineered to wait, essentially lying dormant until they encounter specific environmental cues that trigger the release of glycoprotein payloads. The diversity of potential triggers is schematically illustrated in Figure 6.5, highlighting how these systems can leverage local biochemical or physical conditions to achieve a site-specific release. This strategy is especially appealing for therapeutics that must act in highly localized or temporally controlled ways [108]. This diverse landscape of

delivery strategies, from biomimetic lipid nanoparticles to finely tuned polymeric, metal-based, and stimuli-responsive systems, highlights how each approach contributes to distinct strengths and trade-offs in managing glycoprotein therapeutics (Table 6.2).

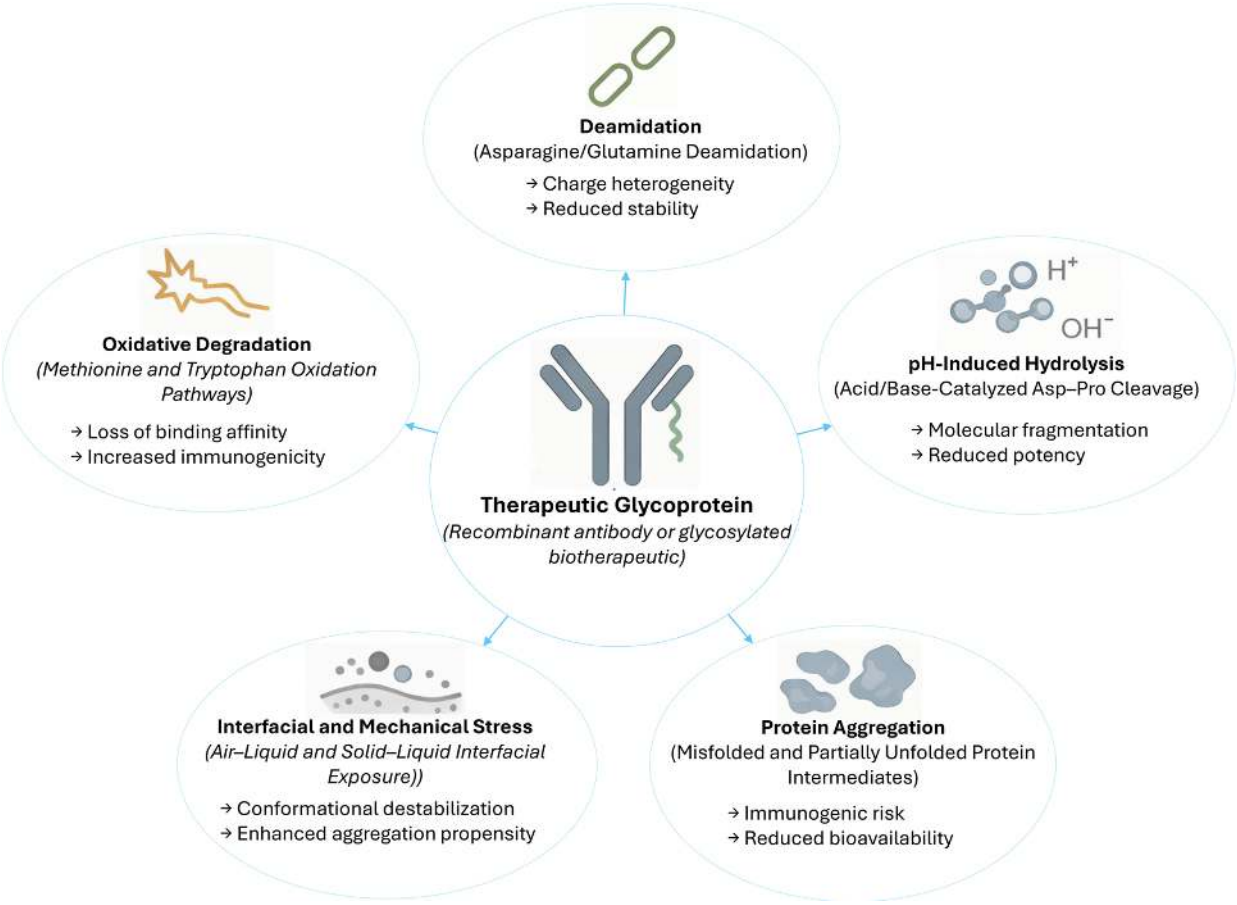

FIGURE 6.5 Chemical and physical degradation pathways of therapeutic glycoproteins. (Illustration of major stress-induced instability mechanisms, including oxidative modification (methionine and tryptophan oxidation), deamidation of asparagine/glutamine residues, pH-dependent Asp–Pro hydrolysis, interfacial/mechanical stress, and aggregation of partially unfolded intermediates. Consequences such as conformational destabilization, charge heterogeneity, reduced potency, and enhanced immunogenicity are delineated, emphasizing formulation and storage challenges in glycoprotein therapeutics.) ⏎

TABLE 6.2

Comparative Overview of Advanced Delivery Systems for Glycoprotein Therapeutics

Carrier Type	Representative Materials	Key Advantages	Main Limitations	Typical Glycoprotein Applications/Ref.
Lipid Nanoparticles (LNPs)	Phospholipids, cholesterol, PEG-lipids	Biomimetic, facilitate membrane fusion, protect labile cargo, customizable surface targeting	Physical instability (fusion, leakage), batch variability, anti-PEG antibody risk	mRNA-encoded glycoprotein vaccines (e.g., SARS-CoV-2 spike), monoclonal antibody formulations under investigation [19, 20, 84, 86]
Polymeric Nanoparticles (PNPs)	PLGA, PLA, chitosan, PEG-copolymers	Tunable release profiles, mucoadhesive (for nasal/oral), scalable functionalization for ligands or antibodies	Residual solvent traces, complex scale-up, require rigorous quality control	Enzyme replacement encapsulation, nasal vaccines, tumor-targeted cytokines [21, 22, 89, 93, 109]
Metal-Based Nanoparticles (MNPs)	Gold, iron oxide, silver	Enable theranostics (imaging + therapy), photothermal, or magnetic responsiveness	Risk of oxidative stress, aggregation, long-term clearance concerns	MRI-visible enzyme therapies, photo-triggered monoclonal antibody delivery [42, 103, 107, 109]

Carrier Type	Representative Materials	Key Advantages	Main Limitations	Typical Glycoprotein Applications/Ref.
Stimuli-Responsive Systems	PNIPAM, pH/enz/redox-cleavable linkers	Release precisely in response to pH, enzymes, temperature, light, magnetic fields	Sophisticated synthesis, regulatory hurdles, lot reproducibility challenges	MMP-triggered tumor enzyme release, heat/light-activated antibody systems [22, 108, 110–113]
Hydrogels and Depots	PEG-based, chitosan, PLGA in situ gels	Local sustained release, reduced systemic toxicity, ease of administration	Limited to local therapies, degradation kinetics must be carefully tuned	Controlled delivery of anti-TNF mAbs, intra-articular enzyme delivery [109]
Microneedles	Silicon, polymers, dissolvable sugars	Painless skin-based delivery, targets dermal APCs, patient self-administration	Limited penetration depth, small payloads, dehydration stability critical	Vaccines with glycoprotein antigens (influenza, SARS-CoV-2) [114, 115]
Hybrid Systems	Lipid-polymer hybrids, metal-polymer composites	Combine advantages (e.g., stability + fusion + responsive triggers)	Complexity in characterization, multi-regulatory pathways	Targeted cancer therapy with antibody+chemo co-loading, simultaneous imaging [92, 116, 117]

6.5.1 pH-Responsive Systems

Consider pH-sensitive nanoparticles as an example of this. Many pathological sites, such as tumors, sites of chronic inflammation, and

endosomal compartments within cells, are slightly acidic compared to the normal physiological pH. By incorporating pH-labile linkers or acid-degradable polymers, these systems remain intact in the bloodstream but release their cargo once they encounter a drop in pH [109, 118–120]. Chitosan derivatives are particularly popular because they exploit their own protonation profiles to swell or dissolve under acidic conditions, thereby freeing encapsulated glycoproteins precisely where needed [116].

6.5.2 Temperature-Responsive Systems

Temperature-sensitive carriers are often constructed around polymers such as poly(N-isopropylacrylamide) (PNIPAM). These materials undergo a sharp phase transition around body-relevant temperatures, which can be tuned to respond to local hyperthermia, whether induced deliberately (via external heating) or occurring naturally in inflamed or neoplastic tissues [119, 121, 122]. The idea is straightforward: keep the glycoprotein safely sequestered under normal conditions but trigger a rapid release when the local temperature exceeds a designated threshold.

6.5.3 Enzyme-Responsive Systems

Enzyme-sensitive nanoparticles take this concept a step further by exploiting disease-specific protease profiles. MMPs and other degradative enzymes are overexpressed in many tumors, and chronic wound sites overexpress matrix metalloproteinases (MMPs) or other degradative enzymes. By crosslinking nanoparticles with peptide sequences cleavable by these enzymes, the carrier effectively “dissolves” in the presence of pathological activity, liberating its glycoprotein cargo exactly where enzyme levels are elevated [112, 123]. This approach clearly reflects the biology of the disease.

6.5.4 Redox- and Light-Responsive Systems

Other designs tap into local redox imbalances; for instance, elevated glutathione concentrations are often found inside cells compared to extracellular environments. Disulfide-bonded nanoparticles can remain stable in circulation, but rapidly break apart upon encountering these reductive conditions, releasing their therapeutic load [117].

Meanwhile, light-responsive systems capitalize on the precision of external triggers. AuNPs functionalized with photolabile linkers can release attached glycoproteins when illuminated by near-infrared light, affording highly localized delivery with minimal systemic disruption [111, 124]. This is particularly appealing in dermatological or superficial tumor contexts, where light penetration is practical.

6.5.5 Magnetic and Ultrasound-Responsive Systems

Platforms that respond to magnetic fields or focused ultrasounds are more ambitious. Magnetic nanoparticles can be guided externally to specific anatomical regions and then subjected to alternating fields that induce localized heating and trigger release [125]. Ultrasound can mechanically disrupt nanoparticle matrices, opening them on demand at deep tissue sites [126].

A common thread among these diverse methods is their shared goal of exerting finer control over where and when therapeutic release occurs. Glycoproteins, whose therapeutic efficacy often hinges on maintaining structural integrity up to the point of delivery, provide a kind of molecular insurance, protecting delicate cargo until environmental signals confirm its time to act.

Of course, this sophistication comes with trade-offs. Stimuli-responsive systems can be more complex to manufacture, may also draw heavier

regulatory scrutiny, and need careful tuning to balance the sensitivity against premature leakage. Still, because of the dependence on these complex molecules, interest in smarter, environment-sensitive carriers continues to grow. In addition to addressing the inherent stability issues, stimuli-responsive carriers leverage pathological cues for on-demand release, as illustrated in Figure 6.6.

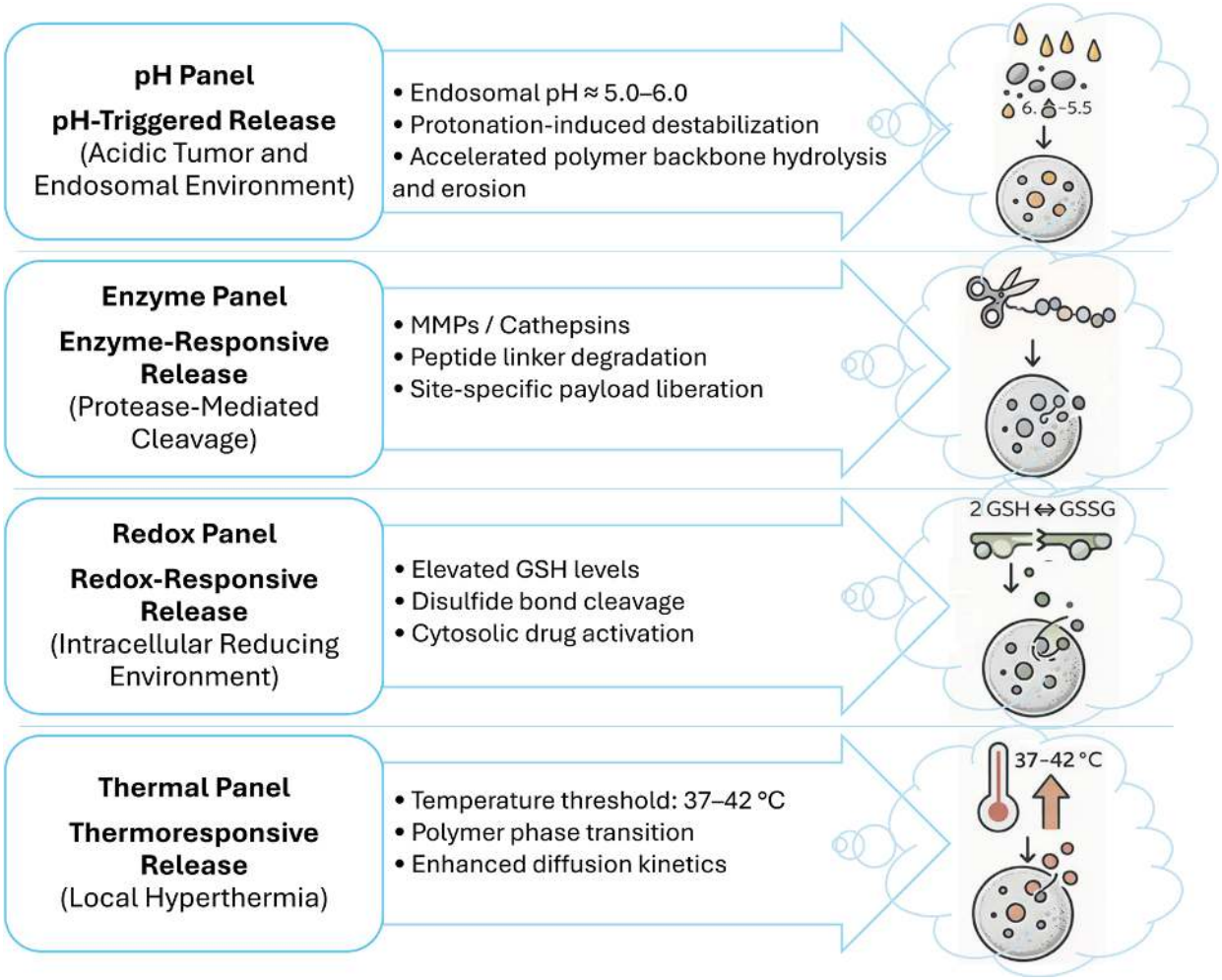

FIGURE 6.6 Stimulus-responsive release mechanisms in smart nanocarrier systems. (Mechanistic panels depicting pH-triggered release within acidic tumor and endosomal compartments, protease-mediated linker cleavage, redox-responsive disulfide bond reduction in glutathione-rich intracellular environments, and thermoresponsive polymer transitions under localized hyperthermia (37–42°C).

These stimuli-dependent strategies enable spatially controlled payload liberation and enhanced intracellular bioavailability.) ⏎

6.6 THERAPEUTIC APPLICATIONS OF ADVANCED GLYCOPROTEIN DELIVERY SYSTEMS

Even so, these intricate delivery platforms, whether lipid-based, polymeric, metal-laden, or stimuli-sensitive, are only as valuable as the clinical problems that they help solve. For glycoproteins, the list of problems is extensive, spanning cancer, infectious diseases, enzyme deficiencies, and so on. The practical significance of these delivery innovations is evident when examining approved glycoprotein therapeutics, which span indications from anemia to oncology and infectious diseases. Selected examples with defined glycan attributes and delivery modalities are listed in Table 6.3. This diversity is illustrated by the wide array of clinical applications (Figure 6.7).

TABLE 6.3

Selected Marketed Glycoprotein Therapeutics: Structural Features, Clinical Uses, and D

Therapeutic Name	Distinctive Glycan Feature
Epoetin alfa (EPO)	Heavily sialylated multi-antennary N-glycans prolong s
Trastuzumab	Fc N-glycosylation critical for FcγR and FcRn engagem
Agalsidase beta	Mannose-6-phosphate residues for CI-MPR lysosomal u
Pfizer-BioNTech mRNA vaccine (BNT162b2)	Encodes glycosylated SARS-CoV-2 spike
Etanercept (Enbrel)	Complex N-glycans on Fc-fused TNF receptor decoy
Darbepoetin alfa (Aranesp)	Engineered hyperglycosylation (5 vs. 3 N-sites vs. EPO
Cetuximab (Erbitux)	Non-human (mouse–human chimeric) N-glycans

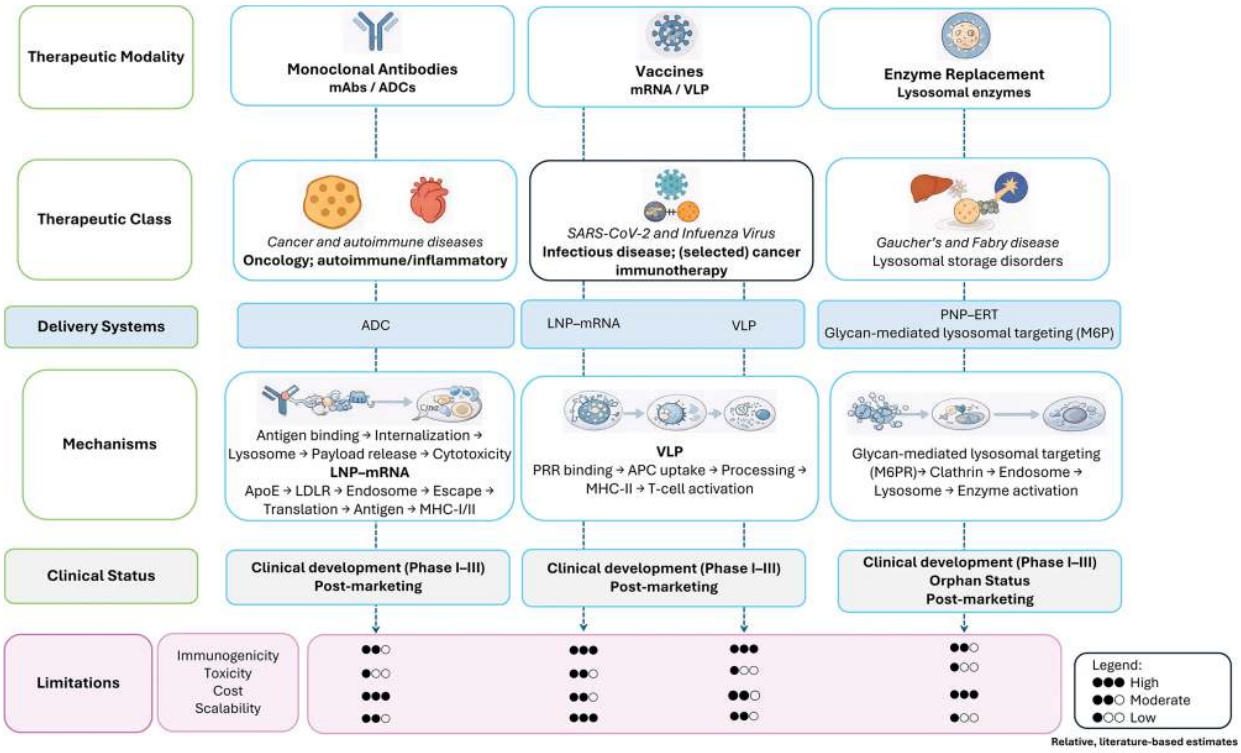

FIGURE 6.7 Clinical applications of therapeutic glycoproteins and advanced delivery platforms. This schematic highlights the key domains of glycoprotein-based therapeutics: monoclonal antibodies employed in cancer and autoimmune diseases, administered as recombinant glycoproteins via intravenous (IV) injection; vaccines targeting *SARS-CoV-2* with lipid nanoparticle (LNP)–mRNA systems and influenza with virus-like particles (VLPs) ± experimental polymeric nanoparticles (PNPs); and ERTs for lysosomal storage disorders such as Gaucher's and Fabry disease, utilizing recombinant glycoproteins (IV) with adjunct investigational PNP carriers (LNP, lipid nanoparticle; VLP, virus-like particle; PNP, polymeric nanoparticle). ⏎

6.6.1 Monoclonal Antibodies

A clear example of the therapeutic power of glycoproteins lies in monoclonal antibodies (mAbs). They have become indispensable in oncology, autoimmune conditions, and even in some infectious diseases. But

their success depends not just on the antibodies themselves. This also depends on how precisely they are delivered [129].

This is exemplified by antibody-drug conjugates (ADCs). They combined the targeting specificity of monoclonal antibodies with the lethality of cytotoxic payloads. Trastuzumab emtansine (T-DM1) is a classic example that directly ferries a chemotherapeutic agent to HER2-positive breast cancer cells and spares much of the surrounding tissue [130, 131]. These combinations have turned mAbs into targeted tools, substantially improving their therapeutic indices.

At the same time, more conventional systems like liposomes and polymers are being engineered to house monoclonals, offering benefits such as prolonged circulation, reduced degradation, and controlled release. This can be particularly useful in multi-agent regimens, where coordinating pharmacokinetics is crucial [110].

Autoimmune diseases have slightly different characteristics. Here, mAbs, such as adalimumab and infliximab, target TNF-α and inhibit excessive inflammatory cascades. While these are typically administered subcutaneously or intravenously without elaborate carriers, there is a growing interest in using hydrogels and local depot systems to sustain release and minimize systemic exposure, an approach that could reduce dosing frequency and side effects in conditions such as rheumatoid arthritis and Crohn's disease [132, 133].

6.6.2 Glycoprotein-Based Vaccines

Vaccines also provide notable applications. Glycoproteins are often the antigens we want to introduce, considering the spike protein in SARS-CoV-2 vaccines. The rapid deployment of lipid nanoparticle-encapsulated mRNA vaccines (such as Pfizer-BioNTech and Moderna) underscores the

importance of advanced delivery systems for stabilizing, protecting, and presenting glycoprotein targets [114, 134].

But it's not confined to mRNA. Virosomes, essentially reconstituted viral envelopes studied with glycoproteins, have been used to present antigens in a format that closely mimics natural infections and promotes robust immunity. The influenza vaccine Inflexal V is a case in point [127, 135]. Microneedle patches are emerging as a promising method for delivering glycoprotein antigens through the skin, leveraging dense networks of immune cells for more potent responses with potentially less discomfort [115, 128].

6.6.3 ERTs

ERT may be the most direct application of glycoprotein delivery. In lysosomal storage disorders, such as Gaucher's or Fabry disease, patients lack the critical glycoprotein enzymes needed to break down cellular substrates. Administering recombinant versions can correct this, provided that the enzymes survive long enough to reach the lysosomes and are taken up efficiently.

Tagging with mannose-6-phosphate has long been the mainstay of enzyme-guidance. New delivery systems have become increasingly sophisticated. Polymeric nanoparticles, for instance, can encapsulate these enzymes, protecting them from serum proteases and facilitating endocytosis [42, 136]. Hydrogels offer another intriguing angle: they slowly release enzymes over time to maintain therapeutic levels with fewer injections [137, 138].

Meanwhile, experimental studies have coupled these enzymes to iron oxide or other nanoparticles, combining ERT with MRI visibility to monitor

delivery in real time, a prime example of how theranostics could evolve in this field [22, 105].

Across these diverse applications, one theme is clear: advanced delivery systems are no longer optional extras; they are becoming increasingly fundamental for realizing the therapeutic potential of glycoproteins. Whether targeting the right cells, protecting delicate structures from premature degradation, or orchestrating the timing of release, these technologies transform what would otherwise be pharmacologically fragile agents into viable and potent treatments.

As more diseases are molecularly deconstructed and matched to glycoprotein-based interventions, from personalized cancer therapies to vaccines for emerging pathogens, the need for delivery strategies that are equally tailored has become more obvious. In this sense, the evolving interplay between glycoprotein engineering and delivery science is less of a luxury and more of an inevitability, shaping the next generation of precision biotherapeutics.

6.7 CHALLENGES AND FUTURE DIRECTIONS

Looking across delivery systems shows considerable optimism in imagining that we are on the cusp of fully mastering these complex molecules. Yet, on closer inspection, it is clear that significant challenges remain, many of which are rooted in biology, regulation, economic pressures, and shifting societal expectations.

6.7.1 Regulatory and Safety Considerations

The regulatory landscape is catching up. Traditional guidelines for biologics focus heavily on ensuring batch consistency and purity, with rigorous

demands for glycosylation profiles, aggregate content, and host cell impurity levels [139, 140]. When delivery systems add additional layers, such as PEG coatings, targeted ligands, or responsive linkers, each has its own safety considerations that require independent validation.

This holds particularly true for nanoparticles and hybrid constructs, which straddle categories. A stimuli-responsive polymer that changes its structure in acidic environments or a metal-core particle used simultaneously for imaging and delivery does not fit neatly into existing pharmacopeial boxes [60, 61]. Regulators are understandably cautious, and developers often navigate protracted review processes to prove long-term biocompatibility and the absence of unforeseen off-target effects.

Cost considerations further compound these challenges. Glycoproteins are already expensive to produce, and layering on advanced delivery often multiplies manufacturing complexity, necessitating new raw materials and more sophisticated quality assurance systems [18, 141]. Indeed, advanced analytical workflows are indispensable for verifying glycoprotein identity, assessing glycoform heterogeneity, and ensuring batch-to-batch equivalence, which are parameters that are central to regulatory oversight. The representative analytical tools and their specific applications are listed in Table 6.4.

TABLE 6.4

Analytical Characterization of Therapeutic Glycoproteins

Analytical Method	Target Attribute	Typical Applications in Glycoprotein QC and R&D	Notes on Interpretation/Challenges

Analytical Method	Target Attribute	Typical Applications in Glycoprotein QC and R&D	Notes on Interpretation/Challenges
Mass Spectrometry (ESI, MALDI)	Intact mass, glycan profiling, peptide mapping	Identifying glycoforms, confirming primary sequence and PTMs	Sensitive to sample prep; may miss low-abundance variants
HPAEC-PAD (High-pH Anion-Exchange Chromatography with Pulsed Amperometric Detection)	Released monosaccharides and oligosaccharides	Quantitation of specific sugars (sialic acid, mannose, etc.)	Requires robust calibration; often coupled with enzymatic digests
Capillary Electrophoresis (CE-SDS, CIEF)	Charge variants, size heterogeneity	Resolves isoforms from deamidation, sialylation, aggregation	Subtle shifts may require orthogonal confirmation
SEC-MALS (Size-Exclusion Chromatography + Multi-Angle Light Scattering)	Aggregation, oligomerization	Accurate molar mass of aggregates vs. monomer	Dilute samples may under-represent aggregates
HILIC (Hydrophilic Interaction LC)	Glycan mapping (released glycans)	Separate glycan species based on polarity; useful for profiling	Co-elution possible; needs MS for confirmation

Analytical Method	Target Attribute	Typical Applications in Glycoprotein QC and R&D	Notes on Interpretation/Challenges
SPR (Surface Plasmon Resonance)	Binding kinetics and affinities (ligand–receptor, FcγR, FcRn)	Validate bioactivity, e.g., ADCC potential, receptor engagement	Requires well-defined immobilization chemistry
DSC (Differential Scanning Calorimetry)	Thermal stability (Tm)	Compare formulations, stress stability studies	Multi-domain unfolding can complicate interpretation
NMR (Nuclear Magnetic Resonance)	Glycan linkage and conformation	Deep structural characterization of glycan branches	Limited by sample concentration and complexity
ELISA/Cell-Based Potency Assays	Functional activity	Confirms retained bioactivity post-production	May mask subtle structural issues that later impact PK

6.7.2 Overcoming Immunogenicity

Immunogenicity remains a challenge. Even the best-controlled manufacturing cannot fully eliminate microheterogeneities in glycosylation, and these subtle differences may be sufficient to tip immune tolerance into antibody formation [23, 57, 58]. Moreover, repeated administration, common with chronic enzyme replacement or oncology protocols, increases the chance that low-level anti-drug antibodies will eventually escalate into neutralizing responses.

Several strategies have been developed to mitigate this problem. PEGylation often helps cloak glycoproteins, reduces immune surveillance, and prolongs their half-life [24]. Engineering expression systems to yield human-like glycosylation, whether by tweaking CHO cell lines or using advanced glycosyltransferase approaches, can also minimize xenoantigen exposure [64, 142]. However, none of these approaches are foolproof or comprehensive.

Some groups have investigated the co-delivery of immune modulators and nanoparticles that simultaneously present glycoproteins and dampen local antigen presentation or T-cell priming. Although promising, these strategies tread a delicate line, balancing the efficacy with the risk of broadly suppressing immune vigilance.

6.7.3 Integration with Personalized Medicine

One of the more promising trajectories is the integration of glycoprotein delivery into personalized medicine. As we learn more about patient-specific molecular signatures, such as HER2 overexpression in breast cancer, PD-L1 status in lung tumors, and glycan receptor patterns in infectious diseases, the case for tailoring both the glycoprotein and its delivery system has grown stronger.

Machine learning tools are beginning to shift through these datasets, helping to predict which patients might respond best to certain formulations or even suggesting optimal nanoparticle compositions for a given immunological milieu. Advances in 3D printing and custom fabrication can produce individualized depot systems or microneedle patches adjusted for patient-specific skin or tissue characteristics.

Realizing this vision depends on building robust pipelines that connect diagnostics, manufacturing, and clinical deployment, a task that is as

logistical and infrastructural as it is scientific. This evolution is demonstrated by the emerging convergence of diagnostics and tailored delivery (Figure 6.8).

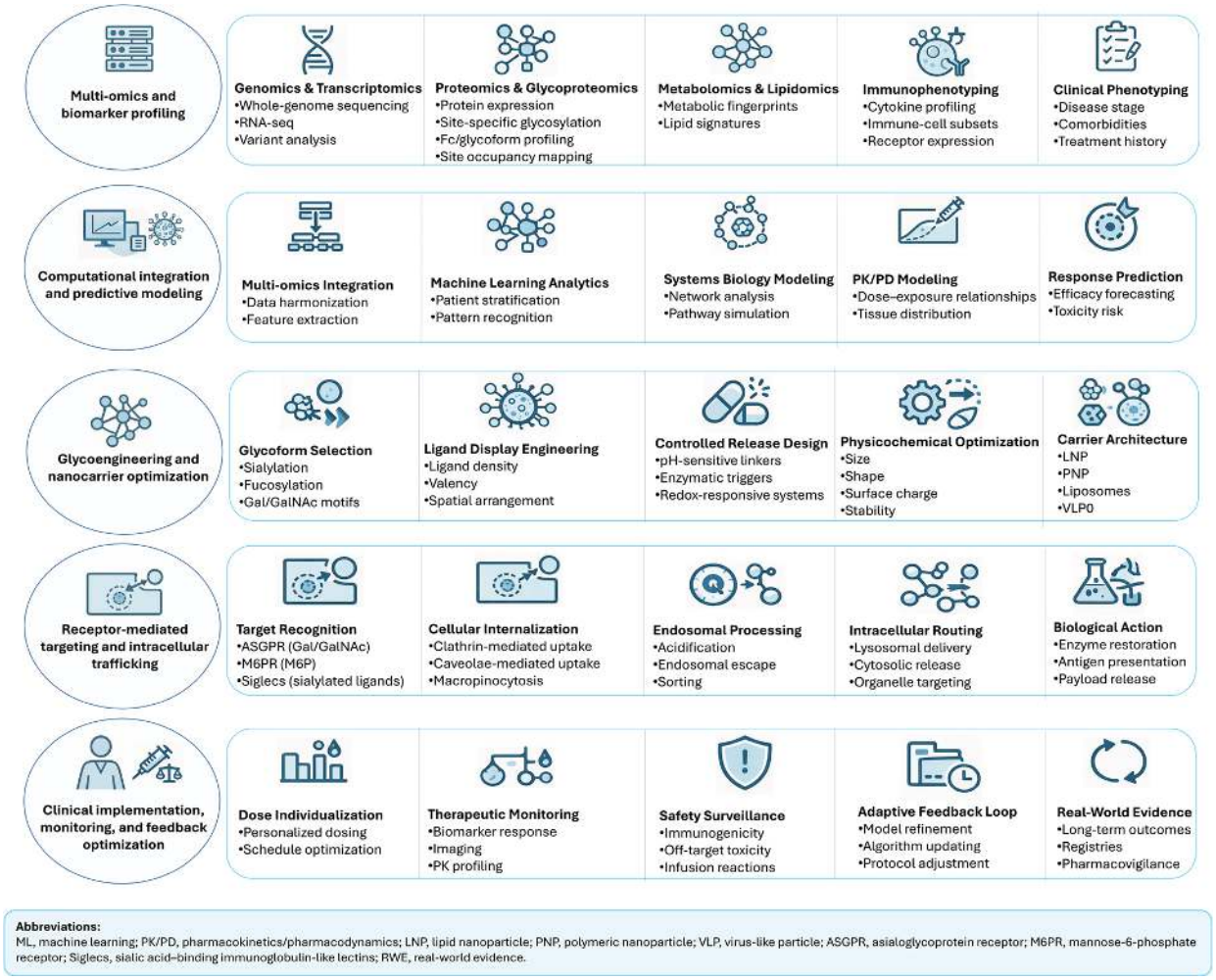

FIGURE 6.8 Multi-omics–guided precision engineering and adaptive clinical optimization of glycoprotein therapeutics. (Systems-level schematic integrating genomics, transcriptomics, proteomics, metabolomics, lipidomics, and immunophenotyping with computational analytics, machine learning–based stratification, and PK/PD modeling. Outputs inform glycoform selection, ligand density engineering, nanocarrier architecture, and controlled-release design. Downstream receptor-mediated targeting (ASGPR, M6PR, Siglecs), intracellular routing, and biological action feed

into clinical monitoring, real-world evidence generation, and adaptive algorithm refinement, establishing a closed-loop precision medicine framework.)

This positions the field as both remarkably advanced and unmistakably in flux. Glycoproteins paired with sophisticated delivery systems are transforming care in areas such as oncology, rare enzyme deficiencies, and vaccine-preventable diseases (VPDs). On the other hand, the persistent challenges of immunogenicity, scale-up consistency, regulatory navigation, and equitable access underscore the extent to which they persist.

Encouragingly, many of these long-standing hurdles are now being approached through more integrative strategies, bridging molecular engineering, material science, and computational design. In that sense, the evolution of glycoprotein therapeutics is not just a story of one class of drugs, but also of how modern biomedicine itself is learning to orchestrate complexity to meet patient needs.

6.8 CONCLUSION

Looking back over the trajectory of glycoprotein therapeutics, the extent to which their promise has depended not only on advances in molecular biology but also on innovations in how we deliver them. From monoclonal antibodies that revolutionize cancer care to ERTs to tackle intractable lysosomal diseases, glycoproteins have steadily expanded the therapeutic frontier. Their biological precision often results in structural fragility, susceptibility to degradation, rapid clearance, and unintended immune responses.

Such demands have driven the evolution of delivery systems from simple liposomes to multifunctional polymeric constructs, metal-based carriers, and sophisticated stimuli-responsive platforms have been consequential. Each

innovation has gradually addressed problems that were once considered insurmountable, such as shielding delicate structures from proteases, steering therapies to precise cellular neighborhoods, and timing release to coincide with pathological triggers.

Many of these solutions raise new questions, even if they answer the old ones. PEGylation extends the half-life but may eventually provoke anti-PEG antibodies. Scaling up glycoengineered proteins improves uniformity yet adds substantial manufacturing and regulatory complexity. Stimuli-responsive systems hold an exquisite theoretical appeal but face practical hurdles in ensuring consistent behavior across patient populations.

The most promising feature is the emerging synergy between these delivery technologies and the broader trends in personalized medicine. As diagnostics become more refined, parsing not only genetic mutations but also glycan signatures, local immune states, and metabolic environments, the case grows stronger for delivery systems as tailored as the therapeutics they carry. AI-guided formulations, bespoke 3D-printed depots, or smart carriers that dynamically adapt to a patient's molecular profile may all move from speculative to standard in the coming decade.

Altogether, these advances suggest that achieving biological precision demands equally careful engineering, often requiring close interplay rather than isolated innovation. We stand a far better chance of unlocking their full therapeutic potential when insights from immunology, materials science, computational modeling, and health systems are integrated [143]. Through these efforts, the field continues to broaden its therapeutic repertoire and extend the boundaries of personalized care.

CONFLICT OF INTEREST

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DATA AVAILABILITY

No new data were generated or analyzed in this study. All information and materials discussed were derived from the published literature and publicly available sources. Further details are available from the corresponding author upon reasonable request.

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REFERENCES

1. Alrohaim A, Mostafa MAH, Saad Alnour TM, et al. Enhancing Drug Efficacy Through Nanoparticle-Based Delivery Systems: A Study on Targeted Cancer Therapy. *International Journal of Surgery*. 2025;111(9):6023–6029.

2. Abdellatif AAH, Sameh Tolba N, Tawfeek HM. Editorial: Nanomedicine and Application; Characterization, Diagnosis, Treatment, and Targeting Cancers. *Drug Development and Industrial Pharmarcy*. 2025;51(9):985–986.
3. Walsh G, Jefferis R. Post-Translational Modifications in the Context of Therapeutic Proteins. *Nature Biotechnology*. 2006;24(10):1241–1252.
4. Helenius A, Aebi M. Roles of N-Linked Glycans in the Endoplasmic Reticulum. *Annual Review of Biochemistry*. 2004;73(1):1019–1049.
5. Abdellatif AAH, Bouazzaoui A, Tawfeek HM, et al. MCT4 Knockdown by Tumor Microenvironment-Responsive Nanoparticles Remodels the Cytokine Profile and Eradicates Aggressive Breast Cancer Cells. *Colloids and Surfaces B: Biointerfaces*. 2024;238:113930.
6. Jelkmann W. Regulation of Erythropoietin Production. *The Journal of Physiology*. 2011;589(6):1251–1258.
7. Jackson CB, Farzan M, Chen B, et al. Mechanisms of SARS-CoV-2 Entry into Cells. *Nature Reviews Molecular Cell Biology*. 2021;23(1):3–20.
8. Qiao S, Zhang S, Ge J, et al. The Spike Glycoprotein of Highly Pathogenic Human Coronaviruses: Structural Insights for Understanding Infection, Evolution and Inhibition. *FEBS Open Bio*. 2022;12(9):1602–1622.
9. Abdellatif AAH, Tawfeek HM, Abdelfattah A, et al. Recent Updates in COVID-19 With Emphasis on Inhalation Therapeutics: Nanostructured and Targeting Systems. *Journal of Drug Delivery Science and Technology*. 2021;63:102435.
10. Bouazzaoui A, Keedwa KM, Sahl SM, et al. Lymphocyte Subsets in Covid-19 Saudi Patients: Infection Caused Significant Reduction in Peripheral Blood Lymphocyte Subset. *Clinical Laboratory*. 2025;71(5):851.
11. Ecker DM, Jones SD, Levine HL. The Therapeutic Monoclonal Antibody Market. *mAbs*. 2015;7(1):9–14.
12. Fu Z, Li S, Han S, et al. Antibody Drug Conjugate: The “Biological Missile” for Targeted Cancer Therapy. *Signal Transduction and Targeted Therapy*. 2022;7(1):93.

13. Bouazzaoui A, Abdellatif AAH. Vaccine Delivery Systems and Administration Routes: Advanced Biotechnological Techniques to Improve the Immunization Efficacy. *Vaccine X*. 2024;19:100500.
14. Gauthier C, El Cheikh K, Basile I, et al. Cation-Independent Mannose 6-Phosphate Receptor: From Roles and Functions to Targeted Therapies. *Journal of Controlled Release*. 2024;365:759–772.
15. Seo J, Oh D-B. Mannose-6-Phosphate Glycan for Lysosomal Targeting: Various Applications from Enzyme Replacement Therapy to Lysosome-Targeting Chimeras. *Animal Cells and Systems*. 2022;26(3):84–91.
16. Solá RJ, Griebenow K. Glycosylation of Therapeutic Proteins. *BioDrugs*. 2010;24(1):9–21.
17. Li C, Wang L-X. Chemoenzymatic Methods for the Synthesis of Glycoproteins. *Chemical Reviews*. 2018;118(17):8359–8413.
18. Sethuraman N, Stadheim TA. Challenges in Therapeutic Glycoprotein Production. *Current Opinion in Biotechnology*. 2006;17(4):341–346.
19. Parvin N, Joo SW, Mandal TK. Enhancing Vaccine Efficacy and Stability: A Review of the Utilization of Nanoparticles in mRNA Vaccines. *Biomolecules*. 2024;14(8):1036.
20. Al Fayez N, Nassar MS, Alshehri AA, et al. Recent Advancement in mRNA Vaccine Development and Applications. *Pharmaceutics*. 2023;15(7):1972.
21. Lemoine D, Préat V. Polymeric Nanoparticles as Delivery System for Influenza Virus Glycoproteins. *Journal of Controlled Release*. 1998;54(1):15–27.
22. Liu G, Lovell JF, Zhang L, et al. Stimulus-Responsive Nanomedicines for Disease Diagnosis and Treatment. *International Journal of Molecular Sciences*. 2020;21(17):6380.
23. Maï NE, Donadio-Andréi S, Iss C, et al. Engineering a Human-Like Glycosylation to Produce Therapeutic Glycoproteins Based on 6-Linked Sialylation in CHO Cells. Glycosylation Engineering of Biopharmaceuticals. *Methods in Molecular Biology*. 2013;988:19–29.
24. Gupta S, Shah B, Fung CS, et al. Engineering Protein Glycosylation in CHO Cells to Be Highly Similar to Murine Host Cells. *Frontiers in Bioengineering and Biotechnology*. 2023;11:1113994.

25. Tvaroška I. The Role of Glycans in Human Immunity—A Sweet Code. *Molecules*. 2025;30(13):2678.

26. Benyair R, Ogen-Shtern N, Lederkremer GZ. Glycan Regulation of ER-Associated Degradation Through Compartmentalization. *Seminars in Cell & Developmental Biology*. 2015;41:99–109.

27. He M, Zhou X, Wang X. Glycosylation: Mechanisms, Biological Functions and Clinical Implications. *Signal Transduction and Targeted Therapy*. 2024;9(1):194.

28. Mousa AM, Almatroudi A, Alwashmi AS, et al. Thyme Oil Alleviates Ova-Induced Bronchial Asthma Through Modulating Th2 Cytokines, IgE, TSLP and ROS. *Biomedicine and Pharmacotherapy*. 2021;140:111726.

29. Charonis AS, Tsilibary EC. Structural and Functional Changes of Laminin and Type IV Collagen After Nonenzymatic Glycation. *Diabetes*. 1992;41(Supplement_2):49–51.

30. Karsdal MA, Nielsen MJ, Sand JM, et al. Extracellular Matrix Remodeling: The Common Denominator in Connective Tissue Diseases. Possibilities for Evaluation and Current Understanding of the Matrix as More Than a Passive Architecture, but a Key Player in Tissue Failure. *ASSAY and Drug Development Technologies*. 2013;11(2):70–92.

31. Méndez-Cobián DA, Guzmán-Silahua S, García-Hernández D, et al. An Overview of Gaucher Disease. *Diagnostics*. 2024;14(24):2840.

32. Al-Allaf FA, Abduljaleel Z, Taher MM, et al. Molecular Dynamics Simulation Reveals Exposed Residues in the Ligand-Binding Domain of the Low-Density Lipoprotein Receptor That Interacts With Vesicular Stomatitis Virus-G Envelope. *Viruses*. 2019;11(11):1063.

33. Bouazzaoui A, Bogari NM, Al-Allaf FA, et al. Anti-E. Coli Immunoglobulin Yolk (IgY): Reduction of Pathogen Receptors and Inflammation Factors Could Be Caused by Decrease in E. Coli Load. *Heliyon*. 2023;9(3):e13876.

34. Abdellatif AAH, Aldalaen SM, Faisal W, et al. Somatostatin Receptors as a New Active Targeting Sites for Nanoparticles. *Saudi Pharmaceutical Journal*. 2018;26(7):1051–1059.

35. Goettig P. Effects of Glycosylation on the Enzymatic Activity and Mechanisms of Proteases. *International Journal of Molecular Sciences*. 2016;17(12):1969.

36. Lee JM, Hammarén HM, Savitski MM, et al. Control of Protein Stability by Post-Translational Modifications. *Nature Communications*. 2023;14(1):201.

37. Ali MM, Zhu S, Amin FR, et al. Molecular Imprinting of Glycoproteins: From Preparation to Cancer Theranostics. *Theranostics*. 2022;12(5):2406–2426.

38. Krammer F. SARS-CoV-2 Vaccines in Development. *Nature*. 2020;586(7830):516–527.

39. Brik A, Ficht S, Wong C. Strategies for the Preparation of Homogenous Glycoproteins. *Current Opinion in Chemical Biology*. 2006;10(6):638–644.

40. Li Y, Fu J, Ling Y, et al. Sialylation on O-Glycans Protects Platelets from Clearance by Liver Kupffer Cells. *Proceedings of the National Academy of Sciences*. 2017;114(31):8360–8365.

41. Svecla M, Nour J, Bladergroen MR, et al. Impact of Asialoglycoprotein Receptor and Mannose Receptor Deficiency on Murine Plasma N-Glycome Profiles. *Molecular & Cellular Proteomics*. 2023;22(9):100615.

42. Abdelkawi A, Slim A, Zinoune Z, et al. Surface Modification of Metallic Nanoparticles for Targeting Drugs. *Coatings*. 2023;13(9):1660.

43. Shen C-Y, Lu C-H, Wu C-H, et al. The Development of Maillard Reaction, and Advanced Glycation End Product (AGE)-Receptor for AGE (RAGE) Signaling Inhibitors as Novel Therapeutic Strategies for Patients With AGE-Related Diseases. *Molecules*. 2020;25(23):5591.

44. folzer E, diepold K, bomans K, et al. Selective Oxidation of Methionine and Tryptophan Residues in a Therapeutic IgG1 Molecule. *Journal of Pharmaceutical Sciences*. 2015;104(9):2824–2831.

45. Gamage CLD, Hageman TS, Weis DD. Rapid Prediction of Deamidation Rates of Proteins to Assess Their Long-Term Stability Using Hydrogen Exchange–Mass Spectrometry. *Journal of Pharmaceutical Sciences*. 2019;108(6):1964–1972.

46. Abdellatif AAH, Ibrahim MA, Amin MA, et al. Cetuximab Conjugated With Octreotide and Entrapped Calcium Alginate-Beads for Targeting Somatostatin Receptors. *Scientific Reports*. 2020;10(1):4736.

47. Masson P, Lushchekina S. Conformational Stability and Denaturation Processes of Proteins Investigated by Electrophoresis Under Extreme Conditions. *Molecules*. 2022;27(20):6861.

48. Behl T, Kaur D, Sehgal A, et al. Exploring the Potential Role of rab5 Protein in Endo-Lysosomal Impairment in Alzheimer's Disease. *Biomedicine and Pharmacotherapy*. 2022;148:112773.
49. Wankhede NL, Kale MB, Upaganlawar AB, et al. Involvement of Molecular Chaperone in Protein-Misfolding Brain Diseases. *Biomedicine and Pharmacotherapy*. 2022;147:112647.
50. Winchester B. Lysosomal Metabolism of Glycoproteins. *Glycobiology*. 2005;15(6):1R–15R.
51. Magnelli PE, Bielik AM, Guthrie EP. Identification and Characterization of Protein Glycosylation Using Specific Endo- and Exoglycosidases. *Journal of Visualized Experiments*. 2011(58):e3749.
52. Abdellatif AAH. Identification of Somatostatin Receptors Using Labeled PEGylated Octreotide, as an Active Internalization. *Drug Dev Ind Pharm*. 2019;45(10):1707–1715.
53. Solá RJ, Griebenow K. Effects of Glycosylation on the Stability of Protein Pharmaceuticals. *Journal of Pharmaceutical Sciences*. 2009;98(4):1223–1245.
54. Mostafa MAH, Khojah HMJ. Nanoparticle-Based Delivery Systems for Phytochemicals in Cancer Therapy: Molecular Mechanisms, Clinical Evidence, and Emerging Trends. *Drug Development and Industrial Pharmacy*. 2025;51(9):1105–1121.
55. Abdellatif AAH, Aldosari BN, Al-Subaiyel A, et al. Transethosomal Gel for the Topical Delivery of Celecoxib: Formulation and Estimation of Skin Cancer Progression. *Pharmaceutics*. 2022;15(1):22.
56. Abdellatif AAH, Mohammed AM, Zayed G, et al. Cyclodextrin/Adamantane-Grafted Polyethylene Glycol-Based Self-Assembling Constructs for Topical Delivery of Ketorolac Tromethamine: Formulation, Characterization, and In Vivo Studies. *AAPS PharmSciTech*. 2022;23(1):45.
57. Harris DP, Vordermeier HM, Arya A, et al. Immunogenicity of Peptides for B Cells Is Not Impaired by Overlapping T-cell Epitope Topology. *Immunology*. 2003;88(3):348–354.
58. Paurević M, Šrajer Gajdošik M, Ribić R. Mannose Ligands for Mannose Receptor Targeting. *International Journal of Molecular Sciences*. 2024;25(3):1370.
59. Facciolà A, Visalli G, Laganà A, et al. An Overview of Vaccine Adjuvants: Current Evidence and Future Perspectives. *Vaccines*. 2022;10(5):819.

60. Gao Y, Joshi M, Zhao Z, et al. PEGylated Therapeutics in the Clinic. *Bioengineering & Translational Medicine*. 2023;9(1):e10600.
61. Gonçalves J, Caliceti P. Optimizing Pharmacological and Immunological Properties of Therapeutic Proteins Through PEGylation: Investigating Key Parameters and Their Impact. *Drug Design, Development and Therapy*. 2024;18:5041–5062.
62. Abdellatif AAH, Tolba NS, Alsharidah M, et al. PEG-4000 Formed Polymeric Nanoparticles Loaded With Cetuximab Downregulate p21 & Stathmin-1 Gene Expression in Cancer Cell Lines. *Life Sciences*. 2022;295:120403.
63. Abdellatif AAH, Tawfeek HM. Development and Evaluation of Fluorescent Gold Nanoparticles. *Drug Development and Industrial Pharmacy*. 2018;44(10):1679–1684.
64. Huang Y, Guo X, Wu Y, et al. Nanotechnology's Frontier in Combatting Infectious and Inflammatory Diseases: Prevention and Treatment. *Signal Transduction and Targeted Therapy*. 2024;9(1):34.
65. Abdel-Bakky MS, Amin E, Ewees MG, et al. Coagulation System Activation for Targeting of COVID-19: Insights into Anticoagulants, Vaccine-Loaded Nanoparticles, and Hypercoagulability in COVID-19 Vaccines. *Viruses*. 2022;14(2):228.
66. Tawfeek HM, Roberts M, El Hamd MA, et al. Glibenclamide Mini-Tablets With an Enhanced Pharmacokinetic and Pharmacodynamic Performance. *AAPS PharmSciTech*. 2018;19(7):2948–2960.
67. Abdellatif AAH, Ahmed F, Mohammed AM, et al. Recent Advances in the Pharmaceutical and Biomedical Applications of Cyclodextrin-Capped Gold Nanoparticles. *International Journal of Nanomedicine*. 2023;18:3247–3281.
68. Othman MH, Zayed GM, Ali UF, et al. Colon-Specific Tablets Containing 5-Fluorouracil Microsponges for Colon Cancer Targeting. *Drug Dev Ind Pharm*. 2020;46(12):2081–2088.
69. Cantz M, Ulrich-Bott B. Disorders of Glycoprotein Degradation. *Journal of Inherited Metabolic Disease*. 1990;13(4):523–537.
70. Abdellatif AAH, Younis MA, Alsharidah M, et al. Biomedical Applications of Quantum Dots: Overview, Challenges, and Clinical Potential. *International Journal of Nanomedicine*.

2022;17:1951–1970.

71. Abdellatif AAH, Younis MA, Alsowinea AF, et al. Lipid Nanoparticles Technology in Vaccines: Shaping the Future of Prophylactic Medicine. *Colloids and Surfaces B: Biointerfaces*. 2023;222:113111.
72. Younis MA, Tawfeek HM, Abdellatif AAH, et al. Clinical Translation of Nanomedicines: Challenges, Opportunities, and Keys. *Advanced Drug Delivery Reviews*. 2022;181:114083.
73. Wielgat P, Rogowski K, Niemirowicz-Laskowska K, et al. Sialic Acid-Siglec Axis as Molecular Checkpoints Targeting of Immune System: Smart Players in Pathology and Conventional Therapy. *International Journal of Molecular Sciences*. 2020;21(12):4361.
74. Younis MA, Alsogaihi MA, Abdellatif AAH, et al. Nanoformulations in the Treatment of Lung Cancer: Current Status and Clinical Potential. *Drug Development and Industrail Pharmacy*. 2025;51(9):1088–1104.
75. Zinsli LV, Stierlin N, Loessner MJ, et al. Deimmunization of Protein Therapeutics – Recent Advances in Experimental and Computational Epitope Prediction and Deletion. *Computational and Structural Biotechnology Journal*. 2021;19:315–329.
76. Stielow M, Witczyńska A, Kubryń N, et al. The Bioavailability of Drugs—The Current State of Knowledge. *Molecules*. 2023;28(24):8038.
77. Laukens B, Visscher CD, Callewaert N. Engineering Yeast for Producing Human Glycoproteins: Where Are We Now? *Future Microbiology*. 2015;10(1):21–34.
78. Harada Y, Ohkawa Y, Maeda K, et al. Glycan Quality Control in and Out of the Endoplasmic Reticulum of Mammalian Cells. *The FEBS Journal*. 2021;289(22):7147–7162.
79. Alhowail AH, Almogbel YS, Abdellatif AAH, et al. Effects of CMF and MET on Glutamate and Dopamine Levels in the Brain, and Their Impact on Cognitive Function. *European Review for Medical and Pharmacological Sciences*. 2022;26(7):2353–2362.
80. Freeze HH, Esko JD, Parodi AJ. Glycans in Glycoprotein Quality Control. In: Varki A, Cummings RD, Esko JD, et al., editors. *Essentials of Glycobiology*. 2nd ed. Cold Spring Harbor (NY) 2009.

81. Chen F-M, Liu X. Advancing Biomaterials of Human Origin for Tissue Engineering. *Progress in Polymer Science*. 2016;53:86–168.

82. Jones D, Allen JE, Yang Y, et al. Accelerators for Classical Molecular Dynamics Simulations of Biomolecules. *Journal of Chemical Theory and Computation*. 2022;18(7):4047–4069.

83. Singh S, Sharma N, Behl T, et al. Promising Strategies of Colloidal Drug Delivery-Based Approaches in Psoriasis Management. *Pharmaceutics*. 2021;13(11):1978.

84. Sela M, Poley M, Mora-Raimundo P, et al. Brain-Targeted Liposomes Loaded With Monoclonal Antibodies Reduce Alpha-Synuclein Aggregation and Improve Behavioral Symptoms in Parkinson's Disease. *Advanced Materials*. 2023;35(51):e2304654.

85. Narayanaswamy R, Torchilin VP. Targeted Delivery of Combination Therapeutics Using Monoclonal Antibody 2C5-Modified Immunoliposomes for Cancer Therapy. *Pharmaceutical Research*. 2021;38(3):429–450.

86. Shi R, Liu X, Wang Y, et al. Long-Term Stability and Immunogenicity of Lipid Nanoparticle COVID-19 mRNA Vaccine Is Affected by Particle Size. *Human Vaccines & Immunotherapeutics*. 2024;20(1):2342592.

87. Miller JL, Lachica R, Sayce AC, et al. Liposome-Mediated Delivery of Iminosugars Enhances Efficacy Against Dengue Virus In Vivo. *Antimicrobial Agents and Chemotherapy*. 2012;56(12):6379–6386.

88. Takada G, Onodera H, Tada K. Delivery of Fungal .BETA.-Galactosidase to Rat Brain by Means of Liposomes. *The Tohoku Journal of Experimental Medicine*. 1982;136(2):219–229.

89. Mosafer J, Sabbaghi A-H, Badiee A, et al. Preparation, Characterization and in Vivo Evaluation of Alginate-Coated Chitosan and Trimethylchitosan Nanoparticles Loaded With PR8 Influenza Virus for Nasal Immunization. *Asian Journal of Pharmaceutical Sciences*. 2019;14(2):216–221.

90. Mohammed HA, Khan RA, Singh V, et al. Solid Lipid Nanoparticles for Targeted Natural and Synthetic Drugs Delivery in High-Incidence Cancers, and Other Diseases: Roles of Preparation Methods, Lipid Composition, Transitional Stability, and Release Profiles in nanocarriers' Development. *Nanotechnology Reviews*. 2023;12(1):20220517.

91. Akhtar N, Mohammed SAA, Singh V, et al. Liposome-Based Drug Delivery of Various Anticancer Agents of Synthetic and Natural Product Origin: A Patent Overview. *Pharmaceutical Patent Analyst*. 2020;9(3):87–116.
92. Yao Y, Zhou Y, Liu L, et al. Nanoparticle-Based Drug Delivery in Cancer Therapy and Its Role in Overcoming Drug Resistance. *Frontiers in Molecular Biosciences*. 2020;7:193.
93. Muntimadugu E, Silva-Abreu M, Vives G, et al. Comparison between Nanoparticle Encapsulation and Surface Loading for Lysosomal Enzyme Replacement Therapy. *International Journal of Molecular Sciences*. 2022;23(7):4034.
94. Mohamed SMM, Aljohani AKB, El-morsy A, et al. Synthesis and Characterization of Sodium alginate/poly(N-Vinylpyrrolidone) Nano-Carrier Loaded With Rebaudioside A and/or Stevioside for Anticancer Drug Delivery. *International Journal of Biological Macromolecules*. 2025;316:144778.
95. Abdellatif AAH, El-Telbany DFA, Zayed G, et al. Hydrogel Containing PEG-Coated Fluconazole Nanoparticles With Enhanced Solubility and Antifungal Activity. *Journal of Pharmaceutical Innovation*. 2018;14(2):112–122.
96. Tan Q, He L, Meng X, et al. Macrophage Biomimetic Nanocarriers for Anti-Inflammation and Targeted Antiviral Treatment in COVID-19. *Journal of Nanobiotechnology*. 2021;19(1):173.
97. Aldosari BN, Abd El-Aal M, Abo Zeid EF, et al. Synthesis and Characterization of Magnetic Ag-Fe(3)O(4)@polymer Hybrid Nanocomposite Systems With Promising Antibacterial Application. *Drug Development and Industrial Pharmacy*. 2023;49(12):723–733.
98. Fernandes RS, Arribada RG, Silva JO, et al. In Vitro and In Vivo Effect of pH-Sensitive PLGA-TPGS-Based Hybrid Nanoparticles Loaded With Doxorubicin for Breast Cancer Therapy. *Pharmaceutics*. 2022;14(11):2394.
99. Salazar VA, Comenge J, Suárez-López R, et al. Gold Nanoparticle Virus-Like Particles Presenting SARS-CoV-2 Spike Protein: Synthesis, Biophysical Properties and Immunogenicity in BALB/c Mice. *Vaccines*. 2024;12(8):829.
100. Abdellatif AAH, Mostafa MAH, Konno H, et al. Exploring the Green Synthesis of Silver Nanoparticles Using Natural Extracts and Their Potential for Cancer Treatment. *3 Biotech*.

2024;14(11):274.

101. Abdellatif AA, Zayed G, El-Bakry A, et al. Novel Gold Nanoparticles Coated With Somatostatin as a Potential Delivery System for Targeting Somatostatin Receptors. *Drug Development and Industrial Pharmacy*. 2016;42(11):1782–1791.

102. Aljohani ASM, Abdellatif AAH, Rasheed Z, et al. Gold-Nanoparticle-Conjugated Citrate Inhibits Tumor Necrosis Factor-Alpha Expression via Suppression of Nuclear Factor Kappa B (NF-kappaB) Activation in Breast Cancer Cells. *Journal of Biomedical Nanotechnology*. 2022;18(2):581–588.

103. Sharma AR, Lee Y-H, Bat-Ulzii A et al. Recent Advances of Metal-Based Nanoparticles in Nucleic Acid Delivery for Therapeutic Applications. *Journal of Nanobiotechnology*. 2022;20(1):501.

104. Moradi N, Muhammadnejad S, Delavari H, et al. Bio-Conjugation of Anti-Human CD3 Monoclonal Antibodies to Magnetic Nanoparticles by Using Cyanogen Bromide: A Potential for Cell Sorting and Noninvasive Diagnosis. *International Journal of Biological Macromolecules*. 2021;192:72–81.

105. Asmat-Campos D, Rojas-Jaimes J, de Oca-Vásquez GM, et al. Biogenic Production of Silver, Zinc Oxide, and Cuprous Oxide Nanoparticles, and Their Impregnation into Textiles With Antiviral Activity Against SARS-CoV-2. *Scientific Reports*. 2023;13(1):9772.

106. Abdellatif AAH, Mostafa MAH, Khojah HMJ, et al. Utilizing Ziziphus spina-christi for Eco-Friendly Synthesis of Silver Nanoparticles: Antimicrobial Activity and Promising Application in Wound Healing. *Nanotechnology Reviews*. 2024;13(1):20240112.

107. Shakil MS, Hasan MA, Sarker SR. Iron Oxide Nanoparticles for Breast Cancer Theranostics. *Current Drug Metabolism*. 2019;20(6):446–456.

108. Wang J, Yao M, Zou J, et al. pH-Sensitive Nanoparticles for Colonic Delivery Anti-miR-301a in Mouse Models of Inflammatory Bowel Diseases. *Nanomaterials*. 2023;13(20):2797.

109. Dartora VFC, Passos JS, Costa-Lotufo LV, et al. Thermosensitive Polymeric Nanoparticles for Drug Co-Encapsulation and Breast Cancer Treatment. *Pharmaceutics*. 2024;16(2):231.

110. Petric Z, Goncalves J, Paixao P. Under the Umbrella of Clinical Pharmacology: Inflammatory Bowel Disease, Infliximab and Adalimumab, and a Bridge to an Era of Biosimilars. *Pharmaceutics*. 2022;14(9):1766.

111. Li M, Li Y, Zheng J, et al. Ultrasound-Responsive Nanocarriers With siRNA and Fe3O4 Regulate Macrophage Polarization and Phagocytosis for Augmented non-Small Cell Lung Cancer Immunotherapy. *Journal of Nanobiotechnology*. 2024;22(1):605.

112. Zhang C, Zhang J, Shi G, et al. A Light Responsive Nanoparticle-Based Delivery System Using Pheophorbide A Graft Polyethylenimine for Dendritic Cell-Based Cancer Immunotherapy. *Molecular Pharmaceutics*. 2017;14(5):1760–1770.

113. Haidari RAA, Abdelkarem FM, Alrehaili BD, et al. Anti-Inflammatory Activity of Sarcoaldosterol A Isolated From Heteroxenia fuscescens: Insights From In Vitro, In Silico, and Spectroscopic Studies. *Chemistry & Biodiversity*. 2025;22(10):e01069.

114. Haach V, Bastos APA, Gava D, et al. A Polyvalent Virosomal Influenza Vaccine Induces Broad Cellular and Humoral Immunity in Pigs. *Virology Journal*. 2023;20(1):181.

115. Solomon M, Muro S. Lysosomal Enzyme Replacement Therapies: Historical Development, Clinical Outcomes, and Future Perspectives. *Advanced Drug Delivery Reviews*. 2017;118:109–134.

116. Metawea ORM, Abdelmoneem MA, Haiba NS, et al. A Novel 'Smart' PNIPAM-Based Copolymer for Breast Cancer Targeted Therapy: Synthesis, and Characterization of Dual pH/temperature-Responsive Lactoferrin-Targeted PNIPAM-Co-AA. *Colloids and Surfaces B: Biointerfaces*. 2021;202:111694.

117. Narayan OP, Dong J, Huang M, et al. Reversible Light-Responsive Protein Hydrogel for on-Demand Cell Encapsulation and Release. *Acta Biomaterialia*. 2025;193:202–214.

118. Abdellatif AAH, Aljutayli A, Mohammed AM. Thermosensitive Chitosan Hydrogel Loaded Cetuximab: a Novel Approach for Parenteral Controlled Delivery. *Drug Development and Industrial Pharmacy*. 2025;51(6):597–605.

119. Abdellatif AAH, Mohammed AM, Saleem I, et al. Smart Injectable Chitosan Hydrogels Loaded With 5-Fluorouracil for the Treatment of Breast Cancer. *Pharmaceutics*. 2022;14(3):661.

120. Abdellatif AAH, Alshubrumi AS, Younis MA. Targeted Nanoparticles: The Smart Way for the Treatment of Colorectal Cancer. *AAPS PharmSciTech*. 2024;25(1):23.

121. Vaghasiya K, Ray E, Singh R, et al. Efficient, Enzyme Responsive and Tumor Receptor Targeting Gelatin Nanoparticles Decorated With Concanavalin-A for Site-Specific and Controlled Drug Delivery for Cancer Therapy. *Materials Science and Engineering: C*. 2021;123:112027.

122. Wang C-Y, Sun M, Fan Z, et al. Intestine Enzyme-Responsive Polysaccharide-Based Hydrogel to Open Epithelial Tight Junctions for Oral Delivery of Imatinib Against Colon Cancer. *Chinese Journal of Polymer Science*. 2022;40(10):1154–1164.

123. Chen C, Zhang Y, Hou Z, et al. Rational Design of Short Peptide-Based Hydrogels With MMP-2 Responsiveness for Controlled Anticancer Peptide Delivery. *Biomacromolecules*. 2017;18(11):3563–3571.

124. Kost J. Ultrasound Induced Delivery of Peptides. *Journal of Controlled Release*. 1993;24(1–3):247–255.

125. Yan H, Yu K, Zhang K, et al. Efficacy and Safety of Trastuzumab Emtansine (T-DM1) in the Treatment of HER2-Positive Metastatic Breast Cancer (MBC): A Meta-Analysis of Randomized Controlled Trial. *Oncotarget*. 2017;8(60):102458–102467.

126. Tsuchikama K, Anami Y, Ha SYY, et al. Exploring the Next Generation of Antibody–Drug Conjugates. *Nature Reviews Clinical Oncology*. 2024;21(3):203–223.

127. Feng Y-X, Hu H, Wong Y-Y, et al. Microneedles: An Emerging Vaccine Delivery Tool and a Prospective Solution to the Challenges of SARS-CoV-2 Mass Vaccination. *Pharmaceutics*. 2023;15(5):1349.

128. Torres-Herrero B, Armenia I, Ortiz C, et al. Opportunities for Nanomaterials in Enzyme Therapy. *Journal of Controlled Release*. 2024;372:619–647.

129. Aboul-Ella H, Gohar A, Ali AA, et al. Monoclonal Antibodies: From Magic Bullet to Precision Weapon. *Molecular Biomedicine*. 2024;5(1):47.

130. Chehelgerdi M, Chehelgerdi M, Allela OQB, et al. Progressing Nanotechnology to Improve Targeted Cancer Treatment: Overcoming Hurdles in Its Clinical Implementation. *Molecular Cancer*. 2023;22(1):169.

131. Osterman MT, Haynes K, Delzell E, et al. Comparative Effectiveness of Infliximab and Adalimumab for Crohn's Disease. *Clinical Gastroenterology and Hepatology*. 2014;12(5):811–817.e3.

132. Patel R, Kaki M, Potluri VS, et al. A Comprehensive Review of SARS-CoV-2 Vaccines: Pfizer, Moderna & Johnson & Johnson. *Human Vaccines & Immunotherapeutics*. 2022;18(1):2002083.

133. Demongeot J, Fougère C. mRNA COVID-19 Vaccines—Facts and Hypotheses on Fragmentation and Encapsulation. *Vaccines*. 2022;11(1):40.

134. Ali H, Akbar M, Iqbal B, et al. Virosome: An Engineered Virus for Vaccine Delivery. *Saudi Pharmaceutical Journal*. 2023;31(5):752–764.

135. Lu H, Zhang S, Wang J, et al. A Review on Polymer and Lipid-Based Nanocarriers and Its Application to Nano-Pharmaceutical and Food-Based Systems. *Frontiers in Nutrition*. 2021;8:783831.

136. Cordeiro AS, Patil-Sen Y, Shivkumar M, et al. Nanovaccine Delivery Approaches and Advanced Delivery Systems for the Prevention of Viral Infections: From Development to Clinical Application. *Pharmaceutics*. 2021;13(12):2091.

137. Kim M, Shin M, Zhao Y, et al. Transformative Impact of Nanocarrier-Mediated Drug Delivery: Overcoming Biological Barriers and Expanding Therapeutic Horizons. *Small Science*. 2024;4(11):2400280.

138. Abdellatif AAH, Mohammed HA, Al-Khalaf AM, et al. Ginkgo biloba Leaves extract's Cosmeceutical Evaluation: A Preliminary Assessments on Human Volunteers Towards Achieving Improved Skin Condition and Rejuvenation. *Drug Development and Industrial Pharmacy*. 2023;49(3):281–292.

139. Mohan T, Verma P, Rao DN. Novel Adjuvants & Delivery Vehicles for Vaccines Development: A Road Ahead. *Indian Journal of Medical Research*. 2013;138(5):779–795.

140. Padmanabhan R, Kheraldine HS, Meskin N, et al. Crosstalk between HER2 and PD-1/PD-L1 in Breast Cancer: From Clinical Applications to Mathematical Models. *Cancers*. 2020;12(3):636.

141. Liao J, Li X, Gan Y et al. Artificial Intelligence Assists Precision Medicine in Cancer Treatment. *Frontiers in Oncology*. 2023;12:998222.

142. Lam EHY, Yu F, Zhu S, et al. 3D Bioprinting for Next-Generation Personalized Medicine. *International Journal of Molecular Sciences*. 2023;24(7):6357.

143. Musa A, Ahmed SR, Hussein S, et al. Prominent Antidiabetic and Anticancer Investigation of Scrophularia deserti Extract: Integration of Experimental and Computational Approaches. *Journal of Molecular Structure*. 2024;1315:138769.

7 Mechanisms of Polypeptide Stability and Degradation

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7.1 INTRODUCTION

The physical and chemical processes together with enzyme action enable the complete breakdown of proteins and peptides into their basic component elements (Gurina & Mohiuddin, 2020; Toldrá et al., 2017). The living cells can degrade and inactivate substances through their physiological functions, while external factors can achieve the same result. Polypeptide stability refers to the capacity of polypeptides to sustain their structure against both environmental and molecular changes which scientists determine through various amino acid sequences and specific storage conditions and pH and moisture content levels (Ahmed et al., 2022; Panja et al., 2020; Zapadka et al., 2017).

7.1.1 Factors Affecting Polypeptide Stability

The polypeptide stability depends on both intrinsic and extrinsic factors which include the sequence of amino acids, the three-dimensional folding of

the structural elements, the external environmental factors and the post-translational modifications (Kuzu et al., 2025; Lee et al., 2023; Panja et al., 2020; Zapadka et al., 2017). Multiple types of interactions are present in the system including non-covalent and covalent interactions together with the chemical and physical characteristics of the surrounding environment (Mandal et al., 2025; Zapadka et al., 2017).

7.1.2 Intrinsic Sequence Properties

The stability of polypeptides is considered to be an unsolved problem because different people understand the term in diverse ways. Scientists from different fields use the protein half-life measurement to evaluate their stability (Morey et al., 2021; Zhou, 2004). Structural biologists use protein structure changes from primary to secondary, tertiary and quaternary levels to evaluate protein stability (Li et al., 2025). Research studies have made great progress toward understanding protein stability together with its disordered state and dynamic behavior.

Intrinsic sequence properties are major determinants of polypeptide stability which affect protein folding, aggregation, and conformational dynamics through the hydrophobicity, the charge polarity and residue patterning of the proteins (Deller et al., 2016; Stollar & Smith, 2020; Bowman et al., 2020; Li et al., 2021; Zeng et al., 2022).

The combination of intrinsic and environmental factors establishes the fundamental stability of polypeptides (Zapadka et al., 2017) which determines the molecular framework through which cellular regulatory system function (Figure 7.1).

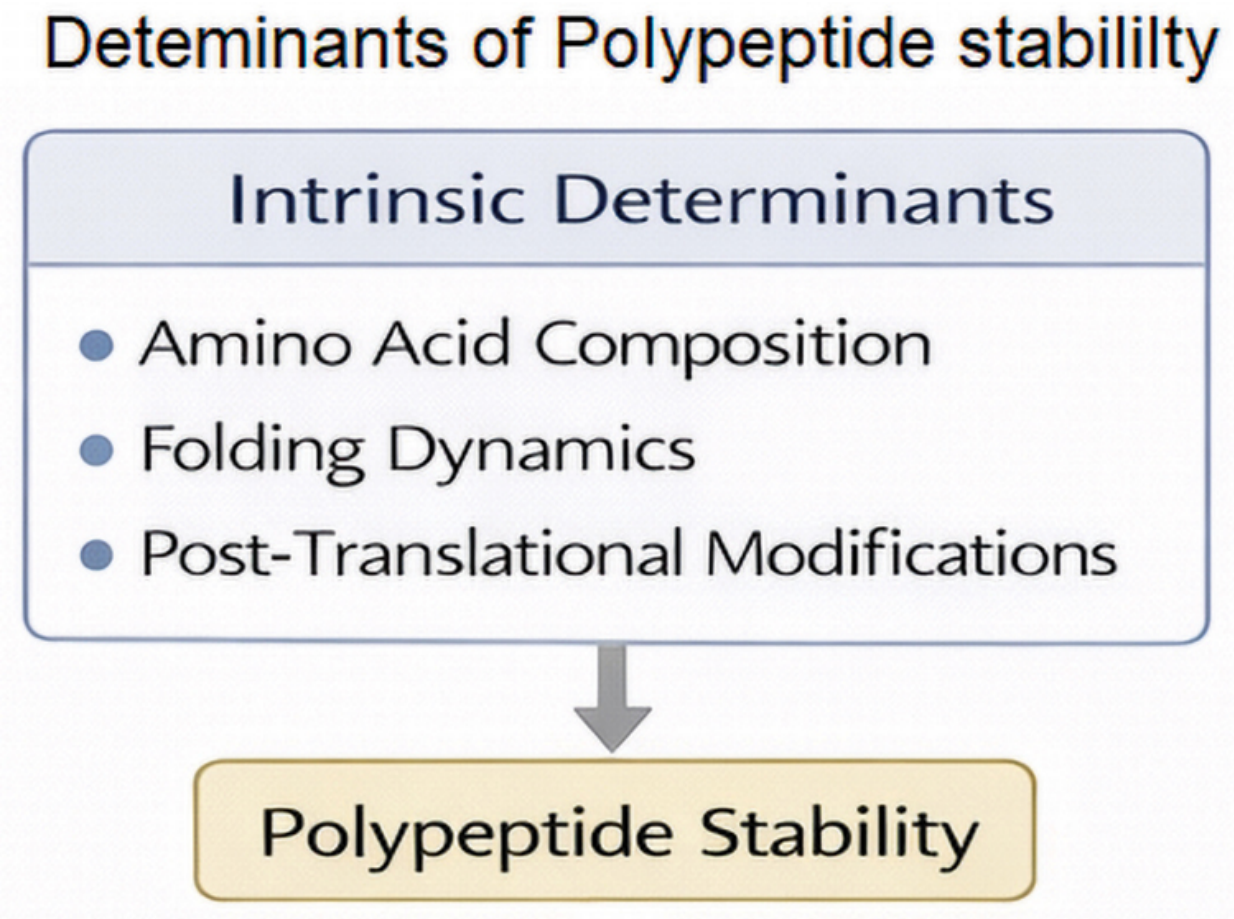

FIGURE 7.1 Determinants of intrinsic and extrinsic polypeptide stability. ⏎

7.1.3 Structural Folding and Conformational Dynamics

Polypeptide stability depends on two factors, the folding process and the subsequent structural changes which occur with time (Pak et al., 2021; Uversky & Uversky, 2014). Folding does not establish a fixed state which marks its complete conclusion because polypeptides enter a series of different conformational states until they reach their active forms. Proteins exhibit dynamic flexibility throughout their entire lifespan after undergoing folding because their structures must adjust to enable ligand attachment, catalytic activity and allosteric control (Balchin et al., 2016). The movements of proteins which enable their functions need energy to proceed

(Li & Ji, 2019). Excessive flexibility can make the protein more prone to both misfolding and degradation to hydrophobic regions due to weakening in the structural elements that maintain the stability (Díaz-Villanueva et al., 2015; Rahban et al., 2022; Yan et al., 2021; Zhu et al., 2020).

Polypeptide folding requires stabilization through hydrogen bonds and hydrophobic interactions while destabilizing forces emerge from both entropy and solvent exposure (Almeida & Souto, 2007; Galano-Frutos & Sancho, 2024). The structural integrity of proteins becomes compromised because small changes in this balance force them to enter a state of partial folding which makes them susceptible to structural damage (Banerjee et al., 2019). The intermediates themselves do not cause disease because they actually perform useful functions which assist in both folding processes and regulatory mechanisms. The cellular quality control mechanisms become overpowered when the conformational dynamics continue to function beyond their normal physiological range (Balch et al., 2014; Pohl & Dikič, 2019).

Proteins can change their structure through structural plasticity, which lets them match various functional requirements, yet this structural flexibility needs strict control. Proteins which possess high levels of conformational flexibility depend on chaperones and binding partners to achieve stability. The relationship between folding and dynamics operates as a dual system which decides whether polypeptides remain active or enter pathways which lead to their degradation (Finka et al., 2015; Jahn & Radford, 2008).

7.1.4 Structural Folding and Conformational Dynamics

The stability of polypeptides depends on two factors which include their initial folding process and the gradual structural transformations that occur

throughout time (Uto et al., 2025; Venkatraman et al., 2001). The process of folding does not create a permanent state which defines its final ending because polypeptides progress through multiple conformational transitions until they achieve their functionally active forms (Ghosh & Ranjan, 2020). Proteins maintain their capacity to change their form during their entire existence which follows the folding process because their structures must modify to permit ligand binding, catalytic function and allosteric modulation. Proteins need energy to execute their functions because their movements rely on energy resources for execution (Corsetti et al., 2024). The condition of excessive flexibility occurs when hydrophobic regions that sustain stability are being damaged, hence makes the protein more vulnerable to both misfolding and degradation.

Polypeptide folding requires hydrogen bonds and hydrophobic interactions to create stabilization which leads to two destabilizing factors that emerge from entropy and solvent exposure (Izzi et al., 2024). The structural integrity of proteins becomes compromised because small changes in this balance force them to enter a state of partial folding making them susceptible to structural damage (Uversky, 2015). The intermediates do not cause disease because they perform functions which help the body both fold proteins and regulate their activities. The cellular quality control mechanisms become overpowered when the conformational dynamics continue to function beyond their normal physiological range (Pohl & Dikič, 2019).

Proteins can change their structure through structural plasticity which lets them match various functional requirements, yet this structural flexibility needs strict control (Ayaz et al., 2021; Haliloglu & Bahar, 2015). The proteins which exhibit high levels of flexible structure require base chaperones together with binding partners for their stabilization (Mchaourab et al., 2009). The folding dynamics between proteins function as a dual

system which determines their active status or which pathway leads to their degradation (Alaei et al., 2021).

7.1.5 Post-Translational Modifications

The cellular mechanism for controlling polypeptide lifespan after creation relies on post-translational modifications as a strong regulatory method (Morimoto, 2019). The PTMs perform their function by changing particular amino acid sites through chemical methods which lead to protein structure stabilization, structural changes and protein destruction signals. The introduction of negative charges from phosphorylation creates new folding patterns which either maintain active protein shapes or make proteins easier to break down for quick elimination (Bah et al., 2014).

The chemical modifications known as acetylation and methylation enable proteins to achieve better stability because they change the specific regions of proteins through which they interact with other molecules (Gibbs et al., 2022). The modifications lead to two different outcomes which protect proteins from destruction by hiding recognition elements or which reveal degrons to signal protein removal (Zhao et al., 2022). Ubiquitination serves as the main PTM which connects protein structural information to their process of proteolytic degradation (Carroll et al., 2020). The process of polyubiquitination sends proteins to undergo degradation, while monoubiquitination functions as a mechanism which controls protein movement and function without causing immediate protein destruction.

The function of PTMs depends on their interaction with other proteins because they do not function as independent elements (Wang et al., 2023; Zhong et al., 2023). Proteins contain multiple modifications whose combination of effects determines their stability in different contexts (Lee et al., 2023; Wang et al., 2022). Cells use the interaction between different

modifications to process environmental signals and metabolic conditions into precise mechanisms which control how long proteins remain active. Through PTMs, stability becomes a dynamic and reversible property which enables cells to adjust their protein content without needing to change their gene expression (Lee et al., 2023).

7.2 CELLULAR MECHANISMS OF POLYPEPTIDE STABILITY

Polypeptide stability inside cells does not occur through natural processes nor does it provide any protection against degradation. The primary sequence of a protein carries structural data which the intracellular systems of a cell use to decide whether the structure will be kept or broken down. Cells function within an environment that contains dense molecular elements while their internal processes move and their energy systems work which makes it hard for most polypeptides to achieve their natural folding process without creating mistakes. The stability of proteins arises through a combination of multiple processes which include folding assistance, protein assembly and proteotoxicity detection which scientists call the proteostasis network (Hipp et al., 2019; Klaips et al., 2018; Kuzu et al., 2025). The network does not create random protein storage; instead, it maintains protein functionality while determining which proteins require protection against degradation.

The coordinated systems of Figure 7.2 demonstrate how cells oversee all stages of polypeptide processing which includes folding and assembly and surveillance and final disposition, while showing how decisions about protein stabilization and degradation operate together throughout the proteostasis network.

Cellular Systems Governing Polypeptide Folding, Surveillance, and Fate

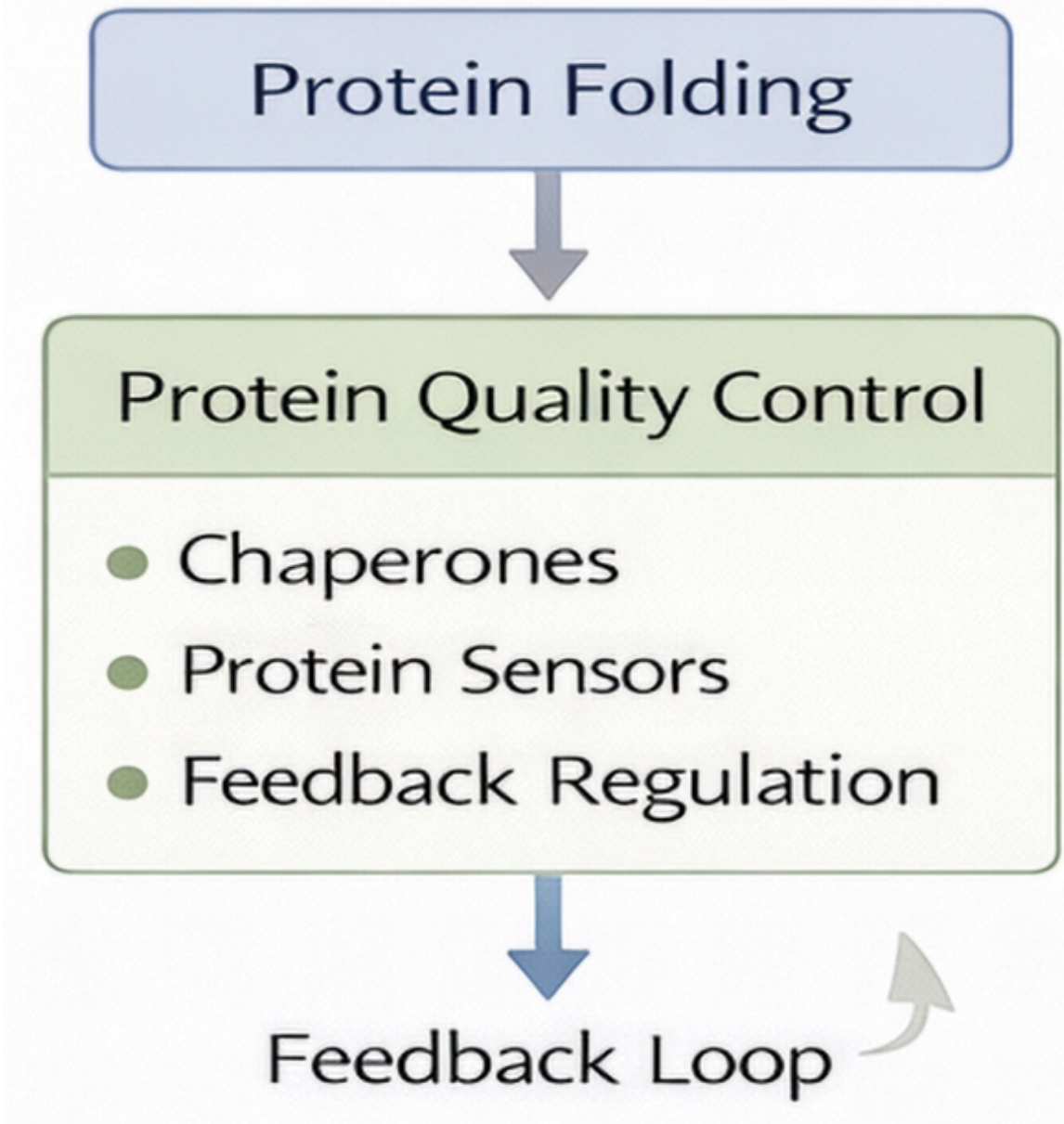

FIGURE 7.2 Cellular mechanisms governing polypeptide stability and fate. ⏎

7.2.1 Molecular Chaperones

Molecular chaperones form the base system which maintains cellular polypeptide stability through their protective functions. The main role of these proteins is to control how proteins fold by blocking non-native interactions which happen during synthesis or stress-induced unfolding (Balchin et al., 2016; Hekmatimoghaddam et al., 2017; Sinnige et al., 2020). Chaperones support polypeptide movement through different shapes by stopping their flow into condensed amigo aggregates.

The Hsp70 family demonstrates this principle through its activities. Hsp70 proteins work at the ribosome-nascent chain interface to bind to hydrophobic segments which become visible as they leave the ribosomal exit tunnel because this moment carries the highest risk of protein aggregation (Chen et al., 2025; Zdancewicz et al., 2025). The J-domain co-chaperones together with nucleotide exchange factors enable repeated folding attempts through their ATP-regulated binding and release cycles which keep proteins in their unfolding state until actual folding starts (Nillegoda et al., 2017). The system accepts that protein folding behavior occurs through probabilistic patterns which guide its function.

The folding process of polypeptides with complicated three-dimensional shapes and their high contact order requires spatial limitations for successful completion. The eukaryotic chaperonin TRiC/CCT provides such an environment by encapsulating substrates within a central chamber, effectively isolating them from the crowded cytosol (Balchin et al., 2016). The structural studies show that TRiC supports proteins which maintain the cytoskeleton and conduct signal transmission because chaperone dependence occurs in specific proteins, not throughout all proteins in the proteome.

Without ATP-dependent systems, small heat shock proteins (sHSPs) function as holdases which respond to stress. The sHSPs under heat shock or oxidative stress conditions make reversible assemblies to capture partially unfolded polypeptides, which prevents permanent aggregation until their normal folding process becomes operational (Díaz-Villanueva et al., 2015; Labbadia & Morimoto, 2015). The cellular mechanism uses this strategy to preserve protein reversibility because it permits protein damage recovery instead of immediately destroying the damaged proteins.

The extended period of chaperone binding functions as a vital testing mechanism. Polypeptides that fail to achieve native structure after repeated cycles are increasingly recognized as terminally defective and routed toward degradation pathways (Hipp et al., 2019). The role of chaperones extends beyond stabilization because they determine the outcome of protein existence.

7.2.2 Protein Complex Assembly

The intrinsic stability of most cellular polypeptides depends on their requirement to form multiprotein complexes. The majority of proteins exist in their native state as incomplete structures which cannot maintain their energy balance until they form bonds with other proteins that will protect their hydrophobic areas. Co-translational assembly processes begin their operation. Ribosome profiling studies have revealed that subunits of protein complexes frequently engage their partners while still being synthesized, which results in reduced time for their unstable intermediate states (Ravichandran et al., 2025). The process requires chaperones to sustain nascent chains in their assembly-ready form while preventing their transition to finished protein structures.

Cells maintain strict surveillance over unassembled subunits. Orphan proteins that fail to find their partners are rapidly targeted for degradation, which helps maintain protein balance while decreasing their probability of forming unwanted protein interactions (Ali et al., 2024; Juszkiewicz & Hegde, 2018). The mechanism plays a vital role in regulatory complexes because extra subunits can create dominant-negative effects which lead to signaling pathway dysregulation.

Assembly-dependent stability provides a method to control protein half-life in a flexible manner. Active complexes enable proteins to achieve their stable form, which results in rapid protein downregulation through complex disassembly instead of transcriptional gene silencing (Jayaraj et al., 2020; Morimoto, 2020; Sala et al., 2017). The regulation process which requires less energy establishes time boundaries for protein synthesis to match actual functional requirements of the cell.

7.2.3 Quality Control Systems

Organisms use cellular quality control systems to assess polypeptide fate by analyzing folding status and assembly state and post-translational molecular signals. Organismal systems operate throughout all cellular regions because proteins undergo different developmental stages in various biological environments (Colalto, 2024). Chaperone-assisted ubiquitination functions as the main mechanism through which quality control operates in the cytosol. E3 ubiquitin ligases identify polypeptides that demonstrate permanent misfolding through their exposed hydrophobic regions and their unusual conformational behavior. Ubiquitination allows for reversible transformation which creates a last opportunity to refold proteins before they undergo permanent cellular commitment.

The endoplasmic reticulum employs a particularly stringent system due to the complexity of secretory proteins. The ER-associated degradation system (ERAD) detects misfolded proteins that exist in the luminal or membrane spaces and then it moves those proteins to the cytosol for their subsequent removal through proteasomal degradation (Needham & Brodsky, 2013). The pathway ensures that only proteins with proper folding progress through the secretory pathway, which protects both extracellular and membrane proteomes.

Mitochondrial quality control presents distinct challenges because cells produce most mitochondrial proteins through cytosolic synthesis before using post-translational mechanisms to import those proteins. Proteins that fail to fold properly after import are degraded by resident proteases, while import failures trigger cytosolic degradation pathways (Klaips et al., 2018; Kuzu et al., 2025). The organization of quality control systems across compartments and their global functions create two types of monitoring systems.

Quality control systems permit certain levels of defects instead of requiring perfect accuracy. The system operates to achieve two goals which include reducing proteotoxic stress and maintaining operational capability through functional diversity protection. That reflects a pragmatic balance rather than rigid perfectionism (Ali et al., 2024; Juszkiewicz & Hegde, 2018).

7.2.4 Regulatory Feedback Mechanisms

Polypeptide stability is dynamically regulated through feedback mechanisms that couple protein fate to cellular state. The most direct example exists within stress-responsive pathways. During proteotoxic stress, the cellular system uses misfolded proteins to create a chaperone storage system which

activates heat shock transcription factors and produces proteostasis elements (Díaz-Villanueva et al., 2015; Labbadia & Morimoto, 2015). The system will reduce negative feedback after it restores folding capacity which stops unnecessary energy consumption.

The unfolded protein response regulates ER folding capacity by managing three essential functions which include transcriptional activation and translational attenuation and degradation pathways (Gao et al., 2024; Hipp et al., 2019). Through stress-induced protein movements, cells establish polypeptide stability by protecting their current proteins which proves that stability exists between synthesis processes and degradation processes.

Feedback loops in individual proteins require post-translational modifications which change interaction networks and degradation patterns. Phosphorylation, acetylation, and ubiquitination serve as molecular switches because they maintain protein stability under certain conditions but they increase protein turnover in different situations (Jayaraj et al., 2020; Morimoto, 2020; Sala et al., 2017). Proteostasis regulation depends on metabolic state and circadian rhythms and aging according to new research which demonstrates that polypeptide stability exists as a systems-level property that reacts to both temporal and environmental changes (Gao et al., 2024; Hipp et al., 2019).

7.3 PATHWAYS OF POLYPEPTIDE DEGRADATION

The endoplasmic reticulum (ER) has a crucial role in the synthesis of cellular protein, its transport, modification, and folding. The cellular and organismal systems need to eliminate excess and incorrectly folded proteins together with their associated polypeptides through degradation processes to maintain proteostasis and protect against harmful effects caused by protein

over-accumulation (Johnston & Samant, 2020; Yang et al., 2025). The cells execute polypeptide degradation through a systematic method which employs the three main pathways: ubiquitin-proteasome system, lysosomal/autophagic pathways (Fregno & Molinari, 2019), and special pathways which include endoplasmic reticulum associated degradation (ERAD), endoplasmic reticulum to lysosome associated degradation (ERLAD), and ribosome-associated quality control (RQC) (Kumari & Brodsky, 2021). The systems protect protein integrity through their ability to remove damaged proteins from cells, while they also help cells respond to stress and prevent disease onset (Chondrogianni et al., 2014). Research activities currently establish the intricate nature of these pathways while also discovering their therapeutic applications (Salomo-Coll et al., 2025).

7.3.1 Ubiquitin-Proteasome System

The increasing evidence links numerous cellular control systems to the process of protein alteration through the polypeptide ubiquitin (Cockram et al., 2021; Pohl & Dikič, 2019; Liao et al., 2024; Spano & Catara, 2023). The cell cycle critical events, class I antigen processing, signal transduction pathways and receptor-mediated endocytosis all need them for their operation. Ubiquitin attachment to a protein causes most of these examples to result in the 26S proteasome system which breaks down the protein (Arkinson et al., 2025). The process begins when ubiquitin attaches to a substrate and the ubiquitinated protein binds to the proteasome which requires the substrate to be unfolded and then deubiquitinated through the proper channels until it reaches the proteasome which will break down the protein into small peptides. The process of protein ubiquitination and deubiquitination involves multiple enzyme families, while the proteasome exists as a collection of particles which have shared features but separate

operational functions (Elu et al., 2022; Liu et al., 2024; Xian et al., 2024). The system exists in two different forms which allow it to recognize different substrates while providing multiple ways to control its activities.

Scientists have classified ubiquitin-mediated polypeptide degradation as a process that happens outside of lysosomes since ancient times. The research from 1980s and 1990s proved evidence which showed that yeast cells without essential lysosomal proteases developed high amounts of ubiquitinated proteins inside their organelles (Galan & Haguenauer-Tsapis, 1997; Wolf & Menssen, 2018). The finding represented a completely original discovery at that particular historical moment.

7.3.2 Autophagy-Lysosome Pathway

Lysosomes operate as signaling centers which control metabolic processes, determine cell development and disease progression. The lysosomes use their autophagy, endocytosis and chaperone-mediated autophagy systems to break down all types of substrates which include long-lived proteins, protein aggregates, damaged organelles and extracellular materials (Yim & Mizushima, 2020). The system serves as an essential mechanism which enables comprehensive material breakdown and waste processing during periods of environmental stress or food scarcity (Fleming et al., 2022; Majeski & Dice, 2004).

The scientific field has experienced a resurgence in interest toward studying autophagy which involves lysosomes breaking down all internal cell components. The autophagic molecular machinery has been discovered which creates multiple studies that show autophagic changes occur in common human diseases like cancer and neurodegenerative disorders and muscular diseases and infectious diseases (Mehrpour et al., 2010; Mizushima et al., 2008). The first genes and protein products responsible for

autophagy were not identified until after a 30-year period which began with their initial description in the 1960s (Levine & Kroemer, 2019; Mizushima et al., 2008). Researchers have established most connections between autophagy and both cellular physiology and disease through studies which demonstrate how autophagy enables cells to eliminate damaged proteins and organelles while maintaining their organelle balance through structural component degradation and its role in supporting both innate and adaptive immune defense (Mizushima et al., 2008; Wang et al., 2024).

Recent studies have increased scientific interest in autophagy which now focuses on understanding how autophagic processes interact with cellular metabolic activities. Autophagy first emerged as a biological process through its connection to protein breakdown which scientists investigated through nutritional state experiments during the initial studies (Gómez-Virgilio et al., 2022; Mortimore & Pösö, 1987; Rabinowitz & White, 2010). The recent discovery of autophagy molecular components now allows scientists to study how autophagy supported cellular metabolic activities throughout history. This article explains how autophagy research has advanced to reveal how cells use their internal energy reserves through autophagic processes. We describe two sections which explain how autophagy failure affects cellular energy balance and how scientists discovered metabolic disorders from impaired autophagy functions.

7.3.3 Mitochondrial Proteases

The mitochondria function as flexible organelles which enable cells to transmit signals and conduct metabolic processes (Kuznetsov et al., 2022). Mitochondria require a specific protein balance because their pathways need both protein creation and protein destruction. Mitochondrial proteases function as specialized enzymes which ensure protein quality control while

they manage mitochondrial biogenesis through their process of destroying damaged proteins and misfolded proteins and excess polypeptides (Table 7.1). The mitochondrial proteases function to protect cells from toxic protein aggregates which cause diseases, while they help cells manage their response to environmental challenges. Mitochondrial proteases utilize their specific functions and substrate preferences to either regulate the complete mitochondrial proteome or control individual protein activities (Liu et al., 2024; Popov, 2020).

TABLE 7.1

The Role of Key Mitochondrial Proteases

Protease	Location	Main Function	Substrate Specificity
m-AAA/i-AAA	Inner membrane	Degradation of membrane/matrix proteins	Misfolded, unassembled, damaged
LON	Matrix	Degradation of misfolded proteins	Unfolded, oxidized, non-aggregated
ClpXP	Matrix	Quality control, stress response	Serine-phosphorylated, damaged
YME1L	Inner membrane	Proteases, stress adaptation	Sequence-specific, accessible signals

7.3.4 Non-Lysosomal Proteolytic Mechanisms

The system for protein quality control consists of two processes lysosomal and non-lysosomal proteolysis which work together to create a networked system that can adapt its operations. The cell's first line of defense uses non-lysosomal proteolytic mechanisms which operate with fast and precise control to execute their protective function, while lysosomes handle permanent storage of degraded materials and long-lasting substances. The

pathways prevent misfolded proteins and damaged proteins and excess proteins and regulatory proteins from leaving the cell. The systems of lysosomal and non-lysosomal proteolysis operate together with both methods protecting the proteome throughout different time periods and throughout different spatial distances while enabling the cell to adapt to environmental changes (Wang et al., 2023; Zhong et al., 2024).

7.3.4.1 Conceptual Distinction and Functional Rationale

The definition of non-lysosomal proteolysis comes from its various protease systems which exist throughout the cell body and nuclear space and mitochondrial space and other cell compartmental areas. The cell uses non-lysosomal pathways to directly target single proteins and small protein groups, while lysosomes need proteins to undergo vesicle transport after being stored. The architectural distinction creates a core functional difference because non-lysosomal proteolysis enables selective regulatory functions, but the system lacks capacity for bulk operations which typical terminal functions deliver (Varshavsky, 2019).

The development of non-lysosomal proteolytic systems shows how eukaryotic proteomes became more complex to handle their increasing protein content. The growing number of proteins and their diverse structures and interdependent control mechanisms increased the requirement for new degradation systems which could fix mistakes while controlling protein amounts throughout the day. The fast elimination of defective proteins stops them from joining protein complexes and prevents harmful interactions and maintains the accuracy of cellular signaling pathways (Hipp et al., 2019).

7.3.4.2 The Proteasome as the Central Non-Lysosomal Hub

The proteasome functions as the fundamental component of non-lysosomal proteolysis because it operates as a multi-unit protease system which performs most eukaryotic cell protein degradation through selective processes. The proteasomal system connects closely with the ubiquitin signaling system because it enables cells to identify specific proteins for disposal based on their current folding status or any present damage or required regulatory period or their incorrect position in the body

The two systems diverge in their substrate preferences yet display distinct differences in their rates of reaction. The proteasomal system performs its degradation process within a time frame of several minutes because this speed enables cells to change their protein content rapidly during times of stress or development or metabolic changes. The process requires this quick pace because protein clearance delays would lead to complete system failures during essential functions which include cell cycle progression and transcriptional regulation and stress adaptation (Hommen et al., 2022.

Proteasomes function as dynamic systems which possess multiple operational states throughout their existence. The cellular state controls three proteasome elements which include their makeup and their distribution throughout the cell and their performance level. Proteasomes redistribute their components and boost their operations and change their functions to handle higher levels of proteotoxic stress during stress situations which shows their dual role as protective systems and waste disposal entities (Hipp et al., 2019).

7.3.4.3 Ubiquitin-Independent Proteasomal Degradation

Proteasomal degradation of most proteins requires ubiquitin tagging, but some proteins can undergo proteolysis without this tagging system.

Substrates in this category demonstrate their capability to interact with the proteasome through their presence of intrinsically disordered regions and exposed hydrophobic segments (Sumikama et al., 2026). The degradation process functions to eliminate regulatory proteins which the body needs to dispose of rapidly to stop signals.

Ubiquitin-independent degradation demonstrates how non-lysosomal proteolytic processes operate because structural weaknesses in proteins act as signals that trigger their destruction. The stability of proteins depends on the combination of sequence characteristics and protein shape changes and their vulnerability to protease attacks rather than the mere presence of ubiquitin.

7.3.4.4 Cytosolic and Nuclear Proteases Beyond the Proteasome

The proteasome system exists as one of several mechanisms through which cytosolic and nuclear proteases carry out their non-lysosomal proteolytic functions. Calpains function as calcium-dependent proteases who execute partial proteolysis instead of total protein breakdown. Calpains utilize their specific cleavage abilities to achieve site-focused substrate control which results in altered protein behavior and distributions and interaction capabilities without total protein destruction (Yoo et al., 2017).

Proteolytic regulation through this mechanism stands in complete opposition to lysosomal digestion because it demonstrates how non-lysosomal proteases function as signaling entities. The process of limited protein cleavage enables cells to activate transcription factors and change their cytoskeletal structure and adjust their synaptic elements. People who experience calpain dysregulation face an increased risk of developing neurodegeneration and muscle wasting and suffering from ischemic damage which demonstrates how regulatory proteolysis exists within a narrow space

that stretches between essential body processes and harmful effects (Metwally et al., 2021.

The nucleus contains special proteolytic functions which control the processes of transcription. Nuclear proteasomes function to destroy transcription factors and chromatin modifiers and cell cycle regulators which enables cells to maintain their ability to switch gene expression on and off. The process of localized degradation enables cells to stop or reduce their transcriptional activation which demonstrates the fundamental role of non-lysosomal proteolysis in creating regulatory systems that control cellular functions (Larsen et al., 2026).

7.3.4.5 Mitochondrial Non-Lysosomal Proteolysis

The mitochondria use their own proteolytic mechanisms to perform protein degradation tasks without needing lysosomes for their operation. The inner mitochondrial membrane and matrix locations of ATP-dependent proteases include Lon and ClpXP proteases which function as mitochondrial protein quality control mechanisms by breaking down misfolded proteins and damaged proteins and proteins that have not been formed into complete structures (Wang et al., 2023).

The organelle's double-membrane structure and its separate genetic material stop mitochondrial proteolysis from happening. The process of importing mitochondrial proteins from the nucleus after their nuclear encoding leads to potential errors during protein folding and protein targeting. Proteases that operate outside lysosomes in mitochondria function as protective barriers which allow only correctly folded and assembled proteins to remain in the organelle.

Mitochondrial proteolysis breakdown results in three major problems which include proteotoxic stress and the loss of oxidative phosphorylation

and the initiation of stress responses that spread throughout the cell. The cellular metabolic pathways which lead to aging and age-related diseases use non-lysosomal proteolysis as their main mechanism (Li et al., 2024.

7.4 PROTEOLYSIS AS A REGULATORY, NOT MERELY DEGRADATIVE, FORCE

The main characteristic that defines non-lysosomal proteolytic mechanisms exists because they design their systems to regulate non-lysosomal proteolysis operations. The body removes defective substrates from the system because their continued presence would cause more damage. The cellular state transitions between different stages depend on the precise timing of cyclins and transcriptional repressors and signaling adaptors and metabolic enzymes degradation processes (Rukhlenko, 2022).

The process of proteolysis in this situation operates in the same way as phosphorylation or transcriptional control does. The process of protein removal from cells through irreversible loss makes cells create directionality toward specific biological outcomes. Non-lysosomal degradation functions as a molecular choice point which changes temporary signals into permanent effects on cellular behavior.

7.5 CROSSTALK BETWEEN LYSOSOMAL AND NON-LYSOSOMAL SYSTEMS

Lysosomal systems function differently from non-lysosomal systems yet their proteolytic pathways establish extensive connections between these two systems. Autophagic pathways receive redirected substrates from proteasomal overload while lysosomal dysfunction triggers cells to increase proteasomal activity according to Kaushik and Cuervo (2015). The system

functions through its dual communication pathways which enable it to maintain stability during periods of changing proteotoxic stress.

The processing of specific substrates demands that both systems operate in a sequential manner. The process of partial proteasomal degradation produces fragments which lysosomes later use to clear their contents or handle their removal. The strategic degradation process uses multiple degradation methods to decrease the buildup of hazardous intermediate substances while demonstrating how different cellular proteolysis systems work together (Moon & Lee, 2018).

7.6 BIOLOGICAL IMPLICATIONS AND DISEASE ASSOCIATIONS

The cellular mechanisms that govern polypeptide stability and degradation extend far beyond housekeeping functions. These mechanisms establish which physical traits organisms will develop and which diseases they will become vulnerable to and which treatments will succeed in their battle against illness. Cells maintain their stability through proteostasis networks which match their genetic makeup and environmental conditions and their metabolic transformations. Biological systems experience perpetual physiological problems and diseases because even the smallest changes to protein stability create widespread effects. The biological effects which arise from changes to polypeptide stability function as molecular failures which extend into systemic disruptions that happen across multiple body tissues. The downstream biological effects that result from polypeptide stability disruption, together with new therapeutic and biotechnological intervention points, are shown in Figure 7.3 as a conceptual framework.

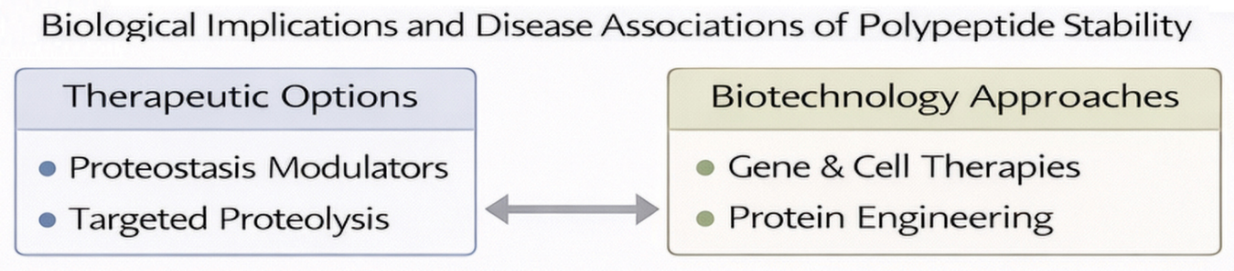

FIGURE 7.3 Major implications of polypeptide. ⏎

7.6.1 PROTEIN MISFOLDING DISORDERS

The most immediate and extensively researched effects of defective polypeptide stability result in protein misfolding disorders. These diseases arise when proteins fail to attain or maintain their native conformations, resulting in the accumulation of misfolded, aggregation-prone species that overwhelm cellular quality control systems. Misfolding does not always cause complete structural destruction because pathogenic proteins maintain their almost native structure while their movement patterns change and their hidden structural elements become visible (Chiti & Dobson, 2017; Knowles et al., 2014).

Neurodegenerative diseases provide particularly compelling examples. In Alzheimer's disease, aberrant folding and aggregation of amyloid-β peptides and tau proteins disrupt neuronal function long before overt neurodegeneration becomes apparent (Hardy & Selkoe, 2002; Selkoe & Hardy, 2016). The proteins use their inherent weaknesses in the proteostasis network which includes aging-based reductions in chaperone function and proteasomal activity to create and maintain their existence within participating neural pathways (Hipp et al., 2019). The same patterns exist in both α-synuclein in Parkinson's disease and huntingtin in Huntington's disease because expanded polyglutamine tracts cause protein structure

collapse which leads to the creation of toxic oligomers (Ross & Poirier, 2004).

The pathogenicity of misfolded proteins depends on their aggregate burden, but other factors also contribute to this disease process. Current research shows that soluble oligomeric intermediates present higher toxic effects on cells than mature fibrils because these intermediates disrupt membrane functions and synaptic communication and internal cellular transport mechanisms (Haass & Selkoe, 2007; Zhang et al., 2023). The discovery has changed treatment methods by moving the focus from clearing aggregates to stabilizing early-stage vulnerable polypeptides.

Protein misfolding causes neurodegenerative diseases and leads to systemic conditions which include cystic fibrosis because the CFTR chloride channel becomes unstable and undergoes early degradation despite having partial operational capacity (Lukacs & Verkman, 2012). The quality control system functions as a dual-purpose mechanism because it prevents defective proteins from building up, yet it removes proteins with partial function which could maintain normal bodily functions. Protein instability leads to disease development because specific polypeptides perform their folding process differently than cellular surveillance systems handle their folding.

7.6.2 Therapeutic Modulation of Protein Stability

The discovery that protein stability functions as a changeable factor for disease development has led to the creation of treatments which restore protein state balance instead of normal gene and protein functions through direct transformation. The methods function at different levels which include both direct polypeptide stabilization and complete cellular folding capacity improvement.

The field uses pharmacological chaperones to demonstrate how scientists create specific methods for protein stabilization. The small molecules create bindings with particular proteins which lead to stabilization of their original or almost original shapes and the proteins develop resistance against destruction routes (Balch et al., 2008). In lysosomal storage disorders such as Fabry disease, pharmacological chaperones have been shown to rescue mutant enzymes by promoting proper folding and lysosomal trafficking, thereby restoring partial enzymatic activity (Lenders et al., 2025).

Scientists now study proteostasis regulators who affect chaperone function and degradation processes and broader stress response systems. The agents restore global folding homeostasis through two mechanisms which include increasing heat shock response signaling and enhancing proteasomal capacity (Díaz-Villanueva et al., 2015; Labbadia & Morimoto, 2015; Margulis et al., 2020). The method creates risks of creating unknown consequences yet shows potential to treat complex diseases which display extensive proteome instability.

The emerging therapeutic approach uses targeted protein degradation to achieve its medicinal effects. The PROTACs technology (proteolysis-targeting chimeras) operates through its system to transfer proteins associated with diseases toward the ubiquitin-proteasome system (Sakamoto et al., 2001). The methods eliminate dangerous proteins by using their destruction systems instead of keeping them safe. The process of protein stability creates a double effect because it maintains health while causing disease through both excessive persistence and premature degradation, which depends on the situation.

The modulation of protein stability requires therapeutic approaches to face the fundamental nature of proteostasis networks which can adapt to different conditions. Cells decrease their long-term productivity through chaperone expression adjustments and degradation rate changes and

translational output modifications which function as compensatory responses to all treatment methods (Hipp et al., 2019). Therapeutic development requires molecular design for successful treatments through system feedback comprehension which helps verify effective outcomes.

7.6.3 Age-Related Protein Degradation Defects

Aging causes a gradual reduction in an organism's ability to maintain proteostasis which results in increased difficulty for maintaining polypeptide stability. The organism experiences this decline because it builds up stress, while its stress response pathways lose their ability to function and its molecular degradation systems become less effective (Brehme et al., 2014; Gao et al., 2024; Kaushik & Cuervo, 2015). The proteins which people managed successfully during their youth period turn into harmful elements for them during their elder years.

Aged cells show reduced capacity for chaperone expression which hampers their ability to combat protein folding problems (Díaz-Villanueva et al., 2015; Labbadia & Morimoto, 2015). The body experiences two problems when its proteasomal system shows decreased function because damaged proteins now stay in the body for longer time periods (Cuervo & Wong, 2014). The changes lead to an environment which allows proteins that have a tendency to form aggregates to exist especially those proteins with regions that have low stability.

The decline of proteostasis in aging people leads to neurodegenerative diseases and metabolic disorders and cardiovascular diseases and immune system dysfunction. The pancreas β-cells develop endoplasmic reticulum stress and insulin resistance through their inability to break down misfolded proteins which links protein stability directly to metabolic health (Ozcan & Tabas, 2012). The process of aging produces different effects on various

proteins in the body. The nuclear pore complex and extracellular matrix contain long-lived proteins which accumulate damage throughout their entire lifetime because these proteins experience structural deterioration (Brehme et al., 2014; Gao et al., 2024; Toyama & Hetzer, 2013). The minor destabilizing events lead to protein persistence which creates severe consequences that reach beyond all initial options because protein persistence produces severe consequences which need early-life proteostasis to prevent additional deterioration.

The lifespan and healthspan of various model organisms increase through interventions which improve protein quality control and include caloric restriction and pharmacological activation of autophagy (Raza, 2024). The results show that healthy aging leads to polypeptide stability protection which creates a situation where aging patterns can be controlled.

7.6.4 Emerging Biotechnological Approaches

The field of biotechnology has reached new heights which enable researchers to study polypeptide stability through advanced methods. High-resolution proteomics now enables researchers to measure protein turnover throughout complete proteomes and discover stability patterns which had remained hidden before (Alamillo et al., 2025; Li et al., 2025). These methods proved traditional protein half-life assumptions wrong by showing that protein half-life changes depending on cell type and developmental stage and environmental conditions.

7.6.4.1 Synthetic Biology

Synthetic biology provides scientists with two different methods which enable them to design proteins that have adjustable stability. Researchers use degron short peptide sequences to create conditional degradation control

systems which enable them to manage protein levels based on predefined external factors (Varshavsky, 2024, 2019). The scientific community adopted these tools to change experimental biology, while these tools become essential for developing therapies that need exact timing control.

7.6.4.2 Machine Learning

Machine learning-assisted protein design enabled scientists to develop new methods which allow them to improve protein stability through design changes that do not affect protein function (Wuyun et al., 2024). Protein development for industrial and biomedical environments benefits from computational predictions which deal with cellular complexity and their experimental validation process.

7.6.4.3 Cell- and Gene-Based Therapies

Cell- and gene-based therapies in medicine and biotechnology require scientists to control protein stability because this process governs treatment success and patient safety. Scientists must develop receptor and enzyme and signaling molecule systems which maintain stability during their therapeutic activity, while their systems need to prevent dangerous effects from extended system operation (Piraner et al., 2024).

The two methods demonstrate a scientific transformation which establishes two new approaches to study polypeptide stability because scientists now regard it as a fundamental design element. Scientists now view this perspective as a way to gain deeper understanding of biological systems and develop more effective therapies which use molecular mechanisms to support bodily functions.

7.7 CONCLUSION

The stability of polypeptides inside living organisms comes from ongoing interactions between different structural components of the proteins. The cell uses its interconnected systems to maintain protein levels through processes that assess protein needs and risk management. The combination of molecular chaperones and assembly-dependent stabilization methods along with quality control pathways and regulatory feedback mechanisms establishes the fate of proteins throughout their entire existence. The biological systems maintain their capacity to adapt through their simultaneous operation which creates a buffer against complex biological systems. The molecular defects that occur during system breakdown lead to a chain reaction which results in diseases that affect the entire body. The understanding of polypeptide stability as a dynamic property that depends on specific contexts transforms protein biology by shifting the focus from fixed protein structures toward their active connections. This perspective establishes a deeper understanding of disease processes while guiding the development of new treatment methods and biotechnological innovations that aim to control protein behavior through controlled processes.

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REFERENCES

Ahmed, T., Sun, X., & Udenigwe, C. (2022). Role of structural properties of bioactive peptides in their stability during simulated gastrointestinal digestion: A systematic review. *Trends in Food Science & Technology*. https://doi.org/10.1016/j.tifs.2022.01.008⏎

Alaei, L., Ashengroph, M., & Moosavi-Movahedi, A. (2021). The concept of protein folding/unfolding and its impacts on human health. *Advances in Protein Chemistry and Structural Biology*, 126, 227–278. https://doi.org/10.1016/bs.apcsb.2021.01.007⏎

Alamillo, L., Black, A., Lam, M., & Lau, E. (2025). Protein turnover dynamics analysis with subcellular spatial resolution. *Bio-Protocol*, 15. https://doi.org/10.21769/bioprotoc.5409⏎

Ali, A., Paracha, S., & Pincus, D. (2024). Preserve or destroy: Orphan protein proteostasis and the heat shock response. *Journal of Cell Biology*, 223(12), e202407123. https://doi.org/10.1083/jcb.202407123⏎

Almeida, A. J., & Souto, E. (2007). Solid lipid nanoparticles as a drug delivery system for peptides and proteins. *Advanced Drug Delivery Reviews*, 59(6), 478–490. https://doi.org/10.1016/j.addr.2007.04.007⏎

Arkinson, C., Dong, K. C., Gee, C. L., Costello, S. M., Soe, A. C., Hura, G. L., Marqusee, S., & Martin, A. (2025). NUB1 traps unfolded FAT10 for ubiquitin-independent degradation by the 26S proteasome. *Nature Structural & Molecular Biology*, 32(9), 1752–1765. https://doi.org/10.1038/s41594-025-01527-3⏎

Ayaz, C., Tepper, L., Brünig, F., Kappler, J., Daldrop, J., & Netz, R. (2021). Non-markovian modeling of protein folding. *Proceedings of the National Academy of Sciences of the United States of America*, 118. https://doi.org/10.1073/pnas.2023856118⏎

Bah, A., Vernon, R., Siddiqui, Z., Krzeminski, M., Muhandiram, R., Zhao, C., Sonenberg, N., Kay, L., & Forman-Kay, J. (2014). Folding of an intrinsically disordered protein by phosphorylation as a regulatory switch. *Nature*, 519, 106–109. https://doi.org/10.1038/nature13999⏎

Balch, W. E., Morimoto, R. I., Dillin, A., & Kelly, J. W. (2008). Adapting proteostasis for disease intervention. *Science*, 319(5865), 916–919. https://doi.org/10.1126/science.1141448⏎

Balch, W. E., Sznajder, J. I., Budinger, S., Finley, D., Laposky, A. D., Cuervo, A. M., Benjamin, I. J., Barreiro, E., Morimoto, R. I., Postow, L., Weissman, A. M., Gail, D., Banks-Schlegel, S., Croxton, T., & Gan, W. (2014). Malfolded protein structure and proteostasis in lung diseases. *American Journal of Respiratory and Critical Care Medicine*, 189(1), 96–103. https://doi.org/10.1164/rccm.201306-1164WS⏎

Balchin, D., Hayer-Hartl, M., & Hartl, F. (2016). In vivo aspects of protein folding and quality control. *Science*, 353. https://doi.org/10.1126/science.aac4354⏎

Banerjee, A., Levy, Y., & Mitra, P. (2019). Analyzing change in protein stability associated with single point deletions in a newly defined protein structure database. *Journal of Proteome Research*, 18(3), 1402–1410. https://doi.org/10.1021/acs.jproteome.9b00048⏎

Bowman, M., Riback, J., Rodríguez, A., Guo, H., Li, J., Sosnick, T., & Clark, P. (2020). Properties of protein unfolded states suggest broad selection for expanded conformational ensembles. *Proceedings of the National Academy of Sciences*, 117, 23356–23364. https://doi.org/10.1073/pnas.2003773117

Brehme, M., Voisine, C., Rolland, T., Wachi, S., Soper, J. H., Zhu, Y., Orton, K., Villella, A., Garza, D., Vidal, M., Ge, H., & Morimoto, R. I. (2014). A chaperome subnetwork safeguards proteostasis in aging and neurodegenerative disease. *Cell Reports*, 9(3), 1135–1150. https://doi.org/10.1016/j.celrep.2014.09.042⏎

Carroll, E., Greene, E., Martin, A., & Marqusee, S. (2020). Site-specific ubiquitination affects protein energetics and proteasomal degradation. *Nature Chemical Biology*, 16, 866–875. https://doi.org/10.1038/s41589-020-0556-3⏎

Chen, X., Hilser, V. J., & Kaiser, C. M. (2025). Arrest peptide profiling resolves co-translational folding pathways and chaperone interactions in vivo. *Nature Communications*, 16(1), 6833. https://doi.org/10.1038/s41467-025-61398-6⏎

Chiti, F., & Dobson, C. M. (2017). Protein misfolding, amyloid formation, and human disease: A summary of progress over the last decade. *Annual Review of Biochemistry*, 86, 27–68. https://doi.org/10.1146/annurev-biochem-061516-045115⏎

Chondrogianni, N., Petropoulos, I., Grimm, S., Georgila, K., Catalgol, B., Friguet, B., Grune, T., & Gonos, E. (2014). Protein damage, repair and proteolysis. *Molecular Aspects of Medicine*, 35, 1–71. https://doi.org/10.1016/j.mam.2012.09.001⏎

Cockram, P., Kist, M., Prakash, S., Chen, S., Wertz, I., & Vučić, D. (2021). Ubiquitination in the regulation of inflammatory cell death and cancer. *Cell Death and Differentiation*, 28, 591–605. https://doi.org/10.1038/s41418-020-00708-5⏎

Colalto, C. (2024). Aspects of complexity in quality and safety assessment of peptide therapeutics and peptide-related impurities. A regulatory perspective. *Regulatory Toxicology and Pharmacology*, 153, 105699. https://doi.org/10.1016/j.yrtph.2024.105699⏎

Corsetti, G., Pasini, E., Scarabelli, T., Romano, C., Singh, A., Scarabelli, C., & Dioguardi, F. (2024). Importance of energy, dietary protein sources, and amino acid composition in the regulation of metabolism: An indissoluble dynamic combination for life. *Nutrients*, 16. https://doi.org/10.3390/nu1615241⏎

Cuervo, A. M., & Wong, E. (2014). Chaperone-mediated autophagy: Roles in disease and aging. *Cell Research*, 24(1), 92–104. https://doi.org/10.1038/cr.2013.153⏎

Deller, M. C., Kong, L., & Rupp, B. (2016). Protein stability: A crystallographer's perspective. *Structural Biology and Crystallization Communications*, 72(2), 72–95. http://doi.org/10.1107/S2053230X15024619⏎

Díaz-Villanueva, J. F., Díaz-Molina, R., & García-González, V. (2015). Protein folding and mechanisms of proteostasis. *International Journal of Molecular Sciences*, 16(8), 17193–17230. https://www.mdpi.com/1422-0067/16/8/17193⏎

Elu, N., Osinalde, N., Ramirez, J., Presa, N., Rodriguez, J. A., Prieto, G., & Mayor, U. (2022). Identification of substrates for human deubiquitinating enzymes (DUBs): An up-to-date review and a case study for neurodevelopmental disorders. *Seminars in Cell & Developmental Biology*. https://doi.org/10.1016/j.semcdb.2022.01.001⏎

Finka, A., Sharma, S., & Goloubinoff, P. (2015). Multi-layered molecular mechanisms of polypeptide holding, unfolding and disaggregation by HSP70/HSP110 chaperones. *Frontiers in Molecular Biosciences*, 2. https://doi.org/10.3389/fmolb.2015.00029⏎

Fleming, A., Bourdenx, M., Fujimaki, M., Karabiyik, C., Krause, G. J., Lopez, A., Martín-Segura, A., Puri, C., Scrivo, A., & Skidmore, J. (2022). The different autophagy degradation pathways and neurodegeneration. *Neuron*, 110(6), 935–966. https://doi.org/10.1016/j.neuron.2022.01.017⏎

Fregno, I., & Molinari, M. (2019). Proteasomal and lysosomal clearance of faulty secretory proteins: ER-associated degradation (ERAD) and ER-to-lysosome-associated degradation (ERLAD) pathways. *Critical Reviews in Biochemistry and Molecular Biology*, 54, 153–163. https://doi.org/10.1080/10409238.2019.1610351⏎

Galan, J., & Haguenauer-Tsapis, R. (1997). Ubiquitin Lys63 is involved in ubiquitination of a yeast plasma membrane protein. *The EMBO Journal*, 16. https://doi.org/10.1093/emboj/16.19.5847⏎

Galano-Frutos, J., & Sancho, J. (2024). Energy, water, and protein folding: a molecular dynamics-based quantitative inventory of molecular interactions and forces that make proteins stable. *Protein Science: A Publication of the Protein Society*, 33. https://doi.org/10.1002/pro.4905⏎

Gao, M., Wu, Y., Yang, X., Wang, J., Hu, X., & Zhou, J. (2024). [Advances of proteostasis network and its stability maintenance mechanism]. *Sheng wu gong cheng xue bao = Chinese Journal of Biotechnology*, 40(2), 434–445. https://doi.org/10.13345/j.cjb.230537⏎

Ghosh, D. K., & Ranjan, A. (2020). The metastable states of proteins. *Protein Science*, 29(7), 1559–1568. https://doi.org/10.1002/pro.3859⏎

Gibbs, D., Bailey, M., & Etherington, R. (2022). A stable start: cotranslational Nt-acetylation promotes proteome stability across kingdoms. *Trends in Cell Biology*. https://doi.org/10.1016/j.tcb.2022.02.004⏎

Gómez-Virgilio, L., Silva-Lucero, M., Flores-Morelos, D., Gallardo-Nieto, J., López-Toledo, G., Abarca-Fernandez, A., Zacapala-Gómez, A., Luna-Muñoz, J., Montiel-Sosa, F., Soto-Rojas, L., Pacheco-Herrero, M., & Cárdenas-Aguayo, M. (2022). Autophagy: A key regulator of homeostasis and disease: an overview of molecular mechanisms and modulators. *Cells*, 11. https://doi.org/10.3390/cells11152262⏎

Gurina, T. S., & Mohiuddin, S. S. (2020). Biochemistry, protein catabolism. In *StatPearls* [Internet]. StatPearls Publishing.⏎

Haass, C., & Selkoe, D. J. (2007). Soluble protein oligomers in neurodegeneration: lessons from the Alzheimer's amyloid beta-peptide. *Nature Reviews Molecular Cell Biology*, 8(2), 101–112. https://doi.org/10.1038/nrm2101⏎

Haliloglu, T., & Bahar, I. (2015). Adaptability of protein structures to enable functional interactions and evolutionary implications. *Current Opinion in Structural Biology*, 35, 17–23. https://doi.org/10.1016/j.sbi.2015.07.007.⏎

Hardy, J., & Selkoe, D. J. (2002). The amyloid hypothesis of Alzheimer's disease: Progress and problems on the road to therapeutics. *Science*, 297(5580), 353–356. https://doi.org/10.1126/science.1072994⏎

Hekmatimoghaddam, S., Zare-Khormizi, M. R., & Pourrajab, F. (2017). Underlying mechanisms and chemical/biochemical therapeutic approaches to ameliorate protein misfolding neurodegenerative diseases. *Biofactors*, 43(6), 737–759. https://doi.org/10.1002/biof.1264⏎

Hipp, M. S., Kasturi, P., & Hartl, F. U. (2019). The proteostasis network and its decline in ageing. *Nature Reviews Molecular Cell Biology*, 20(7), 421–435. https://doi.org/10.1038/s41580-019-0101-y⏎

Hommen, F., Bilican, S., Vilchez, D. 2022. Protein clearance strategies for disease intervention. *Journal of Neural Transmission*, 129(2), 141–172. https://doi.org/10.1007/s00702-021-02431-y.⏎

Izzi, G., Campanile, M., Del Vecchio, P., & Graziano, G. (2024). On the stabilizing effect of aspartate and glutamate and its counteraction by common denaturants. *International Journal of Molecular Sciences*, 25. https://doi.org/10.3390/ijms25179360⏎

Jahn, T., & Radford, S. (2008). Folding versus aggregation: Polypeptide conformations on competing pathways. *Archives of Biochemistry and Biophysics*, 469, 100–117. https://doi.org/10.1016/j.abb.2007.05.015⏎

Jayaraj, G. G., Hipp, M. S., & Hartl, F. U. (2020). Functional modules of the proteostasis network. *Cold Spring Harbor Perspectives in Biology*, 12(1), a033951. https://doi.org/10.1101/cshperspect.a033951⏎

Johnston, H., & Samant, R. (2020). Alternative systems for misfolded protein clearance: life beyond the proteasome. *The FEBS Journal*, 288. https://doi.org/10.1111/febs.15617⏎

Juszkiewicz, S., & Hegde, R. S. (2018). Quality control of orphaned proteins. *Molecular Cell*, 71(3), 443–457. https://doi.org/10.1016/j.molcel.2018.07.001⏎

Kaushik, S., & Cuervo, A. M. (2015). Proteostasis and aging. *Nature Medicine*, 21(12), 1406–1415. https://doi.org/10.1038/nm.4001⏎

Klaips, C., Jayaraj, G., & Hartl, F. (2018). Pathways of cellular proteostasis in aging and disease. *The Journal of Cell Biology*, 217, 51–63. https://doi.org/10.1083/jcb.201709072⏎

Knowles, T. P., Vendruscolo, M., & Dobson, C. M. (2014). The amyloid state and its association with protein misfolding diseases. *Nature Reviews Molecular Cell Biology*, 15(6), 384–396. https://doi.org/10.1038/nrm3810⏎

Kumari, D., & Brodsky, J. (2021). The targeting of native proteins to the endoplasmic reticulum-associated degradation (ERAD) pathway: An expanding repertoire of regulated substrates. *Biomolecules*, 11. https://doi.org/10.3390/biom11081185⏎

Kuznetsov, A. V., Margreiter, R., Ausserlechner, M. J., & Hagenbuchner, J. (2022). The complex interplay between mitochondria, ROS and entire cellular metabolism. *Antioxidants*, 11(10), 1995. https://doi.org/10.3390/antiox11101995⏎

Kuzu, O. F., Granerud, L. J. T., & Saatcioglu, F. (2025). Navigating the landscape of protein folding and proteostasis: From molecular chaperones to therapeutic innovations. *Signal Transduction and Targeted Therapy*, 10(1), 358. https://doi.org/10.1038/s41392-025-02439-w⏎

Labbadia, J., & Morimoto, R. I. (2015). The biology of proteostasis in aging and disease. *Annual Review of Biochemistry*, 84, 435–464. 10.1146/annurev-biochem-060614-033955⏎

Larsen, F. B., Kragelund, B. B., Lindorff-Larsen, K., et al. 2026. Proteasomal control of transcription factors: mechanisms, regulation and dysregulation. *Cellular and Molecular Life Sciences*, 83, 142. https://doi.org/10.1007/s00018-026-06103-6⏎

Lee, J., Hammarén, H., Savitski, M., & Baek, S. (2023). Control of protein stability by post-translational modifications. *Nature Communications*, 14. https://doi.org/10.1038/s41467-023-35795-8⏎

Lenders, M., Menke, E. R., & Brand, E. (2025). Progress and challenges in the treatment of fabry disease. *BioDrugs*, 39(4), 517–535. https://doi.org/10.1007/s40259-025-00723-3⏎

Levine, B., & Kroemer, G. (2019). Biological functions of autophagy genes: A disease perspective. *Cell*, 176, 11–42. https://doi.org/10.1016/j.cell.2018.09.048⏎

Li, D., & Ji, B. (2019). Protein conformational transitions coupling with ligand interactions: Simulations from molecules to medicine. *Medicine in Novel Technology and Devices*, 3, 100026. https://doi.org/10.1016/j.medntd.2019.100026⏎

Li, N., Xie, Y., & Yingling, Y. (2021). Insights into structure and aggregation behavior of elastin-like polypeptide coacervates: all-atom molecular dynamics simulations. *The Journal of Physical Chemistry. B*. https://doi.org/10.1021/acs.jpcb.1c02822

Li, Y., Tian, X., Luo, J., et al. 2024. Molecular mechanisms of aging and anti-aging strategies. *Cell Communication and Signaling*, 22, 285. https://doi.org/10.1186/s12964-024-01663-1⏎

Li, W., Dasgupta, A., Yang, K., Wang, S., Hemandhar-Kumar, N., Chepyala, S., Yarbro, J., Hu, Z., Šalovská, B., Fornasiero, E., Peng, J., & Liu, Y. (2025). Turnover atlas of proteome and phosphoproteome across mouse tissues and brain regions. *Cell*, 188, 2267–2287.e21. https://doi.org/10.1016/j.cell.2025.02.021⏎

Li, X., Perez, R., Ferrie, J., Petersson, E., & Giannakoulias, S. (2025). Accurate prediction of protein tertiary and quaternary stability using fine-tuned protein language models and free energy

perturbation. *International Journal of Molecular Sciences*, 26. https://doi.org/10.3390/ijms26157125

Liao, Y., Zhang, W., Liu, Y., Zhu, C., & Zou, Z. (2024). The role of ubiquitination in health and disease. *MedComm*, 5(10), e736. https://doi.org/10.1002/mco2.736⏎

Liu, B.-H., Xu, C.-Z., Liu, Y., Lu, Z.-L., Fu, T.-L., Li, G.-R., Deng, Y., Luo, G.-Q., Ding, S., & Li, N. (2024). Mitochondrial quality control in human health and disease. *Military Medical Research*, 11(1), 32. https://doi.org/10.1186/s40779-024-00536-5⏎

Liu, F., Chen, J., Li, K., Li, H., Zhu, Y., Zhai, Y., Lu, B., Fan, Y., Liu, Z., Chen, X., Jia, X., Dong, Z., & Liu, K. (2024). Ubiquitination and deubiquitination in cancer: from mechanisms to novel therapeutic approaches. *Molecular Cancer*, 23. https://doi.org/10.1186/s12943-024-02046-3

Lukacs, G. L., & Verkman, A. S. (2012). CFTR: Folding, misfolding and correcting the ΔF508 conformational defect. *Trends in Molecular Medicine*, 18(2), 81–91. https://doi.org/10.1016/j.molmed.2011.10.003⏎

Majeski, A., & Dice, J. (2004). Mechanisms of chaperone-mediated autophagy. *The International Journal of Biochemistry & Cell Biology*, 36(12), 2435–2444. https://doi.org/10.1016/j.biocel.2004.02.013⏎

Mandal, S., Kumar, S., Roy, S., Mahapatra, S., & Das, A. (2025). Interplay between intrinsic propensities of amino acids, backbone hydrogen bonding, and solvent effects governs the secondary structures of peptides. *The Journal of Physical Chemistry Letters*, 12026–12035. https://doi.org/10.1021/acs.jpclett.5c03157⏎

Margulis, B., Tsimokha, A., Zubova, S., & Guzhova, I. (2020). Molecular chaperones and proteolytic machineries regulate protein homeostasis in aging cells. *Cells*, 9. https://doi.org/10.3390/cells9051308⏎

Mchaourab, H., Godar, J., & Stewart, P. (2009). Structure and mechanism of protein stability sensors: Chaperone activity of small heat shock proteins. *Biochemistry*, 48(18), 3828–3837. https://doi.org/10.1021/bi900212j⏎

Mehrpour, M., Esclatine, A., Beau, I., & Codogno, P. (2010). Overview of macroautophagy regulation in mammalian cells. *Cell Research*, 20(7), 748–762. https://doi.org/10.1038/cr.2010.82⏎

Metwally, E., Zhao, G., Zhang, Y. Q. 2021. The calcium-dependent protease calpain in neuronal remodeling and neurodegeneration. *Trends in Neurosciences*, 44(9), 741–752. https://doi.org/10.1016/j.tins.2021.07.003⏎

Mizushima, N., Levine, B., Cuervo, A. M., & Klionsky, D. J. (2008). Autophagy fights disease through cellular self-digestion. *Nature*, 451(7182), 1069–1075. https://doi.org/10.1038/nature06639⏎

Moon, S., Lee, B. H. 2018. Chemically induced cellular proteolysis: An emerging therapeutic strategy for undruggable targets. *Molecules and Cells*, 41(11), 933–942. https://doi.org/10.14348/molcells.2018.0372.⏎

Morey, T., Esmaeili, M., Duennwald, M., & Rylett, R. (2021). SPAAC pulse-chase: A novel click chemistry-based method to determine the half-life of cellular proteins. *Frontiers in Cell and Developmental Biology*, 9. https://doi.org/10.3389/fcell.2021.722560⏎

Morimoto, R. (2019). Cell-nonautonomous regulation of proteostasis in aging and disease. *Cold Spring Harbor Perspectives in Biology*. https://doi.org/10.1101/cshperspect.a034074⏎

Morimoto, R. I. (2020). Cell-nonautonomous regulation of proteostasis in aging and disease. *Cold Spring Harbor Perspectives in Biology*, 12(4), a034074. https://doi.org/10.1101/cshperspect.a034074⏎

Mortimore, G. E., & Pösö, A. R. (1987). Intracellular protein catabolism and its control during nutrient deprivation and supply. *Annual Review of Nutrition*, 7, 539–564. https://doi.org/10.1146/annurev.nu.07.070187.002543⏎

Needham, P. G., & Brodsky, J. L. (2013). How early studies on secreted and membrane protein quality control gave rise to the ER associated degradation (ERAD) pathway: The early history of ERAD. *Biochimica et Biophysica Acta*, 1833(11), 2447–2457. https://doi.org/10.1016/j.bbamcr.2013.03.018⏎

Nillegoda, N. B., Stank, A., Malinverni, D., Alberts, N., Szlachcic, A., Barducci, A., De Los Rios, P., Wade, R. C., & Bukau, B. (2017). Evolution of an -protein network driving protein disaggregation in eukaryotes. *Elife*, 6, e24560. https://doi.org/10.7554/eLife.24560⏎

Ozcan, L., & Tabas, I. (2012). Role of endoplasmic reticulum stress in metabolic disease and other disorders. *Annual Review of Medicine*, 63, 317–328. https://doi.org/10.1146/annurev-med-043010-144749⏎

Pak, M., Markhieva, K., Novikova, M., Petrov, D., Vorobyev, I., Maksimova, E., Kondrashov, F., & Ivankov, D. (2021). Using AlphaFold to predict the impact of single mutations on protein stability and function. *PLOS ONE*, 18. https://doi.org/10.1371/journal.pone.0282689⏎

Panja, A., Maiti, S., & Bandyopadhyay, B. (2020). Protein stability governed by its structural plasticity is inferred by physicochemical factors and salt bridges. *Scientific Reports*, 10. https://doi.org/10.1038/s41598-020-58825-7⏎

Piraner, D., Abedi, M., Gonzalez, M., Chazin-Gray, A., Lin, A., Zhu, I., Ravindran, P., Schlichthaerle, T., Huang, B., Bearchild, T., Lee, D., Wyman, S., Jun, Y., Baker, D., & Roybal, K. (2024). Engineered receptors for soluble cellular communication and disease sensing. *Nature*, 638, 805–813. https://doi.org/10.1038/s41586-024-08366-0⏎

Pohl, C., & Dikič, I. (2019). Cellular quality control by the ubiquitin-proteasome system and autophagy. *Science*, 366, 818–822. https://doi.org/10.1126/science.aax3769⏎

Popov, L. D. (2020). Mitochondrial biogenesis: An update. *Journal of Cellular and Molecular Medicine*, 24(9), 4892–4899. https://doi.org/10.1111/jcmm.15194⏎

Rabinowitz, J., & White, E. (2010). Autophagy and metabolism. *Science*, 330, 1344–1348. https://doi.org/10.1126/science.1193497⏎

Rahban, M., Zolghadri, S., Salehi, N., Ahmad, F., Haertlé, T., Rezaei-Ghaleh, N., Sawyer, L., & Saboury, A. (2022). Thermal stability enhancement: Fundamental concepts of protein engineering strategies to manipulate the flexible structure. *International Journal of Biological Macromolecules*. https://doi.org/10.1016/j.ijbiomac.2022.06.154⏎

Ravichandran, A., Puri, A., Bhate, S. H., Habibullah, B. I., Singh, G., & Das, R. (2025). Structure-guided engineering of protein stability through core hydrophobicity. *Protein Science*, 34(12), e70360. https://doi.org/10.1002/pro.70360⏎

Raza, S. (2024). Autophagy and metabolic aging: Understanding and future applications. *Biochimica et Biophysica Acta Molecular Cell Research*, 1871(6), 119753. https://doi.org/10.1016/j.bbamcr.2024.119753⏎

Ross, C. A., & Poirier, M. A. (2004). Protein aggregation and neurodegenerative disease. *Nature Medicine*, 10, S10–S17. https://doi.org/10.1038/nm1066⏎

Rukhlenko, O. S., Halasz, M., Rauch, N., et al. 2022. Control of cell state transitions. *Nature* 609, 975–985. https://doi.org/10.1038/s41586-022-05194-y⏎

Sakamoto, K. M., Kim, K. B., Kumagai, A., Mercurio, F., Crews, C. M., & Deshaies, R. J. (2001). Protacs: Chimeric molecules that target proteins to the -F box complex for ubiquitination and degradation. *Proceedings of the National Academy of Sciences of the United States of America*, 98(15), 8554–8559. https://doi.org/10.1073/pnas.141230798⏎

Sala, A. J., Bott, L. C., & Morimoto, R. I. (2017). Shaping proteostasis at the cellular, tissue, and organismal level. *The Journal of Cell Biology*, 216(5), 1231–1241. https://doi.org/10.1083/jcb.201612111⏎

Salomo-Coll, C., Jiménez-Moreno, N., & Wilkinson, S. (2025). Lysosomal degradation of ER client proteins by ER-phagy and related pathways. *Journal of Molecular Biology*, 169035. https://doi.org/10.1016/j.jmb.2025.169035⏎

Selkoe, D. J., & Hardy, J. (2016). The amyloid hypothesis of Alzheimer's disease at 25 years. *EMBO Molecular Medicine*, 8(6), 595–608. https://doi.org/10.15252/emmm.201606210⏎

Sinnige, T., Yu, A., & Morimoto, R. I. (2020). Challenging proteostasis: Role of the chaperone network to control aggregation-prone proteins in human disease. *Advances in Experimental Medicine and Biology*, 1243, 53–68. https://doi.org/10.1007/978-3-030-40204-4_4⏎

Spano, D., & Catara, G. (2023). Targeting the ubiquitin-proteasome system and recent advances in cancer therapy. *Cells*, 13(1), 29. https://doi.org/10.3390/cells13010029⏎

Stollar, E. J., & Smith, D. P. (2020). Uncovering protein structure. *Essays in Biochemistry*, 64(4), 649–680. https://doi.org/10.1042/EBC20190042⏎

Sumikama, R., Shibata, Y., Wu, R., & Murata, S. (2026). Recent insights into mechanisms regulating the proteasome: Implications for human disease. *Molecular and Cellular Biology*, 46(5), 487–504. https://doi.org/10.1080/10985549.2025.2587085⏎

Toldrá, F., Reig, M., Aristoy, M., & Mora, L. (2017). Generation of bioactive peptides during food processing. *Food Chemistry*, 267, 395–404. https://doi.org/10.1016/j.foodchem.2017.06.119⏎

Toyama, B. H., & Hetzer, M. W. (2013). Protein homeostasis: Live long, won't prosper. *Nature Reviews of Molecular Cell Biology*, 14(1), 55–61. https://doi.org/10.1038/nrm3496⏎

Uto, T., Suenaga, H., & Kaneko, Y. (2025). Solvent-induced stabilization and folding pathways of α-helical peptides: A computational investigation using steered molecular dynamics. *Chemistry*, e02163. https://doi.org/10.1002/chem.202502163⏎

Uversky, A. V., & Uversky, V. N. (2014). Amino acid code for potein folding, misfolding, and non-folding. In M. Ryadnov & E. Farkas (Eds.), *Amino Acids, Peptides and Proteins* (Vol. 39). The Royal Society of Chemistry. https://doi.org/10.1039/9781849739962-00192⏎

Uversky, V. (2015). Functional roles of transiently and intrinsically disordered regions within proteins. *The FEBS Journal*, 282. https://doi.org/10.1111/febs.13202⏎

Varshavsky, A. (2019). N-degron and C-degron pathways of protein degradation. *Proceedings of National Academy of Sciences of the United States of America*, 116(2), 358–366. https://doi.org/10.1073/pnas.1816596116⏎

Varshavsky, A. (2024). N-degron pathways. *Proceedings of National Academy of Sciences of the United States of America*, 121(39), e2408697121. https://doi.org/10.1073/pnas.2408697121⏎

Venkatraman, J., Shankaramma, S., & Balaram, P. (2001). Design of folded peptides. *Chemical Reviews*, 101(10), 3131–52. https://doi.org/10.1021/cr000053z⏎

Wang, H., Inoue, A., Lei, Y., Wu, H., Hong, L., & Cheng, X. W. (2023). Cathepsins in the extracellular space: Focusing on non-lysosomal proteolytic functions with clinical implications. *Cellular Signalling*, 103, 110531. https://doi.org/10.1016/j.cellsig.2022.110531⏎

Wang, R., Lan, C., Benlagha, K., Câmara, N., Miller, H., Kubo, M., Heegaard, S., Lee, P., Yang, L., Forsman, H., Li, X., Zhai, Z., & Liu, C. (2024). The interaction of innate immune and adaptive immune system. *MedComm*, 5. https://doi.org/10.1002/mco2.714⏎

Wang, S., Osgood, A., & Chatterjee, A. (2022). Uncovering post-translational modification-associated protein-protein interactions. *Current Opinion in Structural Biology*, 74, 102352. https://doi.org/10.1016/j.sbi.2022.102352⏎

Wolf, D. H., & Menssen, R. (2018). Mechanisms of cell regulation–proteolysis, the big surprise. *FEBS Letters*, 592(15), 2515–2524. https://doi.org/10.1002/1873-3468.13109⏎

Wuyun, Q., Chen, Y., Shen, Y., Cao, Y., Hu, G., Cui, W., Gao, J., & Zheng, W. (2024). Recent progress of protein tertiary structure prediction. *Molecules*, 29(4), 832. https://doi.org/10.3390/molecules29040832⏎

Xian, Y., Ye, J., Tang, Y., Zhang, N., Peng, C., Huang, W., & He, G. (2024). Deubiquitinases as novel therapeutic targets for diseases. *MedComm*, 5(12), e70036. https://doi.org/10.1002/mco2.70036⏎

Yan, S., Xu, J., Zhang, S., & Li, Y. (2021). Effects of flexibility and surface hydrophobicity on emulsifying properties: Ultrasound-treated soybean protein isolate. *Lwt - Food Science and Technology*, 142, 110881. https://doi.org/10.1016/j.lwt.2021.110881⏎

Yang, Y., Peng, H., Meng, D., Fa, Z., Yao, C., Lin, X., Schick, J., & Jin, X. (2025). Stress management: How the endoplasmic reticulum mitigates protein misfolding and oxidative stress by the dual role of glutathione peroxidase 8. *Biomolecules*, 15. https://doi.org/10.3390/biom15060847⏎

Yim, W., & Mizushima, N. (2020). Lysosome biology in autophagy. *Cell Discovery*, 6. https://doi.org/10.1038/s41421-020-0141-7⏎

Yoo, Y. J., Feng, Y., Kim, Y. H., Yagonia, C. F. J. 2017. Specificity of enzymes. In *Fundamentals of Enzyme Engineering*. Springer. https://doi.org/10.1007/978-94-024-1026-6_10⏎

Zapadka, K. L., Becher, F. J., Gomes dos Santos, A., & Jackson, S. E. (2017). Factors affecting the physical stability (aggregation) of peptide therapeutics. *Interface Focus*, 7(6). https://doi.org/10.1098/rsfs.2017.0030⏎

Zdancewicz, S., Maldosevic, E., Malezyna, K., & Jomaa, A. (2025). NAC couples protein synthesis with nascent polypeptide myristoylation on the ribosome. *The EMBO Journal*, 44(22), 6320–6342. https://doi.org/10.1038/s44318-025-00548-4⏎

Zeng, X., Ruff, K., & Pappu, R. (2022). Competing interactions give rise to two-state behavior and switch-like transitions in charge-rich intrinsically disordered proteins. *Proceedings of the National Academy of Sciences of the United States of America*, 119. https://doi.org/10.1101/2022.01.11.475920⏎

Zhang, Y., Chen, H., Li, R., Sterling, K., & Song, W. (2023). Amyloid β-based therapy for Alzheimer's disease: Challenges, successes and future. *Signal Transduction and Targeted Therapy*, 8(1), 248. https://doi.org/10.1038/s41392-023-01484-7⏎

Zhao, L., Zhao, J., Zhong, K., Tong, A., & Jia, D. (2022). Targeted protein degradation: Mechanisms, strategies and application. *Signal Transduction and Targeted Therapy*, 7. https://doi.org/10.1038/s41392-022-00966-4⏎

Zhong, G., Chang, X., Xie, W., & Zhou, X. (2024). Targeted protein degradation: Advances in drug discovery and clinical practice. *Signal Transduction and Targeted Therapy*, 9(1), 308. https://doi.org/10.1038/s41392-024-02004-x⏎

Zhong, Q., Xiao, X., Qiu, Y., Xu, Z., Chen, C., Chong, B., Zhao, X., Hai, S., Li, S., An, Z., & Dai, L. (2023). Protein posttranslational modifications in health and diseases: Functions, regulatory mechanisms, and therapeutic implications. *MedComm*, 4. https://doi.org/10.1002/mco2.261⏎

Zhou, P. (2004). Determining protein half-lives. In *Signal Transduction Protocols* (pp. 67–77). Springer. https://doi.org/10.1385/1-59259-816-1:067⏎

Zhu, Y., Fu, S., Changling, W., Qi, B., Teng, F., Li, Y., & Jiang, L. (2020). The investigation of protein flexibility of various soybean cultivars in relation to physicochemical and conformational properties. *Food Hydrocolloids*. https://doi.org/10.1016/j.foodhyd.2020.105709⏎

8 Clinical Application of Peptides in Disease Management

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8.1 INTRODUCTION

From their early empirical applications, such as the use of venom-derived compounds in traditional medicine, to the pivotal isolation of insulin in 1921, peptides have progressively evolved into rationally engineered therapeutic agents (see Figure 8.1). Peptides are a structurally diverse class of biomolecules, defined by covalent peptide bonds linking amino acid residues in sequences, typically ranging from 10 to 100 residues [1–4]. Structurally analogous to proteins, peptides are distinguished by their lower molecular weights, generally below 10 kDa, and serve a wide range of physiological roles. These include endogenous hormones, neurotransmitters, ion channel ligands, growth factors, and innate immune effectors with significant antimicrobial properties [1, 2]. Nonetheless, their integration into mainstream pharmacotherapy has highlighted persistent tensions between biological promise and translational feasibility, particularly in terms of manufacturing complexity and economic viability. Therapeutic peptides

occupy an intermediary niche between small-molecule drugs and large biologics, offering distinct pharmacological advantages and drawbacks [5] (Table 8.1).

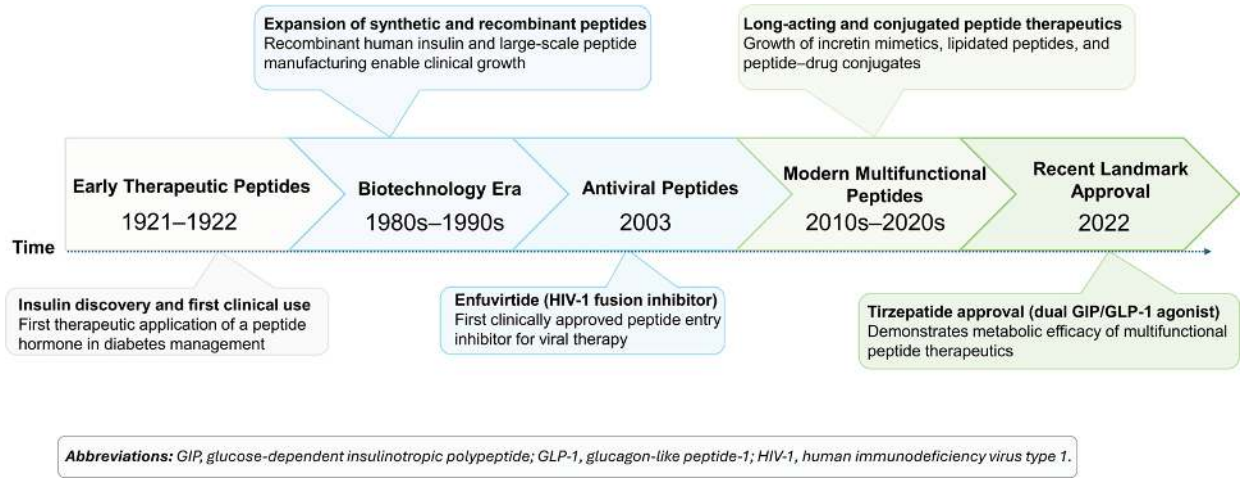


FIGURE 8.1 Historical evolution of therapeutic peptides. Chronological progression of peptide therapeutics from the clinical introduction of insulin (1921–1922) to contemporary multifunctional peptide platforms. Milestones include recombinant peptide biotechnology expansion, antiviral entry inhibitors (e.g., enfuvirtide), long-acting and conjugated peptide strategies, and recent landmark approvals such as tirzepatide, illustrating the transition toward metabolically and structurally engineered peptide systems. ⏎

TABLE 8.1

Comparative Properties of Peptide Therapeutics, Small Molecules, and Monoclonal Antibodies ⏎

Property	Small Molecules	Peptides	Monoclonal Antibodies (mAbs)
Molecular Weight (MW)	Typically, < 900 Da [6].	~1–5 kDa [7]	> 150 kDa [8]

Property	Small Molecules	Peptides	Monoclonal Antibodies (mAbs)
Oral Bioavailability	High (30–90%) [9]	Low (<2%), unless specially formulated [10]	None; degraded in the gastrointestinal tract
Plasma Half-life	Short (typically hours)	Short to moderate (minutes to hours)	Long (typically days to weeks)
Target Specificity	Moderate; may bind multiple targets	High; often receptor or enzyme-specific	Very high; typically binds a single antigen
Blood–Brain Barrier (BBB) Penetration	Often crosses passively (lipophilic drugs)	Generally poor; rare exceptions via structural design [11]	Absent; does not cross BBB without modification
Route of Administration	Primarily oral	SC, IM, IV, nasal; few oral (e.g., SNAC-enabled)	IV or SC injection only
Immunogenicity Risk	Low to moderate; usually well tolerated	Moderate; dependent on sequence, origin, and formulation	High; especially for chimeric or non-humanized antibodies
Production Cost	Low (chemical synthesis; scalable)	Medium (synthetic or recombinant processes)	Very high (requires mammalian cell culture and purification)
Structural Complexity	Low; chemically well-defined	Moderate; sensitive to hydrolysis and aggregation	Very high; requires correct folding and glycosylation
Regulatory Pathway	Well-established (including generics/ANDA)	Requires novel CMC, stability, and delivery data	Stringent biologics pathway (BLA submission under FDA/EMA)

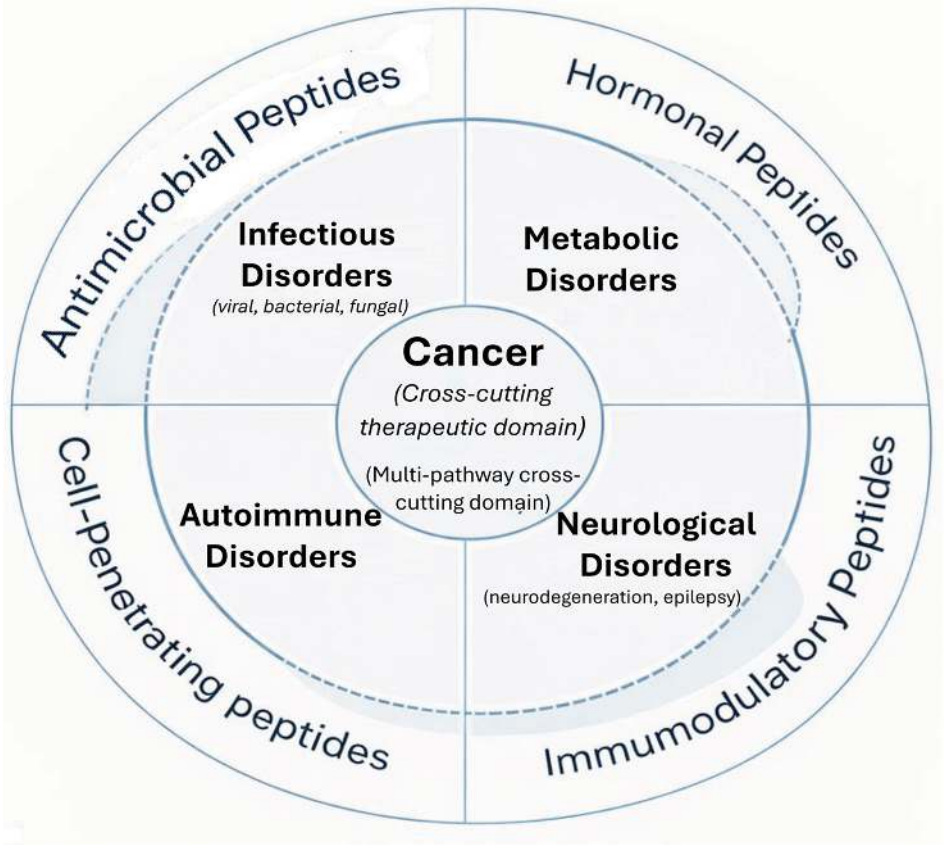
Over the past two decades, significant advancements in peptide synthesis and biological characterization have reinforced the importance of peptides as

therapeutic candidates. Their high specificity and modular structure enable selective targeting across numerous biological pathways, rendering them suitable for diverse disease contexts (see Table 8.2). Peptide-based therapeutics are currently under investigation or are in use for conditions such as cancer, infectious diseases, metabolic syndromes, and cardiovascular dysfunction. Their pharmacological advantages include reduced immunogenicity, ease of structural modification, and depending on composition moderate membrane permeability. However, their broader clinical utility is frequently limited by inherent liabilities such as rapid enzymatic degradation and short plasma half-life [12]. Therapeutic peptide development frequently builds upon endogenous peptide scaffolds, leveraging their naturally high target affinities and minimal off-target interactions. Notably, peptides often demonstrate higher clinical approval rates than small molecules, a trend largely attributed to their target fidelity and biological compatibility [2]. A central challenge to peptide drug development is poor oral bioavailability, primarily due to enzymatic degradation and limited epithelial permeability within the gastrointestinal tract, which is primarily attributed to enzymatic degradation in the gastrointestinal tract and limited translocation across the intestinal epithelium. Even after absorption, peptides are prone to rapid plasma hydrolysis, which contributes to a short systemic half-life and limited therapeutic exposure. These pharmacokinetic barriers necessitate non-oral administration routes and often require advanced formulation strategies to maintain efficacy. Consequently, recent efforts have focused on developing delivery systems that enhance stability and prolong systemic exposure, thereby improving therapeutic profiles (see Figure 8.2) [13].

TABLE 8.2

Examples of Clinically Approved or Investigational Peptide Therapeutics by Indication

Platform	Mechanism	Pharmacokinetic Advantage	Clinical Status
PEGylation	Covalent attachment of polyethylene glycol (PEG) chains to peptide termini or lysine residues [14]	↑ Circulation half-life, ↓ renal clearance, ↓ immunogenicity	Approved (e.g., PEG–interferon-α, Pegvisomant)
SNAC (sodium N-[8-(2-hydroxybenzoyl) amino] caprylate)	Enhances transcellular absorption via membrane fluidization and localized protease inhibition [15]	↑ Oral bioavailability; enables systemic peptide delivery via stomach	Approved (e.g., Oral semaglutide – *Rybelsus*®)
Liposomes	Encapsulation of peptides within phospholipid bilayers {118}	Protection from proteolysis; tunable tissue targeting via formulation	Investigational (active in oncology, antimicrobial R&D pipelines)
Fusion proteins	Genetic fusion with albumin or Fc domain of IgG to exploit FcRn recycling [16]	↑ Plasma half-life, ↓ dosing frequency	Approved (e.g., Dulaglutide, Albiglutide)
Implant systems	Biodegradable or polymer-based subcutaneous depots for sustained peptide release [17]	Zero-order or near-zero-order kinetics; ↑ patient compliance	Approved (e.g., *Zoladex*® – goserelin acetate depot implant)



Dotted arcs indicate predominant mechanistic, pharmacodynamic, and translational overlaps between peptide classes and disease domains.

Abbreviations:
AMPs, antimicrobial peptides; CPPs, cell-penetrating peptides; GLP-1, glucagon-like peptide-1.

FIGURE 8.2 Therapeutic landscape of peptide modalities. Conceptual mapping of major therapeutic applications of peptide-based drugs, including endocrine, antimicrobial, antiviral, oncologic, and metabolic indications. The schematic highlights the expanding pharmacological scope of peptides beyond hormone replacement toward multifunctional and targeted therapeutic platforms. ⏎

While peptides are widely valued for their precision and tolerability, the ongoing debate surrounds their scalability and economic feasibility relative to small-molecule drugs and monoclonal antibodies. This dual tension of biochemical sophistication versus pragmatic scalability frames much of contemporary discourse on peptide-based interventions.

8.2 PEPTIDE-BASED THERAPEUTIC AGENTS

The modern trajectory of peptide therapeutics began with the landmark discovery of insulin, followed by a steady expansion driven by natural compound isolation, synthetic refinement, and recombinant engineering, all contributing to the growth of peptides within contemporary drug development. Currently, more than 80 peptide-based drugs have received regulatory approval worldwide, including a notable subset of non-insulin agents, such as oxytocin, vasopressin, and others with neuroendocrine or metabolic indications [18–20]. Over 200 candidates are currently in clinical evaluation, with 600 compounds undergoing preclinical testing, an indication of increasing pharmaceutical interest.

These figures suggest growing investment, yet they also reveal how peptides still lag behind more dominant platforms in terms of absolute volume and late-stage development (Figure 8.4) [13, 21]. While encouraging, this output remains modest relative to the expansive pipelines of small molecules and the rapid rise in monoclonal antibody therapeutics. The development of peptide-based agents continues to be shaped by a dual reality: high pharmacological precision on the one hand, and delivery-related challenges on the other (Table 8.3).

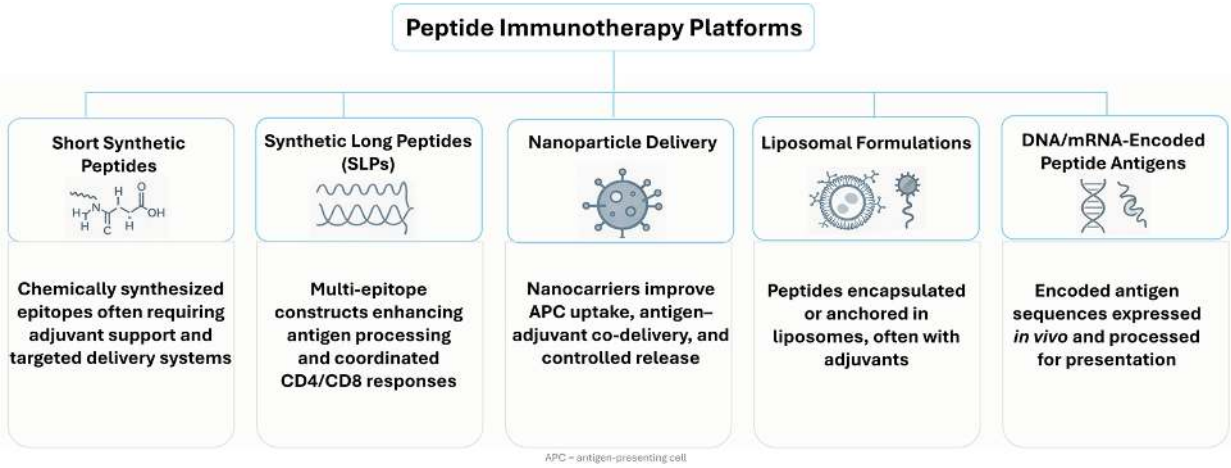


FIGURE 8.4 Peptide immunotherapeutic strategies. Mechanistic overview of peptide-based immunomodulatory platforms, including vaccine epitopes, immune checkpoint modulators, tumor-targeting peptides, and T-cell engaging constructs. The diagram illustrates the integration of antigen specificity, immune activation, and delivery optimization. ⏎

TABLE 8.3

Representative Classes of Therapeutic Peptides: Origins, Structures, and Pharmacological Roles ⏎

Peptide Class	Biological Origin	Structural Feature	Pharmacological Role	Example Drugs
Hormonal	Endogenous peptides (e.g., pancreatic, pituitary hormones)	Short linear or mildly cyclic; often with disulfide bridges	Mimic or supplement physiological hormones	Insulin, GLP-1 analogs (e.g., Exenatide, Liraglutide) [18, 19]
Antimicrobial (AMPs)	Evolutionarily conserved sequences from amphibians, insects, mammals	Amphipathic α-helix or β-sheet structures	Direct microbial lysis via membrane disruption	Pexiganan (topical), LL-37 [22, 23]
Tumor-targeting	Derived from venoms or rationally designed synthetic peptides	Cyclic RGD motifs; disulfide-bonded loops	Target integrins or tumor vasculature (e.g., αvβ3 receptors)	Cilengitide (αvβ3 integrin antagonist) [24].

Peptide Class	Biological Origin	Structural Feature	Pharmacological Role	Example Drugs
Cell-penetrating (CPPs)	Synthetic or derived from viral/bacterial proteins	Short, cationic; rich in arginine and lysine residues	Facilitate intracellular delivery of drugs/nucleic acids	TAT-peptide conjugates, penetratin-based systems [25, 26].
Immunomodulatory	Naturally occurring thymic peptides or cytokine fragments	Short sequences (5–20 amino acids); may be C-terminally amidated	Enhance, modulate, or restore immune function	Thymosin alpha-1 (Zadaxin®) for hepatitis B/C [15].

The search for precise and well-tolerated therapies continues to draw attention to peptides, both those found in nature and those redesigned in the laboratory. In recent years, peptide research has expanded rapidly, covering conditions as different as antibiotic-resistant infections, cancers, and metabolic diseases [4, 27].

Perhaps the strongest appeal of peptide drugs lies in their selectivity. Because they fit their targets so closely, these molecules often trigger fewer unwanted side effects [22, 28]. Unlike many small molecules, peptides are metabolized through clearer routes and rarely wander into off-target territory. Yet, their promise is tempered by practical difficulties. Many peptides are swiftly degraded by enzymes or cleared from the bloodstream before they can act [13, 14, 21].

That fragility makes oral dosing unreliable and shortens their window of action.

Chemists have countered these problems by reshaping peptide structures to last longer in circulation. They tweak backbones and side chains or

sometimes close a flexible chain into a stable ring. These adjustments make the molecules far tougher against enzymatic attack without sacrificing activity [15].

The reward is a sturdier, longer-lived therapeutic.

Peptides now expand to touch almost every medical field, from infection control to oncology and diabetes, demonstrating how smart molecular design can turn a simple chain of amino acids into a versatile class of drugs.

8.2.1 Antimicrobial Peptides

Natural peptide antibiotics form the backbone of infection control, especially when bacteria outsmart conventional drugs. Bacitracin, colistin, gramicidin D, and vancomycin remain the best-known examples; each strikes pathogens on multiple fronts, damaging cell membranes or crippling essential enzymes. However, their systemic use remains limited by notable cytotoxicity, necessitating topical or narrowly defined applications, which restrict their use primarily to topical formulations. In some instances, these compounds have been reoriented toward other therapeutic domains, such as the use of dactinomycin in oncology [29, 30]. Like other antimicrobial categories, peptide antibiotics are not immune to the escalating threat of resistance driven by microbial genetic plasticity [6, 16]. The accelerated evolution of microbial genomes continues to undermine the structural stability and therapeutic longevity of both established and emerging antimicrobial peptides (AMPs). Most clinically relevant antimicrobial peptides exhibit cyclic structures, which enhance both their stability and specificity, and gramicidin D is a notable exception. Linear peptides often demonstrate greater potency *in vitro*, but are generally less stable and more cytotoxic, thus limiting their clinical applicability, and reduce their effective lifetimes [9].

Though cyclization confers resistance to enzymatic degradation, it may reduce conformational flexibility, potentially impairing efficacy against rapidly mutating microbial targets in the face of rapidly mutating microbial targets, a dilemma that continues to provoke debate in antimicrobial peptide design (Figure 8.3).

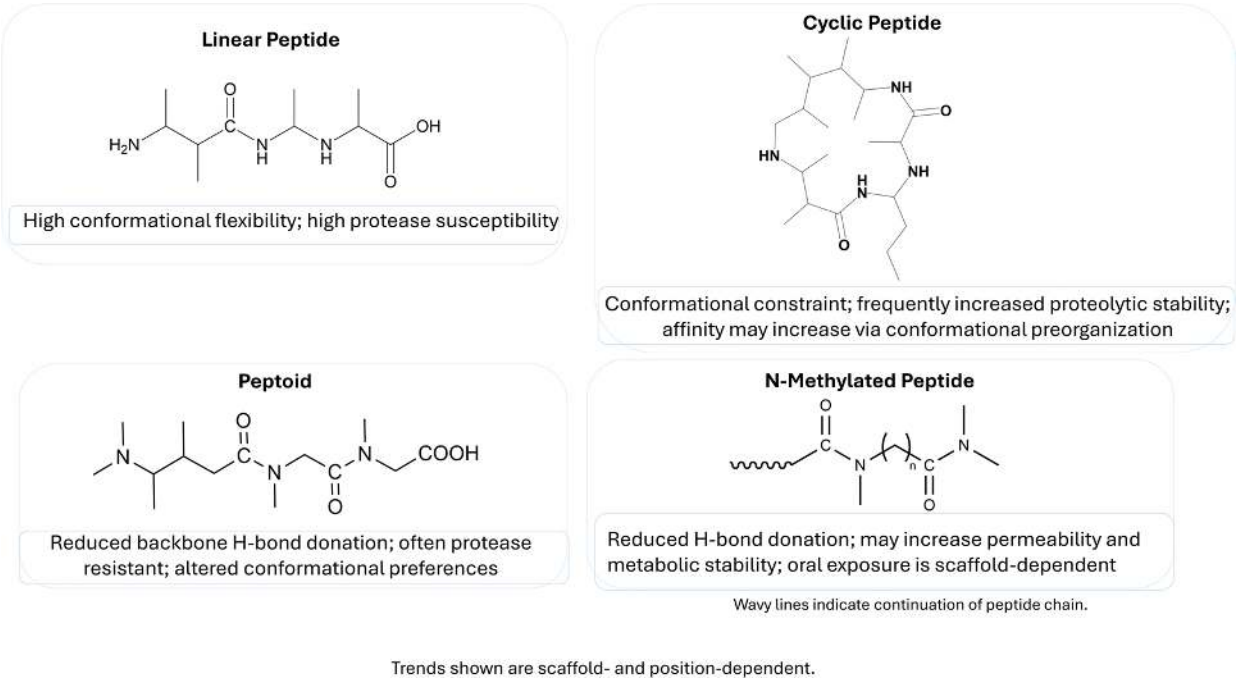


FIGURE 8.3 Structural categories of therapeutic peptides. Schematic representation of major peptide architectures, including linear peptides, cyclic peptides, stapled helices, peptidomimetics, and conjugated formats. Structural topology directly influences conformational rigidity, metabolic stability, receptor selectivity, and pharmacokinetic behavior. ⏎

Lantibiotics are polycyclic peptides characterized by the inclusion of lanthionine and methyllanthionine amino acids cyclized through a thioether linker [7]. They are synthesized by Gram-positive bacteria as defense molecules against competitor Gram-positive bacteria, inhibiting their cell membrane and cell wall synthesis. These compounds have emerged as

promising solutions against resistant bacterial infections [10]. Furthermore, there are many antibacterial polypeptides in clinical trials, of which PL-18 is the most recently reported polypeptide for bacterial infection, where *in vivo* and *in vitro* studies revealed strong activity against both gram-positive and gram-negative bacteria, with MIC values of 1–4 μM [31]. Among the recent polymyxin derivatives, SPR206 (EVER 206) stands out for a simple reason: it was built to keep the antibacterial power of the older drugs while sparing the kidneys. In laboratory tests, it shows strong activity against difficult, multidrug- and extensively drug-resistant Gram-negative species—*Pseudomonas aeruginosa, Acinetobacter baumannii*, and members of the *Enterobacteriaceae* family [8]. A related compound, QPX9003 (BRII 693), is being advanced as an intravenous treatment for infections caused by *Acinetobacter* and *Pseudomonas* species. The goal is the same: broad antimicrobial coverage with far less renal toxicity. Phase-I studies are underway in the United States, and partner groups in China are testing it against resistant *E. coli* and *Pseudomonas aeruginosa* [32]. Murepavadin, a synthetic 14-residue cyclic peptide modeled after protegrin I, takes a different approach. It locks onto the outer-membrane protein LptD, an essential conduit for lipopolysaccharide transport, and in doing so cripples the bacterial cell envelope. Clinical trials are assessing its value against infections caused by *Klebsiella pneumoniae, A. baumannii, P. aeruginosa*, and *Enterobacter* spp. [33]. Another candidate, OMN6, evolved from the *cecropin A* scaffold and has been redesigned to attack carbapenem-resistant *A. baumannii* and other Gram-negative strains [34].

Beyond bacteria. The same design logic is now being used against viruses. Peptides can interrupt viral life cycles by blocking conserved host–pathogen interactions, offering a flexible platform for future antivirals.

8.2.2 Antiviral Peptides

Viral diseases remain a global concern, so researchers continue to search for peptide-based approaches that can interfere with replication at multiple points in the cycle [35]. A number of natural peptides already show this capacity, acting at stages that range from viral entry to genome release. Short peptide sequences, which are often engineered from viral envelopes or fusion proteins, have been modified to function as targeted inhibitors. Natural antiviral peptide derivatives are usually tolerant to degradation, denaturation, and extreme temperature and pH conditions [15]. Insights gained from HIV-focused peptide research have been extended to other viral pathogens, revealing broader antiviral activity in select candidates [36]. Enfuvirtide, a synthetic peptide inhibitor of HIV-1, binds to the gp41 envelope protein and disrupts the conformational changes required for membrane fusion [37]. The 16-amino-acid peptide flufirvitide, obtained from the surface glycoprotein of influenza virus, is an effective inhibitor of influenza viral infections [38, 39]. Certain peptides have shown potential to inhibit SARS-CoV-2 entry by interfering with host receptor binding or membrane fusion processes. They showed the ability to block the angiotensin-converting enzyme 2 (ACE2) receptor to prevent viral invasion [40, 41]. Mucroporin-M1, a modified peptide originally inspired by scorpion venom, has been shown to bind the ACE2 receptor, hinting that it could slow or block viral entry [42, 43].

A related compound, structurally akin to human defensin HD5, also interferes with ACE2 binding.

Another peptide, RTD-1, forms a stable cyclic structure and was first isolated from rhesus-monkey leukocytes. In mouse models of SARS-CoV infection, RTD-1 reduced disease severity, not by attacking the virus directly but by calming the host's immune response. Treated animals displayed increased IL-6, keratinocyte chemoattractant, and G-CSF levels in lung

tissue, markers that suggest a dampening of the cytokine storm during viral clearance [43–45].

The fusion-blocking peptide EK1 takes a different route. Composed of 34 amino acids, it latches onto the HR1 region of coronavirus spike proteins, preventing the membrane-fusion step required for viral entry. Chemists later boosted its potency by attaching a small lipid tail—cholesterol or palmitic acid—through flexible linkers, which strengthened its grip on the viral–host interface and markedly increased antiviral activity [10, 46].

A further candidate, Aviptadil, a synthetic version of vasoactive intestinal peptide (VIP), is being explored for its ability to limit cytokine-driven damage in the lungs of SARS-CoV-2 patients. Aviptadil targets VPAC1, VPAC2, and PACAP-R1 G protein-coupled receptors expressed in alveolar type II (AT-II) cells, which are the primary targets of SARS-CoV-2. Through this interaction, aviptadil mitigates cytokine-induced apoptosis, preserves surfactant secretion, and contributes to the maintenance of alveolar function while also directly inhibiting viral replication. Although intravenous aviptadil received emergency authorization in Georgia, its approval was denied in the United States. Nonetheless, clinical trials exploring inhalable formulations are ongoing in the United States, Turkey, and Germany [47].

Despite these mechanistic advances, peptide antivirals have not supplanted small-molecule therapies for major indications, such as HIV or HCV. This shortfall often results in a critical pharmacoeconomic question: can peptides maintain therapeutic plasma levels affordably over prolonged regimens, especially when competing with well-entrenched, orally available, small molecules?

8.2.3 Antineoplastic Peptides

In oncology, peptides are under active investigation both as direct cytotoxic agents and as vectors for selective drug delivery, particularly through conjugation with chemotherapeutics [48, 49]. Interest in antineoplastic peptides has intensified following the identification of cytotoxic sequences from natural sources, including spider (*Lycosa erythrograntha*) venom-derived compounds or the synthetic derivative Lye TxI-b [50]. For example, a Bax-inhibiting peptide derived from the Ku70 protein exhibits cell-penetrating properties and pro-apoptotic modulation [51]. Didemnin B, a cyclic depsipeptide of marine origin, was among the earliest peptide-based compounds to enter clinical oncology trials, targeting hematologic malignancies [52].

Kahalalide F is a natural peptide that was first isolated from the sea slug *Elysia rufescens* and is a C75 cyclic tridecapeptide that contains several unusual amino acids such as Z-dehydroaminobutyric acid. Kahalalide F, a cyclic peptide isolated from *Elysia rufescens*, induces necrosis-like cell death, likely via suppression of downstream effectors in the PI3K/Akt pathway, including Akt, which is thought to be the main point of action in kahalalide F-induced apoptosis. The anti-tumor activity of kahalalide F is currently being investigated in Phase II clinical trials in patients with melanoma, hepatic carcinoma, and NSCLC [47].

Carfilzomib, a second-generation epoxyketone proteasome inhibitor, has a tetrapeptide backbone that facilitates selective and irreversible binding to the β5 subunit. These attributes stem from specific structural modifications of the epoxomicin scaffold. Mechanistically, the peptide backbone of carfilzomib enables precise binding within the proteasome's active site, whereas its epoxyketone moiety forms a covalent bond with the catalytic threonine residue, leading to irreversible inhibition of the β5 subunit's proteolytic activity [53].

Magainin II, a naturally occurring peptide from *Xenopus laevis*, exhibits both antimicrobial effects and cytotoxicity toward select tumor cells [54].

Cell targeting peptides, A distinct subclass of antineoplastic peptides includes those designed to selectively target overexpressed receptors or tumor-specific antigens in cancer cells. Many therapeutic peptides work by homing in on specific molecular receptors that are overexpressed on tumor cells, among them the epidermal growth factor receptor (EGFR), integrins, and G protein–coupled receptors (GPCRs) [48].

By binding these targets, a peptide can help drag an attached drug inside the cell or fine-tune the receptor's own signaling activity.

A classic illustration of this concept is the Arg-Gly-Asp (RGD) motif, which confers strong affinity for integrins involved in tumor adhesion and invasion. Cilengitide and lunasin are two well-studied members of this group. Lunasin, a 43-amino-acid peptide first isolated from soybeans, shows distinct anticancer behavior through epigenetic actions, and it can inhibit histone H3 and H4 acetylation, trigger apoptosis, and suppress caspase-3 expression in living systems [55, 56].

Not every peptide acts by killing tumor cells directly; many serve instead as delivery guides, steering therapeutic cargos to the right tissue. GE11, for instance, binds EGFR with nanomolar strength and has been adapted as a targeting head for numerous conjugated drugs [57, 58]. Another example, the 17-residue BR2 peptide, selectively transports pro-apoptotic single-chain antibody fragments into colon-cancer cells [59]. Similar targeting motifs have been integrated into metal-based chemotherapeutic constructs to boost tumor selectivity and overall efficacy [60, 61].

Even with these advances, short biological half-lives and protease sensitivity still limit clinical performance [62]. To counter this, chemists often introduce structural constraints, the most reliable of which is peptide cyclization. By covalently joining the peptide's N- and C-termini,

cyclization shields both ends from enzymatic cleavage and locks the chain into a defined conformation that interacts more predictably with its biological target [63].

In cancer therapeutics, cyclic RGD peptides have drawn attention as potent peptidomimetics, acting as high-affinity antagonists of α_vβ_3 and α_vβ_5 integrins. These receptors are typically overexpressed in cancers such as melanoma, colon carcinoma, and breast tumors, making cyclic RGD scaffolds valuable both for targeted therapy and for drug-delivery design [24].

Still, tumor environments vary widely in oxygen tension, pH, and stromal composition, often blunting single-agent efficacy [64]. Still, translating these mechanistic advantages into robust clinical outcomes is fraught with obstacles, including tumor microenvironment heterogeneity and rapid systemic clearance, which has historically limited peptide exposure at neoplastic sites [64, 65].

8.2.4 Anti-obesity Peptides

The worldwide prevalence of overweight and obesity has risen steadily, by roughly one-fifth since the early 1990s. Lifestyle measures such as dietary restraint and regular exercise still form the foundation of management, yet in most people they achieve only modest losses of a few percentage points in body weight. For this reason, physicians often combine behavioral therapy with pharmacologic or peptide-based approaches aimed at sustaining longer-term metabolic change. Interest in bioactive peptides as anti-obesity agents has expanded rapidly over the past decade. These small sequences can influence appetite, lipid turnover, and adipose-tissue remodeling, and their discovery has encouraged the design of peptidomimetic analogues that are both more potent and metabolically stable [66]. Several candidates have

shown meaningful activity in experimental obesity models [67, 68]. Soy-derived peptides are among the earliest examples. The tetrapeptide Val-His-Val-Val, for instance, stimulates lipolysis in skeletal muscle exposed to a high-fat diet [51], A related glycinin-derived pentapeptide, Leu-Pro-Tyr-Pro-Arg, reduces circulating triglycerides and cholesterol without disturbing overall protein balance [69]. Another innovative line of work targets the blood supply of white adipose tissue. The peptide motif CKGGRAKDC binds prohibitin, a membrane protein enriched in adipose vasculature, and can deliver pro-apoptotic payloads directly to fat-cell capillaries. In pre-clinical studies, such targeted delivery triggered regression of existing white fat deposits and improved metabolic parameters, with no notable systemic toxicity [70].

Because prohibitin is also present in human adipose vessels, this mechanism could inform future therapies for obesity management.

The synthetic derivative adipotide exemplifies this strategy. It consistently reduced adipose mass in animal studies [71, 72], although an early human trial ended in 2019 for unspecified safety concerns [73].

Mechanistically, anti-obesity peptides operate through diverse routes, from incretin-mimetic agents that heighten satiety to molecules that dismantle adipose vasculature, explaining their different clinical outcomes and safety profiles. Tirzepatide, a recent dual agonist of the GIP and GLP-1 receptors, illustrates this integrative logic. By imitating the actions of both incretin hormones released after meals, tirzepatide improves glucose handling and energy balance in patients with type 2 diabetes. GLP-1 promotes insulin secretion, suppresses glucagon release, and delays gastric emptying, thereby contributing to enhanced postprandial glycemic control.

Concurrently, GIP receptor activation augments the hypoglycemic response, producing a synergistic interaction that differentiates tirzepatide from incretin-based monotherapy agents. In addition to glycemic regulation,

tirzepatide has shown pronounced efficacy in promoting weight reduction, which is an essential therapeutic asset for individuals with type 2 diabetes complicated by obesity. Since September 2021, multiple clinical trials have assessed the safety and therapeutic efficacy of tirzepatide. Preliminary data indicate that tirzepatide may surpass existing GLP-1 receptor agonists in achieving combined glycemic and weight-related endpoints, potentially reshaping therapeutic paradigms in metabolic diseases.

Elevated levels of low-density lipoprotein cholesterol (LDL-C) constitute a primary risk factor for an increased incidence of cardiovascular disease (CVD). Conversely, high-density lipoprotein (HDL) facilitates reverse cholesterol transport and shuttles excess cholesterol from the peripheral tissues to the liver for excretion. Apolipoprotein A-I (ApoA-I), composed of ten amphipathic α-helices, constitutes the primary protein component of HDL and has been extensively studied for its anti-atherogenic properties [74]. ApoA-I serves as a structural template for designing mimetic peptides with diverse functional properties [75–77]. For instance, deficiencies or mutations in ApoA-I are associated with Tangier disease, a rare genetic disorder characterized by the near-absence of HDL cholesterol [78, 79]. Several ApoA-I mimetic peptides, such as D-4F, L-4F, and L37pA, exhibit high lipid-binding specificity and facilitate nonreceptor-mediated cholesterol efflux [80]. Since 2014, glucagon-like peptide-1 receptor agonists (GLP-1RAs), such as liraglutide and semaglutide, have modulated glycemic control by enhancing insulin secretion, suppressing glucagon release, slowing gastric emptying, collectively promoting satiety, and reducing caloric intake. Following the approval of liraglutide, additional GLP-1 agonists and novel peptide-based agents have been used clinically for obesity management [81].

In pediatric populations, where obesity has reached an alarming prevalence, attention has increasingly turned to pharmacological

interventions, particularly GLP-1 receptor agonists. Emerging data suggest that GLP-1Ras, exenatide, liraglutide, and semaglutide may offer clinically meaningful weight reduction and metabolic improvements in both children and adults.

Semaglutide, the most recently approved agent in this class, has demonstrated notable weight loss effects in pediatric trials with safety profiles comparable to those of adult cohorts. In cases where behavioral modification proves inadequate, GLP-1RAs may serve as adjunctive options for pediatric obesity management. Longitudinal studies are essential to clarify extended safety profiles and explore inequities in therapeutic access across pediatric populations [82].

However, regulators remain circumspect, particularly when novel vascular targets are involved, highlighting the need for rigorous long-term safety monitoring as these therapies advance.

8.2.5 Cardiovascular Peptides

The story of cardiovascular peptides began almost 40 years ago with the identification of atrial natriuretic peptide (ANP)—the first hormone found to originate directly from the heart. Soon after came brain natriuretic peptide (BNP) and C-type natriuretic peptide (CNP), extending the concept that the myocardium does far more than pump blood; it also regulates vascular tone and fluid balance.

Recombinant ANP and BNP have since been tested clinically, where they can improve hemodynamic parameters in acute decompensated heart failure and have been incorporated into treatment protocols [83, 84]. For chronic disease, **angiotensin-receptor neprilysin inhibitors (ARNIs)** represent a validated therapeutic class, although whether BNP remains a reliable biomarker during ARNI therapy is still debated [85].

Another key player, endothelin, was first isolated from porcine aortic endothelial cells and is recognized as one of the most potent endogenous vasoconstrictors. Experimental models show clear benefits from endothelin-receptor antagonists, yet large trials have failed to demonstrate survival gains or fewer hospitalizations [86]. Nonetheless, these antagonists have proven to be highly effective in the management of pulmonary arterial hypertension and are currently endorsed in clinical guidelines for this indication [87].

The vasodilator adrenomedullin, a 52-amino-acid peptide discovered in human pheochromocytoma tissue, is abundantly expressed in the heart. It limits fibroblast proliferation, suppresses collagen synthesis, and counteracts hypertrophy while boosting contractility [88, 89]. Intravenous infusions can acutely improve cardiac hemodynamics in patients with heart failure [90].

Another fascinating molecule is ghrelin, first identified in the rat stomach as the natural ligand of the growth-hormone secretagogue receptor (GHS-R) [91]. Beyond stimulating appetite and growth-hormone release, ghrelin confers cardiovascular benefits: prolonged administration improves left-ventricular function, reduces maladaptive remodeling, and helps preserve skeletal muscle mass and exercise tolerance in both experimental models and human studies [92, 93].

Across the natriuretic peptide family, ANP, BNP, and CNP remain central to cardiovascular and renal homeostasis, balancing blood volume, pressure, and vascular tone [94, 95]. Preclinical data also point to additional candidates. For instance, vasoactive intestinal peptide (VIP) infusion raises myocardial VIP levels and reverses established fibrosis in rats [89], whereas the cyclic peptide RD808 selectively blocks β_1-adrenergic receptors, reducing receptor-mediated myocardial injury in murine models [96, 97].

Designing peptide therapies for the heart often requires balancing strong hemodynamic effects against drawbacks such as rapid desensitization or

accelerated renal clearance. Despite these hurdles, cardiovascular peptides continue to redefine the heart as both a mechanical and endocrine organ. Many remain promising, but their translation to durable medicines still depends on extending plasma half-life and preventing receptor down-regulation through improved delivery technologies. Future work will likely need to reconcile these short biological half-lives with chronic dosing realities, especially in diseases like heart failure, where lifelong management is the rule rather than the exception.

8.3 INNOVATIONS AND DELIVERY STRATEGIES (NEXT-GENERATION THERAPEUTIC MODALITIES)

These next-generation strategies can be conceptually divided into three domains: peptide immunogens (vaccines), peptide-drug conjugates (PDCs), and advanced delivery constructs, each devised to circumvent the distinct pharmacological bottlenecks inherent in classical peptide therapies.

8.3.1 Vaccines-Based Peptides

Peptide vaccines are subunits composed of short amino acid sequences derived from pathogens or tumor antigens that stimulate cellular (T cell) or humoral (antibody) immune responses [98]. They are chemically synthesized according to the amino acid sequence of a known or predicted segment of an antigenic epitope in the antigenic gene of a pathogen [99]. These are short peptides or synthetic long-chain peptides of more than 20 amino acids. Long-chain peptides are more stable and immunogenic, as they are processed by antigen-presenting cells that activate CD4+ and CD8+ T cells to elicit stronger and longer-lasting immunity [100]. Peptide vaccines have the advantage of avoiding biological contamination as they are synthetic, stable, and water-soluble, and can be designed to target multiple

epitopes, yet face formulation and immunogenicity hurdles (Table 8.4) [101].

TABLE 8.4

Peptide Vaccine Platforms: Types, Mechanisms, Applications, and Developmental Challenges

Platform Type	Application	Mechanism/Target	Key Challenges
Synthetic epitope peptides	Viral infections, cancers	Target MHC class I/II to activate CD8+/CD4+ T-cell response	Low immunogenicity, short *in vivo* half-life [101, 102].
Long peptide vaccines	Cancer immunotherapy	Deliver multiple epitopes for enhanced T-cell priming	Requires endosomal processing; complex synthesis [103].
Nanoparticle-based peptides	Infectious diseases, autoimmunity	Promote uptake and cross-presentation by APCs	Variability in particle size, regulatory concerns [104, 105].
Liposome-conjugated peptides	HIV, HBV, tumor vaccines	Improve peptide delivery and stability	Cost of production; physicochemical instability [106]
Peptide–adjuvant conjugates	Tuberculosis, emerging viral threats	Stimulate innate immunity while enhancing antigen response	Cytokine overstimulation; formulation compatibility [107, 108].
DNA/RNA–encoded peptides	Emerging pathogens (e.g., SARS-CoV-2)	Encode peptides in host cells to induce endogenous expression	Cold-chain logistics; variability in host expression [109, 110].

The impetus for tumor-targeting peptide vaccines lies in the mutational burden that differentiates malignant cells from healthy cells, creating

neoantigenic landscapes that can be precisely probed by T-cell receptors. There are many tumor cells with various mutations, some of which are presented to the surface of tumor cells by MHC, which are then specifically recognized by the TCR of T cells, thus directly killing tumor cells, which are called neoantigens. If specific tumor cell neoantigens are identified and used as amino acid sequences to develop cancer vaccines, they can activate tumor-specific T-cell responses against tumor cells [102]. Peptide vaccines have the advantages of being safe and easily produced. Current challenges include poor immunogenicity, as they are formed of short amino acid sequences lacking the complex tertiary structure of the full antigen, which reduces their ability to trigger a robust immune response. They have short half-lives *in vivo* owing to their susceptibility to enzymatic degradation and peptide vaccines targeting specific human leukocyte antigen (HLA) molecules, which limits their efficacy to individuals with compatible HLA types. Many short peptides lack CD4+ epitopes, which hinders the development of sustained cellular and humoral immunity [101]. To improve efficacy and stability, longer synthetic peptides of more than 20 amino acids enable presentation by antigen-presenting cells, which activate both CD8+ and CD4+ T cells [103]. Conjugation of peptides and virus-like peptides (VLPs) or nanoparticles similar to natural pathogens will also enhance efficacy, stabilize conformational epitopes, augment antibody binding, and enhance immunogenicity (Figure 8.4 [101, 103]).

Prophylactic peptide vaccines are designed for pathogenicity by priming the immune system against specific antigens before exposure or the development of diseases such as cancer. They are short-chain amino acids that mimic parts of disease-associated proteins, eliciting the desired immune response [104, 105]. Prophylactic peptide vaccines can be tailored to target specific pathogens or tumor antigens, thereby reducing off-target effects and the risk of autoimmunity [104, 106]. They have been investigated for

diseases such as HIV, hepatitis, allergies [105], lung cancer (targeting Ddx21-mutant peptide) [11], breast cancer (targeting the GP2 protein stimulates cytotoxic T cells), and lung cancer (targeting the mutant KARS will prevent premalignant lung lesions in preclinical models) [105].

8.3.2 Peptide-Drug Conjugates (PDCs)

Over the last few decades, new therapeutic strategies have emerged, including unprecedented drug conjugate therapies for increased potency and a broader range of targetable tumors. Peptide-drug conjugates (PDCs) are a new hope for targeted therapy, with the main advantages of enhanced tissue penetration, easier chemical synthesis, and lower production costs. With the problems arise from antibody-drug conjugates (ADCs) as complex pharmacokinetics, inevitable side effects, and ineffective payloads release, related to the monoclonal antibody component. PDCs are a new type of drug conjugate that pair a cell-targeting peptide with a drug molecule to enhance drug targeting, improve efficacy, and reduce adverse effects. Compared to antibody-drug conjugates (ADC), PDC rely on a peptide chain of approximately 10 amino acids to target tumor cells. The therapeutic effects of PDCs are noteworthy, but they also encounter problems, such as poor stability, short blood circulation time, and slow clinical development [111, 112]. For example, the PDC melphalan (flufenamide) has received FDA accelerated approval for treating multiple myeloma, becoming the second PDC drug introduced to the market worldwide. This triggered interest in PDC drugs, although melphalan was withdrawn from the US market based on phase 3 results, which showed inferior overall survival [110].

Even so, PDCs remain in early phase trials for many indications, with questions lingering about their long-term stability, tissue distribution, and immunogenic profiles compared with better-characterized ADCs.

8.4 ADVANTAGES AND DISADVANTAGES OF THERAPEUTIC PEPTIDES

Peptides offer meaningful advantages over larger biologics, including a reduced tendency to provoke immune reactions and generally more economical production costs, while maintaining a high degree of target specificity and affinity [21]. Compared to monoclonal antibodies, peptides offer improved tissue penetration and lower production costs, yet their markedly shorter half-lives frequently necessitate parenteral administration, which is a persistent deterrent to patient compliance in chronic conditions. Peptides occupy an unusual middle ground in drug design: they are small enough to be chemically customized, yet complex enough to engage targets that small molecules often cannot reach. In contrast to bulky macromolecules, their structures can be tweaked to cross cell membranes, opening the door to intracellular delivery [113]. Even with these advantages, most peptide therapeutics still struggle with less-than-ideal pharmacokinetics. Absorption tends to be poor, proteolytic enzymes rapidly dismantle them, and they disappear from circulation within minutes, limitations that continue to restrict their clinical utility [114].

Another difficulty lies outside the laboratory. Regulatory frameworks built around either small molecules or large biologics often fail to fit peptides neatly. These agents sit awkwardly between the two worlds, forcing regulators to adopt evaluation methods and safety benchmarks that were never designed for hybrid pharmacologies.

One-way researchers have tried to bypass these barriers are through peptidomimetics, synthetic analogues that imitate the form and function of native peptides while resisting enzymatic breakdown [115]. The combination of modern structural biology, recombinant DNA tools, and synthetic chemistry has accelerated discovery in this area. Today, peptide-based

therapeutics are being explored for metabolic, cardiovascular, respiratory, and infectious diseases alike [74], Yet, significant challenges remain. Peptides are notoriously fragile inside the body; their flexible, linear chains are easy prey for hydrolytic and proteolytic enzymes. Factors such as length, hydrophobic balance, and charge distribution all influence how—or whether—they can cross a cell membrane [19, 116]. Consequently, most candidates in clinical testing still act on extracellular receptors, including GPCRs, GnRH, and GLP-1 receptors [19].

Because natural peptides lack rigid secondary and tertiary structures, they degrade quickly and clear from circulation soon after administration [117].

Improving them requires more than clever chemistry—it demands a genuinely interdisciplinary design strategy that strengthens half-life, solubility, and bioactivity while optimizing delivery.

Chemical modification has already delivered some success. Techniques such as PEGylation and encapsulation have improved stability and plasma residence (Figure 8.5), making these molecules more practical for drug development. Still, one fundamental problem persists: their inherent vulnerability to digestive enzymes continues to block efficient oral delivery. Overcoming that single barrier remains one of the key frontiers in peptide therapeutics [113].

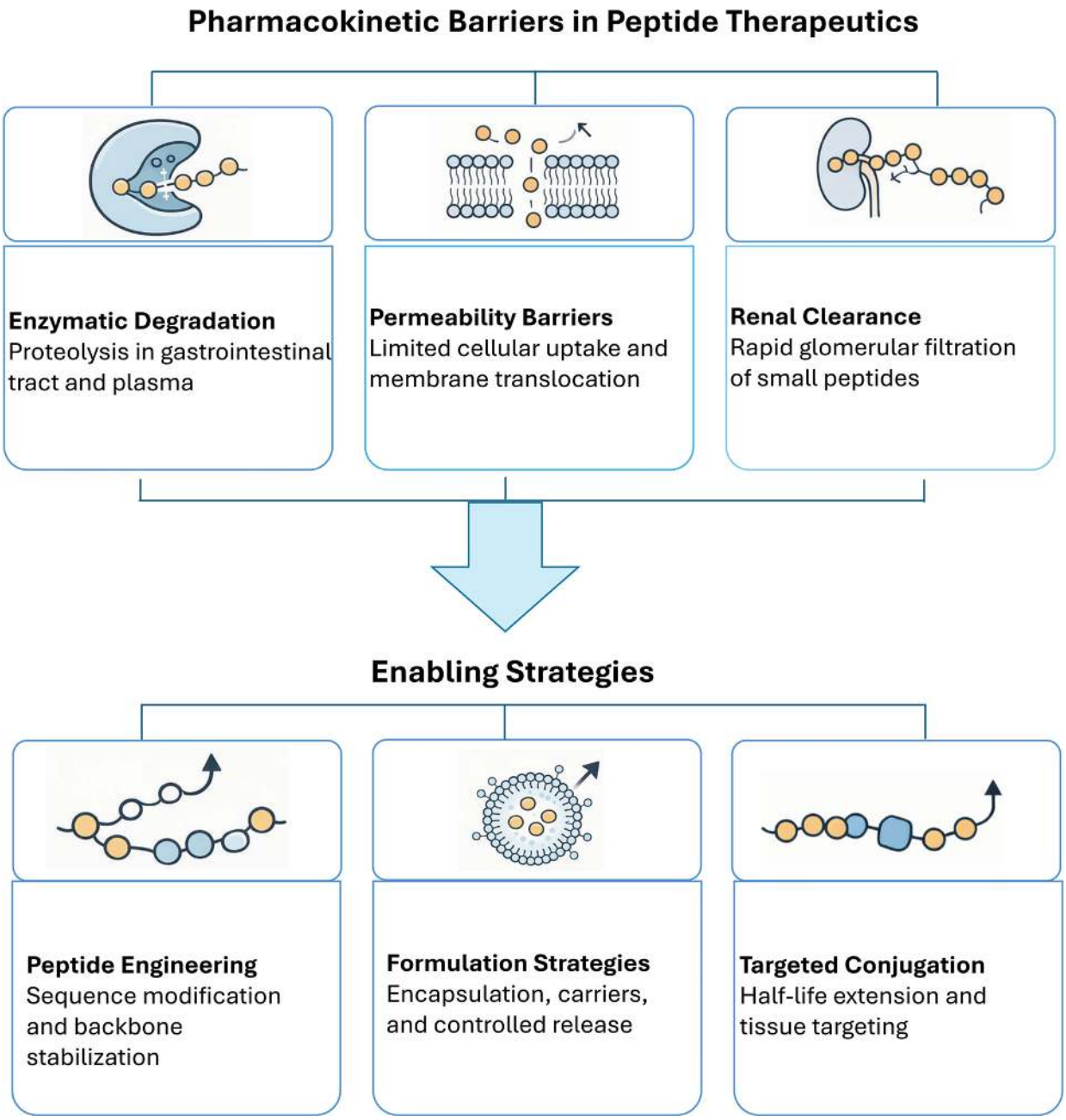


FIGURE 8.5 Pharmacokinetic barriers and enabling strategies in peptide therapeutics. Overview of principal pharmacokinetic limitations of peptide drugs—enzymatic degradation, membrane impermeability, and rapid renal clearance—and corresponding enabling strategies including backbone engineering, carrier-based delivery, and half-life extension technologies. ⏎

8.5 ENHANCING DRUG DELIVERY OF THERAPEUTIC PEPTIDES

Chemical modification of peptides has led to notable improvements in their biological activity, and plasma stability is being developed to enhance bioavailability (Figure 8.5), rendering them more amenable to pharmaceutical development. Despite these advancements, a fundamental limitation persists: the inherent susceptibility of peptides to enzymatic hydrolysis, particularly by gastric enzymes, severely restricts their oral bioavailability. Consequently, most peptide-based therapeutics are currently administered via the parenteral route.

Peptide drugs often struggle to survive long enough in the body to reach their intended targets. In response, researchers have been rethinking how these molecules are delivered rather than how they are made. Current efforts center on extending stability and keeping active concentrations in circulation for longer periods.

Several practical solutions have gained ground: encapsulating peptides within liposomes or biodegradable microspheres; PEGylating them to increase molecular size and limit renal filtration; linking them to carrier proteins; and even embedding them in small subcutaneous implants that release the drug gradually [118]. Each of these strategies, though different in mechanism, shares a single goal—shielding fragile peptides from enzymatic breakdown so that more of the dose remains available to exert its therapeutic effect (Table 8.5) [119].

TABLE 8.5

Pharmacokinetic Challenges and Modern Mitigation Strategies for Peptide Drugs

Barrier	Cause	Clinical Consequence	Biopharmaceutical Solution	Example Drug

Barrier	Cause	Clinical Consequence	Biopharmaceutical Solution	Example Drug
Gastrointestinal (GI) proteolysis	Exposure to digestive enzymes (e.g., pepsin, trypsin, chymotrypsin)	Extensive peptide degradation before systemic absorption	Co-administration of protease inhibitors; enteric or SNAC coatings	Oral semaglutide (uses SNAC for local protection)
Poor intestinal permeability	High molecular weight, polarity, and lack of passive diffusion	Extremely low oral bioavailability (<2%)	Permeation enhancers; cell-penetrating peptides (CPPs); SNAC carriers	TAT-conjugated peptide cargo (preclinical)
Renal filtration and clearance	Peptides <30 kDa rapidly eliminated via glomerular filtration	Short plasma half-life (minutes to hours)	PEGylation; albumin fusion; biodegradable microspheres	PEG–interferon-α; Dulaglutide (albumin fusion)
Enzymatic degradation in circulation	Circulating proteases in plasma and tissues	Requires frequent dosing; limited duration of action	Structural modifications (e.g., cyclization, D-amino acids)	Octreotide, Lanreotide (stable cyclic analogs of somatostatin)

Barrier	Cause	Clinical Consequence	Biopharmaceutical Solution	Example Drug
Hepatic first-pass metabolism	Enzymatic degradation via gut-liver axis (CYPs, peptidases)	Reduced bioavailability and therapeutic efficacy	Subcutaneous/intramuscular delivery; SNAC-mediated absorption bypass	Oral semaglutide (avoids first-pass via gastric absorption with SNAC)

8.5.1 Peptide PEGylation

To overcome the rapid clearance, low half-life, and immunogenicity of peptides, PEGs are linked to peptide molecules either via cleavable ester bonds or via a non-hydrolyzable amide bond to obtain a non-biodegradable, non-toxic, low-immunogenic polymer. PEG linkers are now well established for extending peptide half-life and improving therapeutic profiles. PEGylation increases the effective molecular weight of peptides with reduced renal clearance, protects against protease digestion by increasing steric hindrance, and helps increase absorption by increasing the water solubility of the target peptide [120]. These advantages make PEGylation a promising approach for enhancing the activity of therapeutic peptides [121]. In the application of PEGylation, polypeptides provide excellent candidates with adequate biocompatibility and biodegradability, with tunable hydrophilicity. Long-term PEG use is correlated with vacuole formation in cells and hypersensitivity reactions, which is one of the drawbacks of PEGylation [122]. Over 200 peptides, including G17DT, have been used in clinical trials for cancer [123].

8.5.2 Cell-Penetrating Peptides (CPPs)

Cell-penetrating peptides (CPPs) are a class of peptides consisting usually of 30-amino-acid residues or less that are directly able to cross the cell membrane and enter the cell. They are positively charged peptide fragments of variable amino acid lengths, are rich in basic amino acid residues such as arginine and lysine, and have an α-helical spatial conformation in their secondary structures. Using these properties, more penetrating and efficient membrane penetrating peptides PEp-1 and MPG have been synthesized and successfully carry active substances such as small molecule drugs, peptides, proteins, siRNAs, and nanoparticles) into cells to exert biological activities, and some scholars call these short peptides as protein transduction domains or Trojan horse peptides. Cell-penetrating peptides can effectively promote transdermal absorption of biomolecular drugs and are of great value in targeted formulations, transdermal drug delivery formulations, and cosmetics, which can significantly improve the bioavailability of drugs.

8.5.3 Co-formulation with Permeation Enhancers

Covalent small-molecule drugs are often praised for their practical advantages in pharmacology. Compared with noncovalent compounds, covalent inhibitors tend to offer stronger and more sustained activity, along with better pharmacokinetic profiles and longer half-lives. This usually means that fewer doses are needed, which improves patient adherence and simplifies treatment. The success of these small molecules has encouraged similar ideas in peptide drug design, especially in efforts to make oral formulations a reality, which has been a challenge for researchers for decades [25]. A good example of this is semaglutide, a GLP-1 receptor agonist that has been modified by attaching a C18 fatty acid chain. This simple chemical change makes a substantial difference: the drug now lasts

long enough in circulation to be administered just once a week and shows far greater plasma stability than its earlier counterparts [26, 124]. This is a reminder that even small structural adjustments can have a large effect on therapeutic performance and patient convenience.

Recently, formulation chemistry has opened another path forward. Researchers have created an oral version of semaglutide by combining it with sodium N-[8-(2-hydroxybenzoyl) amino] caprylate (SNAC). In this case, SNAC does more than simply act as an additive. It protects the peptide in the acidic environment of the stomach and helps it move across the gastric wall by making it slightly more lipid-like. Together, these two effects enable sufficient drug to reach the bloodstream, which until recently seemed almost impossible for a peptide of this size [125, 126].

The development of oral semaglutide demonstrates how chemistry and formulation design can work together to solve long-standing delivery problems. This suggests that similar co-formulation methods could be applied to other peptide drugs in the future, provided that their structural and pharmacological features are compatible [127–130] (Figure 8.6 [131, 132]).

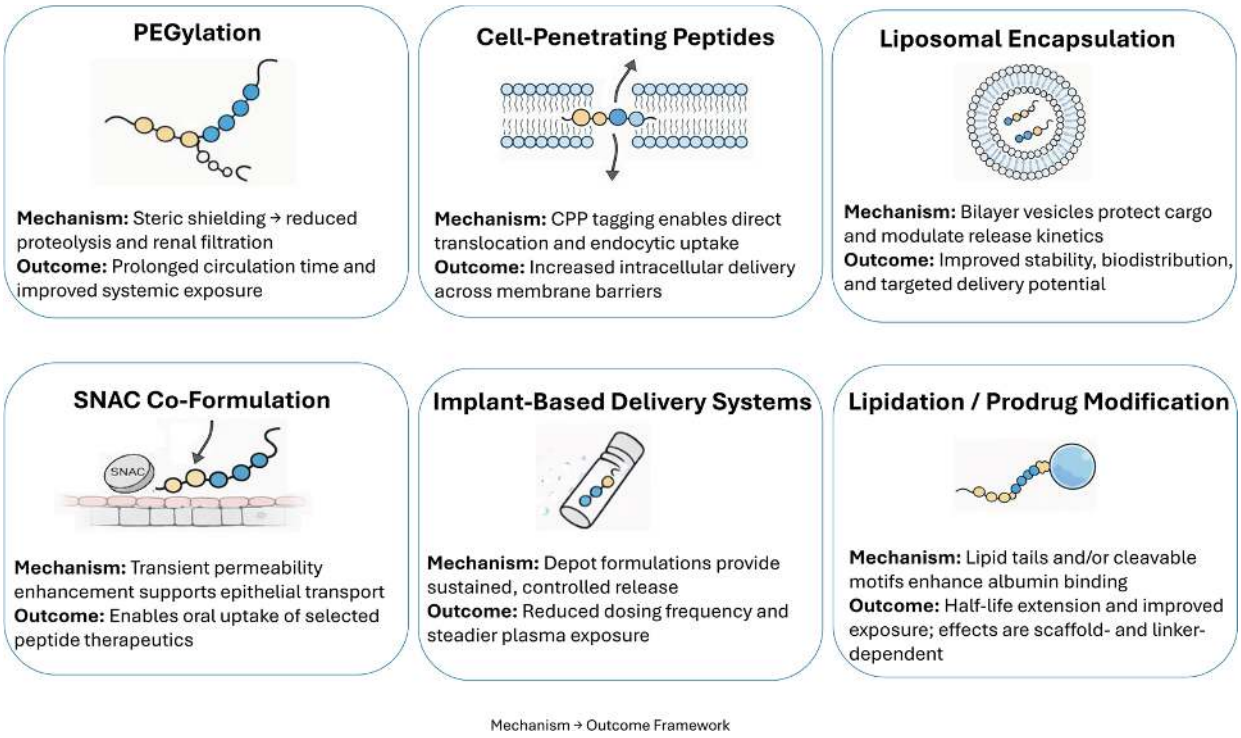


FIGURE 8.6 Advanced delivery technologies for peptide therapeutics. Schematic overview of major formulation and chemical modification strategies used to improve the pharmacokinetic performance and bioavailability of peptide drugs, including PEGylation, cell-penetrating peptide (CPP) conjugation, liposomal encapsulation, SNAC co-formulation, implant-based sustained-release systems, and lipidation/prodrug modification. Each panel summarizes the principal mechanistic basis and corresponding therapeutic outcome associated with the respective delivery approach. PEG, polyethylene glycol; CPP, cell-penetrating peptide; SNAC, sodium N-(8-[2-hydroxybenzoyl]amino) caprylate. ⏎

Parallel efforts have explored alternative delivery routes such as pulmonary inhalation, transdermal patches, and implantable infusion pumps. These approaches have been particularly promising for the development of inhalable insulin and microimplantable pump systems designed to provide controlled and sustained peptide administration [17, 130, 133]. Sophisticated delivery platforms, while scientifically compelling, also introduce layers of

manufacturing and quality control complexity that can lengthen time to approval and elevate costs.

8.6 PEPTIDE THERAPEUTICS OF THE FUTURE (CUTTING-EDGE DESIGN STRATEGIES)

Therapeutic peptides have undergone significant innovation through progress in peptide design, synthesis, and delivery. These innovations address limitations such as poor stability and bioavailability, expanding the therapeutic potential of peptides in diverse fields such as metabolic disorders, oncology, and infectious diseases [11]. This innovation unfolds along several key lines of research designed to optimize pharmacokinetics and target selectivity (Table 8.6).

TABLE 8.6

Structural Modification Techniques and Their Effects on Peptide Behavior

Modification	Site of Change	Effect on Stability	PK/PD Impact	Common Applications
Cyclization	Peptide backbone (head-to-tail or side-chain cyclization)	↑ Conformational rigidity, ↑ resistance to proteolysis [18]	↑ Plasma half-life, ↑ receptor selectivity	Octreotide [134–136], Cyclosporine, Oxytocin analogs
D-amino acid substitution	L-amino acids replaced by D-amino acids at strategic sites	↑ Enzymatic stability, ↓ recognition by endogenous peptidases [19]	↑ Oral bioavailability, ↑ systemic exposure	Modified GLP-1 agonists, Somatostatin mimetics [135–137]

Modification	Site of Change	Effect on Stability	PK/PD Impact	Common Applications
N-methylation	Amide nitrogen (N) of the peptide backbone	↓ Hydrogen bond donation, ↑ conformational constraint [21]	↑ Membrane permeability, ↑ oral bioavailability	Cyclosporine, N-methylated cyclic peptides
Peptoids	Replacement of α-carbon with nitrogen in the backbone	↑ Resistance to proteolytic enzymes, ↓ immunogenic potential [27]	Modified biodistribution, extended duration of action	Peptoid-based antimicrobials, immunotherapy adjuvants
Halogenation	Aromatic residues (Phe, Tyr, Trp side chains)	↑ Lipophilicity, modulated oxidative metabolism [22]	Enhanced membrane passage, altered receptor binding kinetics	Halogenated CNS-active peptides, anticancer peptide leads

8.7 NOVEL PEPTIDE DESIGN

8.7.1 CYCLIZATION

Cyclization is a widely adopted and chemically straightforward strategy for converting linear peptides into cyclic structures that confer a range of pharmacological benefits. This structural modification enhances peptide stability, improves binding affinity and specificity, and facilitates greater cellular permeability, which can significantly increase the therapeutic potential of both peptides and peptidomimetics [138]. The rigid and preorganized geometry of cyclic peptides reduces the entropic penalty upon target binding, resulting in more efficient and selective molecular interactions.

Cyclic peptides have demonstrated considerable utility in various biomedical applications, including modulation of protein–protein interactions, RNA binding, enzyme inhibition, diagnostic imaging, and targeted drug delivery. Given their enhanced pharmacodynamic and pharmacokinetic properties, the use of macrocyclic peptides in drug development is expected to increase substantially [139]. Recent innovations in macrocyclization methodologies have yielded peptide derivatives with significantly improved therapeutic profiles. For instance, cyclic peptides engineered to disrupt the PD-1/PD-L1 immune checkpoint exhibited a 34-fold increase in blocking potency and a four-fold improvement in serum stability relative to their linear counterparts. These findings underscore the value of cyclization as a powerful design principle, particularly in cases where detailed structural information about peptide–receptor interactions is limited [140].

8.7.2 D-Amino-Acid Substitution

Endogenous peptides and proteins are constructed exclusively from l-amino acid residues, rendering them susceptible to rapid degradation by proteases that have evolved to recognize this stereochemistry. Substituting selected residues with their d-enantiomers confers marked resistance to these enzymes, thus enhancing the proteolytic stability of therapeutic peptides [141]. D-substitution can also attenuate the formation of anti-PEG antibodies and diminish the systemic toxicity of certain peptide-PEG conjugates *in vivo* [142]. Such substitutions may be introduced either globally or at specific positions; nonetheless, attendant conformational changes can alter target engagement. Partial D-substitution of the antimicrobial peptide W3R6 (yielding D-Arg-W3R6) preserved antimicrobial potency and improved

stability, whereas complete replacement with D-residues (D-W3R6) reduced biological activity despite similar gains in protease resistance [143].

8.7.3 Peptoids and N-Methylation

Peptoids form a distinctive branch of peptidomimetics in which the side chains are attached to the backbone nitrogen, producing N-substituted glycine residues. This subtle architectural change has important consequences: it shields the backbone from proteolytic attack and, in doing so, greatly extends molecular stability. It also simplifies synthesis, since peptoids can be assembled efficiently through standard solid-phase methods [144]. Incorporating such residues into collagen-mimetic peptides (CMPs) has produced triple-helical analogues that remain stable under conditions where ordinary collagen fragments rapidly unfold. These synthetic helices are now being explored for applications in regenerative medicine and biomaterial engineering [145].

Large-scale combinatorial screens have uncovered several biologically active members of this family. One notable compound, AEC5, is a tripeptoid with striking antifungal activity against *Cryptococcus neoformans*—it can clear fungal cultures within a few hours [146, 147]. Using a related one-bead-one-compound (OBOC) approach, researchers have also identified cyclic peptoids that inhibit cyclophilin D, a mitochondrial regulator linked to neurodegenerative pathways. Among these, peptoid I11 stood out for its strong anti-inflammatory effect, low toxicity, and ability to cross the blood–brain barrier, pointing to its potential in modulating mitochondrial function within neurons [148]. Because of their rigid backbones and resistance to enzymatic breakdown, peptoids frequently outperform standard peptides in discovery platforms. Their expanding chemical space is now shaping the next generation of peptidomimetic scaffolds (Table 8.7).

TABLE 8.7

Emerging Peptidomimetic Classes and Notable Examples

Tool/Methodology	Principle	Application in Peptide Design	Clinical Impact
In silico structure–activity relationship (SAR) modeling	Predicts biological activity using molecular descriptors and 3D conformations [32]	Lead optimization; prediction of toxicity, solubility, and receptor selectivity [135]	Enhances preclinical predictability; reduces need for extensive animal testing
Molecular docking and dynamics simulations	Simulates peptide–target binding and interaction dynamics [33, 149]	Refines binding affinity; models conformational flexibility	Supports rational design of selective binders (e.g., integrin-targeting peptides) [65, 150]
Machine learning for de novo peptide design	AI algorithms (e.g., neural networks, transformers) generate novel sequences [34]	Accelerates identification of stable, bioavailable, target-specific peptides	Actively used in discovery of antimicrobial, metabolic, and anticancer peptides
Peptide microarrays	Immobilized peptide libraries enable high-throughput binding studies [35]	Epitope mapping; optimization of diagnostic or vaccine peptides [151]	Applied in neoantigen screening and immunopeptidomics
Solid-phase peptide synthesis (SPPS)	Stepwise chemical assembly of peptides on resin support [36]	Enables rapid and diverse peptide synthesis, including post-translational mimics	Foundation of commercial and custom peptide manufacturing

Tool/Methodology	Principle	Application in Peptide Design	Clinical Impact
Phage display libraries	Bacteriophage vectors display peptides for target-based screening [37].	High-throughput discovery of ligands, inhibitors, and diagnostic tools	Origin of several approved biologics (e.g., Adalimumab, evolocumab)
Click chemistry and peptide stapling	Covalent constraints enhance peptide helicity and stability [38]	Improves protease resistance, bioactivity, and cell permeability	Stapled peptides in clinical trials (e.g., MDM2 inhibitors, inflammatory targets)
CRISPR-guided peptide engineering	Uses CRISPR/Cas systems to guide intracellular expression of peptide variants [39]	Programmable, *in vivo* production of therapeutic peptides	Still experimental; promising for gene-regulated peptide therapies

Among the chemical approaches used to reinforce peptide improve pharmacokinetic behavior and stability, N-methylation of the peptide backbone has consistently proven one of the most effective. Adding an N-methyl group introduces extra steric bulk and limits backbone flexibility, frequently biasing the molecule toward a cis-like conformation that favors receptor engagement. What appears to be a minor modification can, in practice, produce marked benefits for greater permeability across biological membranes, a longer plasma half-life, and commonly more favorable pharmacokinetic profile [63]. Beyond structural stabilization, stepwise N-methylation has also become a useful analytical probe for understanding how peptides interact with their receptors under physiological conditions. Work on human urotensin II analogs illustrates this clearly: slight alterations in N–C bond geometry can redirect receptor affinity and signaling selectivity by reshaping local hydrogen-bonding networks [152]. Likewise, experiments

with N-methylated derivatives of angiotensin-converting enzyme (ACE), neutral endopeptidase (NEP), and aminopeptidase N (APN) have yielded molecules of noticeably higher potency in preclinical models of hypertension, cardiac hypertrophy, and fibrotic disorders [153].

Despite its promise, the method has long been constrained by synthetic hurdles. N-methylated amino acid precursors are scarce and coupling reactions tend to proceed inefficiently when these residues are incorporated during peptide-chain assembly. Recent innovations have begun to ease these obstacles. Chemoenzymatic methods using borosin-type methyltransferases now permit site-specific methylation on a catalytic scaffold; once modification is complete, the peptide can be detached to afford high-yield, position-defined analogs [137]. These advances have transformed what was once a labor-intensive procedure into a more practical tool for medicinal chemists [154].

8.7.4 Halogenation

Running parallel to methylation, halogenation offers an adaptable strategy for fine-tuning the physicochemical and pharmacological profiles of peptide therapeutics. The deliberate introduction of halogen atoms, iodine, bromine, chlorine, or fluorine, can shift a molecule's polarity, lipophilicity, and non-covalent interaction pattern in predictable yet powerful ways. As a result, halogenated derivatives could exhibit improved membrane penetration, greater receptor selectivity, and occasionally reduced off-target activity [155]. A frequently cited example involves RGD-based peptides, in which substitution of tryptophan residues with halogenated counterparts markedly enhances binding affinity and specificity for the integrin α_vβ_3 receptor [156]. Comparable effects have been noted in antimicrobial peptoids bearing chlorine or bromine, which tend to display stronger activity against Gram-

positive organisms. By contrast, fluorination, despite its theoretical appeal, usually provides only modest improvements in potency. Nevertheless, halogenation must be approached with care. Replacing polar side chains with bulky halogens can increase hydrophobicity and aggregation, sometimes leading to poor solubility and loss of therapeutic efficacy [157].

Thus, any advantage in stability or selectivity must be weighed against these physical drawbacks to preserve an acceptable pharmacological profile [133, 158]. Such chemical fine-tuning exemplifies how peptide optimization now straddles classical medicinal chemistry and biopolymer engineering, demanding interdisciplinary insights rarely seen even a decade ago (Figure 8.7).

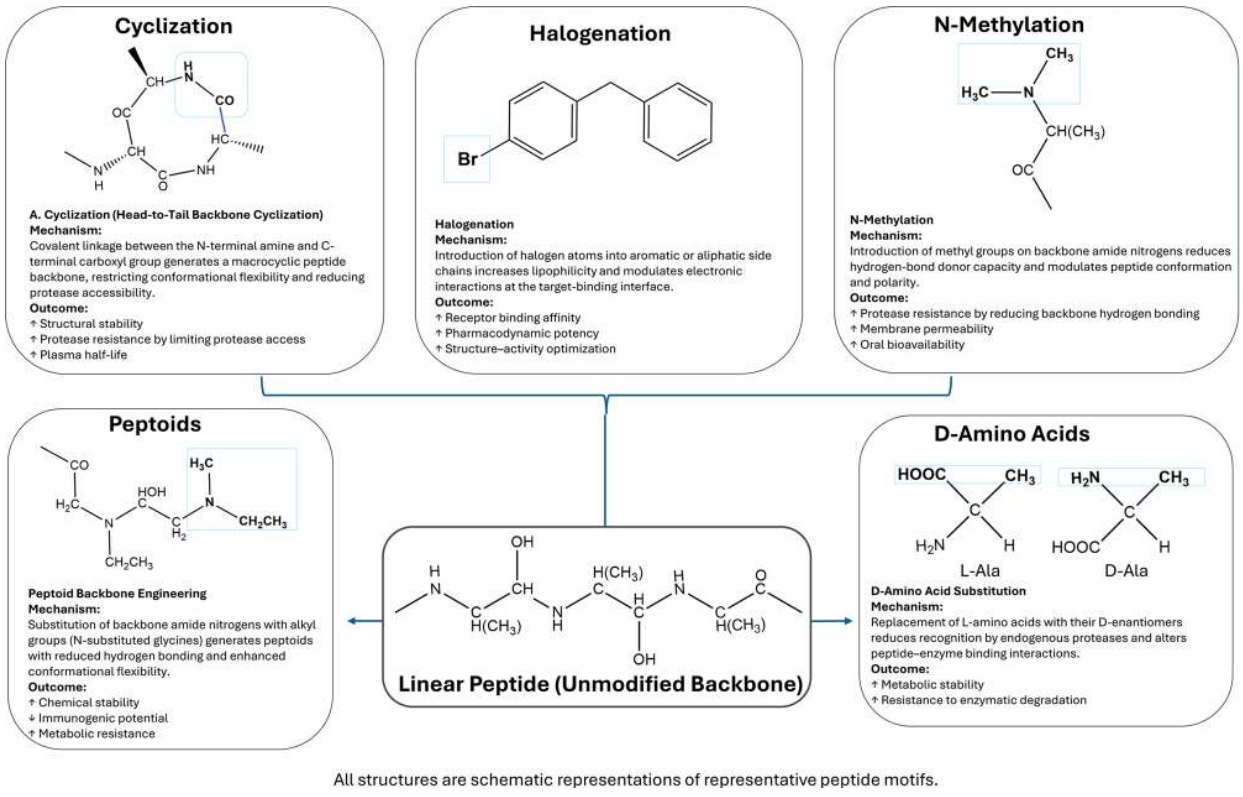


FIGURE 8.7 Chemical and backbone modification strategies for improving peptide stability and pharmacokinetic performance. Schematic overview of principal chemical and structural modifications used to enhance the metabolic stability, membrane permeability, and systemic exposure of peptide

therapeutics. The central panel shows an unmodified linear peptide backbone. Surrounding panels illustrate representative modification approaches, including head-to-tail cyclization, halogenation, backbone N-methylation, peptoid engineering, and D-amino-acid substitution. These modifications modulate molecular conformation, limit protease accessibility, and optimize target interactions, thereby improving pharmacokinetic and pharmacodynamic properties. All structures are schematic representations of representative peptide motifs. ⏎

The following bar chart illustrates the number of approved and pipeline-stage drugs across three major therapeutic classes. Small molecules dominate both categories, with approximately 2,000 approved drugs and an estimated 7,500 in development. Monoclonal antibodies and biologics follow with around 100 approved therapies and 1,000 in clinical or preclinical stages. Peptides show a growing presence, with about 100 approved and 400 pipeline candidates. The figure emphasizes the relative maturity of small molecules, the expanding biologics space, and the emerging interest in peptide-based therapeutics (Figure 8.8).

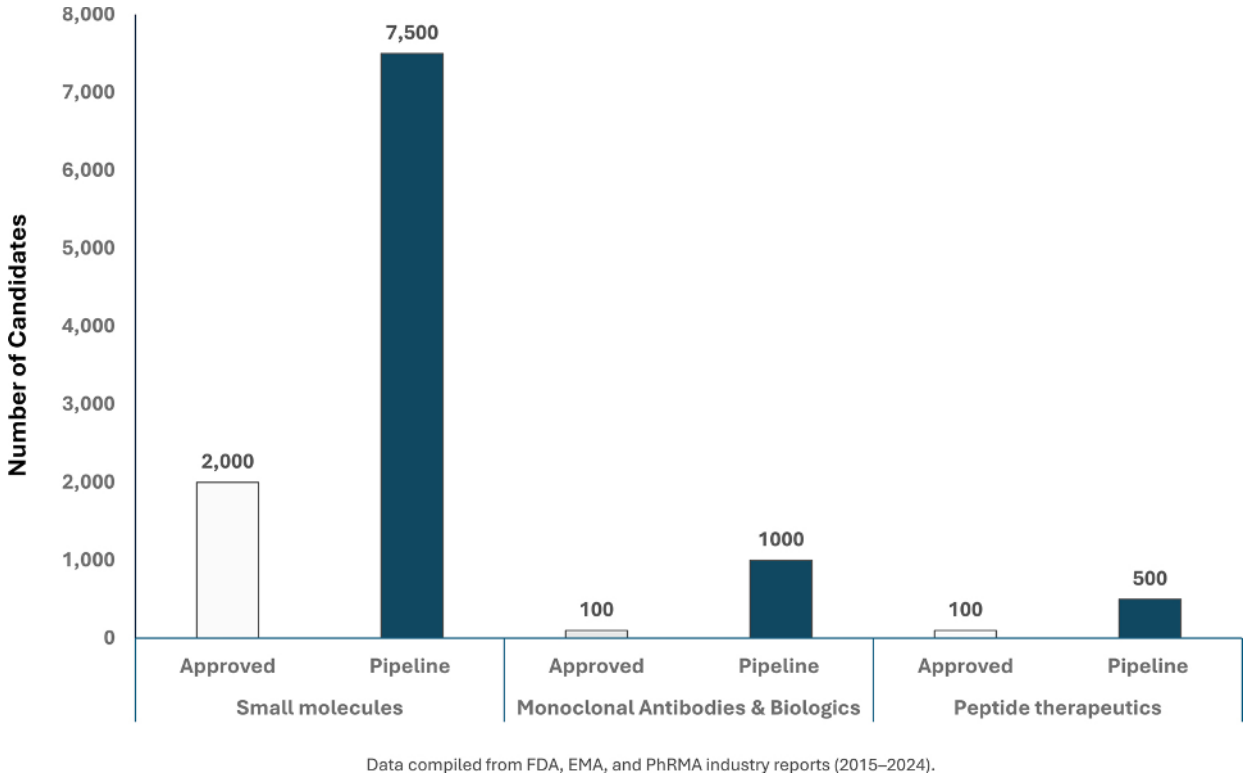


FIGURE 8.8 Distribution of approved and investigational drug candidates across major therapeutic modalities. Comparative overview of small-molecule agents, monoclonal antibodies and biologics, and peptide therapeutics, highlighting quantitative disparities between regulatory approvals and active development pipelines. Pipeline data encompass preclinical and clinical-stage candidates (Phases I–III) and were compiled from major regulatory and pharmaceutical industry reports. ⏎

Finally, it should be remembered that such molecular refinements seldom come without broader implications. Peptides engineered for heightened metabolic stability or altered degradation pathways often require expanded toxicological evaluation and prolonged safety testing before clinical approval. These additional steps inevitably extend development timelines and raise manufacturing costs. The field therefore continues to balance chemical creativity with translational feasibility, a defining tension in the evolution of modern peptide-based therapeutics.

8.8 FUTURE PERSPECTIVES

The landscape of peptide therapeutics is undergoing rapid and far-reaching transformations. Emerging modalities, most notably peptide-based vaccines and peptide–drug conjugates, are redefining how molecular precision can be leveraged for disease-specific targeting, with especially strong promise in oncology and infectious diseases. Breakthroughs in synthetic biology, combinatorial chemistry, and machine-learning-assisted design are expected to accelerate the identification of entirely new peptide scaffolds and refine the mapping of structure–activity relationships with unprecedented accuracy.

Looking ahead, the deliberate integration of halogenation, backbone N-methylation, and peptoid frameworks into rational drug design strategies may help dissolve long-standing pharmacokinetic barriers, giving rise to orally bioavailable and protease-resistant peptide therapeutics. Parallel progress in delivery technologies, from adhesive microneedle patches and smart transdermal matrices to miniature implantable depots, promises to improve patient adherence while broadening therapeutic exposure windows.

However, technological progress alone is insufficient. For peptides to reach their clinical potential, innovation must proceed in tandem with regulatory harmonization. The challenge is to ensure that new structural modifications, while improving stability and efficacy, do not inadvertently increase immunogenicity or off-target toxicity. In this sense, the future of peptide therapeutics will depend as much on policy alignment and translational foresight as on laboratory ingenuity.

As the field continues to weave molecular engineering with clinical pragmatism, peptides appear to be destined to occupy a defining, though continually contested, position within the expanding arsenal of personalized medicine (Figure 8.9).

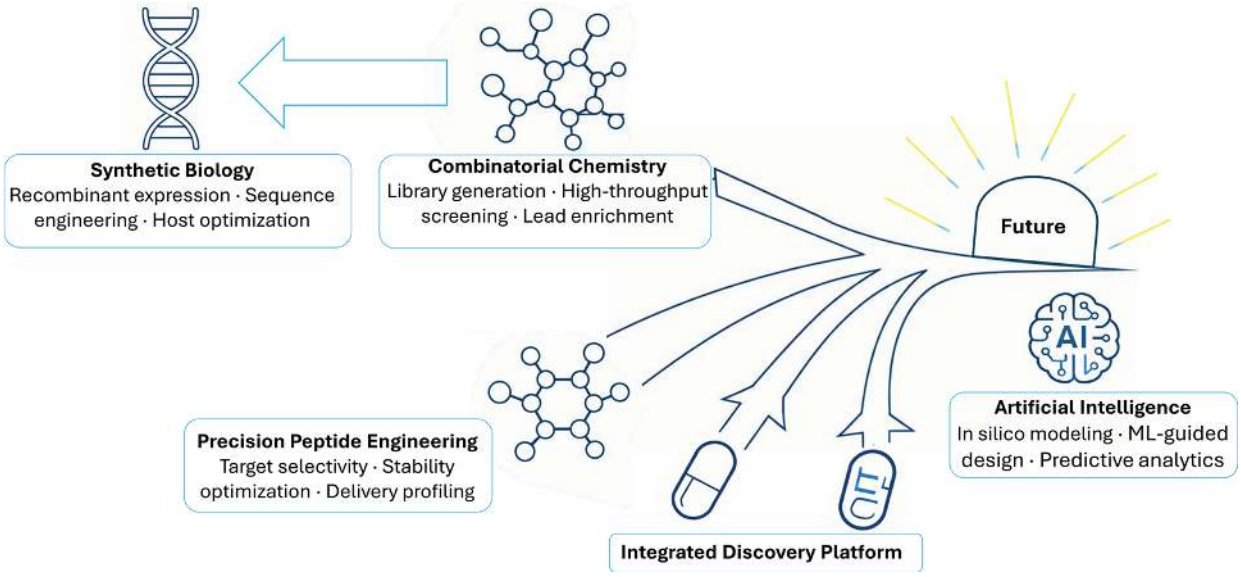


FIGURE 8.9 Integrated technological framework for next-generation peptide discovery and optimization. Conceptual schematic illustrating the convergence of synthetic biology, combinatorial chemistry, precision peptide engineering, and artificial intelligence within an integrated discovery platform. Recombinant expression systems and sequence engineering enable scalable peptide production and host optimization. Combinatorial library generation coupled with high-throughput screening facilitates rapid lead enrichment. Precision engineering strategies refine target selectivity, structural stability, and delivery characteristics. Artificial intelligence–driven in silico modeling and machine learning–guided design accelerate predictive optimization and translational prioritization. The coordinated integration of these platforms defines a systems-level approach shaping the future of peptide therapeutics. ⏎

8.9 CONCLUSION

Peptides are now among the most versatile and most adaptable classes of contemporary therapeutics. Motivated by inherent specificity, adaptable bioactivity, and structural plasticity peptides developed exceptionally well-suited to address wide range of clinical issues. As demonstrated throughout this chapter, peptide-based interventions extend far beyond classical

hormone replacement therapies, such as insulin. They include antimicrobial and antiviral agents, anticancer constructs, metabolic regulators, and cardiovascular modulators, each reflecting the growing confidence in peptide-centered drug design.

The ongoing refinement of delivery and formulation strategies, including PEGylation, cell-penetrating conjugates, nano-co-formulations, and permeation enhancers, has further enhanced their therapeutic potential. Equally transformative are the structural innovations of recent years, including cyclization, D-amino-acid substitution, and the creation of stable peptidomimetics that blur the boundary between small molecules and biologics.

Taken together, these advances point to a future in which peptides serve not merely as supplements to traditional drugs but as frontline therapeutics capable of matching biological complexity with molecular precision.

CONFLICT OF INTEREST

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DATA AVAILABILITY

No new data were generated or analyzed in this study. All information and materials discussed were derived from the published literature and publicly available sources. Further details are available from the corresponding author upon reasonable request.

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REFERENCES

1. Giordano C, Marchio M, Timofeeva E, Biagini G: Neuroactive Peptides as Putative Mediators of Antiepileptic Ketogenic Diets. *Front Neurol* 2014, **5**:63.⏎
2. Davda J, Declerck P, Hu-Lieskovan S, Hickling TP, Jacobs IA, Chou J, Salek-Ardakani S, Kraynov E: Immunogenicity of Immunomodulatory, Antibody-Based, Oncology Therapeutics. *J Immunother Cancer* 2019, 7(1):105.⏎
3. Bouazzaoui A, Abdellatif AAH: Vaccine Delivery Systems and Administration Routes: Advanced Biotechnological Techniques to Improve the Immunization Efficacy. *Vaccine X* 2024, **19**:100500.
4. Bouazzaoui A, Abdellatif AAH, Al-Allaf FA, Bogari NM, Al-Dehlawi S, Qari SH: Strategies for Vaccination: Conventional Vaccine Approaches Versus New-Generation Strategies in Combination with Adjuvants. *Pharmaceutics* 2021, **13**(2):329.⏎
5. Abdel-Bakky MS, Amin E, Ewees MG, Mahmoud NI, Mohammed HA, Altowayan WM, Abdellatif AAH: Coagulation System Activation for Targeting of COVID-19: Insights into

Anticoagulants, Vaccine-Loaded Nanoparticles, and Hypercoagulability in COVID-19 Vaccines. *Viruses* 2022, **14**(2):307.

6. Samreen, Ahmad I, Malak HA, Abulreesh HH: Environmental Antimicrobial Resistance and Its Drivers: A Potential Threat to Public Health. *J Glob Antimicrob Resist* 2021, **27**:101–111.
7. Ongey EL, Neubauer P: Lanthipeptides: Chemical Synthesis Versus in Vivo Biosynthesis as Tools for Pharmaceutical Production. *Microb Cell Fact* 2016, **15**(1):97.
8. Brown P, Abbott E, Abdulle O, Boakes S, Coleman S, Divall N, Duperchy E, Moss S, Rivers D, Simonovic M *et al*: Design of Next Generation Polymyxins with Lower Toxicity: The Discovery of SPR206. *ACS Infect Dis* 2019, **5**(10):1645–1656.
9. Molchanova N, Hansen PR, Franzyk H: Advances in Development of Antimicrobial Peptidomimetics as Potential Drugs. *Molecules* 2017, **22**(9):1430.
10. Vila J, Moreno-Morales J, Balleste-Delpierre C: Current Landscape in the Discovery of Novel Antibacterial Agents. *Clin Microbiol Infect* 2020, **26**(5):596–603.
11. Zheng B, Wang X, Guo M, Tzeng CM: Therapeutic Peptides: Recent Advances in Discovery, Synthesis, and Clinical Translation. *Int J Mol Sci* 2025, **26**(11):5131.
12. Qian H. Current Status and Trends in Research and Development of Polypeptide Drugs. In: Yu B, Li N, Fu C, editors. *Privileged Scaffolds in Drug Discovery*. Amsterdam, The Netherlands: Academic Press. 2023, pp. 861–874.
13. Wang L, Wang N, Zhang W, Cheng X, Yan Z, Shao G, Wang X, Wang R, Fu C: Therapeutic Peptides: Current Applications and Future Directions. *Signal Transduct Target Ther* 2022, **7**(1):48.
14. Grauer A, König B: Peptidomimetics – A Versatile Route to Biologically Active Compounds. *European Journal of Organic Chemistry* 2009, **2009**(30):5099–5111.
15. Al-Azzam S, Ding Y, Liu J, Pandya P, Ting JP, Afshar S: Peptides to Combat Viral Infectious Diseases. *Peptides* 2020, **134**:170402.
16. O'Neill J: Tackling drug-resistant infections globally: Final report and recommendations; Government of the United Kingdom. London, UK: Review on Antimicrobial Resistance. 2016, p. 80.

17. Kim ES, Plosker GL: AFREZZA (R) (Insulin Human) Inhalation Powder: A Review in Diabetes Mellitus. *Drugs* 2015, **75**:1679–1686.

18. Erak M, Bellmann-Sickert K, Els-Heindl S, Beck-Sickinger AG: Peptide Chemistry Toolbox - Transforming Natural Peptides into Peptide Therapeutics. *Bioorg Med Chem* 2018, **26**(10):2759–2765.

19. Lau JL, Dunn MK: Therapeutic Peptides: Historical Perspectives, Current Development Trends, and Future Directions. *Bioorg Med Chem* 2018, **26**(10):2700–2707.

20. Al Musaimi O, Al Shaer D, Albericio F, de la Torre BG: 2022 FDA TIDES (Peptides and Oligonucleotides) Harvest. *Pharmaceuticals (Basel)* 2023, **16**(3):471.

21. Muttenthaler M, King GF, Adams DJ, Alewood PF: Trends in Peptide Drug Discovery. *Nat Rev Drug Discov* 2021, **20**(4):309–325.

22. Enayathullah MG, Parekh Y, Banu S, Ram S, Nagaraj R, Kumar BK, Idris MM: Gramicidin S and Melittin: Potential Anti-Viral Therapeutic Peptides to Treat SARS-CoV-2 Infection. *Sci Rep* 2022, **12**(1):3446.

23. Bobde SS, Alsaab FM, Wang G, Hoek ML: Ab Initio Designed Antimicrobial Peptides Against Gram-Negative Bacteria. *Front Microbiol* 2021, **12**:3460.

24. Desgrosellier JS, Cheresh DA: Integrins in Cancer: Biological Implications and Therapeutic Opportunities. *Nat Rev Cancer* 2010, **10**(1):9–22.

25. Lonsdale R, Ward RA: Structure-Based Design of Targeted Covalent Inhibitors. *Chem Soc Rev* 2018, **47**:3816–3830.

26. Cosmi F, Laini R, Nicolucci A: Semaglutide and Cardiovascular Outcomes in Patients with Type 2 Diabetes. *N Engl J Med* 2017, **376**(9):890.

27. Sims EK, Carr ALJ, Oram RA, DiMeglio LA, Evans-Molina C: 100 Years of Insulin: Celebrating the Past, Present and Future of Diabetes Therapy. *Nat Med* 2021, **27**(7):1154–1164.

28. Bobde SS, Alsaab FM, Wang G, Van Hoek ML: Ab Initio Designed Antimicrobial Peptides Against Gram-Negative Bacteria. *Front Microbiol* 2021, **12**:715246.

29. Abdellatif AAH, Mostafa MAH, Khojah HMJ, Al Haidari RA, Tawfeek HM, Soliman GM, Al Thagfan SS, Faris TM, Tolba NS: Utilizing Ziziphus Spina-christi for Eco-Friendly Synthesis of Silver Nanoparticles: Antimicrobial Activity and Promising Application in Wound Healing. *Nanotechnol Rev* 2024, **13**(1):20230112.
30. Kaur R, Kanotra M, Sood A, Abdellatif AAH, Bhatia S, Al-Harrasi A, Aleya L, Vargas-De-La-Cruz C, Behl T: Emergence of Nutriments as a Nascent Complementary Therapy Against Antimicrobial Resistance. *Environ Sci Pollut Res Int* 2022, **29**(33):49568–49582.
31. Zheng S, Tu Y, Li B, Qu G, Li A, Peng X, Li S, Shao C: Antimicrobial Peptide Biological Activity, Delivery Systems and Clinical Translation Status and Challenges. *J Trans Med* 2025, **23**(1):112.
32. Rossino G, Marchese E, Galli G, Verde F, Finizio M, Serra M, Linciano P, Collina S: Peptides as Therapeutic Agents: Challenges and Opportunities in the Green Transition Era. *Molecules* 2023, **28**(20):7165.
33. Martin-Loeches I, Dale GE, Torres A: Murepavadin: A New Antibiotic Class in the Pipeline. *Expert Rev Anti Infect Ther* 2018, **16**(4):259–268.
34. Mandel S, Michaeli J, Nur N, Erbetti I, Zazoun J, Ferrari L, Felici A, Cohen-Kutner M, Bachnoff N: OMN6 a Novel Bioengineered Peptide for the Treatment of Multidrug Resistant Gram Negative Bacteria. *Sci Rep* 2021, **11**(1):6603.
35. Nathanson N: The human toll of viral diseases. In: *Viral Pathogenesis*. 3rd ed. San Diego, CA: Academic Press, 2016, pp. 3–16.
36. Ahmadi K, Farasat A, Rostamian M, Johari B, Madanchi H: Enfuvirtide, an HIV-1 Fusion Inhibitor Peptide, can Act as a Potent SARS-CoV-2 Fusion Inhibitor: An in Silico Drug Repurposing Study. *J Biomol Struct Dyn* 2022, **40**(12):5566–5576.
37. Dando TM: Perry CM: Enfuvirtide. *Drugs* 2003, **63**(24):2755–2766; discussion 2767–2758.
38. Bai Y, Jones JC, Wong SS, Zanin M: Antivirals Targeting the Surface Glycoproteins of Influenza Virus: Mechanisms of Action and Resistance. *Viruses* 2021, **13**(4):624.
39. Freitas ED, Bataglioli RA, Oshodi J, Beppu MM: Antimicrobial Peptides and Their Potential Application in Antiviral Coating Agents. *Colloids Surf B Biointerfaces* 2022, **217**:112693.

40. Zhang Q, Chen X, Li B, Lu C, Yang S, Long J, Chen H, Huang J, He B: A Database of Anti-Coronavirus Peptides. *Sci Data* 2022, **9**(1):294.

41. Madhavan M, AlOmair LA, Ks D, Mustafa S: Exploring Peptide Studies Related to SARS-CoV to Accelerate the Development of Novel Therapeutic and Prophylactic Solutions Against COVID-19. *J Infect Public Health* 2021, **14**(8):1106–1119.

42. Souza FR, Moura PG, Costa RKM, Silva RS, Pimentel AS: Absolute Binding Free Energies of Mucroporin and Its Analog Mucroporin-M1 With the Heptad Repeat 1 Domain and RNA-Dependent RNA Polymerase of SARS-CoV-2. *J Biomol Struct Dyn* 2023, **41**(14):6957–6968.

43. Mahendran ASK, Lim YS, Fang CM, Loh HS, Le CF: The Potential of Antiviral Peptides as COVID-19 Therapeutics. *Front Pharmacol* 2020, **11**:575444.

44. Bouazzaoui A, Keedwa KM, Sahl SM, Al-Hashmi MS, Alzabeedi KH, Albshri MH, Bukhari MA, Qabani FH, Mula IA, Badr OI *et al*: Lymphocyte Subsets in Covid-19 Saudi Patients: Infection Caused Significant Reduction in Peripheral Blood Lymphocyte Subset. *Clin Lab* 2025,**71**(5).

45. Abdellatif AAH, Tawfeek HM, Abdelfattah A, El-Saber Batiha G, Hetta HF: Recent Updates in COVID-19 with Emphasis on Inhalation Therapeutics: Nanostructured and Targeting Systems. *J Drug Deliv Sci Technol* 2021, **63**:102435.

46. Xia S, Liu M, Wang C, Xu W, Lan Q, Feng S, Qi F, Bao L, Du L, Liu S *et al*: Inhibition of SARS-CoV-2 (Previously 2019-Ncov) Infection by a Highly Potent Pan-Coronavirus Fusion Inhibitor Targeting Its Spike Protein That Harbors a High Capacity to Mediate Membrane Fusion. *Cell Res* 2020, **30**(4):343–355.

47. Mehta Y, Mehta C, Chandrasekaran A: Aviptadil: A Multifaceted Approach to Mitigating Hypoxemia in Acute Respiratory Distress Syndrome. *Respir Med Case Rep* 2024, **48**:101992.

48. Li CM, Haratipour P, Lingeman RG, Perry JJP, Gu L, Hickey RJ, Malkas LH: Novel Peptide Therapeutic Approaches for Cancer Treatment. *Cells* 2021, **10**(11):2908.

49. Chiangjong W, Chutipongtanate S, Hongeng S: Anticancer Peptide: Physicochemical Property, Functional Aspect and Trend in Clinical Application (Review). *Int J Oncol* 2020, **57**(3):678–696.

50. Abdel-Salam MAL, Cravalho-Tavares J, Cravalho AT, Kitten GT, Nyffeler J, Daias FF, Reis PVMD: Amino Acid Availability and Cellular Regulation Mechanisms. *Aminoacids* 2019, **51**(3):433–449.

51. Luan X, Wu Y, Shen Y-W, Zhang H, Zhou Y-D, Chen H-Z, Nagle DG, Zhang W-D: Natural Products Report. *Nat Prod Rep* 2020, **38**(1):7–17.

52. Potts MB, McMillan EA, Rosales TI, Kim HS, Ou YH, Toombs JE, Brekken RA, Minden MD, MacMillan JB, White MA: Mode of Action and Pharmacogenomic Biomarkers for Exceptional Responders to Didemnin B. *Nat Chem Biol* 2015, **11**(6):401–408.

53. Jayaweera SPE, Wanigasinghe Kanakanamge SP, Rajalingam D, Silva GN: Carfilzomib: A Promising Proteasome Inhibitor for the Treatment of Relapsed and Refractory Multiple Myeloma. *Front Oncol* 2021, **11**:740796.

54. Gallucci E, Micelli S, Picciarelli V: Multi-Channel and Single-Channel Investigation of Protein and Peptide Incorporation into BLM. *Membr Sci Technol* 2003, **7**:413–447.

55. Wan X, Liu H, Sun Y, Zhang J, Chen X, Chen N: Lunasin: A Promising Polypeptide for the Prevention and Treatment of Cancer. *Oncol Lett* 2017, **13**:3997–4001.

56. Rusdi N, Purwaningsih E, Hestiantoro A, Elya B, Kusmardi K, Kusmardi K: In Vivo Antimammary Tumor Effects of Soybean Extract with Targeted Lunasin. *Pharmacogn J* 2021, **13**:1269–1276.

57. Li K, Pang L, Pan X, Fan S, Wang X, Wang Q, Dai P, Gao W, Gao J: GE11 Modified PLGA/TPGS Nanoparticles Targeting Delivery of Salinomycin to Breast Cancer Cells. *Technol Cancer Res Treat* 2021, **20**:15330338211004954.

58. Ren H, Gao C, Zhou L, Liu M, Xie C, Lu W: EGFR-Targeted Poly(ethylene Glycol)-Distearoylphosphatidylethanolamine Micelle Loaded With Paclitaxel for Laryngeal Cancer: Preparation, Characterization and in Vitro Evaluation. *Drug Deliv* 2015, **22**(6):785–794.

59. Lim KJ, Sung BH, Shin JR, Lee YW, Kim DJ, Yang KS, Kim SC: A Cancer Specific Cell-Penetrating Peptide, BR2, for the Efficient Delivery of an Scfv into Cancer Cells. *PLoS One* 2013, **8**(6):e66084.

60. Muggia FM, Bonetti A, Hoeschele JD, Rozencweig M, Howell SB: Platinum Antitumor Complexes: 50 Years Since Barnett Rosenberg's Discovery. *J Clin Oncol* 2015, **33**(35):4219–4226.

61. Miller RP, Tadagavadi RK, Ramesh G, Reeves WB: Mechanisms of Cisplatin Nephrotoxicity. *Toxins (Basel)* 2010, **2**(11):2490–2518.

62. Mostafa MAH, Khojah HMJ, Ohta T: Isolation and Identification of Novel Selective Antitumor Constituents, Sidrin and Sidroside, from Zizyphus Spina-christi. *Saudi Pharm J* 2023, **31**(6):1019–1028.

63. Li Y, Li W, Xu Z: Improvement on Permeability of Cyclic Peptide/Peptidomimetic: Backbone N-Methylation as A Useful Tool. *Mar Drugs* 2021, **19**(6):10–3390.

64. Younis MA, Tawfeek HM, Abdellatif AAH, Abdel-Aleem JA, Harashima H: Clinical Translation of Nanomedicines: Challenges, Opportunities, and Keys. *Adv Drug Deliv Rev* 2022, **181**:114083.

65. Abdellatif AAH, Bouazzaoui A, Tawfeek HM, Younis MA: MCT4 Knockdown by Tumor Microenvironment-Responsive Nanoparticles Remodels the Cytokine Profile and Eradicates Aggressive Breast Cancer Cells. *Colloids Surf B Biointerfaces* 2024, **238**:113930.

66. Agwa RH, Alkully TH, Alghamdi TA, Alghamdi RF, Alzahrani AM, Alghamdi WS, et al. Prevalence of Obesity and its Relationship with Lifestyle Patterns among Adults in Al-Baha, Saudi Arabia: A cross-sectional study. *Medicine*. 2026, 105(3):e47038.

67. Musa A, Ahmed SR, Hussein S, Youssif KA, El-Ghorab AH, Haidari RAA, Mostafa MAH, Almaghrabi M, Aldakhil TY, Mohamed MA *et al*: Prominent Antidiabetic and Anticancer Investigation of Scrophularia deserti Extract: Integration of Experimental and Computational Approaches. *J Mol Struct* 2024, **1315**:137923.

68. Mohammad FS, Das U, Samanta SK, Irfan Z, Gopinath SCB, Mostafa MAH, Al-Haidari RA, Abdellatif AAH, Shehata AM, Gouda MM: Evaluation of Sechium edule Fruit Attenuation Impact on the Cardiomyopathy of the STZ-Induced Diabetic Rats. *Heliyon* 2024, **10**(9):e30456.

69. Marthandam Asokan S, Hung TH, Chiang WD, Lin WT: Lipolysis-Stimulating Peptide from Soybean Protects Against High Fat Diet-Induced Apoptosis in Skeletal Muscles. *J Med Food*

2018, **21**(3):225–232.

70. Kumar MS: Peptides and Peptidomimetics as Potential Antiobesity Agents: Overview of Current Status. *Front Nutr* 2019, **6**:11.

71. Barnhart KF, Christianson DR, Hanley PW, Driessen WH, Bernacky BJ, Baze WB, Wen S, Tian M, Ma J, Kolonin MG *et al*: A Peptidomimetic Targeting White Fat Causes Weight Loss and Improved Insulin Resistance in Obese Monkeys. *Sci Transl Med* 2011, **3**(108):108ra112.

72. Kolonin MG, Saha PK, Chan L, Pasqualini R, Arap W: Reversal of Obesity by Targeted Ablation of Adipose Tissue. *Nat Med* 2004, **10**(6):625–632.

73. Staquicini FI, Cardo-Vila M, Kolonin MG, Trepel M, Edwards JK, Nunes DN, Sergeeva A, Efstathiou E, Sun J, Almeida NF *et al*: Vascular Ligand-Receptor Mapping by Direct Combinatorial Selection in Cancer Patients. *Proc Natl Acad Sci U S A* 2011, **108**(46):18637–18642.

74. Fisher EA, Feig JE, Hewing B, Hazen SL, Smith JD: HDLF: Dysfunction, and Reverse Cholesterol Transport. *Arterioscler Thromb Vasc Biol* 2012, **32**:2813–2820.

75. Davidson WS, Hazlett T, Mantulin WW, Jonas A: The Role of Apolipoprotein AI Domains in Lipid Binding. *Proc Natl Acad Sci U S A* 1996, **93**(24):13605–13610.

76. Stoekenbroek RM, Stroes ES, Hovingh GK: ApoA-I Mimetics. *Handb Exp Pharmacol* 2015, **224**:631–648.

77. Takata K, Imaizumi S, Kawachi E, Yahiro E, Suematsu Y, Shimizu T, Abe S, Matsuo Y, Nakajima K, Yasuno T *et al*: The ApoA-I Mimetic Peptide FAMP Promotes Recovery from Hindlimb Ischemia Through a Nitric Oxide (NO)-Related Pathway. *Int J Cardiol* 2016, **207**:317–325.

78. Schaefer EJ, Santos RD, Asztalos BF: Marked HDL Deficiency and Premature Coronary Heart Disease. *Curr Opin Lipidol* 2010, 21(4):289–297.

79. Uehara Y, Chiesa G, Saku K: High-Density Lipoprotein-Targeted Therapy and Apolipoprotein A-I Mimetic Peptides. *Circ J* 2015, **79**(12):2523–2528.

80. Suematsu Y, Kawachi E, Idemoto Y, Matsuo Y, Kuwano T, Kitajima K, Imaizumi S, Kawamura A, Saku K, Uehara Y *et al*: Anti-Atherosclerotic Effects of an Improved Apolipoprotein A-I

Mimetic Peptide. *Int J Cardiol* 2019, **297**:111–117.

81. White CR, Datta G, Wilson L, Palgunachari MN, Anantharamaiah GM. The apoA-I Mimetic Peptide 4F Protects Apolipoprotein A-I from Oxidative Damage. *Chem. Phys. Lipids.* 2019, 219:28–35.
82. Kavarian PN, Mosher TL, Abu El Haija M: Use of Glucagon-Like-Peptide 1 Receptor Agonist in the Treatment of Childhood Obesity. *Curr Opin Pediatr* 2024, **36**(5):542–546.
83. Nishikimi T, Maeda N, Matsuoka H: The Role of Natriuretic Peptides in Cardioprotection. *Cardiovasc Res* 2006, **69**(2):318–328.
84. Miyazaki T, Otani K, Chiba A, Nishimura H, Tokudome T, Takano-Watanabe H, Matsuo A, Ishikawa H, Shimamoto K, Fukui H *et al*: A New Secretory Peptide of Natriuretic Peptide Family, Osteocrin, Suppresses the Progression of Congestive Heart Failure After Myocardial Infarction. *Circ Res* 2018, **122**(5):742–751.
85. McMurray JJ, Packer M, Desai AS, Gong J, Lefkowitz MP, Rizkala AR, Rouleau JL, Shi VC, Solomon SD, Swedberg K *et al*: Angiotensin-Neprilysin Inhibition Versus Enalapril in Heart Failure. *N Engl J Med* 2014, **371**(11):993–1004.
86. Davenport AP, Hyndman KA, Dhaun N, Southan C, Kohan DE, Pollock JS, Pollock DM, Webb DJ, Maguire, JJ: Endothelin. *Pharmacol Rev* 2016, **68**:357–418.
87. Rosenkranz S, Lang IM, Blindt R, Bonderman D, Bruch L, Diller GP, Felgendreher R, Gerges C, Hohenforst-Schmidt W, Holt S *et al*: Pulmonary Hypertension Associated With Left Heart Disease: Updated Recommendations of the Cologne Consensus Conference 2018. *Int J Cardiol* 2018, **272S**:53–62.
88. Kitamura K, Kangawa K, Kawamoto M, Ichiki Y, Nakamura S, Matsuo H, Eto T: Adrenomedullin: A Novel Hypotensive Peptide Isolated from Human Pheochromocytoma. *Biochem Biophys Res Commun* 1993, **192**(2):553–560.
89. Nishikimi T, Matsuoka H: Cardiac Adrenomedullin: Its Role in Cardiac Hypertrophy and Heart Failure. *Curr Med Chem Cardiovasc Hematol Agents* 2005, **3**(3):231–242.
90. Yoshizawa T, Sakurai T, Kamiyoshi A, Ichikawa-Shindo Y, Kawate H, Iesato Y, Koyama T, Uetake R, Yang L, Yamauchi A *et al*: Novel Regulation of Cardiac Metabolism and Homeostasis

by the Adrenomedullin-Receptor Activity-Modifying Protein 2 System. *Hypertension* 2013, **61**(2):341–351.

91. Kojima M, Hosoda H, Date Y, Nakazato M, Matsuo H, Kangawa K: Ghrelin is a Growth-Hormone-Releasing Acylated Peptide from Stomach. *Nature* 1999, **402**(6762):656–660.
92. Hosoda H, Kojima M, Kangawa K: Biological, Physiological, and Pharmacological Aspects of Ghrelin. *J Pharmacol Sci* 2006, **100**(5):398–410.
93. Kishimoto I, Tokudome T, Hosoda H, Miyazato M, Kangawa K: Ghrelin and Cardiovascular Diseases. *J Cardiol* 2012, **59**(1):8–13.
94. Kuwahara K: The Natriuretic Peptide System in Heart Failure: Diagnostic and Therapeutic Implications. *Pharmacol Ther* 2021, **227**:107863.
95. Burnett JC Jr.: Atrial Natriuretic Peptide, Heart Failure and the Heart as an Endocrine Organ. *Clin Chem* 2019, **65**(12):1602–1603.
96. Dong Y: Cyclic Peptide RD808 Reduces Myocardial Injury Induced by beta1-Adrenoreceptor Autoantibodies. *Heart Vessels* 2019, **34**:1040–1051.
97. Gao HR, Gao HY: Cardiovascular Functions of Central Corticotropin-Releasing Factor Related Peptides System. *Neuropeptides* 2019, **75**:18–24.
98. Schokrpur S, White MG, Roland CL, Patel SP, Immuno-Oncology: New Insights into Targets and Therapies. *Surg Oncol Clin N Am* 2024, **33**(2):265–278.
99. Melief CJ, van der Burg SH: Immunotherapy of Established (pre)malignant Disease by Synthetic Long Peptide Vaccines. *Nat Rev Cancer* 2008, **8**(5):351–360.
100. Bojarska J, Wolf WM: Short Peptides as Powerful Arsenal for Smart Fighting Cancer. *Cancers* 2024, **16**(19):3321.
101. Azmi F, Ahmad Fuaad AA, Skwarczynski M, Toth I: Recent Progress in Adjuvant Discovery for Peptide-Based Subunit Vaccines. *Hum Vaccin Immunother* 2014, 10(3):778–796.
102. Tsung K, Norton JA: In Situ Vaccine, Immunological Memory and Cancer Cure. *Hum Vaccin Immunother* 2016, **12**(1):117–119.

103. Malonis RJ, Lai JR, Vergnolle O: Peptide-Based Vaccines: Current Progress and Future Challenges. *Chem Rev* 2020, **120**(6):3210–3229.

104. Lekshmy M, Dhanya CR, Smrithi JS, Sindhurani JA, Vandanamthadathil JJ, Veettil JT, Anila L, Lathakumari VS, Nayar AM, Madhavan M: Peptide Vaccines as Therapeutic and Prophylactic Agents for Female-Specific Cancers: The Current Landscape. *Pharmaceuticals (Basel)* 2023, **16**(7): 1054.

105. Linhart B, Narayanan M, Focke-Tejkl M, Wrba F, Vrtala S, Valenta R: Prophylactic and Therapeutic Vaccination With Carrier-Bound Bet v 1 Peptides Lacking Allergen-Specific T Cell Epitopes Reduces Bet v 1-Specific T Cell Responses via Blocking Antibodies in a Murine Model for Birch Pollen Allergy. *Clin Exp Allergy* 2014, **44**(2):278–287.

106. Crews DW, Dombroski JA, King MR: Prophylactic Cancer Vaccines Engineered to Elicit Specific Adaptive Immune Response. *Front Oncol* 2021, **11**:626463.

107. Zheng S, Tu Y, Li B, Qu G, Li A, Peng X, Li S, Shao C: Antimicrobial Peptide Biological Activity, Delivery Systems and Clinical Translation Status and Challenges. *J Transl Med* 2025, **23**(1):292.

108. Yang Y, Wang S, Ma P, Jiang Y, Cheng K, Yu Y, Jiang N, Miao H, Tang Q, Liu F *et al*: Drug Conjugate-Based Anticancer Therapy - Current Status and Perspectives. *Cancer Lett* 2023, **552**:215997.

109. Wang M, Liu J, Xia M, Yin L, Zhang L, Liu X, Cheng Y: Peptide-Drug Conjugates: A New Paradigm for Targeted Cancer Therapy. *Eur J Med Chem* 2024, 265:115997.

110. Olivier T, Prasad V: The Approval and Withdrawal of Melphalan Flufenamide (Melflufen): Implications for the State of the FDA. *Transl Oncol* 2022, **18**:101374.

111. Yang Y, Wang S, Ma P, Jiang Y, Cheng K, Yu Y, Jiang N, Miao H, Tang Q, Liu F *et al*: Drug Conjugate-Based Anticancer Therapy - Current Status and Perspectives. *Cancer Lett* 2023, **552**:215969.

112. Wang M, Liu J, Xia M, Yin L, Zhang L, Liu X, Cheng Y: Peptide-Drug Conjugates: A New Paradigm for Targeted Cancer Therapy. *Eur J Med Chem* 2024, **265**:116119.

113. Xiao W, Jiang W, Chen Z, Huang Y, Mao J, Zheng W, Hu Y, Shi J: Advance in Peptide-Based Drug Development: Delivery Platforms, Therapeutics and Vaccines. *Signal Transduct Target Ther* 2025, **10**(1):58.

114. Ding B, Zhu Z, Guo C, Li J, Gan Y, Yu M: Oral Peptide Therapeutics for Diabetes Treatment: State-of-the-Art and Future Perspectives. *Acta Pharmaceutica Sinica B* 2024, **14**(5):2006–2025.

115. Lee YS: Peptidomimetics and Their Applications for Opioid Peptide Drug Discovery. *Biomolecules* 2022, 12(9):1295.

116. Craik DJ, Fairlie DP, Liras S, Price D: The Future of Peptide-Based Drugs. *Chem Biol Drug Des* 2013, **81**(1):136–147.

117. Diao L, Meibohm B: Pharmacokinetics and Pharmacokinetic-Pharmacodynamic Correlations of Therapeutic Peptides. *Clin Pharmacokinet* 2013, **52**(10):855–868.

118. Mohamed SMM, Aljohani AKB, El-morsy A, Al Haidari RA, Alamri MAA, Maqnas YA, Alsibaee AM, Mostafa MAH, Keshek DE, Mohamed MG *et al*: Synthesis and Characterization of Sodium alginate/poly(N-Vinylpyrrolidone) Nano-Carrier Loaded With Rebaudioside A and/or Stevioside for Anticancer Drug Delivery. *Int J Biol Macromol* 2025, 316:138792.

119. Mitragotri S, Burke PA, Langer R: Overcoming the Challenges in Administering Biopharmaceuticals: Formulation and Delivery Strategies. *Nat Rev Drug Discov* 2014, **13**(9):655–672.

120. Alrohaim A, Mostafa MAH, Saad Alnour TM, Alghamdi S, Almuraikhi N, Mirdad DSA, Alghamdi H, Senbel AM: Enhancing Drug Efficacy Through Nanoparticle-Based Delivery Systems: a Study on Targeted Cancer Therapy. *Int J Surg* 2025, 111(9):6023–6029.

121. Benincasa M, Zahariev S, Pelillo C, Milan A, Gennaro R, Scocchi M: PEGylation of the Peptide Bac7(1e35) Reduces Renal Clearance While Retaining Antibacterial Activity and Bacterial Cell Penetration Capacity. *Eur J Med Chem* 2015, **95**:210–219.

122. Chen H, Zhang Q: Polypeptides as Alternatives to PEGylation of Therapeutic Agents. *Expert Opin Drug Deliv* 2024, **21**(1):1–12.

123. Gilliam AD, Watson SA: G17DT: An Antigastrin Immunogen for the Treatment of Gastrointestinal Malignancy. *Expert Opin Biol Ther* 2007, **7**(3):397–404.

124. Knudsen LB, Lau J: The Discovery and Development of Liraglutide and Semaglutide. *Front Endocrinol (Lausanne)* 2019, **10**:155.

125. Buckley ST, Baekdal TA, Vegge A, Maarbjerg SJ, Pyke C, Ahnfelt-Ronne J, Madsen KG, Scheele SG, Alanentalo T, Kirk RK *et al*: Transcellular Stomach Absorption of a Derivatized Glucagon-Like Peptide-1 Receptor Agonist. *Sci Transl Med* 2018, **10**(467):7047.

126. Bucheit JD, Pamulapati LG, Carter N, Malloy K, Dixon DL, Sisson EM: Oral Semaglutide: A Review of the First Oral Glucagon-Like Peptide 1 Receptor Agonist. *Diabetes Technol Ther* 2020, **22**(1):10–18.

127. Abdellatif AAH, Mohammed AM, Saleem I, Alsharidah M, Al Rugaie O, Ahmed F, Osman SK: Smart Injectable Chitosan Hydrogels Loaded with 5-Fluorouracil for the Treatment of Breast Cancer. *Pharmaceutics* 2022, **14**(3):557.

128. Tawfeek HM, Abou-Taleb DAE, Badary DM, Ibrahim M, Abdellatif AAH: Pharmaceutical, Clinical, and Immunohistochemical Studies of Metformin Hydrochloride Topical Hydrogel for Wound Healing Application. *Arch Dermatol Res* 2020, **312**(2):113–121.

129. Aldosari BN, Tawfeek HM, Abdellatif AAH, Almurshedi AS, Alfagih IM, AlQuadeib BT, Abbas AYA, Mohammed HM, Hassan YA, Fayed MH *et al*: Comparative Study of Lepidium sativum Orally Administered Seeds, Hydrogel and Atorvastatin on Obesity of Rats Fed on a High Fat Diet. *Drug Dev Ind Pharm* 2024, **50**(7):605–618.

130. Abdellatif AAH, Al-Subaiyel A, Mohammed AM: Thermosensitive Polymers-Based Injectable Hydrogels: A Quantitative Validations Design Utilized for Controlled Delivery of Gefitinib Anticancer Drug. *Eur Rev Med Pharmacol Sci* 2023, **27**(6):2646–2658.

131. Eldor R, Arbit E, Corcos A, Kidron M: Glucose-Reducing Effect of the ORMD-0801 Oral Insulin Preparation in Patients With Uncontrolled Type 1 Diabetes: A Pilot Study. *PLoS One* 2013, **8**(4):e59524.

132. Biermasz NR: New Medical Therapies on the Horizon: Oral Octreotide. *Pituitary* 2017, **20**(1):149–153.

133. Abdellatif AAH, Aljutayli A, Mohammed AM: Thermosensitive Chitosan Hydrogel Loaded Cetuximab: A Novel Approach for Parenteral Controlled Delivery. *Drug Dev Ind Pharm* 2025, **51**(6):597–605.

134. Abdellatif AAH: Identification of Somatostatin Receptors Using Labeled PEGylated Octreotide, as an Active Internalization. *Drug Dev Ind Pharm* 2019, **45**(10):1707–1715.

135. Abdellatif AAH, Abou-Taleb HA, Abd El Ghany AA, Lutz I, Bouazzaoui A: Targeting of Somatostatin Receptors Expressed in Blood Cells Using Quantum Dots Coated With Vapreotide. *Saudi Pharm J* 2018, **26**(8):1162–1169.

136. Abdellatif AAH, Aldalaen SM, Faisal W, Tawfeek HM: Somatostatin Receptors as a New Active Targeting Sites for Nanoparticles. *Saudi Pharm J* 2018, 26(7):1051–1059.

137. Abdellatif AA, Zayed G, El-Bakry A, Zaky A, Saleem IY, Tawfeek HM: Novel Gold Nanoparticles Coated With Somatostatin as a Potential Delivery System for Targeting Somatostatin Receptors. *Drug Dev Ind Pharm* 2016, **42**(11):1782–1791.

138. Zorzi A, Deyle K, Heinis C: Cyclic Peptide Therapeutics: Past, Present and Future. *Curr Opin Chem Biol* 2017, **38**:24–29.

139. Choi JS, Joo SH: Recent Trends in Cyclic Peptides as Therapeutic Agents and Biochemical Tools. *Biomol Ther (Seoul)* 2020, **28**(1):18–24.

140. Fetse J, Zhao Z, Liu H, Mamani UF, Mustafa B, Adhikary P, Ibrahim M, Liu Y, Patel P, Nakhjiri M *et al*: Discovery of Cyclic Peptide Inhibitors Targeting PD-L1 for Cancer Immunotherapy. *J Med Chem* 2022, **65**(18):12002–12013.

141. Domhan C, Uhl P, Kleist C, Zimmermann S, Umstatter F, Leotta K, Mier W, Wink M: Replacement of l-Amino Acids by d-Amino Acids in the Antimicrobial Peptide Ranalexin and Its Consequences for Antimicrobial Activity and Biodistribution. *Molecules* 2019, **24**(16):2987.

142. Sylvestre M, Lv S, Yang LF, Luera N, Peeler DJ, Chen BM, Roffler SR, Pun SH: Replacement of L-Amino Acid Peptides With D-Amino Acid Peptides Mitigates Anti-PEG Antibody Generation Against Polymer-Peptide Conjugates in Mice. *J Control Release* 2021, **331**:142–153.

143. Li Y, Liu T, Liu Y, Tan Z, Ju Y, Yang Y, Dong W: Antimicrobial Activity, Membrane Interaction and Stability of the D-Amino Acid Substituted Analogs of Antimicrobial Peptide W3R6. *J*

Photochem Photobiol B 2019, **200**:111645.

144. Eckhauser ML: The Neodymium-YAG Laser and Gastrointestinal Malignancy. *Arch Surg* 1990, **125**(9):1152–1154.

145. Kessler JL, Kang G, Qin Z, Kang H, Whitby FG, Cheatham TE, 3rd, Hill CP, Li Y, Yu SM: Peptoid Residues Make Diverse, Hyperstable Collagen Triple-Helices. *J Am Chem Soc* 2021, **143**(29):10910–10919.

146. Spicer SK, Subramani A, Aguila AL, Green RM, McClelland EE, Bicker KL: Toward a Clinical Antifungal Peptoid: Investigations into the Therapeutic Potential of AEC5. *Biopolymers* 2019, **110**(6):e23276.

147. Haidari RAA, Abdelkarem FM, Alrehaili BD, Alhaddad A, El-Sayed AAA, Samman WA, Mostafa MAH: Anti-Inflammatory Activity of Sarcoaldosterol A Isolated From Heteroxenia fuscescens: Insights From In Vitro, In Silico, and Spectroscopic Studies. *Chem Biodivers* 2025, 22(10): e01069.

148. Hyun S, Park N, Nam SH, Cheon DH, Lee Y, Lim HS, Yu J: One-Bead-One-Compound Screening Approach to the Identification of Cyclic Peptoid Inhibitors of Cyclophilin D as Neuroprotective Agents from Mitochondrial Dysfunction. *Chem Commun (Camb)* 2021, **57**(19):2388–2391.

149. Abdelkarem FM, Nafady AM, Allam AE, Mostafa MAH, Al Haidari RA, Hassan HA, Zaki MEA, Assaf HK, Kamel MR, Zidan SAH *et al*: A Comprehensive in Silico Study of New Metabolites from Heteroxenia fuscescens with SARS-CoV-2 Inhibitory Activity. *Molecules* 2022, **27**(21):7308.

150. Abdellatif AAH, Sameh Tolba N, Tawfeek HM: Editorial: Nanomedicine and Application; Characterization, Diagnosis, Treatment, and Targeting Cancers. *Drug Dev Ind Pharm* 2025, **51**(9):985–986.

151. Tawfeek HM, Abdellatif AAH, Dennison TJ, Mohammed AR, Sadiq Y, Saleem IY: Colonic Delivery of Indometacin Loaded PGA-Co-PDL Microparticles Coated with Eudragit L100–55 from Fast Disintegrating Tablets. *Int J Pharm* 2017, **531**(1):80–89.

152. Merlino F, Billard E, Yousif AM, Di Maro S, Brancaccio D, Abate L, Carotenuto A, Bellavita R, d'Emmanuele di Villa Bianca R, Santicioli P *et al*: Functional Selectivity Revealed by N-Methylation Scanning of Human Urotensin II and Related Peptides. *J Med Chem* 2019, **62**(3):1455–1467.

153. Savitha MN, Suvilesh KN, Siddesha JM, Milan Gowda MD, Choudhury M, Velmurugan D, Umashankar M, Vishwanath BS: Combinatorial Inhibition of Angiotensin Converting Enzyme, Neutral Endopeptidase and Aminopeptidase N by N-Methylated Peptides Alleviates Blood Pressure and Fibrosis in Rat Model of Dexamethasone-Induced Hypertension. *Peptides* 2020, **123**:170180.

154. Zheng Y, Ongpipattanakul C, Nair SK: Bioconjugate Platform for Iterative Backbone N-Methylation of Peptides. *ACS Catal* 2022, **12**(22):14006–14014.

155. Sana B, Ke D, Li EHY, Ho T, Seayad J, Duong HA, Ghadessy FJ: Halogenation of Peptides and Proteins Using Engineered Tryptophan Halogenase Enzymes. *Biomolecules* 2022, **12**(12):10–3390.

156. Kemker I, Schroder DC, Feiner RC, Muller KM, Marion A, Sewald N: Tuning the Biological Activity of RGD Peptides with Halotryptophansdagger. *J Med Chem* 2021, **64**(1):586–601.

157. Molchanova N, Nielsen JE, Sorensen KB, Prabhala BK, Hansen PR, Lund R, Barron AE, Jenssen H: Halogenation as a Tool to Tune Antimicrobial Activity of Peptoids. *Sci Rep* 2020, **10**(1):14805.

158. Tawfeek HM, Roberts M, El Hamd MA, Abdellatif AAH, Younis MA: Glibenclamide Mini-Tablets with an Enhanced Pharmacokinetic and Pharmacodynamic Performance. *AAPS PharmSciTech* 2018, **19**(7):2948–2960.

9 Regulatory Challenges and Strategies in Polypeptide Therapeutics

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9.1 INTRODUCTION

Peptides differ in a significant way from small-molecule drugs, constituting a great challenge to their regulation. They offer their own obstacles in having a complex manufacturing process, variable structures, and properties of rapid biotransformation in the body together with a likelihood of eliciting immune responses. Thus, the regulations of peptide drugs need to strike a careful balance between encouraging innovation and ensuring safety and efficacy. Plus, these regulations have to keep up with the fast pace of new scientific discoveries and technological progress.

9.1.1 International Regulatory Bodies and Guidelines

When it comes to the rules governing peptide treatments, major international bodies like the U.S. Food and Drug Administration (FDA), the European Medicines Agency (EMA), Japan's Pharmaceuticals and Medical Devices Agency (PMDA), and China's National Medical Products Administration

(NMPA) are the key players shaping the landscape (Table 9.1). Each of these agencies offers specific guidance, outlines the steps needed for approval, and sets requirements for monitoring these treatments after they've been released to the public, all tailored to biological therapies and peptide-based drugs. In the United States, the FDA typically assesses peptide therapies through its Center for Drug Evaluation and Research (CDER), while biologics are usually handled by the Center for Biologics Evaluation and Research (CBER). Deciding which center takes the lead often depends on factors like the length of the peptide, how it's made (chemically synthesized, for example), and whether it comes from recombinant DNA technology or solid-phase peptide synthesis. Over in the European Union, the EMA uses its centralized process for granting marketing authorization to peptides that are considered biologics, under the rules set out by the EMA (EMA n.d.). The harmonization efforts worldwide, led by ICH and WHO, aim to standardize the guidelines for peptide drug development, mostly under GMP, stability studies, and bioequivalence requirements. Nevertheless, differences persist across regulatory regions concerning drug descriptions as "chemical drugs" versus "biologics," again leading to distinctions in the clinical trial expectations and manufacturing documentation (Morin et al., 2023; Wijesinghe et al., 2022; WHO, n.d.a, n.d.b).

TABLE 9.1

Regulatory Frameworks for Peptide Therapeutics

Regulatory Body	Scope of Oversight	Key Guidelines	Recent Developments
FDA	U.S. market approval	21 CFR Part 312	Guidance updated for complex generics and biologics

Regulatory Body	Scope of Oversight	Key Guidelines	Recent Developments
EMA	EU market approval	EMA/CHMP/ICH/437	Harmonization with ICH standards
ICH	International standards	Q6B, Q11	Revisions for biologics standardization
WHO	Global Health Standards	TRS No. 1003	Emphasis on quality in low-resource settings
TGA	Australian market	TGA Guidelines	Implementation of risk-based assessments
PMDA	Japanese market	PMDA Guidelines	Immunogenicity testing focus
Health Canada	Canadian market	Health Canada Guidelines	Real-world evidence integration
Swissmedic	Swiss market	Swissmedic Guidelines	Focus on personalized medicine approaches

9.1.2 Classification of Polypeptide-Based Drugs

Peptide drugs are usually classified according to their lengths, structures, and origins. Short-chain peptides (less than 40 amino acids), particularly those prepared by chemical synthesis methods, tend to be regulated as small-molecule drugs, while long-chain polypeptides (greater than 40 amino acids) or those derived *via* recombinant DNA technology are classified as biologics. Therefore, this classification determines whether the drug will and/or can follow either the New Drug Application (NDA) pathway or the Biologics License Application (BLA) pathway in the United States.

The EMA treats such synthetic peptides differently in certain cases, sometimes treating them as chemical entities if they fall below an arbitrary molecular weight cut-off, while the recombinant and larger ones are classified as biologics. Complications of hybrid classification arise with

therapeutic peptides conjugated to lipids, nanoparticles, or polyethylene glycol (PEG) in which the peptide component is coupled to advanced drug delivery systems (Li et al., 2021). Such constructions usually require additional scrutiny under combination product regulations. These requirements make biosimilar peptides tougher compared with small-molecule generics; they will have to justify their comparability in terms of structure, function, and even clinical endpoints, considering the possibility of immunogenicity and variability (Grudzinska-Goebel et al., 2025). The approval of insulin analog biosimilars has set precedents for peptide regulatory science, but currently, discussions are ongoing about how to expand similar frameworks to next-generation peptides (e.g., stapled peptides, cyclic peptides) (Lau & Dunn, 2018).

9.1.3 Approval Pathways for Biologics and Peptide Therapies

Pathway approval for the peptide therapeutics depends on jurisdiction, classification, and therapeutic indication. In the United States, a chemically synthesized peptide can follow the 505(b)(1) NDA pathway, while recombinant peptides generally require the 351(a) BLA pathway. A shorter route like the 505(b)(2) allows referring to existing literature or previous findings, easing the approval process for modified peptide analogs.

Most peptide biologics, especially oncological, immunological, or rare disease treatments, fall within the centralization procedure of the EMA. This procedure provides for conditional marketing authorizations based on surrogate endpoints for earlier approvals in the case of life-threatening diseases. In Japan, PMDA often requires additional bridging studies, reflecting ethnic and pharmacogenomic considerations, when foreign clinical trial data are submitted (Ichimaru et al., 2010; Naito, 2000). China, on the other hand, recently instituted priority review procedures for novel synthetic

peptide drugs, focusing on major unmet medical needs in infectious disease and oncology (Tan et al., 2025; Zou et al., 2023; Su et al., 2022). Even regulatory authorities worldwide are looking at real-world evidence (RWE) more and more often to support peptide approvals, particularly when long-term safety data are required post-marketing. Adaptive licensing models, sometimes termed "staggered approval," are gaining momentum for peptides targeting rare diseases to allow earlier access for patients while still requiring further submission of data (Debnath et al., 2025; Li et al., 2024; Yoo, 2023).

9.1.4 Harmonization Across Global Regulatory Systems

The harmonization of peptide regulatory frameworks remains a major objective but is continually challenged by the discrepancies in definitions, approval timelines, and clinical requirements. While ICH guidelines, for instance, ICH Q5 through Q12, have harmonized issues such as viral safety testing, stability studies, and comparability exercises, agencies may differ on how to interpret immunogenicity risk or what constitutes acceptable analytical similarity thresholds (Anderson et al., 2025; Grudzinska-Goebel et al., 2025; González-González et al., 2022; McMahon et al., 2021; Raghavan & McCombie, 2017). The FDA and the EMA have developed some degree of commonality through mutual recognition of GMP inspections, but requirements in clinical development diverge. For instance, the FDA frequently mandates supplementary pediatric studies under the Pediatric Research Equity Act (Sellers et al., 2022), whereas EMA operates under its Paediatric Regulation, resulting in contradictions in pediatric peptide drug development (Chinmayi et al., 2023).

To address these gaps, the international working groups are indicating a move toward regulatory convergence for evaluating peptide drugs,

especially with regard to pharmacovigilance and biosimilar evaluation. Between these two domains, the WHO Prequalification Programme plays a considerable role in establishing and assuring global access to high-quality peptide medicines, in particular, in regions of low- and middle-income countries where regulatory resources are limited (Broojerdi et al., 2024; Morin et al., 2023; Blaschke et al., 2019; Ahonkhai et al., 2016; WHO, n.d.a, n.d.b).

9.2 MANUFACTURING COMPLEXITY AND QUALITY CONTROL

Manufacturing polypeptide therapeutics is a very technically challenging process (Table 9.2) since synthesis approaches require diversity and also use sensitive peptides-to-modifications. Both main routes—namely, solid-phase peptide synthesis (SPPS) and recombinant DNA expression—have regulations. SPPS allows for a high level of precision in the design of the amino acid sequence but is mainly characterized by chemical heterogeneity due to incomplete coupling reactions and side-product formation, further complicating the purification and quality assurance (Jayakrishnan et al., 2024; Ahonkhai et al., 2016). In contrast, recombinant methods may cause the introduction of post-translational modifications (PTMs), such as glycosylation, which should be stringently monitored for consistency.

TABLE 9.2

Manufacturing Challenges and Strategies

Challenge	Impact on Quality	Regulatory Concern	Mitigation Strategy

Challenge	Impact on Quality	Regulatory Concern	Mitigation Strategy
Peptide aggregation	Reduced stability	Compliance with ICH Q6B	Use stabilizers, lyophilization
Impurities from synthesis	Safety risks	Adherence to GMP	Advanced purification techniques
Batch-to-batch variability	Inconsistent efficacy	Consistency in production	Continuous manufacturing adoption
Scalability	Cost implications	Economic feasibility	Develop scalable synthesis methods
Documentation complexity	Approval delays	Timely submission	Streamlined regulatory documentation
Raw material variability	Process robustness	GMP compliance	Standardized supplier qualification
Equipment compatibility	Process deviation	Validation requirements	Equipment qualification and monitoring
Formulation reproducibility	Bioavailability	Stability guidelines	Use excipients, optimize formulation

Regulatory requirements include demonstrating Good Manufacturing Practices (GMP) in process validation, contamination control, and reproducibility (Table 9.2**)** (WHO annex 3). For those peptides, a slight alteration in synthesis or purification yields a markedly different biological activity, stability, or immunogenicity. For example, differences in aggregation or isomerization occurring during manufacture can substantially alter therapeutic efficacy (Hach et al., 2024). In particular, for conjugated peptide formulations, such as PEGylated or lipidated peptides, increased

regulatory commitment exists as a result of heterogeneity in conjugation sites, leading to varied pharmacokinetics (Wijesinghe et al., 2022).

Another layer of complication comes in scaling peptide production from the laboratory to industrial production. Regulatory entities usually flag the lot-to-lot variability and thus require very extensive analytical comparability data. Continuous manufacturing platforms that have emerged hold promise in addressing this variability of a batch; however, clear criteria for their evaluation must be set down by the regulators before they can be implemented on a broader scale.

9.3 STABILITY, STORAGE, AND FORMULATION PROBLEMS

The stability of all polypeptide drugs is a major issue facing life science developers. Hydrolysis, oxidation, deamidation, and even aggregation become other threats during transportation and storage. Instability is a concern for approval by regulators and long-term impacts on post-marketing performance. Conversely, as such a peptide usually requires cold-chain delivery and storage for integrity-giving havoc to the cost and accessibility, they become a nightmare, particularly in low-resource settings (Stepanova et al., 2023). Formulation design is a key bottleneck for regulatory acceptance. The selection of excipients, pH buffers, and stabilizers must be justified by stability-indicating assays showing consistency in shelf-life and potency. Regulatory authorities are now increasingly demanding forced degradation studies mimicking stress conditions (light, heat, agitation) that would predict long-term stability of the peptides (Elsayed et al., 2025; EMA, 2022).

Specific challenges also come with delivery for the unique peptide formulations. Many therapeutic peptides have inadequate oral bioavailability due to enzymatic deactivation within the gastro-intestinal tract, which forces

the choice of parenteral administration, or alternatively, novel delivery technologies such as nanoparticle encapsulation, intranasal delivery, and transdermal patches (Omidian & Cubeddu, 2025; Peng et al., 2023; Li et al., 2021; Richard, 2017; Mitragotri et al., 2014). Regulators must carefully assess whether any of these new delivery systems indeed affect the pharmacokinetics, biodistribution, or immunogenicity of the peptide (Grudzinska-Goebel et al., 2025). A case in point would be oral peptide formulations that involve the use of permeation enhancers and the subsequent safety concerns pertaining to the disruption of intestinal barrier function, which will require new toxicology studies before regulatory approval (Mehrotra et al., 2023; Maher et al., 2021; McCartney et al., 2016). All in all, regulatory review regarding peptide stability and formulation needs a thorough characterization with a complete package of analytical stability data, excipient safety validation, and *in vivo* delivery assessments. This paints a picture not just for safety, but also for world accessibility and pricing.

9.4 SAFETY, IMMUNOGENICITY, AND TOXICITY CONCERNS

The hurdle of immunogenicity can be one of the greatest regulatory hurdles in the area of peptide therapeutics. The minor variation in the sequence, conformation, or aggregation of a peptide can elicit an unwanted immune reaction that may range from mild hypersensitivity to anaphylaxis. Peptides are generally considered less immunogenic when compared to monoclonal antibodies; however, combination with carriers such as PEG and incorporation in nanoparticles can modify the immune recognition profile of peptides (Grudzinska-Goebel et al., 2025; Omidian & Cubeddu, 2025; Tiwari et al., 2025; Li et al., 2021). Therefore, a very comprehensive

immunogenicity risk assessment is required by agencies. It includes all the *in vitro* assays like dendritic cell activation, silico prediction of T-cell epitopes, and *in vivo* preclinical immunotoxicity studies. Immunogenicity prediction is not an exact science; post-marketing surveillance frequently indicates adverse immune reactions that did not appear during trials (see Table 9.3 about various risks, method for assessment, along with ICH guideline).

TABLE 9.3

Immunogenicity and Safety Assessment

Immunogenicity Risk	Assessment Method	Regulatory Requirement	Recent Findings
Anti-drug antibodies	ELISA, bridging assays	ICH E10, E17	Longer peptides show increased incidence
Cross-reactivity	Epitope mapping	ICH E6, E2E	Observed in monoclonal peptides
Complement activation	Hemolysis assays	ICH S6	Detected in lipid-conjugated peptides
Anaphylactic reactions	Skin tests	ICH E6	Rare but significant in vaccines
Cytokine release	*In vitro* immune assays	FDA Immunogenicity Guidance	Predictive of clinical risk
Autoimmune potential	Animal models	ICH S6(R1)	Requires longitudinal assessment
Toxic metabolites	LC-MS/MS	EMA Guidelines	Novel peptides can generate reactive species
Off-target effects	*In silico* modeling	ICH E16	Reduced with design optimization

Toxicology assessment for peptides also faces the same regulatory hurdles. Although peptides generally break down to natural amino acids and

thereby lessen their systemic toxicity, their metabolite or conjugate accumulation in tissues might cause organ-specific toxicity. For example, the repeated use of lipidated peptides has been linked with liver and kidney buildup in the preclinical models (Myšková et al., 2023; Menacho-Melgar et al., 2019). Thus, extended toxicology studies are always required by regulators, especially in the case of chronic administration. There are problems, however, in extrapolating trial results to broader populations. Differences may exist among subgroups of patients, such as children, the elderly, and those who are immunocompromised, in their different responses to peptide therapy. Regulatory authorities often demand that dedicated subgroup analyses or post-authorization safety studies capture the long-term impacts of using these drugs in the real world (Chinmayi et al., 2023).

9.5 CLINICAL TRIAL DESIGN AND ENDPOINT DEFINITION

It will usually be necessary for clinical development of peptide therapies to resolve economic issues related to trial design, endpoint selection, and statistical rigor. Peptides, unlike small molecules, frequently target very complicated biological networks, making it no longer probable that one can identify clear surrogate markers of efficacy. For instance, a well-known example is that metabolic peptides such as GLP-1 analogs mandate long-term cardiovascular outcome trials to establish safety and benefits beyond glycemic control (Ceasovschih et al., 2025; Li et al., 2024; Tariq et al., 2024). Trial design is also hampered by the short half-life of many peptides, requiring either multiple doses or depot formulations. Regulators typically require extensive PK/PD modeling, justifying the choice of a particular dosing regimen and minimizing variability in exposure. Although adaptive trial designs are gaining acceptance, certain regulatory authorities, including

the FDA, still uphold traditional RCTs as providing the best evidence in this regard (FDA, 2019). Choosing and defining appropriate endpoints is still another hurdle. Generally, in rare diseases or oncology indications, regulators may accept surrogate biomarkers such as progression-free survival or biochemical markers. However, chronic conditions like obesity or diabetes demand long-term endpoints (e.g., morbidity or mortality), which substantially extend timelines and costs of trials (CDC, 2025).

Additionally, global regulatory divergences complicate trial planning. Whereas the FDA or EMA may grant acceptance to adaptive designs and real-world data integration, regulators from emerging markets may prefer the conventional phase III trials, causing a setback in the harmonization of approvals (FDA, 2019). Such inconsistencies act as major roadblocks for global clinical programs, especially on the path to developing global peptide biosimilars.

9.6 STRATEGIES FOR OVERCOMING REGULATORY BARRIERS

As polypeptide therapeutics are confronted by a plethora of regulatory impediments, a multi-faceted scientifically robust strategy will have to be implemented. The challenges of manufacture, stability, immunogenicity, and clinical design were discussed in Section 2. Here, we discuss the possible forward-looking approaches that can be adopted by developers, regulators, and stakeholders toward facilitating the undertakings of a safe and efficacious peptide development. The major strategic levers are advanced analytics, risk-based pharmacovigilance, adaptive trials, and embedded regulatory science partnerships (Figure 9.1) (Wang et al., 2022).

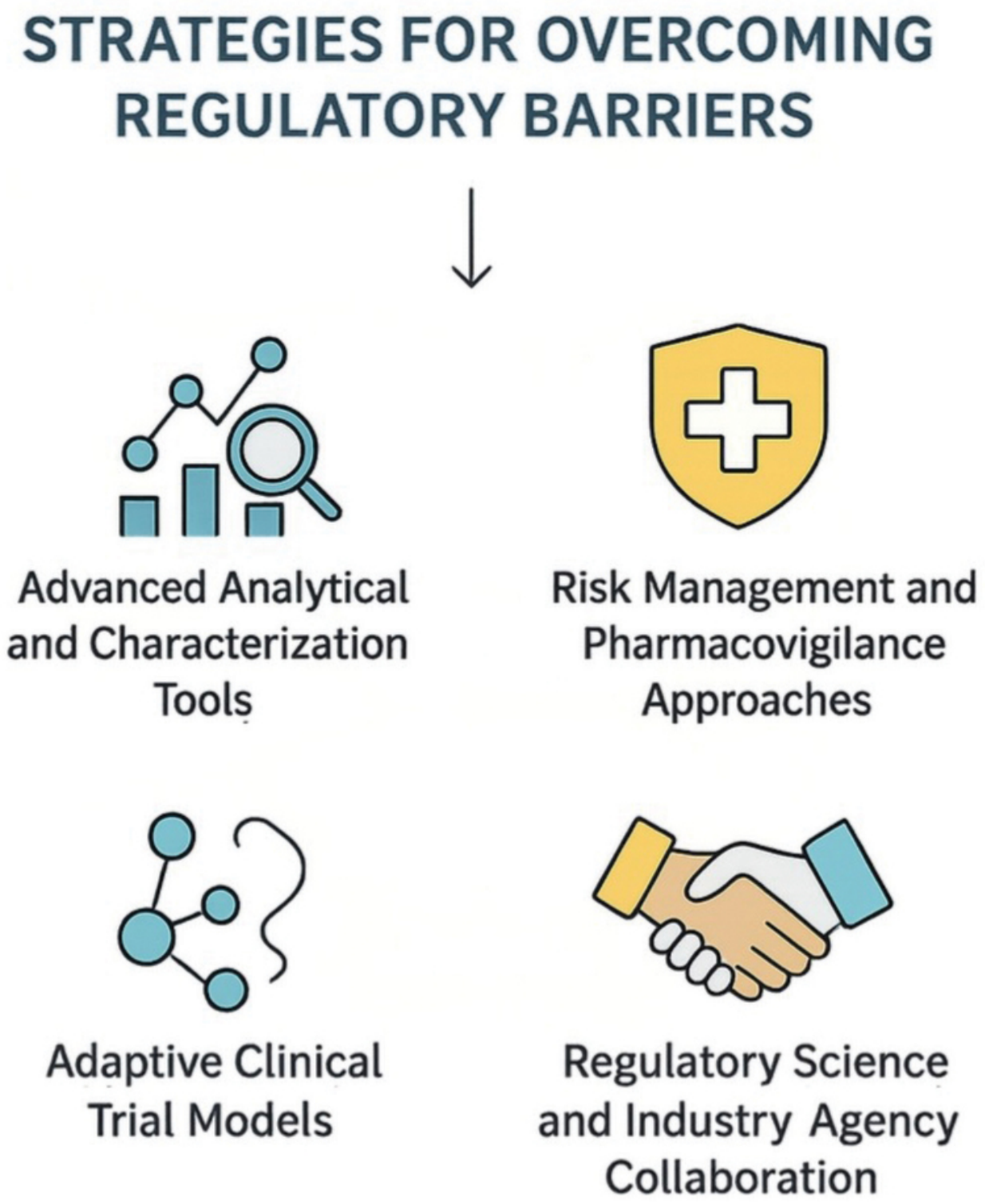

FIGURE 9.1 Descriptions of the major strategies to address regulatory issues in pharmaceutical development. The major strategies are: Complex analytical techniques for product characterization

while establishing assurances to the regulatory body for the stability, quality, and efficacy. Safety assessments for real-world use and continuous risk monitoring and assessment throughout the lifecycle of the product. Trials with flexible formats that can adapt rapidly to address potentially concerning regulatory gaps in an effective and timely manner. ⏎

9.6.1 Advanced Analytical and Characterization Tools

The main strategy proposed for overcoming regulatory scrutiny is the employment of high-resolution and complementary analytical techniques to provide deep characterization of peptide structures, impurities, degradation pathways, and batch consistency. Increasingly, regulators expect data packages to provide evidence of subtle molecular variants and complement discussions related to the mechanistic understanding of product behavior (Elsayed et al., 2025; Kuril et al., 2024; Li et al., 2024; Rizvi et al., 2024).

9.6.1.1 High-Resolution Mass Spectrometry and Multi-dimensional Separation

Analysis of all important impurity types in peptides, including isomers as well as deamidated, epimerized, and oxidized ones, will require state-of-the-art LC-MS/MS, ion-mobility spectrometry (IMS), and high-resolution mass spectrometry (HRMS) (Patel et al., 2025; Mathay et al., 2023; Klepach et al., 2022). Combined separation using LC and IMS separates conformers and aggregates, revealing structural heterogeneity that could impact immunogenicity. Kuril et al. (2024) argue that, in the case of comparability, sensitive detection of low-level impurities is necessary for generic or follow-on peptide submissions.

9.6.1.2 NMR, Circular Dichroism, and Hydrogen-Deuterium Exchange

Nuclear Magnetic Resonance (NMR) spectroscopy, circular dichroism (CD) (Oyama et al., 2026; Guo et al., 2025), and hydrogen-deuterium exchange

mass spectrometry (HDX-MS) cast light on the peptide secondary/tertiary structure, dynamics, and stability under stress conditions (Arora et al., 2025; Krishnamurthy et al., 2025). These techniques assist in differentiating the conformational isoforms which cannot be differentiated by mass alone (Klepach et al., 2022). Differential scanning calorimetry (DSC) and thermogravimetric analysis (TGA) further assess thermal stability and folding energetics, which would be key to justifying storage conditions (Patel et al., 2025; Mathay et al., 2023; Sooram et al., 2023).

9.6.1.3 Process Analytical Technology (PAT) and Real-time Monitoring

The integration of PAT in peptide manufacture would include real-time monitoring of critical process parameters (CPPs) and critical quality attributes (CQAs) for tighter control over synthesis and purification (FDA's PAT framework) (Elsayed et al., 2025). For coupling efficiency, reaction completion, and impurity formation, in-line UV, Raman spectroscopy, or near-infrared (NIR) sensors could support process understanding and regulatory acceptability (Elsayed et al., 2025).

9.6.1.4 Data Analytics, AI, and Predictive Modeling

To manage the complexity of peptide variants and large analytical datasets, machine learning (ML) and artificial intelligence (AI) models are getting increasingly applied. For example, deep generative models can anticipate peptide structural behavior, stability, and even immunogenic potential (Ekambaram & Dokholyan, 2025; Hashemi et al., 2024; Goles et al., 2024). Embedding these predictions in regulatory submissions serves to rationalize method selection choice and impurity risk thresholds. Specifically, in a generative peptide design context, a contrastive diffusion model (MMCD) integrates structure with sequence to suggest modifications having desirable

properties (Wang et al., 2024). Similarly, peptide-specific adapted language models (e.g., PepDoRA) could predict functional and safety profiles and thus help prioritize candidates (Du et al., 2025).

9.6.1.5 Control Strategy Frameworks and Lifecycle Management

Developers, in addition to analytics, should articulate a control strategy systematizing the analytical, manufacturing, and stability controls for peptides. An effective control strategy would demonstrate to regulatory bodies that the developer has foreseen and implemented risk mitigation measures throughout the product lifecycle. This further entails impurity thresholds (for example, identify impurities ≥0.10% and qualify ≥0.5%) to be correlated with immunogenic risk frameworks (Puig & Shubow, 2025; Li et al., 2024; De Groot et al., 2023). In contrast, evolving plans for lifecycle management of the ICH Q12 type could allow changes in assays or specifications after approval with demonstrably strong justification for comparability (Elsayed et al., 2025). The combination of advanced, orthogonal analytical and modeling tools will create a scientifically justified characterization package that reduces regulatory uncertainty and increases confidence in making decisions regarding specifications, shelf-life, and comparability.

9.6.2 Risk Management and Pharmacovigilance Approaches

A properly functioning pharmacovigilance (PV) program and risk management will take the uncertainties of preclinical work and bridge them to real-world safety evaluations, specifically for immunogenicity and rare adverse events (Pack et al., 2025).

9.6.2.1 Risk Assessment and Immunogenicity Mitigation Pre-Launch

The assessment of immunogenicity should be risk-based and adopted by developers as early as in development, using advanced tools of in silico epitope prediction, in vitro assays (e.g., dendritic cell activation, T-cell proliferation), and animal models, which are being more broadly characterized in terms of potential immune liabilities (Grudzinska-Goebel et al., 2025). Such findings should guide mitigation strategies, including sequence de-immunization, pegylation shielding, or tolerance induction regimens. When potentially novel impurities or aggregates are present (e.g., >0.5%), regulatory feedback would expect additional immunotoxicity evaluation (Puig & Shubow, 2025; De Groot et al., 2023).

9.6.2.2 Benefit-Risk Framework and Post-Marketing Plans

A benefit-risk framework considering the target disease context must be submitted. For those peptides developed for more serious or unmet medical needs, regulators may give some latitude within the safety margins, provided some post-marketing commitments exist. Immunogenicity monitoring (e.g., anti-drug antibodies, neutralizing antibodies), cross-reactivity testing, and exposure-response correlation over time must be included in any Post-Authorization Safety Studies (PASS) or Risk Management Plans (RMPs), which usually come as an obligation during the approval of biologics (Rogers-Crovak et al., 2024; EMA, 2022; FDA, 2019).

9.6.2.3 Real-World Evidence (RWE) and Adaptive Surveillance

The capture of real-world data (e.g., data derived from electronic health records, registries) offers an opportunity to build long-term safety monitoring and early detection of rare events. Regulators are more than willing to consider RWE for label extension or safety signal assessment provided the data maintain acceptable quality and have been generated under

sufficient methodological rigor (Fetse et al., 2023; Lamers, 2022; Apostolopoulos et al., 2021; Mann et al., 2021). Adaptive pharmacovigilance systems assess safety outcome thresholds to trigger an in-depth analysis or label change in a predetermined manner.

9.6.2.4 Lifecycle Learning and Feedback Loops

A learning health system supports continuing feedback from manufacturing/QA to clinical and PV, facilitating successive refinements. Such regulatory submissions that will decrease uncertainty in steps—i.e., by starting with surrogate endpoints but moving on to definitive outcomes—become more believable when supported by strong surveillance plans. In the gradual or conditional approval context, iterative safety data review through PV is a key enabler for regulatory flexibility (Fetse et al., 2023; Lamers, 2022; Apostolopoulos et al., 2021; Mann et al., 2021).

9.6.3 Adaptive Clinical Trial Models

Conventional fixed-sample clinical trials seldom offer the requisite agility for present-day peptide therapeutics, especially in rare disease, precision medicine, or rapidly advancing indications. Adaptive design trials, a regulatory-friendly approach, endow a strategic advantage, as do platform trials.

9.6.3.1 Seamless Phase II/III Designs and Dose Escalation

Combining phases (seamless phase II/III designs) minimizes timelines and resource burden, allowing passage from dose-finding to confirmatory efficacy within a single protocol. Increasingly, regulators accept these approaches, provided that they are planned beforehand and justified with a statistical rationale (Rogers-Crovak et al., 2024; EMA, 2022; FDA, 2019).

For peptides with narrow therapeutic windows, adaptive dose-escalation designs (Bayesian, model-based, etc.) are efficient and enhance patient safety through real-time updates on dose assignments.

9.6.3.2 Basket, Umbrella, and Master Protocols

Adopting platform trial frameworks (umbrella for multiple subtypes of a disease, basket for a single molecular target across diseases) increases efficiency in examining peptide agents across heterogeneous populations. Such designs allow for shared control arms and dynamic cohort expansion or contraction. Regulatory agencies have started endorsing these platforms; however, those approaches must manage rigorously the type I error statistical controls (Li et al., 2024; Fetse et al., 2023; Lamers, 2022; Apostolopoulos et al., 2021; Mann et al., 2021).

9.6.3.3 Adaptive Enrichment and Biomarker Guided Arms

Adaptive trials may consider biomarker-driven enrichment based on interim results to modify patient selection criteria favoring responders. Enrichment of populations may improve efficacy detection, thereby reducing sample size for peptides, whereby the biological mechanism is clearly defined. Regulators are more positive about these designs when prespecified and scientifically justified (Hua et al., 2025).

9.6.3.4 Interim Analyses, Sample Size Re-estimation, and Futility Stopping

Having interim analyses with sample size re-estimation gives room for modification of the trial in view of accumulating data to extend enrollment or even stop for early futility with optimized use of resources. Newer guidelines issued by regulatory bodies are now favoring the use of such

adaptive strategies as long as a thoroughly maintained control over statistical error is warranted (Rogers-Crovak et al., 2024; EMA, 2022; FDA, 2019).

9.6.3.5 Hybrid Design Incorporating RWE/External Controls

Hybrid designs with the integration of external or historical controls from observational cohorts or prior trials may mitigate the burden on the control arm, especially in rare diseases. Such external comparators would be accepted by regulators to alleviate the placebo burden and foster rapid enrollment, provided that they use appropriate covariate matching and adjustment (Fetse et al., 2023; Lamers, 2022; Apostolopoulos et al., 2021; Mann et al., 2021). The astute application of adaptive models will significantly decrease peptide developers' trial duration, thus optimizing resource utilization and yielding solid regulatory-grade evidence.

9.6.4 Regulatory Science and Industry-Agency Collaboration

Peptide therapeutics could be seen as undergoing an advanced stage of assessment into trial models in close collaboration with innovators, regulators, and an extended scientific community.

9.6.4.1 Early Regulatory Advice and Engagement

Early-stage scientific advice, pre-IND consulting, and concurrent scientific advice with regulatory agencies help align development plans with expectations. This alignment may de-risk submission pathways, minimize review cycles, or prevent major recommendation issues from arising (Rogers-Crovak et al., 2024; EMA, 2022; FDA, 2019). For peptides with new modalities (e.g., conjugated systems, intracellular delivery), joint workshops and innovation meetings may clarify acceptable evidence approaches.

9.6.4.2 Regulatory Scientific Research Consortia

Industry-regulatory consortia in peptide therapeutics may generate common reference datasets, established best practices, and consensus on testing thresholds. Such consortia help contain uncertainty and foster harmonized standards. For example, a benchmarking exercise on impurity control across companies could assist regulators in defining thresholds for acceptability (Pack et al., 2025).

9.6.4.3 Pilot Programs and Submission of Case Studies

Whenever possible, regulators tend to admit the existence of pilot programs for new modalities by means of special pathways (e.g., FDA's Emerging Technology Program). Developers may propose different pilot peptides regarding testing new manufacturing or analytical methodologies under surveillance review in increments until gains in some precedent (Fetse et al., 2023; Lamers, 2022; Apostolopoulos et al., 2021; Mann et al., 2021). Publishing case-study outcomes (successful regulatory acceptance) will contribute to a common experience in peptide therapeutic issues.

9.6.4.4 International Harmonization and Recognition

Collaborations in the working groups of ICH and regional harmonization activities, in addition to mutual recognition of inspections or data, are likely to facilitate multinational peptide development. Harmonized guidelines would be useful in setting impurity thresholds, and immunogenicity and post-marketing surveillance would aid in reducing regulatory fragmentation. By working with global bodies such as WHO prequalification or using reliance models, duplication can be minimized (Fetse et al., 2023; Morin et al., 2023; Lamers, 2022; Mann et al., 2021; Apostolopoulos et al., 2021; WHO, n.d.a, n.d.b).

9.6.4.5 Training and Capacity Development of Regulators

Given the specialized nature of peptides, capacity development within regulatory agencies becomes very critical. Collaborative training programs, joint evaluation toolboxes, and peptide-specific regulatory science curricula would keep regulators abreast of emerging methodologies, thus enhancing the quality and consistency of reviews.

9.7 FUTURE PERSPECTIVES AND POLICY DIRECTIONS

From the research done on data till October in the year 2023. Polypeptide therapeutics find themselves almost at an inflection point: advances in peptide chemistry, delivery technologies, and mathematical and computational design currently converge with changing paradigms in regulation and a hot policy debate concerning affordability, access, and ethical allocation. This final section of the manuscript examines both the near and the mid-term horizon for peptide medicines, considering future innovations in technology (such as next-generation peptides and delivery systems) toward individualized therapies, and the much larger ethical, economic, and policy challenges that will be involved in shaping development, approval, and access to these agents. The analysis emphasizes practical policy options that different stakeholders in regulation, industry, payers, and academia might pursue to maximize patient benefit while upholding rigorous safety and efficacy standards.

9.7.1 Next-Generation Peptides and New Emerging Therapeutic Modalities

Indeed, the panorama of peptide medicines goes beyond both classical linear peptides and towards the capability of designer architectures to overcome some of the conventional limitations such as poor stability, restricted cell

permeability, and short half-life. Next-generation classes include stapled peptides, peptidomimetics, cyclic and constrained peptides, *D*-amino-acid-substituted peptides, and peptide-small molecule hybrids. These engineered modalities improve better target binding, protease resistance, and intracellular targeting of protein-protein interactions previously considered as "undruggable" (Lombardi et al., 2025; Zheng et al., 2025; Li et al., 2024; Lau & Dunn, 2018). Some of them already proceed into clinical evaluation, showing that modifications such as stapling and backbone cyclization can dramatically extend the systemic half-life and improve pharmacokinetics (Omidian & Cubeddu, 2025; Hach et al., 2024).

Advances in peptidomimetics and the development of non-canonical amino acids have resulted in molecules with improved oral bioavailability and proteolytic stability, helping to create new therapeutic niches. Such constructs often require developing new analytical characterization strategies to document nonstandard residues and stereochemistry and have accompanying toxicology approaches adapted to metabolites that differ from conventional amino acid breakdown products (Lombardi et al., 2025; He et al., 2023; Bolleddula et al., 2022). Therefore, adaptability of the regulatory frameworks to appropriate-case assessments should also be enacted in the future, with more emphasis given to scientific justification rather than rigid comparison to historical comparators in risk-benefit analysis.

From a translational perspective, it is about time that industry and regulators come together and begin to create a public corpus of case studies documenting model successful development and approval pathways for these modalities, along with their analytical packages, immunogenicity risk assessments, and manufacturing controls. Such an endeavor can reduce the development uncertainty for the developer and promote regulatory convergence (Ball et al., 2022; Lombardo et al., 2017).

9.7.2 Innovations in Delivery Systems and Formulation

Access to peptide therapeutics has invariably depended on innovations in delivery. Traditionally reliant on parenteral administration, the field has begun to look beyond this to oral and other non-invasive routes made possible through absorption enhancers, protease inhibitors, mucoadhesive carriers, and nanoparticle encapsulation technologies (Omidian & Cubeddu, 2025; Li et al., 2021; Lombardo et al., 2017). In recent times, oral bioavailability has been better realized through peptide formulations taking the form of GLP-1 receptor agonists, making the case for the promise but also the complexity surrounding permeation enhancers' safety and that of their long-term effects on mucosal integrity. Therefore, in the evaluation of the mechanism of action, regulatory evaluation must also take into account toxicology studies and post-marketing surveillance.

Nanotechnology-based delivery, such as lipid nanoparticles and polymeric carriers, constitutes yet another path of transformation through which peptides are protected from degradation and made available for targeted delivery (Omidian & Cubeddu, 2025; Li et al., 2025, 2024, 2021). However, the same developments raise new questions in the field of regulation concerning nanoparticle clearance, immune activation, and tissue accumulation (Grudzinska-Goebel et al., 2025). It is very necessary to develop standardized assays along with validation tools and cross-industry reference materials that ensure reproducible regulatory evaluation and, therefore, global harmonization (Table 9.4).

TABLE 9.4

Delivery Systems and Regulatory Considerations

Delivery System	Regulatory Challenge	Guideline Reference	Recent Innovations
Oral peptides	Low bioavailability	ICH Q1A, Q3D	Absorption enhancers
Nanoparticles	Toxicity assessment	ICH S9, S6	Biodegradable carriers
Lipid-based systems	Stability	ICH Q5C, Q6B	Stable liposome formulations
Transdermal patches	Skin irritation	ICH Q3A, Q5C	Penetration enhancers
Injectable depot	Injection site reactions	FDA Guidance	Long-acting sustained release
Inhalation systems	Lung deposition	EMA Inhalation Guideline	Nano-spray aerosols
Hydrogel carriers	Biocompatibility	ICH Q8	Controlled release matrices

9.7.3 Trends in Personalized and Precision Medicine

The intrinsic nature of peptide therapeutics is modular and highly adaptable in an effort to fit with specificity into individualized medicine. Rapid synthesis and design cycles allow biomarker-based patient stratification and adaptive enrichment to be introduced into clinical trials (Barcelos et al., 2025; Chinmayi et al., 2023). The transformation of companion diagnostics into imaging and biomarker panels involves identifying response cohorts and monitoring target engagement to enhance trial productivity and therapeutic performance (Li et al., 2025, 2024). However, precision peptide therapeutics are also confronted with regulatory and economic hurdles. Drug-diagnostic co-approval requirements complicate timelines, while targeting narrow populations may challenge reimbursement models (Fetse et al., 2023; Lamers, 2022; Apostolopoulos et al., 2021; Mann et al., 2021). Policy solutions include harmonizing combined review processes for diagnostics;

creating incentives for data-sharing mechanisms for biomarker data; and piloting value-based reimbursement systems that tie costs to real-world outcomes (Fetse et al., 2023; Apostolopoulos et al., 2021; Lamers, 2022; Mann et al., 2021). Table 9.5 summarizes recent developments in the field in light of regulatory hurdles and the Persianized approach involved.

TABLE 9.5

Personalized Medicine and Regulatory Challenges

Personalized Approach	Regulatory Hurdle	Guideline Reference	Recent Developments
Companion diagnostics	Approval pathway	ICH E2E, E6	Streamlined FDA/EMA processes
Biomarker-based dosing	Dose optimization	ICH E10, E17	Integration of pharmacogenomics
Patient-specific formulations	Manufacturing flexibility	ICH Q7, Q10	Modular and small-batch manufacturing
Adaptive N-of-1 trials	Statistical validation	ICH E9(R1)	FDA supportive guidance
Genetic stratification	Patient selection	ICH E16	Predictive biomarkers used
Immunogenicity profiling	Safety assurance	ICH S6(R1)	Integrated preclinical-clinical approach

9.7.4 Ethical, Economic, and Policy Issues

To be sustainable, peptide-based therapeutics will need to confront ethical and cost-related issues. Production and development costs tend to be high, because of which therapies often become relatively challenging to afford and gain equal access (Wijesinghe et al., 2022). Even though biosimilar competition opens up access for a few biologics, evidence shows that without intentionally driven policy mechanisms, portions of savings may not

be brought down to patients directly (Fetse et al., 2023; Lamers, 2022; Apostolopoulos et al., 2021; Mann et al., 2021). Mechanisms such as indication-based pricing, value-based contracts, and public negotiation are able to help strike that balance between innovation incentives and affordability.

Ethically, all regulators would be obliged to uphold transparency alongside manufacturers concerning the clinical benefits of or limitations of the products. Accelerated approvals would also involve binding post-marketing responsibilities to be coupled with open-access registries to capture long-term safety and effectiveness data. Moreover, equity is addressed by ensuring global initiatives such as WHO prequalification, reliance pathways, and technology transfer for access to low- and middle-income countries (Morin et al., 2023; WHO, n.d.a, n.d.b).

From the standpoint of policy, government and regulatory bodies should prioritize the following:

1. Facilitation of global regulatory harmonization to expedite access.
2. To outline clear pathways for new peptide modalities & delivery systems.
3. To promote affordability via innovative procurement and pricing models.
4. Building a regulatory science infrastructure with shared scientific resources.
5. To ensure ethical stewardship of accelerated and conditional approvals.

9.8 CONCLUSION

The next decade is a crucial window of opportunity for transforming peptide therapeutics into precision medicines that are widely available. Their success hinges on global regulatory alignment, the fostering of delivery innovation, an acceptance of the precision approach, and embracing affordability and equity in the core of policy. If these preconditions are satisfied, polypeptide therapeutics will deliver tangible public health returns across various diseases and patient populations.

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USE OF GENERATIVE AI TOOL

In preparing this work, the author utilized ChatGPT 3.5 to enhance the clarity and readability of the text and sentence improvement. After employing ChatGPT 3.5, the author carefully reviewed and edited the content as necessary. The author assumes full accountability for the publication's content.

REFERENCES

Ahonkhai, V., Martins, S., Portet, A., Lumpkin, M., & Hartman, D. (2016). Speeding access to vaccines and medicines in low- and middle-income countries: A case for change and a framework for optimized product market authorization. *PLoS ONE*, *11*(11), e0166515. https://doi.org/10.1371/journal.pone.0166515⏎

Anderson, J., Burgwin, C., & Hamilton, D. (2025). Retrospective evaluation of cycled resin in viral clearance studies—A multiple company collaboration—Post ICH Q5A(R2) review. *PDA Journal of Pharmaceutical Science and Technology*, *79*, 320–336. https://doi.org/10.5731/pdajpst.2024-003024⏎

Apostolopoulos, V., Bojarska, J., Chai, T. T., Elnagdy, S., Kaczmarek, K., Matsoukas, J., et al. (2021). A global review on short peptides: Frontiers and perspectives. *Molecules*, *26*(2), 430. https://doi.org/10.3390/molecules26020430⏎

Arora, K., Celejewski, K., & Narang, D. (2025). Advancing structural biology and protein-ligand interactions through hydrogen/deuterium exchange mass spectrometry (HDX-MS). *Methods in Molecular Biology*, *2905*, 181–192. https://doi.org/10.1007/978-1-0716-4418-8_12⏎

Ball, K., Bruin, G., Escandón, E., Funk, C., Pereira, J. N., Yang, T. Y., et al. (2022). Characterizing the pharmacokinetics and biodistribution of therapeutic proteins: An industry white paper. *Drug Metabolism and Disposition*, *50*(6), 858–866. https://doi.org/10.1124/dmd.121.000463⏎

Barcelos, M. P., Brambila, B., Novo-Mansur, M. T. M., & Tomich de Paula da Silva, C. H. (2025). Therapeutic peptides: Innovation and potential at the frontier of medicine and pharmacology. In C. A. Taft & S. R. Lazaro (Eds.), *Computational intelligence in drug development* (Series in BioEngineering, p. 6). Springer. https://doi.org/10.1007/978-3-032-07366-2_6⏎

Blaschke, T., Lumpkin, M., & Hartman, D. (2019). The World Health Organization prequalification program and clinical pharmacology in 2030. *Clinical Pharmacology & Therapeutics*, *107*, 68–71. https://doi.org/10.1002/cpt.1680⏎

Bolleddula, J., Brady, K., Bruin, G., Lee, A., Martin, J. A., Walles, M., et al. (2022). Absorption, distribution, metabolism, and excretion of therapeutic proteins: Current industry practices and

future perspectives. *Drug Metabolism and Disposition*, *50*(6), 837–845. https://doi.org/10.1124/dmd.121.000461⏎

Broojerdi, A., Salvati, A., Abdelfattah, M., Dehaghi, R., Sillo, H., & Gaspar, R. (2024). WHO-listed authorities (WLA) framework: Transparent evidence-based approach for promoting regulatory reliance towards increased access to quality-assured medical products. *Frontiers in Medicine*, *11*, Article 1467229. https://doi.org/10.3389/fmed.2024.1467229⏎

CDC. (2025). Fast facts: Health and economic costs of chronic conditions. https://www.cdc.gov/chronic-disease/data-research/facts-stats/index.html [Accessed 11/1/2026]⏎

Ceasovschih, A., Banjanin, N., Bednarek, A., Beqiraj, A., Cherska, M., Ejubović, M., et al. (2025). Atherosclerotic features in patients with heart failure. *Archives of Medical Science*, *21*(4), 1107–1129. https://doi.org/10.5114/aoms/208372⏎

Chinmayi, C. S., Pratheksha, S., Lavanya, M., Parvathaneni, M., & Kalaivanan, R. (2023). An overview and comparison of regulatory pathways and guidelines for pediatric study plans in the US and EU. *Journal of Medicinal Chemistry & Science*, *6*(12), 3057–3071⏎

De Groot, A. S., Roberts, B. J., Mattei, A., Lelias, S., Boyle, C., & Martin, W. D. (2023). Immunogenicity risk assessment of synthetic peptide drugs and their impurities. *Drug Discovery Today*, *28*(10), 103714. https://doi.org/10.1016/j.drudis.2023.103714⏎

Debnath, A., Mazumder, R., Mazumder, A., Tyagi, P., & Singh, R. (2025). Challenges and progress of orphan drug development for rare diseases. *Current Pharmaceutical Biotechnology*. https://doi.org/10.2174/0113892010371761250616112614⏎

Du, Z., Caragea, D., Guo, X., & Li, Y. (2025). PepBERT: Lightweight language models for bioactive peptide representation. *bioRxiv*. https://doi.org/10.1101/2025.04.08.647838⏎

Ekambaram, S., & Dokholyan, N. V. (2025). Peptide-based drug design using generative AI. *Chemical Communications*, *2025*(d), 04998a. https://doi.org/10.1039/d5cc04998a⏎

Elsayed, Y. Y., Kühl, T., & Imhof, D. (2025). Regulatory guidelines for the analysis of therapeutic peptides and proteins. *Journal of Peptide Science*, *31*(3), e70001. https://doi.org/10.1002/psc.70001⏎

EMA. (2022). ICH guideline Q14 on analytical procedure development. https://scholar.google.com/scholar_lookup?title=ICH%20Guideline%20Q14%20on%20Analytical%20Procedure%20Development&publication_year=2022⏎

EMA. (n.d.). Marketing authorization guidance documents. https://www.ema.europa.eu/en/human-regulatory-overview/marketing-authorisation/marketing-authorisation-guidance-documents [Accessed 13/1/2026]⏎

FDA. (2019). Adaptive design clinical trials for drugs and biologics: Guidance for industry. https://www.fda.gov/regulatory-information/search-fda-guidance-documents/adaptive-design-clinical-trials-drugs-and-biologics-guidance-industry [Accessed 11/1/2026]⏎

Fetse, J., Kandel, S., Mamani, U. F., & Cheng, K. (2023). Recent advances in the development of therapeutic peptides. *Trends in Pharmacological Sciences*, *44*(7), 425–441. https://doi.org/10.1016/j.tips.2023.04.003⏎

Giri, T., Sakharwade, S., Subbappa, P., Chinnakadoori, S., & Sharma, N. (2025). Regulatory considerations in synthetic peptide characterization: Techniques and compliance. *Separation Science Plus*, 8(6), e70057. https://doi.org/10.1002/sscp.70057

Goles, M., Daza, A., Cabas-Mora, G., Sarmiento-Varón, L., Sepúlveda-Yañez, J., Anvari-Kazemabad, H., et al. (2024). Peptide-based drug discovery through artificial intelligence: Towards an autonomous design of therapeutic peptides. *Briefings in Bioinformatics*, *25*(4), bbae275. https://doi.org/10.1093/bib/bbae275⏎

González-González, O., Ramirez, I., Ramirez, B., O'Connell, P., Ballesteros, M., Torrado, J., & Serrano, D. (2022). Drug stability: ICH versus accelerated predictive stability studies. *Pharmaceutics*, *14*(11), 2324. https://doi.org/10.3390/pharmaceutics14112324⏎

Grudzinska-Goebel, J., Benstein, K., Bloem, K., Cowan, K. J., Gorovits, B., Jadhav, M., et al. (2025). Immunogenicity risk assessment for tailored mitigation and monitoring of biotherapeutics during development: Recommendations from the European Immunogenicity Platform. *Frontiers in Immunology*, *16*, 1581153. https://doi.org/10.3389/fimmu.2025.1581153⏎

Guo, W., Makarov, A. A., Buevich, A. V., & Jiang, Y. (2025). Strategy for improving circular dichroism spectra deconvolution accuracy for macrocyclic peptides in drug discovery. *Journal of Pharmaceutical and Biomedical Analysis*, *252*, 116476. https://doi.org/10.1016/j.jpba.2024.116476

Hach, M., Engelund, D. K., Mysling, S., Mogensen, J. E., Schelde, O., Haselmann, K. F., et al. (2024). Impact of manufacturing process and compounding on properties and quality of follow-on GLP-1 polypeptide drugs. *Pharmaceutical Research*, *41*(10), 1991–2014. https://doi.org/10.1007/s11095-024-03771-6

Hashemi, S., Vosough, P., Taghizadeh, S., & Savardashtaki, A. (2024). Therapeutic peptide development revolutionized: Harnessing the power of artificial intelligence for drug discovery. *Heliyon*, *10*(22), e40265. https://doi.org/10.1016/j.heliyon.2024.e40265

He, M. M., Zhu, S. X., Cannon, J. R., Christensen, J. K., Duggal, R., Gunduz, M., et al. (2023). Metabolism and excretion of therapeutic peptides: Current industry practices, perspectives, and recommendations. *Drug Metabolism and Disposition*, *51*(11), 1436–1450. https://doi.org/10.1124/dmd.123.001437

Hua, K., Hong, H., & Wang, X. (2025). Biomarker-guided adaptive enrichment design with threshold detection for clinical trials with time-to-event outcome. *Journal of Biopharmaceutical Statistics*, *35*(6), 1209–1226. https://doi.org/10.1080/10543406.2025.2489291

Ichimaru, K., Toyoshima, S., & Uyama, Y. (2010). Effective global drug development strategy for obtaining regulatory approval in Japan in the context of ethnicity-related drug response factors. *Clinical Pharmacology & Therapeutics*, *87*(3), 362–366. https://doi.org/10.1038/clpt.2009.285

Jayakrishnan, A., Rosli, W., Tahir, A., Razak, F., Kee, P., Ng, H., et al. (2024). Evolving paradigms of recombinant protein production in pharmaceutical industry: A rigorous review. *Science*, 6(1), 9. https://doi.org/10.3390/sci6010009

Klepach, A., Tran, H., Mohammed, F. A., & ElSayed, M. E. H. (2022). Characterization and impact of peptide physicochemical properties on oral and subcutaneous delivery. *Advanced Drug Delivery Reviews*, *186*, 114322. https://doi.org/10.1016/j.addr.2022.114322

Krishnamurthy, S., Musgaard, M., Tehan, B. G., Jazayeri, A., & Liko, I. (2025). The evolving role of hydrogen/deuterium exchange mass spectrometry in early-stage drug discovery. *Current Opinion in Structural Biology*, *92*, 103051. https://doi.org/10.1016/j.sbi.2025.103051⏎

Kuril, A. K., Saravanan, K., & Subbappa, P. K. (2024). Analytical considerations for characterization of generic peptide product: A regulatory insight. *Analytical Biochemistry*, *694*, 115633. https://doi.org/10.1016/j.ab.2024.115633⏎

Lamers, C. (2022). Overcoming the shortcomings of peptide-based therapeutics. *Future Drug Discovery*, *4*, FDD75. https://doi.org/10.4155/fdd-2022-0005⏎

Lau, J. L., & Dunn, M. K. (2018). Therapeutic peptides: Historical perspectives, current development trends, and future directions. *Bioorganic & Medicinal Chemistry*, *26*(10), 2700–2707. https://doi.org/10.1016/j.bmc.2017.06.052⏎

Li, L., Li, R., Jiang, Y., Chao, J., & Liao, H. (2021). Advances in hydrocarbon stapled peptides via ring-closing metathesis: Synthetic strategies, structural diversity, and therapeutic applications. *Chembiochem*, *26*(23), e202500527. https://doi.org/10.1002/cbic.202500527⏎

Li, X., Jian, M., Sun, Y., Zhu, Q., & Wang, Z. (2021). The peptide functionalized inorganic nanoparticles for cancer-related bioanalytical and biomedical applications. *Molecules*, *26*(11), 3228. https://doi.org/10.3390/molecules26113228

Li, Y., Wu, M., Fu, Y., Xue, J., Yuan, F., Qu, T., Rissanou, A. N., Wang, Y., et al. (2024). Therapeutic stapled peptides: Efficacy and molecular targets. *Pharmacological Research*, *203*, 107137. https://doi.org/10.1016/j.phrs.2024.107137⏎

Lombardi, L., Genio, V. D., Albericio, F., & Williams, D. R. (2025). Advances in peptidomimetics for next-generation therapeutics: Strategies, modifications, and applications. *Chemical Reviews*, *125*(15), 7099–7166. https://doi.org/10.1021/acs.chemrev.4c00989⏎

Lombardo, F., Desai, P. V., Arimoto, R., Desino, K. E., Fischer, H., Keefer, C. E., et al. (2017). In silico absorption, distribution, metabolism, excretion, and pharmacokinetics (ADME-PK): Utility and best practices. *Journal of Medicinal Chemistry*, *60*(22), 9097–9113. https://doi.org/10.1021/acs.jmedchem.7b00487⏎

López-Sánchez, A., Rodríguez-Mejia, K., Cuero-Amu, K., Ardila-Chantré, N., Reyes-Calderón, J., González-López, N., et al. (2024). A new methodology for synthetic peptides purification and counterion exchange in one step using solid-phase extraction chromatography. *Processes*, *13*(1), 27. https://doi.org/10.3390/pr13010027

Maher, S., Geoghegan, C., & Brayden, D. J. (2021). Intestinal permeation enhancers to improve oral bioavailability of macromolecules: Reasons for low efficacy in humans. *Expert Opinion on Drug Delivery*, *18*(2), 273–300.

Mann, S. P., Treit, P. V., Geyer, P. E., Omenn, G. S., & Mann, M. (2021). Ethical principles, constraints, and opportunities in clinical proteomics. *Molecular & Cellular Proteomics*, *20*, 100046. https://doi.org/10.1016/j.mcpro.2021

Mathay, M., Keller, A., & Bruce, J. E. (2023). Studying protein-ligand interactions by protein denaturation and quantitative cross-linking mass spectrometry. *Analytical Chemistry*, *95*(25), 9432–9436. https://doi.org/10.1021/acs.analchem.2c04501

McCartney, F., Gleeson, J. P., & Brayden, D. J. (2016). Safety concerns over the use of intestinal permeation enhancers: A mini-review. *Tissue Barriers*, *4*(2), e1176822.

McMahon, M., Abbott, A., Babayan, Y., Carhart, J., Chen, C., Debie, E., et al. (2021). Considerations for updates to ICH Q1 and Q5C stability guidelines: Embracing current technology and risk assessment strategies. *The AAPS Journal*, *23*(6), 107. https://doi.org/10.1208/s12248-021-00641-6

Mehrotra, S., Bg, P. K., Nayak, P. G., Joseph, A., & Manikkath, J. (2023). Recent progress in the oral delivery of therapeutic peptides and proteins: Overview of pharmaceutical strategies to overcome absorption hurdles. *Advanced Pharmaceutical Bulletin*, *14*(1), 11.

Menacho-Melgar, R., Decker, J. S., Hennigan, J. N., & Lynch, M. D. (2019). A review of lipidation in the development of advanced protein and peptide therapeutics. *Journal of Controlled Release*, *295*, 1–12. https://doi.org/10.1016/j.jconrel.2018.12.032

Mitra, M., DeMarco, S., Holub, B., Thiruneelakantapillai, L., & Thackaberry, E. (2020). Development of peptide therapeutics: A nonclinical safety assessment perspective. *Regulatory Toxicology and*

Pharmacology, *117*, 104766. https://doi.org/10.1016/j.yrtph.2020.104766

Mitragotri, S., Burke, P. A., & Langer, R. (2014). Overcoming the challenges in administering biopharmaceuticals: formulation and delivery strategies. *Nature Reviews Drug Discovery*, *13*(9), 655–672.

Moderna, J. K. (n.d.). Comprehensive review of regulatory intelligence and its framework. https://www.pda.org/pda-letter-portal/home/full-article/a-comprehensive-review-of-regulatory-intelligence-and-its-framework [Accessed 11/1/2026]

Morin, S., Segafredo, G., Piccolis, M., Das, A., Das, M., Loffredi, N., et al. (2023). Expanding access to biotherapeutics in low-income and middle-income countries through public health non-exclusive voluntary intellectual property licensing: Considerations, requirements, and opportunities. *The Lancet Global Health*, *11*(1), e145–e154. https://doi.org/10.1016/S2214-109X(22)00460-0

Myšková, A., Sýkora, D., Kuneš, J., & Maletínská, L. (2023). Lipidization as a tool toward peptide therapeutics. *Drug Delivery*, *30*(1), 2284685. https://doi.org/10.1080/10717544.2023.2284685

Naito, C. (2000). Necessity and requirements of bridging studies and their present status in Japan. *International Journal of Clinical Pharmacology & Therapeutics*, *38*(2), 80–86. https://doi.org/10.5414/cpp38080

Omidian, H., & Cubeddu, L. X. (2025). Peptide-functionalized nanomedicine: Advancements in drug delivery, diagnostics, and biomedical applications. *Molecules*, *30*(7), 1572. https://doi.org/10.3390/molecules30071572

Oyama, T., Suzuki, S., & Akao, K. I. (2026). Circular dichroism spectroscopy in protein engineering and pharmaceutical development: Applications in structural characterization and quality assessment. *Protein Expression and Purification*, *237*, 106826. https://doi.org/10.1016/j.pep.2025.106826

Pack, B. W., Manheim, J., Chahrour, O., Wood, B., Zhang, L., Vogel, U., et al. (2025). Therapeutic peptides control strategy: Perspective on current industry practices. *Organic Process Research & Development*, *29*(2), 270–280. https://doi.org/10.1021/acs.oprd.4c00386

Pack, B. W., Siegel, R. W., Cornwell, P. D., Ferrante, A., Roepke, D. A., Hodsdon, M. E., et al. (2025). A phase-appropriate risk assessment strategy in support of the safety of peptide and oligonucleotide-related impurities. *AAPS Journal*, *27*(2), 56. https://doi.org/10.1208/s12248-025-01023-y

Patel, M., Parikh, D., Parihar, A., Prajapati, B., Patel, M., Salave, S., et al. (2025). Protein and peptide therapeutics: Stability challenges, regulatory demands, and innovative formulation solutions for enhanced clinical effectiveness. *Protein and Peptide Letters*, 32(7), 490–507. https://doi.org/10.2174/0109298665375151250626124048⏎

Peng, H., Wang, J., Chen, J., Peng, Y., Wang, X., Chen, Y., et al. (2023). Challenges and opportunities in delivering oral peptides and proteins. *Expert Opinion on Drug Delivery*, *20*(10), 1349–1369.⏎

Puig, M., & Shubow, S. (2025). Immunogenicity of therapeutic peptide products: bridging the gaps regarding the role of product-related risk factors. *Frontiers in Immunology*, *16*, 1608401. https://doi.org/10.3389/fimmu.2025.1608401⏎

Raghavan, R., & McCombie, R. (2017). ICH Q5E comparability of biotechnological/biological products subject to changes in their manufacturing processes (Chapter 15). In A. Teasdale, D. Elder, & R. W. Nims (Eds.), *ICH quality guidelines: An implementation guide* (pp. 409–432). John Wiley & Sons. https://doi.org/10.1002/9781118971147.ch15⏎

Richard, J. (2017). Challenges in oral peptide delivery: Lessons learnt from the clinic and future prospects. *Therapeutic Delivery*, *8*(8), 663–684.⏎

Rizvi, S. F. A., Zhang, H., & Fang, Q. (2024). Engineering peptide drug therapeutics through chemical conjugation and implication in clinics. *Medical Research Reviews*, *44*(6), 2420–2471. https://doi.org/10.1002/med.22046⏎

Rogers-Crovak, J., Delaney, E., & Detlefsen, D. (2024). Recommendation for clarifying FDA policy in evaluating “sameness” of higher order structure for generic peptide therapeutics. *The AAPS Journal*, *27*(1), 8. https://doi.org/10.1208/s12248-024-00994-8⏎

Sellers, J., Mihaescu, C., Ayalew, K., Kronstein, P., Yu, B., Ning, Y., et al. (2022). Descriptive analysis of good clinical practice inspection findings from U.S. Food and Drug Administration and

European Medicines Agency. *Therapeutic Innovation & Regulatory Science*, *56*, 753–764. https://doi.org/10.1007/s43441-022-00417-w⏎

Sharma, K., Sharma, K., Sharma, A., & Jain, R. (2022). Peptide-based drug discovery: Current status and recent advances. *Drug Discovery Today*, 28(1), 103464. https://doi.org/10.1016/j.drudis.2022.103464

Sooram, B., Gupta, N., Chethireddy, V. R., Tripathi, T., & Saudagar, P. (2023). Applications of differential scanning calorimetry in studying folding and stability of proteins. In P. Saudagar & T. Tripathi (Eds.), *Protein folding dynamics and stability*. Springer. https://doi.org/10.1007/978-981-99-2079-2_3⏎

Stepanova, M., Nikiforov, A., Tennikova, T., & Korzhikova-Vlakh, E. (2023). Polypeptide-based systems: From synthesis to application in drug delivery. *Pharmaceutics*, *15*(11), 2641. https://doi.org/10.3390/pharmaceutics15112641⏎

Su, L., Liu, S., Li, G., Xie, C., Yang, H., Liu, Y., et al. (2022). Trends and characteristics of new drug approvals in China, 2011-2021. *Therapeutic Innovation & Regulatory Science*, *57*, 343–351. https://doi.org/10.1007/s43441-022-00472-3⏎

Tan, R., Hua, H., Zhou, S., Yang, Z., Yang, C., Huang, G., et al. (2025). Current landscape of innovative drug development and regulatory support in China. *Signal Transduction and Targeted Therapy*, *10*, 220. https://doi.org/10.1038/s41392-025-02267-y⏎

Tariq, S., Ali, M. A., Hassan Iftikhar, H. M., Fareh, A. M., Shah, S. Q. A., Perveen, F., & Zaman, T. (2024). Long-term cardiovascular outcomes of glucagon-like peptide-1 (GLP-1) receptor agonists in type 2 diabetes: A systematic review. *Cureus*, *16*(11), e73705. https://doi.org/10.7759/cureus.73705⏎

Tiwari, A., et al. (2025). PEGylation as a tool to alter immunological properties of nanocarriers. In R. K. Tekade & N. K. Jain (Eds.), *PEGylated nanocarriers in medicine and pharmacy*. Springer. https://doi.org/10.1007/978-981-97-7316-9_6⏎

Wang, L., Wang, N., Zhang, W., Cheng, X., Yan, Z., Shao, G., et al. (2022). Therapeutic peptides: Current applications and future directions. *Signal Transduction and Targeted Therapy*, *7*(1), 48.

https://doi.org/10.1038/s41392-022-00904-4⏎

Wang, Y., Xuan, L., Feng, H., Zhankun, X., & Zhang, W. (2024). A multi-modal contrastive diffusion model for therapeutic peptide generation. In AAAI *c*onference. https://www.aaai.org⏎

Wang, Z., Xiong, Z., Huang, F., & Zhang, W. (2024). A multi-modal contrastive diffusion model for therapeutic peptide generation. *arXiv*. https://arxiv.org/html/2312.15665v2

Wijesinghe, A., Kumari, S., & Booth, V. (2022). Conjugates for use in peptide therapeutics: A systematic review and meta-analysis. *PLoS One*, *17*(3), e0255753. https://doi.org/10.1371/journal.pone.0255753⏎

World Health Organization. (n.d.a). Enhancing access through efficient regulation, technical assistance, and openness. https://extranet.who.int/prequal/sites/default/files/media_document/2.21%20Supporting%20countries%20towards%20faster%20and%20easier%20access%20to%20testing%20commodities.pdf [Accessed 13/1/2026]⏎

World Health Organization. (n.d.b). WHO health technology access programme. https://www.who.int/initiatives/who-health-technology-access [Accessed 13/1/2026]⏎

Xiao, W., Jiang, W., Chen, Z., Huang, Y., Mao, J., Zheng, W., et al. (2025). Advances in peptide-based drug development: Delivery platforms, therapeutics, and vaccines. *Signal Transduction and Targeted Therapy*, *10*, 67. https://doi.org/10.1038/s41392-024-02107-5

Yongkang, W., Xuan, L., Feng, H., Zhankun, X., & Zhang, W. (2024). A multi-modal contrastive diffusion model for therapeutic peptide generation. In AAAI *c*onference. https://www.aaai.org

Yoo, H. (2023). Development of orphan drugs for rare diseases. *Clinical and Experimental Pediatrics*, *67*, 315–327. https://doi.org/10.3345/cep.2023.00535⏎

Zheng, B., Wang, X., Guo, M., & Tzeng, C. M. (2025). Therapeutic peptides: Recent advances in discovery, synthesis, and clinical translation. *International Journal of Molecular Sciences*, *26*(11), 5131. https://doi.org/10.3390/ijms26115131⏎

Zou, L., Qi, Y., Jiang, Y., Tang, L., Du, Y., Zhao, B., et al. (2023). Criteria and regulatory considerations for the conditional approval of innovative antitumor drugs in China: From the

perspective of clinical reviewers. *Cancer Communications*, *43*, 171–176. https://doi.org/10.1002/cac2.12400⏎

10 Personalized Medicine Personalized MedicinePersonalized Medicine

Customizing Polypeptide Formulations

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10.1 INTRODUCTION TO PERSONALIZED MEDICINE

Precision medicine, sometimes referred to as personalized medicine, is a new idea in the field of medicine that focusses on tailoring each patient's medical treatment to their unique characteristics. Precision medicine takes into account each patient's unique biological profile in order to maximize the safety and efficacy of medical interventions, as opposed to the conventional, classical methods of a one-size-fits-all model (Delpierre & Lefèvre, 2023). The following are some of the omics technology advancements that have significantly spurred this change: Understanding differences in a person's genetic composition that predispose them to risk or how they react to

treatment can be achieved through the comprehensive decoding of their DNA, which is known as genomics (Recharla et al., 2023), The study of RNA transcripts by gene, or transcriptomics, is used to describe gene expression and its fluctuations during illness or in response to medical interventions (Pelaia et al., 2023), Proteomics is the large-scale study of proteins that offers a better understanding of the impact of proteins and their interactions on cell functioning and well-being (Sun et al., 2024), Metabolomics is the systematic study of small molecules (metabolites) involved in metabolism that can reveal a patient's condition (Lin et al., 2024).

Genomic medicine is inferior to personalized medicine. In order to optimize and customize medical treatments for each patient, personalized medicine integrates different kinds of biological data. A crucial element of the approach is pharmacogenomics, which customizes medication treatments based on a person's genetic data. It increases the efficiency of medications and reduces the possibility of adverse effects, making treatment safer and more effective (Pirmohamed, 2023) In practice, personalized medicine enables the application of particular treatments in addition to risk prediction. This suggests that medical professionals will be able to identify patients who are most at risk of contracting specific illnesses and treat them using specific techniques based on their genetic profile. These tactics are progressively being used in various medical specialties, including neurology, endocrinology, and oncology particularly (Sarhangi et al., 2024).

In order to evaluate sophisticated clinical data and develop individualized treatment plans, personalized medicine significantly depends on modern technology. Among the important technologies are mobile health devices that provide real-time health information and facilitate ongoing patient monitoring, mass spectrometry that quantifies and recognizes proteins as well as metabolites with high sensitivity, and high-throughput

sequencing that quickly identifies and analyses genetic information in detail (Nilius et al., 2024). The following technologies have revealed a great deal of individual molecular and cellular variations. With this knowledge of distinctions, physicians can now more accurately predict a patient's risk of developing a disease and create treatment plans specific to each patient's needs in order to further improve outcomes and encourage individualized care (Molla & Bitew, 2024).

Additionally, polypeptide customization through precision medicine has the potential to revolutionize the way peptides are made and utilized in therapy. Small chains of amino acids called polypeptides play important roles in many biological processes and are becoming increasingly utilized as medications due to their low toxicity, specificity, and effectiveness. Despite their potential, modifying these molecules for specific patients is still a significant scientific and technological undertaking (Kobeissy et al., 2023).

Precision medicine provides an integrated framework which utilizes cutting-edge tools and technology to customize treatments to each patient's distinct biological profile in order to prevent this. The most significant of these is the application of additive manufacturing, specifically 3D printing, which will enable the creation of precise biomaterials. Customized drug delivery systems that adjust to the distinct physiological and pathological features of each patient can be designed and manufactured using these technologies (de León et al., 2023).

Additionally, 3D printing can create custom advancing toward a time when therapeutic approaches are not only enhanced but also more tailored to the requirements of each patient such as tissues and organ replacements that are unique to each patient, which could greatly improve results. Precision medicine transcends the division between customized therapy solutions and generic peptide-based therapies by facilitating such customization (Borthakur et al., 2026).

The potential of precision medicine to customize polypeptides relies on its ability to leverage patient-specific data to improve the outcome of treatment. As nanotechnology and bioanalytical techniques improve, precision medicine will offer us increasingly better ways of preparing personalized polypeptide therapy. This will make medical therapy more effective and safer overall (Calabretta et al., 2020).

10.2 BIOLOGICAL AND THERAPEUTIC POTENTIAL OF POLYPEPTIDES

Polypeptides are essential to contemporary therapeutics because of their biodegradability and biocompatibility, which make them appropriate for drug delivery and therapeutic applications. These artificial polymers can be designed to create a variety of supramolecular structures, including vesicles, micelles, and nanoparticles, which help deliver medications to particular bodily locations (Song et al., 2017).

Their suitability for biomedical applications, such as gene and drug delivery, bio-imaging, and tissue engineering, is further enhanced by their chemical diversity and capacity to form secondary structures. Polypeptides have several important therapeutic uses in drug delivery systems, where they are used as carrier molecules to enhance the pharmacokinetics and effectiveness of medicinal substances (Yuan et al., 2025).

Polypeptide drug conjugates have emerged as a promising strategy to enhance therapeutic efficacy by improving pharmacokinetic profiles, enabling targeted drug delivery, and facilitating controlled release mechanisms. Together, these characteristics reduce off-target toxicity and increase treatment efficacy (Zhang et al., 2021). Additionally, they might contain responsive to stimuli polypeptides, which alter their structure or behavior as they respond to environmental changes. A few examples of these

changes include redox or pH variations, as well as site-specific drug release. This ability to react significantly reduces systemic toxicity while simultaneously improving therapeutic efficacy (Zhang et al., 2021). Through direct *in vivo* synthesis of synthetic polypeptides, host-defense peptides have been created in oncology to have the same effect as cancer cell apoptosis. This anti-tumor strategy has demonstrated high levels of cytotoxicity on a variety of cancer cell populations with minimal hemolysis activity, demonstrating the agent's effectiveness and safety (Shen et al., 2024).

Furthermore, the extent of the *in vivo* half-life of therapeutic peptides and proteins, as well as their usefulness and therapeutic efficacy, depends heavily on synthetic polypeptides. One noteworthy example is the XTEN (TM) polypeptide, which has a unique structure that has been engineered to increase half-life, bioavailability, and reduce dosage (Podust et al., 2016). These systems depend on polypeptides, which, because of their tendency to be biocompatible and biodegradable carriers, have an extensive amount of potential to improve the pharmacokinetic characteristics of therapeutic agents. These advancements show how important polypeptide technologies are to the creation of contemporary medicines, particularly in the areas of cancer therapy, targeted drug delivery, and improving the stability and effectiveness of medications. The emergence of omics technologies revolutionized personalized medicine, particularly in the creation of medications based on polypeptides. Figure 10.1 summarizes the biological and therapeutic potential of polypeptides.

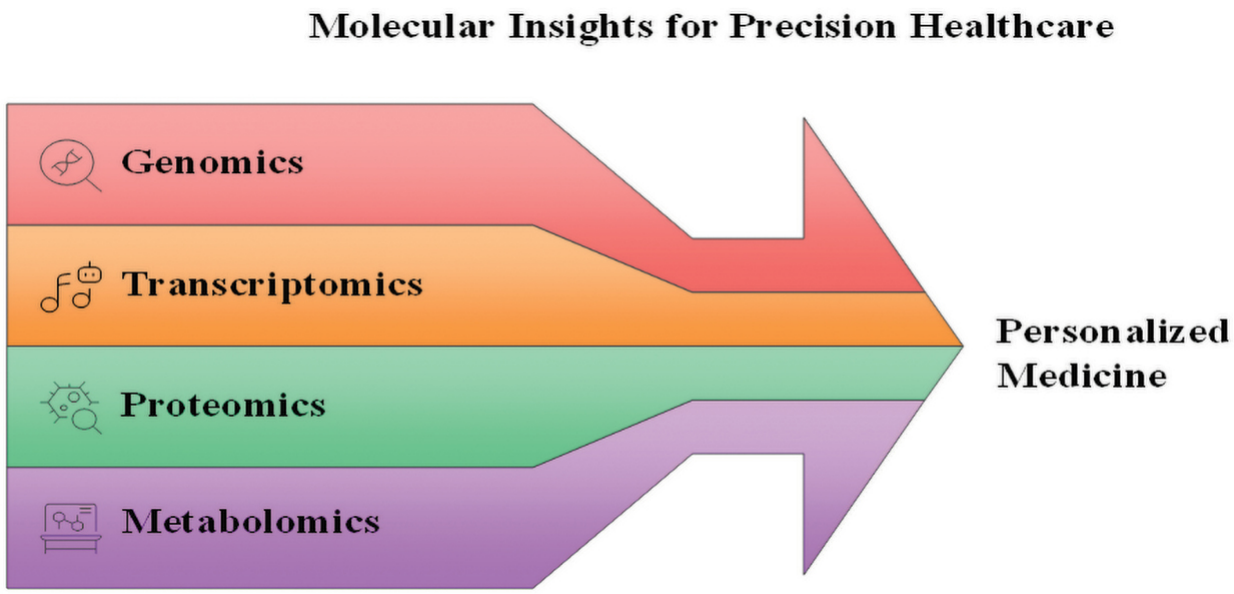

FIGURE 10.1 Biological and therapeutic potential of polypeptides. ⏎

10.3 OMICS-DRIVEN PERSONALIZATION: GENOMIC, PROTEOMIC, AND METABOLOMIC INSIGHTS

Clinicians and researchers can now use genomic, proteomic, and metabolomic data to develop extremely personalized treatment strategies that take account of interpatient heterogeneity in disease pathophysiology and the response of the patient to treatment (Molla & Bitew, 2024). The main role of genomics in this new science is the identification of genetic polymorphisms that affect the polypeptide drug metabolism, response, and toxicity. To provide a hypothetical example, the pharmacokinetics of GLP-1 receptor agonists administered to treat type 2 diabetes are dramatically affected by genetic variations of a gene DPP4 in the coding of dipeptidyl peptidase-4. DPP4 variants are observed in patients, with different enzyme activity, and dosing schedules must be customized, thereby producing optimum and therapeutic effect (Gummadi & Guddati, 2021). On the same note, pharmacogenomic research also led to the conclusion that single

nucleotide polymorphisms (SNPs) occur in the ABCB1 gene (ATP-Binding Cassette Subfamily B Member 1) to alter the transport and availability of peptide-based anticancer drugs, and concluded on the necessity of genetic screening before the treatment and application of anticancer drugs. The findings also indicate that there is a necessity for genomic profiling to determine the selection and optimization of polypeptide therapy in individual patients (Ankathil, 2017).

Proteomics augments genomic information by providing dynamic mapping of disease-related protein expression patterns so that new areas of treatment can be identified. The proteomic analysis conducted by mass spectrometry has played a central role in discovering tumor antigens like HER2 in breast and PD-L1 in melanoma that can be attacked using precision-engineered conjugated polypeptides (Al-Amrani et al., 2021). Recent developments in high-throughput proteomics have also allowed the discovery of peptide biomarkers to discover the diseases early. Specifically, a group of serum peptides in 2023 was described in relation to early-stage Alzheimer's, where pre-symptomatic intervention opportunities are present (Thanasukarn et al., 2025). Proteomic analysis of tumor microenvironments has also demonstrated per-patient heterogeneity in receptor expression, which has been used to design multi-target peptide-drug conjugates to avoid resistance mechanisms. These uses demonstrate how proteomics bridges the gap between functional pathology and genetic predisposition, enabling more targeted polypeptide therapeutic design (Zhou et al., 2024).

By identifying disease-disturbed metabolic pathways and using this information to inform the design of polypeptides that restore balance, metabolomics also makes personalized medicine possible. For example, branched-chain amino acid (BCAA) profiling has revealed insulin resistance metabolic markers and made it possible to create peptide analogues with improved stability and binding specificity (Rizo-Roca et al., 2025).

Metabolomic analysis of tumor isolate exosomes in oncology has revealed individual metabolite profiles for peptide drugs that predict response. In the year 2024, it was demonstrated that mitochondrial-targeting peptides, which take advantage of metabolic weaknesses, were more effective in patients with increased lactate in tumor microcompartments. This method is also crucial in the response to therapy and toxicity evaluation (Kohli & Poulogiannis, 2025). Real-time metabolomic evaluation of patients on polypeptide therapies is going to identify early indications for negative outcomes, such as renal or hepatic impairment, to allow for early intervention. Clinicians can then create comprehensive treatment interventions that take into account the multifactorial nature of complex disease by integrating metabolomic data with genomic and proteomic information (Petrak et al., 2024).

The integration of multi-omics into clinical practice is also dependent on advanced computational techniques capable of handling large, heterogeneous datasets. Machine learning (ML) and artificial intelligence (AI) algorithms have become indispensable in this respect, enabling the prediction of the optimal polypeptide sequences, dosage, and combinations for an individual patient. For example, AI platforms like Deep Peptide have already been used to design de novo polypeptides with high binding affinity for disease-related targets with time and cost savings over traditional drug discovery Xu et al. (2021). Machine learning models trained on omics data can also predict patient-specific adverse effects, such as immunogenic responses to peptide therapeutics, by analyzing past datasets of clinical trials. While these enhancements have occurred, standardization of omics data across institutions and interoperability of disparate analytical platforms are still challenges. Initiatives like the European Union's 1+ Million Genomes Project and the U.S. All of US Research Program are attempting to address

the difficulties by developing centralized repositories for harmonized omics data (Li et al., 2024).

Pragmatic and ethical issues take center stage as omics-informed personalization enters the mainstream. Data privacy concerns, in particular regarding the storing and sharing of sensitive genetic information, demand robust cybersecurity measures and open consent protocols (Pina et al., 2024). Access to personalized polypeptide therapy is also a matter of concern, considering that variations in health infrastructure and funding might limit access to omics technology in low-resource settings. Furthermore, the environmental context that regulates omics-based therapies is evolving, with regulatory agencies like the FDA and EMA working constantly to establish standards for the validation of multi-omics biomarkers and AI-derived therapeutic algorithms (FDA, 2024). Transcending these challenges will be crucial to realizing the full potential of omics-directed personalized medicine (Marques & Costa, 2024). Figure 10.2 depicts the omic-driven approach of personalization.

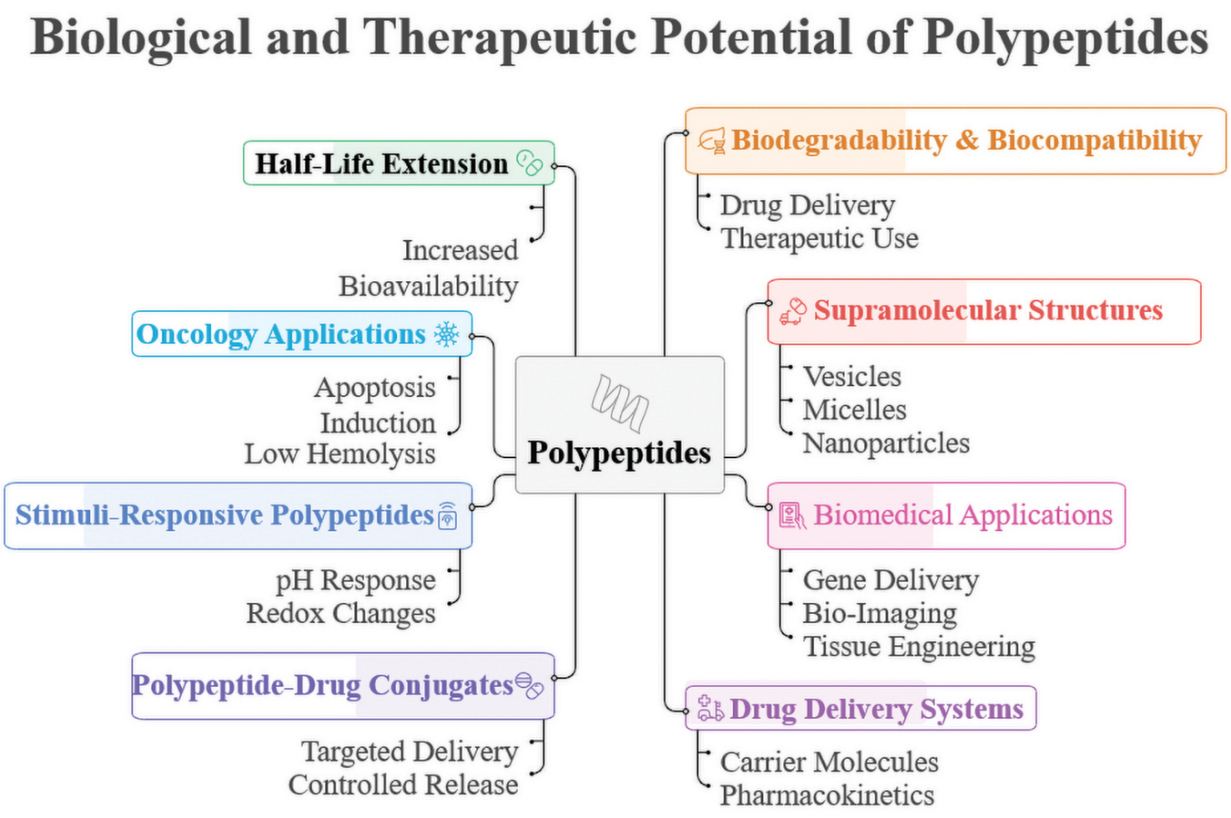

FIGURE 10.2 Molecular insights for precision medicine. ⏎

10.4 TECHNOLOGIES FOR POLYPEPTIDE SYNTHESIS AND MODIFICATION

Design of polypeptide therapeutics relies heavily on advanced synthesis and modification technologies enabling full control over peptide structure, activity, and stability. The most recent decade has seen tremendous progress in chemical and biological approaches to synthesis, as well as in new methods of modifying polypeptides in order to maximize their therapeutic potential. These technologies have allowed the alleviation of the previously limited applications of Peptides that include low bioavailability, low half-lives, and immunogenicity to enable their application on the threshold of personalized medicine (Wang et al., 2022).

Although solid-phase peptide synthesis (SPPS) was invented by Merrifield back in the 1960s, it continues to represent the backbone of peptide synthesis as applied in research and medical practice. This procedure can be used to synthesize amino acids stepwise on insoluble resin support that has the possibility of achieving maximum yield (Rahman et al., 2023). SPPS has been designed over the past couple of years to alleviate the issue of increased efficiency and scalability. In another example, it took several days to perform an operation that could be carried out in hours using automated flow-chemistry systems, without decreasing the amount of reagents consumed but leading to an increased yield (Collins et al., 2023). Protecting groups and new couple reagents have also made the synthesis of challenging sequences of sterically hindered or aggregation-prone residues easier. The developments have proved especially helpful in the synthesis of complex peptides containing post-translational modifications or non-natural amino acids which are significant parameters in drug discovery (Nandhini et al., 2023).

Recombinant DNA technology is a potent supplement to chemical synthesis in the case of longer polypeptides or those in which the required intricate combination of folding patterns is too difficult to achieve chemically. Escherichia coli and Pichia pastoris are considered microbial expression systems that lead to high-level and authentic expression of therapeutic peptides (Dağcı & Unver, 2023). Evolution of yeast expression systems has also allowed the utility to yield mammalian-like glycosylation patterns of humanized glycopeptides that, unlike the case before, can evade the constraint regarding generating biologically active eukaryotic peptides (Gündüz Ergün et al., 2019). Advances were also achieved in the cell culture of mammals, where CRISPR genome editing generated cell lines that are able to express disulfide-conjugated peptides in their native form. These biotechnologies of biological synthesis are particularly valuable in the

manufacture of peptide hormones and growth factors that require precise three-dimensional structures to be active (Ansori et al., 2023).

Chemical modification methods have also evolved, e.g., the capacity to engineer peptide derivatives with enhanced pharmacological profiles. PEGylation, or the covalent attachment of polyethylene glycol chains, remains one of the most highly effective techniques for increasing peptide half-life, as indicated by the therapeutic effectiveness of PEGylated G-CSF and interferon therapies (Gao & Joshi, 2024). More recent site-specific PEGylation techniques using click chemistry or enzyme-catalyzed conjugation have increased product homogeneity and maintained bioactivity. Another promising approach is lipid conjugation, fatty-acid-modified peptides with increased membrane permeability and prolonged circulation half-lives. The GLP-1 analog semaglutide with a C18 fatty diacid chain is one of the success stories of this approach with once-weekly administration in the clinic (Makharadze et al., 2025).

Cyclization is another potent modification approach yielding improved peptide stability and target binding. Among these methods for cyclization are the head-to-tail cyclization, the disulfide bridging, and stapled peptide technologies (Zhang et al., 2019). Stapled peptides, which include the use of unnatural amino acids to form hydrocarbon “staples,” have been very effective for the targeting of historically “undruggable” intracellular protein-protein interactions. Some stapled peptide p53-MDM2 interaction inhibitors are undergoing cancer therapy clinical trials with enhanced cellular penetration and proteolytic stability compared to linear analogs. Similarly, macrocyclic peptides discovered using phage display or mRNA display technologies have led to stable inhibitors of challenging targets like membrane proteins and transcription factors (Li et al., 2024). New technologies are also progressively expanding the scope of peptide engineering. Non-canonical amino acid (ncAA) incorporation with

ribosomes enables the addition of specialist chemical functionality that could be used to enable new conjugation strategies or modulate peptide behavior (Huang et al., 2025). Expansion into peptide nucleic acid (PNA) conjugates has developed new gene-targeted therapeutics, and peptide-drug conjugates (PDCs) are still emerging as targeted delivery vehicles for cytotoxic compounds. Advances in computational peptide design, in the form of machine learning models trained on activity and structural data, are assisting in accelerating the discovery of optimized peptide therapeutics (Armstrong et al., 2025).

Despite these technologies, there are still obstacles to overcome in order to bring peptide therapies from the bench to the patient's bedside. Manufacturing problems frequently arise when synthesis processes are scaled up, especially for highly modified peptides. Particularly for complex products like PEGylated peptides, analytical characterization of peptide purity and presentation requires the development of sophisticated equipment and methods. Regulations governing the approval of new peptide modalities are constantly changing, and developers must adapt to new characterization and quality control standards (Wang et al., 2025). Figure 10.3 summarizes the technologies used for Polypeptide Synthesis and Modification.

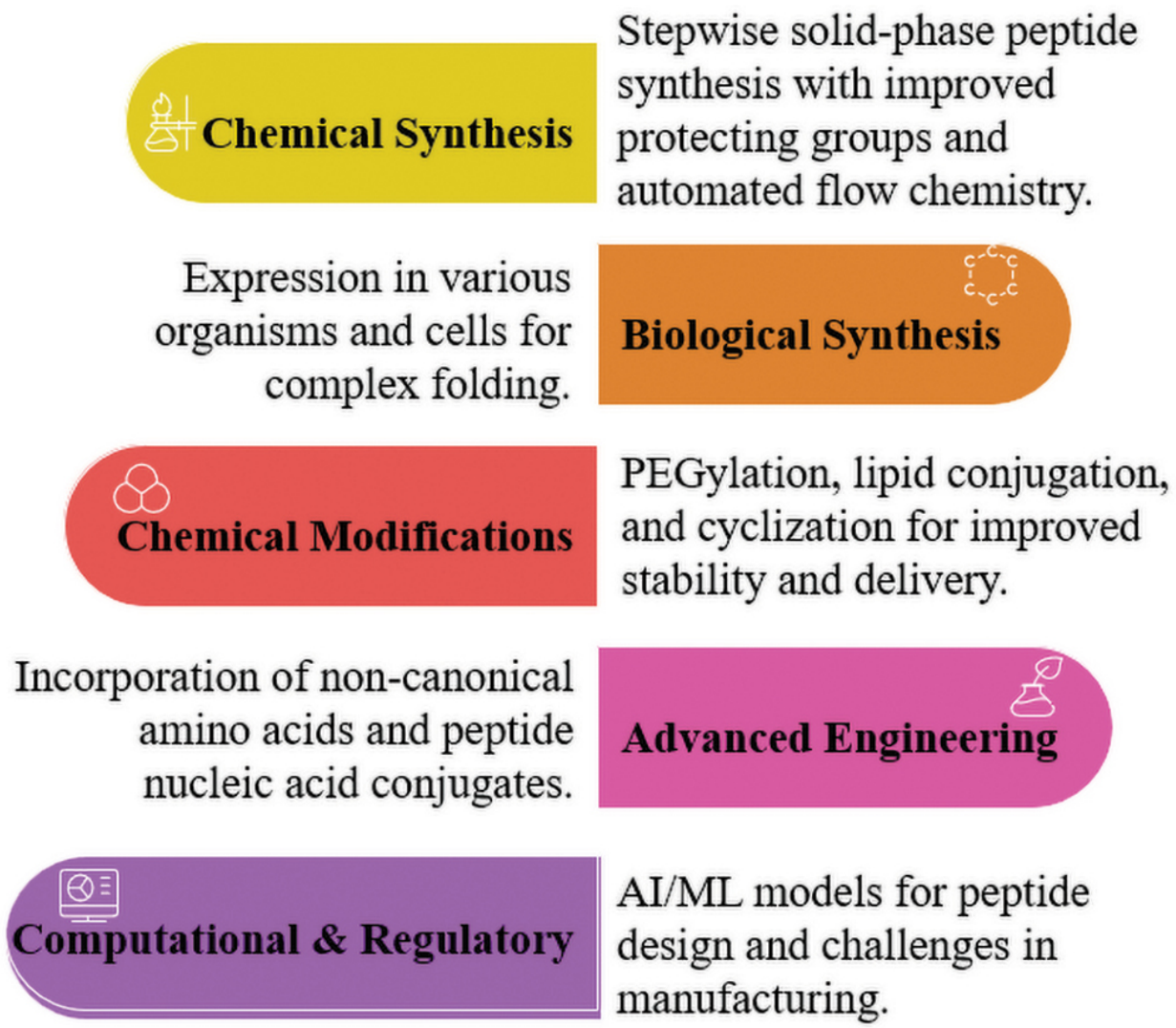

FIGURE 10.3 Peptide synthesis methods.

10.5 TECHNIQUES FOR CUSTOMIZATION IN POLYPEPTIDE FORMULATIONS

A new era of precision medicine, in which treatment plans are carefully developed based on the unique characteristics of each patient, has been brought about by the development of customized polypeptide therapies. This method makes use of contemporary technologies to improve delivery systems, customize peptide sequences, and treat biological problems unique

to each patient. These approaches are based on the potential for entire-molecule profiling, which uses genomic, proteomic, and metabolomic data to customize treatments to each patient's unique pathophysiology (Marques & Costa, 2024). The more precise example is the tumor neoantigens detected in tumors on next-generation sequencing, which have been noted in glioblastoma and melanoma clinical trials and can guide the development of customized cancer vaccines. Peptide replacements that can be precisely tailored to replace nonfunctional proteins are also available to patients with rare hereditary diseases. CRISPR gene editing optimizes the sequence (Ahmed et al., 2024). Other than optimization of sequences, there have been dramatic efforts of optimizing peptide stability and bioavailability to accommodate idiosyncratic metabolism. Backbone alterations, including D-amino acid replacement, methylation, and cyclization, have proven an excellent strategy to boost circulation half-lives without affecting biological activity (Evans & King, 2020). The success of the GLP-1 agonist analogue semaglutide, a fatty acid within the side chain modification, is a remarkable example of how smart systems can twist the pharmacokinetics of peptides to deliver once-weekly dosing in diabetes treatment. Using machine learning algorithms to evaluate very large numbers of peptide conformations and their fates under metabolic conditions has also aided the process of determining the most appropriate sites of modification (Mahapatra & Karuppasamy, 2022). The computational approach will be especially useful in going through the fine line of peptide stability and immunogenicity so that personalized formulations do not trigger undesirable immune responses but create the maximum therapeutic effect possible. The patient-specific polypeptide formulations also have targeted delivery systems, and they allow avoiding physiological and anatomical barriers in a patient. Drug loading can be performed to develop patient-specific nanocarrier platforms such as lipid nanoparticles and other polymeric micelles, which survive the

microenvironment of the disease and attain enhanced tissue permeation and cellular entry within the body (Mattei et al., 2024). Indicatively, the peptide-drug conjugates with incorporated tumor-homing signals have proven exceptionally selective in targeting the desired delivery of chemotherapeutics to the cancer cells, wherein the levels of toxicity on the normal tissues are strikingly low, and it has been attested by recent clinical trials in breast cancer and ovarian cancer (Jadhav et al., 2025). Transdermal and oral drug delivery systems have also made significant advances, with microneedle arrays and permeability enhancers making non-invasive delivery of peptides previously restricted to injection feasible. These developments impact pediatric and geriatric patients most, where differentiated delivery approaches can significantly enhance compliance and quality of life (Liu et al., 2025).

Even the convergence of real-time tracking technologies with polypeptide formulations has been advanced one step further in this journey of personalization. Dynamic dose adjustment relies on patient-specific treatment responses. In vivo monitoring with biosensors-enabled smart delivery systems can feed data streams into precision dosing algorithms (Ho et al., 2020). This closed-loop approach has been particularly promising for diabetes care, where continuous glucose monitoring systems in real time are applied along with algorithm-based insulin delivery to create personalized patient regimens. Similarly, biomarker-monitoring wearable sensors that monitor cytokines or cardiac peptides allow for real-time adjustment of immunotherapies and cardiovascular interventions, reducing side effects while increasing outcomes (Borel et al., 2024).

With these developments, however, the translation of personalized polypeptide preparations into scalable, cost-efficient, and regulator-approved treatments presents grave challenges. Manufacture of patient-specific therapies needs to employ flexible manufacturing platforms with high-

throughput synthesis and quality control, where new technologies like microfluidic peptide synthesis and 3D bioprinting hold promise to meet these demands (Wang et al., 2025). Access remains a critical issue, particularly for orphan diseases, but decentralized production plans and automation improvements can lower costs. Healthcare regulating agencies are gradually adapting to these innovations, with the FDA and EMA developing new paradigms for evaluating customized therapies, including less bureaucratic pathways for changes to approved peptide scaffolds (Ghadessi & Di, 2023).

Future developments could further optimize the personalization of peptide medications through the use of polypeptide engineering, multi-omics profiling, artificial intelligence, and innovative delivery technologies. In order to design, synthesize, and manufacture customized peptide therapies in clinically acceptable time frames, future research investigates the possibility of fully automated, AI-driven systems that integrate patient data (Parvin et al., 2025). Treatment regimens for complex and diverse diseases like cancer and autoimmune diseases, where a one-size-fits-all approach is ineffective, could be revolutionized by such technology. As technology develops, customized polypeptide formulations will be used as an anchor treatment in precision medicine that is more individualized and customized to the patient's unique biological signature (Sarhangi et al., 2022). Table 10.1 gives a summary of the customization techniques in Polypeptide Formulations.

TABLE 10.1

Personalization Strategies in Polypeptide Formulations

Category	Themes	Key Strategies	Examples of Applications	References

Category	Themes	Key Strategies	Examples of Applications	References
Personalization	Personalized Design in treating cancer and Rare Diseases	Tailoring sequences with omics data; CRISPR-assisted correction; peptide substitutions	Tumor neoantigen vaccines (melanoma, glioblastoma); gene-editing for defective proteins	(Yang et al., 2025), (Bhattacharyya et al., 2024)
Molecular Optimization	• Peptide Stability and Bioavailability • Pharmacokinetic Optimization	Backbone alterations (D-amino acids, cyclization); side-chain modifications	Longer peptide half-life; GLP-1 analogue semaglutide for diabetes	(Shi & Di, 2022) (Xiao et al., 2025)
AI and Computational Tools	AI and Computational Tools	Machine learning for stability and immunogenicity; AI-driven automated synthesis and design	Prediction of modification sites; rapid personalized therapies for cancer and autoimmune diseases	(Ogidi et al., 2025) (Ma et al., 2024)
Targeted and Controlled Delivery	• Targeted Delivery Systems • Tumor Targeting • Non-Invasive Delivery • Smart Systems and Biosensors	Nanocarriers, peptide-drug conjugates, microneedles, wearable biosensors	Breast/ovarian cancer trials; diabetes (CGM + insulin pumps); improved compliance in pediatrics/geriatrics	(Wang et al., 2018) (Liang* et al., 2024)

* Molecular optimization, AI/computational tools, and targeted/controlled delivery are billed as complementary techniques. Wang et al. (2018), is an early foundational reference. The rest of the citations indicate the position/contexts expected for 2024 and 2025. ⏎

10.6 ROLE OF PREDICTIVE ANALYTICS AND ARTIFICIAL INTELLIGENCE IN POLYPEPTIDE THERAPEUTICS

Drug development, drug discovery, and personalized medicine therapy planning have all been transformed by artificial intelligence (AI) and predictive analytics in polypeptide therapeutics. Researchers' ability to create peptides, predict clinical efficacy, and track treatment plans for specific patients is being altered by new developments in computational techniques (Paul et al., 2021). To find novel peptide sequences with high specificity at the target site and fewer off-target effects, the AI algorithms leverage massive data sets, such as genomic, proteomic, and clinical data. Structural biology has now developed to the point where machine learning models, trained on assembly databases, are now able to predict peptide-protein interactions with high accuracy and can dramatically increase the prediction of lead drug candidates (Gholap & Omri, 2025). As an example, we can cite deep learning applications such as AlphaFold and Rosetta Fold repurposed to the design of peptides to specifically target disease-relevant proteins, omitting the conventional trial-and-error drug discovery path. These types of computational interventions are also of particular value in regulating difficult-to-target protein-protein interventions, which have proved difficult to address in the past by small-molecule or biologic intervention (Ocana et al., 2025).

Besides drug discovery, AI can be used in the rationalization of targeted medicine polypeptide formulation. Prediction models analyze patient-specific determinants including genetic variation, metabolic profiles, and

disease biomarkers that are obtained experimentally with a perspective of suggesting ideal sequences, dosages, and the form of peptides to be delivered. This strategy has been especially appealing in the field of oncology, where AI tools allow tumor sequencing data to be paired with peptide-drug libraries to make patient-specific neoantigens to design cancer vaccines (Vora & Gholap, 2023). Machine learning algorithms can even be used to predict how a patient will respond to peptide hormones such as insulin or GLP-1 analogs based on a patient's genomic and clinical profile, which can be used to improve prescribing dosage regimens even in a metabolic disease process (Villikudathil et al., 2024). Real-world data collection, wearables, and continuous monitoring systems improve these predictive capabilities, and dynamic feedback systems help to improve treatment plans over time. An excellent illustration of this transformative potential is the creation of closed-loop diabetes treatment systems that combine AI-driven insulin delivery with continuous glucose monitoring (Mansour et al., 2024).

Important issues with polypeptide development, such as stability, immunogenicity, and bioavailability, are also addressed by predictive analytics. Improvements in plasma half-life and preservation of biological function through structural alterations can also be predicted by machine learning algorithms built on peptide degradation patterns (Zheng et al., 2025). The algorithms recommend stabilization interventions like cyclization or D-amino acid substitution while taking sequence composition, post-translational modifications, and possible sites of proteolytic cleavage into account. The prediction of immunogenicity is another extremely useful application, in which software applications search through peptide sequences to identify potential T-cell epitopes that are most likely to trigger negative immune responses (Yang et al., 2025). When it comes to chronic medications that require frequent administration, where immunogenicity

may restrict long-term activity, this is extremely beneficial. Additionally, by predicting absorption rates and estimating interactions with biological barriers, predictive models are used to customize peptide formulations for all delivery routes, from oral to transdermal (Varanko et al., 2020).

Digital health platforms that integrate data and provide decision support enable the clinical application of AI-peptide therapies. To provide personalized treatment recommendations, the platforms combine data from electronic health records, real-time monitoring, and multi-omics. Hospitals are now testing AI clinical decision support systems to help doctors choose or switch peptide-based treatment in complex cases with comorbidities (Bajwa et al., 2021). Large populations of patients can be processed by the systems to identify subgroups that may benefit from particular peptide formulations or combinations of treatments. For instance, clustering evaluations have enabled more individualized treatment strategies by identifying unique patient phenotypes in autoimmune diseases with varying responses to peptide immunomodulators. These kinds of digital platforms also take into account pharmacoeconomic factors, which assist physicians in customizing therapeutic efficacy while cutting costs, hence making customized peptide treatments affordable (Danieli et al., 2024). However, there are still many obstacles in the way of the widespread use of AI for polypeptide treatments. Interoperability and data quality problems are ongoing, and heterogeneous data sources are often non-standardized. Data privacy and algorithmic bias ethics must be continuously addressed, especially when handling private genomic information (Wubineh et al., 2024).

Future developments in AI and novel technologies may further transform polypeptide therapies. Drug development times could be greatly shortened by using quantum computing in peptide folding model simulations. Fully automated design-make-test-analyze cycles for peptide optimization may be

possible by combining generative AI with robotics-based synthesis platforms (Sudabathula & Researcher, 2025). Clinicians may be able to test a variety of peptide treatment approaches without ever using them thanks to digital twin technology, which creates virtual patient models to simulate therapy. Truly customized polypeptide medicine where therapy isn't just tailored to patient subsets but is best adjusted in real time to each patient's dynamic biology will become more and more feasible as those technologies develop. At this junction of biological knowledge and computational power, polypeptide medications hold the promise of more efficient, focused, and economical treatments for a variety of illnesses (Meijer et al., 2023). Figure 10.4 depicts the role of artificial intelligence in peptide design.

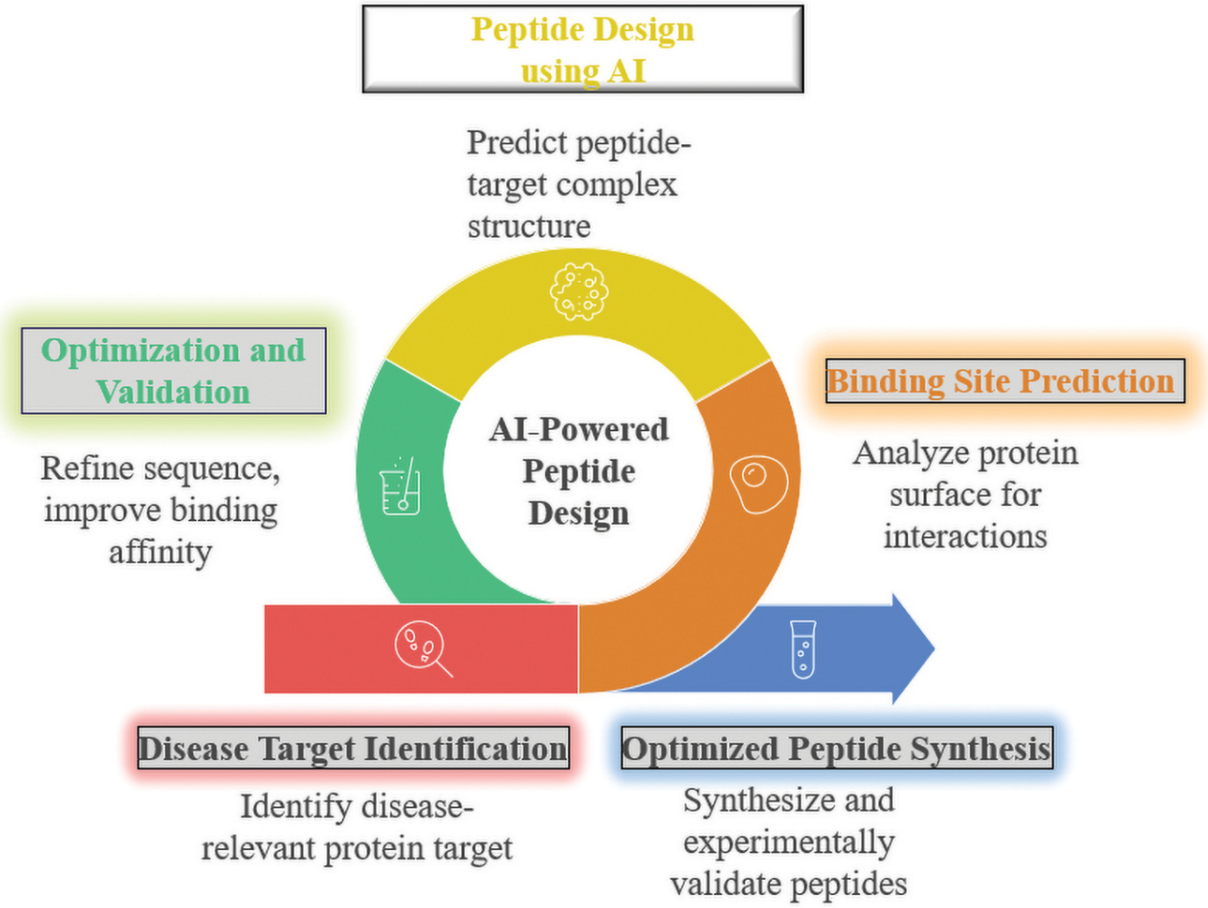

FIGURE 10.4 Peptide design using AI. ⏎

10.7 CASE STUDIES AND CLINICAL USES OF SPECIFIC POLYPEPTIDE TREATMENTS

Numerous medical specialties have seen incredible success stories as a result of the translation of personalized polypeptide therapies from the bench to the bedside, highlighting their transformative influence in clinical practice. Neoantigen-targeted peptide vaccines have become a ground-breaking strategy in oncology, with multiple clinical trials showing better outcomes in aggressive cancers. When compared to standard immunotherapy alone, a paradigm trial of melanoma patients who received customized peptide vaccines against tumor mutational profiles showed a markedly improved progression-free survival (Chen et al., 2020). Likewise, peptide-drug conjugates directed by CD19 and CD22 have demonstrated previously unheard-of response rates in relapsed B-cell lymphoma in hematologic malignancies, with some achieving total remission following the failure of several lines of conventional therapy. These results suggest that tumor heterogeneity and drug resistance mechanisms that usually restrict conventional chemotherapies may be overcome by polypeptide customizations (Wang et al., 2025).

Customized peptide formulations have led to equally dramatic advancements in metabolic disorders. Precision DPP4 polymorphism-guided genetically engineered GLP-1 analogues were shown to provide better glycemic control and fewer side effects than conventional formulations in a multicenter trial conducted in type 2 diabetics (Zein & Raffaello, 2022). At the same time, brittle diabetic patients have experienced a significant decrease in hypoglycemic episodes, including, for the first time in their

disease course, near-normal glycemic variability, thanks to insulin analogues whose shapes have been altered to accommodate continuous glucose monitoring profiles. These examples show how the long-standing issue of high interpatient variability in drug response that has plagued the treatment of metabolic diseases can be resolved through peptide individualization (Kwon & Moon, 2025).

Another area where polypeptide personalized therapies have had a significant impact is in genetic rare diseases. Children who were given customized peptide conjugates that were specifically made to replace defective Lamin A proteins have shown a remarkable clinical improvement in progeria, a deadly, premature aging disease. In order to treat previously incurable neurologic disorders, conjugated peptides containing enzymes in lysosomal storage diseases are made to target particular mutations and cross the blood-brain barrier. The potential of customizing polypeptides to treat formerly "undruggable" genetic diseases through molecular targeting is demonstrated by these success cases (Lamis et al., 2022).

Novel peptide technologies have also revolutionized autoimmune diseases. Multiple sclerosis patients have developed immune tolerance as a result of antigen-specific immunotherapy using engineered peptide sequences; some have seen a decrease in disability and a reduction in lesion burden (Kammona & Kiparissides, 2020). In rheumatoid arthritis, tailored peptide vaccines that target a person's own citrullinated peptide pattern have shown promise in controlling autoimmune reactions without causing general immunosuppression. These drugs demonstrate that, in contrast to conventional immunosuppressants, polypeptide therapy can regulate immunity with greater specificity (Jin et al., 2024).

Even the clinical use of personified peptide therapies was ironically strengthened by the COVID-19 pandemic. Spike-generated peptides were quickly and precisely developed to combat novel threats, and they were

effectively used as preventative and therapeutic measures in patients with compromised immune systems. The event served as a useful lesson in how peptides can be used to build an agile platform that can respond to novel pathogen strains. This could be equally crucial for pandemic preparations in the future (Madhavan et al., 2021).

However, it has been discovered that all of these achievements create significant difficulties in the field. Emergency applications may be limited by lead times and the relatively high complexity of producing patient-specific peptides (Shi & McHugh, 2023). Although output-based compensation models are also being tested, current models are not yet prepared to pay for the high cost of appropriately tailored treatments. As the number of customized peptide products rises, regulatory frameworks are still changing as organizations work to balance innovation and patient safety (Koleva-Kolarova et al., 2022).

Together, these clinical examples show that customized polypeptide treatments can have effects that are impossible to achieve with traditional methods, especially in complicated, diverse illnesses. These case studies are undoubtedly just the beginning of a larger revolution in precision medicine, as manufacturing technologies advance and computational design methodologies develop. The growing body of clinical data clearly shows that over the next ten years, polypeptide personalization will remain the foundation of therapeutic approaches in many medical specialties (Zheng et al., 2025).

10.8 PERSONALIZED POLYPEPTIDE THERAPEUTICS: OBSTACLES AND PROSPECTS

Before customized polypeptide treatments can have their full clinical impact, they must overcome significant scientific, technological, and socioeconomic

obstacles, which outweighs their enormous potential. Since the manufacturing of patient-specific peptides necessitates flexible, small-scale manufacturing schemes that differ greatly from mass production for conventional pharmaceuticals, production issues rank among the most significant of these challenges. The quick turnaround times required for personalized medicines are difficult for modern good manufacturing practice (cGMP) facilities, especially when it comes to the treatment of severe diseases such as cancer, in which time management is crucial (Baral & Choi, 2025). These issues may be resolved by healthcare manufacturing technologies and modular automated synthesis modules; artificial intelligence (AI)-controlled robotic systems and microfluidic peptide synthesizers are two possible remedies. However, significant investment in infrastructure and regulatory changes will be required to have such strategies available (Urrea & Kern, 2025).

Economic considerations are another major obstacle since developing and manufacturing personalized polypeptide therapies usually come with astronomically high costs compared to conventional treatments. Current reimbursement systems in most healthcare systems are poorly equipped to handle therapies that have effects on small populations of patients or even just one patient (Koleva-Kolarova et al., 2022). Innovative pricing models like outcome-based pricing and amortization across indications are being explored to improve access. The cost of related diagnostics and multi-omics profiling needed for personalization also contributes to these economic costs, although decreased sequencing costs and the development of more targeted biomarker panels could potentially mitigate this cost burden in the future (Molla & Bitew, 2024).

Scientific challenges remain in certain regions of polypeptide individualization. The accurate prediction of immunogenicity is also far from optimal, with neutralizing antibodies elicited by some patients against

therapeutic peptides after advanced computational screening. Delivery across biological barriers, particularly the blood-brain barrier for neuro-related uses remains to limit the majority of potential peptide treatments (Harris & Cohen, 2024). Additionally, the intrinsic dynamic character of the majority of disease conditions requires therapy that can match evolving pathologies, prompting exploration of "smart" polypeptide systems capable of real-time response to shifting physiology. Innovative approaches like stimulus-responsive peptide materials and evolutionary peptide design algorithms are currently being used to overcome such scientific obstacles, but much more work needs to be done before these solutions are developed to the level of clinical availability (Singh et al., 2025). Intellectual property issues provide additional complications because traditional patent regimes will be unable to protect customized therapies that can involve private compositions of public-domain peptide sequences and patient-specific data (Wilson & Booth, 2024).

Looking ahead, several emerging trends will reshape the next generation of customized polypeptide drugs. Timelines for drug discovery will be completely transformed by the use of quantum computer-based simulations of peptide folding. Advances in synthetic biology could make live therapies possible, wherein engineered cells generate customized peptides in response to biomarkers unique to each patient (Xiao et al., 2025). Before implementing various peptide treatment regimens in the clinic, clinicians may be able to model them using digital twin technology to simulate therapy using virtual patient models. But what will be most revolutionary is the ongoing convergence of AI and experimental platforms, which could allow for highly automated make-design-test-analyze loops that could produce customized polypeptide drugs from concept to clinic in previously unthinkable timescales (Giansanti & Morelli, 2025).

As technology advances, ethics will become more important. Inequities of equitable access must be addressed in order not to make personalized medicine available solely to wealthy people or to healthcare systems (Mennella et al., 2024). Issues of privacy on data pertaining to genomics and health data required for personalization must be brought under robust governance regimes. In addition, the psychological effect of genuinely individualized therapies such as increased patient expectations and possible differences in outcomes must be taken into account as these treatments move into mainstream application (Khan et al., 2025).

The next decade will probably find personalized polypeptide treatments move from being extraordinary exceptions to standard clinical choices for numerous illnesses. As manufacturing becomes more decentralized and automated, as computational tools grow more sophisticated, and as regulatory pathways adapt, these therapies may fundamentally alter our approach to treating complex diseases (Faiyazuddin et al., 2025). In order to create living, responsive therapeutic systems that do away with the distinction between drug and patient, the ultimate vision is a medical model in which polypeptide drugs are continuously tailored to each patient's changing biological condition, both at the beginning of treatment and throughout the course of the disease. Even though there are still enormous obstacles to overcome, the unprecedented progress made thus far indicates that this future may be closer than we think (Su et al., 2024). Figure 10.5 enlightens the obstacles in adapting polypeptide personalized therapies.

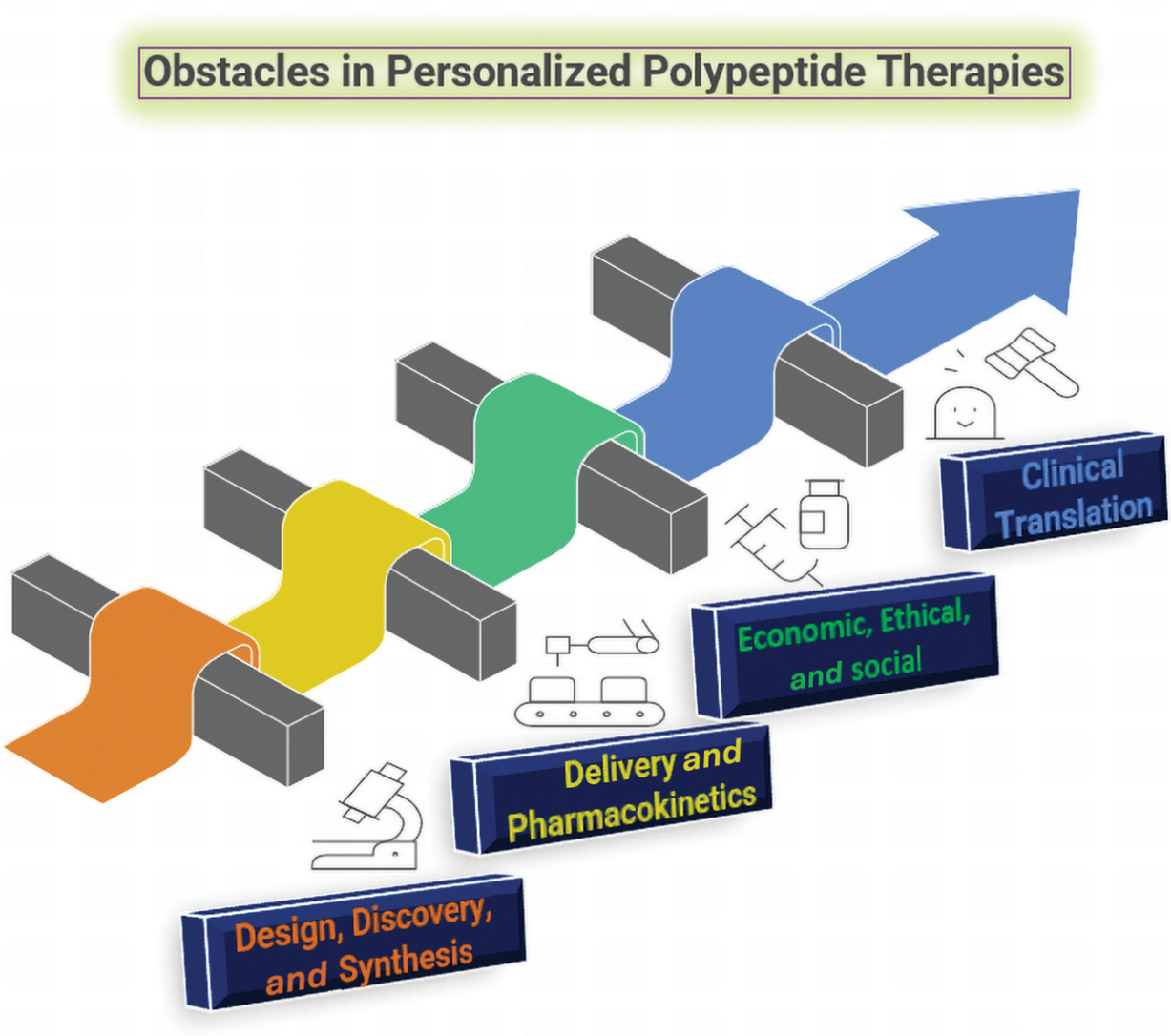

FIGURE 10.5 Obstacles in personalized polypeptide therapies.

10.9 CONCLUSION

Precision medicine is at the forefront of custom-designed polypeptide therapeutics, which offer highly customized treatments that are remarkably accurate and optimally matched to the patient's specific needs. From cancer vaccines to the treatment of metabolic diseases, advancements in peptide engineering, AI-driven design, and multi-omics integration have enabled the

creation of treatments that surpass traditional boundaries in drug development (Hashemi et al., 2024). Clinical results demonstrate that they can even improve in situations where traditional approaches fall short. Cost-effectiveness, availability on a parity basis, and manufacturing scalability are still issues that need to be resolved. Developments in synthetic biology, closed-loop delivery systems, and real-time adaptive therapy made possible by continuous efforts to build regulatory infrastructures will be essential to advancements (Dzau et al., 2021). Once these platforms mature, personalized polypeptides will be as commonplace as novel therapies, revolutionizing medical treatment modalities. This environment is dedicated to offering genuinely patient-centered solutions, changing healthcare from mass market to precision and from reactive to proactive. Personalized polypeptide medicine will redefine what is possible in treatments by using interdisciplinary approaches to address present constraints, ushering in a time when each therapy is as distinct as the patient for whom it is designed (Mathur & Sutton, 2017).

Declaration: ChatGPT 3.5 was utilized for grammar and sentence refinement.

REFERENCES

Ahmed, S., Mazhar, M. S., & Shabbir, M. F. (2024). Neoantigen-based cancer vaccines: Current innovations, challenges and future directions in personalized immunotherapy. *Cancer Immunology Connect*, *1*(1), 1–10.

Al-Amrani, S., Al-Jabri, Z., Al-Zaabi, A., Alshekaili, J., & Al-Khabori, M. (2021). Proteomics: Concepts and applications in human medicine. *World Journal of Biological Chemistry*, *12*(5), 57–69. https://doi.org/10.4331/wjbc.v12.i5.57

Ankathil, R. (2017). ABCB1 genetic variants in leukemias: Current insights into treatment outcomes. *Pharmacogenomics and Personalized Medicine*, *10*, 169–181. https://doi.org/10.2147/pgpm.s105208

Ansori, A. N., Antonius, Y., Susilo, R. J., Hayaza, S., Kharisma, V. D., Parikesit, A. A., Zainul, R., Jakhmola, V., Saklani, T., Rebezov, M., Ullah, M. E., Maksimiuk, N., Derkho, M., & Burkov, P. (2023). Application of CRISPR-Cas9 genome editing technology in various fields: A review. *Narra J*, *3*(2), e184. https://doi.org/10.52225/narra.v3i2.184

Armstrong, A., Coburn, F., Nsereko, Y., & Al Musaimi, O. (2025). Peptide-drug conjugates: A new Hope for cancer. *Journal of Peptide Science*, *31*(8), e70040.

Bajwa, J., Munir, U., Nori, A., & Williams, B. (2021). Artificial intelligence in healthcare: Transforming the practice of medicine. *Future Healthcare Journal*, *8*(2), e188–e194. https://doi.org/10.7861/fhj.2021-0095

Baral, K. C., & Choi, K. Y. (2025). Barriers and strategies for oral peptide and protein therapeutics delivery: Update on clinical advances. *Pharmaceutics*, *17*(4), 397. https://www.mdpi.com/1999-4923/17/4/397

Bhattacharyya, P., Mehndiratta, K., Maiti, S., & Chakraborty, D. (2024). Rare genetic disorders in India: Current status, challenges, and CRISPR-based therapy. *Journal of Biosciences*, *49*(1), 28.

Borel, A.-L., Lablanche, S., Waterlot, C., Joffray, E., Barra, C., Arnol, N., Amougay, H., & Benhamou, P.-Y. (2024). Closed-loop insulin therapy for people with type 2 diabetes treated with an insulin pump: A 12-week multicenter, open-label randomized, controlled, crossover trial. *Diabetes Care*, *47*(10), 1778–1786. https://doi.org/10.2337/dc24-0623

Borthakur, P. P., Das, A., Sahariah, J. J., Pramanik, P., Baruah, E., & Pathak, K. (2026). Revolutionizing patient care: 3D printing for customized medical devices and therapeutics. *Biomedical Materials & Devices*, *4*, 1275–1302. https://doi.org/10.1007/s44174-025-00324-2

Calabretta, M. M., Zangheri, M., Lopreside, A., Marchegiani, E., Montali, L., Simoni, P., & Roda, A. (2020). Precision medicine, bioanalytics and nanomaterials: Toward a new generation of personalized portable diagnostics. *Analyst*, *145*(8), 2841–2853.

Chen, X., Yang, J., Wang, L., & Liu, B. (2020). Personalized neoantigen vaccination with synthetic long peptides: Recent advances and future perspectives. *Theranostics*, *10*(13), 6011–6023. https://doi.org/10.7150/thno.38742

Collins, J. M., Singh, S. K., White, T. A., Cesta, D. J., Simpson, C. L., Tubb, L. J., & Houser, C. L. (2023). Total wash elimination for solid phase peptide synthesis. *Nature Communications*, *14*(1), 8168. https://doi.org/10.1038/s41467-023-44074-5

Dağcı, İ., & Unver, Y. (2023). *Production of Recombinant Protein Using Pichia Pastoris Expression System*.

Danieli, M. G., Brunetto, S., Gammeri, L., Palmeri, D., Claudi, I., Shoenfeld, Y., & Gangemi, S. (2024). Machine learning application in autoimmune diseases: State of art and future prospectives. *Autoimmunity Reviews*, *23*(2), 103496. https://doi.org/10.1016/j.autrev.2023.103496

de León, E. H.-P., Valle-Pérez, A. U., Khan, Z. N., & Hauser, C. A. (2023). Intelligent and smart biomaterials for sustainable 3D printing applications. *Current Opinion in Biomedical Engineering*, *26*, 100450.

Delpierre, C., & Lefèvre, T. (2023). Precision and personalized medicine: What their current definition says and silences about the model of health they promote. Implication for the development of personalized health. *Frontiers in Sociology*, *8*, 1112159.

Dzau, V. J., McClellan, M. B., McGinnis, J. M., Marx, J. C., Sullenger, R. D., & ElLaissi, W. (2021). Vital directions for health and health care: Priorities for 2021: Commentary describes a collection of articles in the vital directions for health and health care series. *Health Affairs*, *40*(2), 197–203.

Evans, B. J., & King, A. T. (2020). Methods to enhance the metabolic stability of peptide-based PET radiopharmaceuticals. *Molecules*, *25*(10), 2314. https://doi.org/10.3390/molecules25102314

Faiyazuddin, D., Rahman, S., Anand, G., Siddiqui, R., Mehta, R., Khatib, M., Bawankule, S., Quazi Syed, Z., Hussain, A., & Sah, R. (2025). The impact of artificial intelligence on healthcare: A comprehensive review of advancements in diagnostics, treatment, and operational efficiency. *Health Science Reports*, *8*(6), e70312. https://doi.org/10.1002/hsr2.70312

Gao, Y., & Joshi, M. (2024). PEGylated therapeutics in the clinic. *Bioengineering & Translational Medicine*, *9*(1), e10600. https://doi.org/10.1002/btm2.10600⏎

Ghadessi, M., & Di, J. (2023). Decentralized clinical trials and rare diseases: A drug information association innovative design scientific working group (DIA-IDSWG) perspective. *Orphanet Journal of Rare Diseases*, *18*(1), 79. https://doi.org/10.1186/s13023-023-02693-7⏎

Gholap, A. D., & Omri, A. (2025). Advances in artificial intelligence-envisioned technologies for protein and nucleic acid research. *Drug Discovery Today*, *30*(5), 104362. https://doi.org/10.1016/j.drudis.2025.104362⏎

Giansanti, D., & Morelli, S. (2025). Exploring the potential of digital twins in cancer treatment: A narrative review of reviews. *Journal of Clinical Medicine*, *14*(10), 3574. https://www.mdpi.com/2077-0383/14/10/3574⏎

Gummadi, A. C., & Guddati, A. K. (2021). Genetic polymorphisms in pharmaceuticals and chemotherapy. *World Journal of Oncology*, *12*(5), 149–154. https://doi.org/10.14740/wjon1405⏎

Gündüz Ergün, B., Hüccetoğulları, D., Öztürk, S., & Celik, E. (2019). Established and upcoming yeast expression systems: Embryological, cellular, and genetic methods. In A. S. Badran & J. D. Keasling (Eds.), *Yeast Systems Biology* (Vol. 1923, pp. 1–74). Springer. https://doi.org/10.1007/978-1-4939-9024-5_1⏎

Harris, C. T., & Cohen, S. (2024). Reducing immunogenicity by design: Approaches to minimize immunogenicity of monoclonal antibodies. *Drugs*, *38*(2), 205–226. https://doi.org/10.1007/s40259-023-00641-2⏎

Hashemi, S., Vosough, P., Taghizadeh, S., & Savardashtaki, A. (2024). Therapeutic peptide development revolutionized: Harnessing the power of artificial intelligence for drug discovery. *Heliyon*, *10*, e40265. https://doi.org/10.1016/j.heliyon.2024.e40265⏎

Ho, D., Quake, S. R., McCabe, E. R. B., Chng, W. J., Chow, E. K., Ding, X., Gelb, B. D., Ginsburg, G. S., Hassenstab, J., Ho, C. M., Mobley, W. C., Nolan, G. P., Rosen, S. T., Tan, P., Yen, Y., & Zarrinpar, A. (2020). Enabling technologies for personalized and precision medicine. *Trends in Biotechnology*, *38*(5), 497–518. https://doi.org/10.1016/j.tibtech.2019.12.021⏎

Huang, Y., Zhang, P., Wang, H., Chen, Y., Liu, T., & Luo, X. (2025). Genetic code expansion: Recent developments and emerging applications. *Chemical Reviews*, *125*(2), 523–598. https://doi.org/10.1021/acs.chemrev.4c00216⏎

Jadhav, K., Abhang, A., Kole, E. B., Gadade, D., Dusane, A., Iyer, A., Sharma, A., Rout, S. K., Gholap, A. D., Naik, J., Verma, R. K., & Rojekar, S. (2025). Peptide–drug conjugates as next-generation therapeutics: Exploring the potential and clinical progress. *Bioengineering*, *12*(5), 481. https://www.mdpi.com/2306-5354/12/5/481⏎

Jin, X., Dong, T., Wang, Q., Xie, Y., Fang, X., Wei, C., Liu, S., Zheng, X., Wang, P., Zhu, D., Cao, L., Dong, S., Fang, K., Zhong, C., Wang, J., Hu, F., & Li, Z. (2024). A citrullinated antigenic vaccine in treatment of autoimmune arthritis. *Science Bulletin (Beijing)*, *69*(18), 2920–2929. https://doi.org/10.1016/j.scib.2024.02.042⏎

Kammona, O., & Kiparissides, C. (2020). Recent advances in antigen-specific immunotherapies for the treatment of multiple sclerosis. *Brain Sciences*, *10*(6), 333. https://doi.org/10.3390/brainsci10060333⏎

Khan, A., Barapatre, A. R., Babar, N., Doshi, J., Ghaly, M., Patel, K. G., Nawaz, S., Hasana, U., Khatri, S. P., Pathange, S., Pesaru, A. R., Puvvada, C. S., Billoo, M., & Jamil, U. (2025). Genomic medicine and personalized treatment: A narrative review. *Annals of Medicine and Surgery (London)*, *87*(3), 1406–1414. https://doi.org/10.1097/ms9.0000000000002965⏎

Kobeissy, F., Goli, M., Yadikar, H., Shakkour, Z., Kurup, M., Haidar, M. A., Alroumi, S., Mondello, S., Wang, K. K., & Mechref, Y. (2023). Advances in neuroproteomics for neurotrauma: Unraveling insights for personalized medicine and future prospects. *Frontiers in Neurology*, *14*, 1288740.⏎

Kohli, M., & Poulogiannis, G. (2025). Harnessing the power of metabolomics for precision oncology: Current advances and future directions. *Cells*, *14*(6), 402. https://www.mdpi.com/2073-4409/14/6/402⏎

Koleva-Kolarova, R., Buchanan, J., Vellekoop, H., Huygens, S., Versteegh, M., Mölken, M. R., Szilberhorn, L., Zelei, T., Nagy, B., Wordsworth, S., & Tsiachristas, A. (2022). Financing and reimbursement models for personalised medicine: A systematic review to identify current models

and future options. *Appl Health Econ Health Policy*, *20*(4), 501–524. https://doi.org/10.1007/s40258-021-00714-9

Kwon, S. Y., & Moon, J. S. (2025). Advances in continuous glucose monitoring: Clinical applications. *Endocrinology and Metabolism*, *40*(2), 161–173. https://doi.org/10.3803/EnM.2025.2370

Lamis, A., Siddiqui, S. W., Ashok, T., Patni, N., Fatima, M., & Aneef, A. N. (2022). Hutchinson-Gilford progeria syndrome: A literature review. *Cureus*, *14*(8), e28388.

Li, Y., Wu, M., Fu, Y., Xue, J., Yuan, F., Qu, T., Rissanou, A. N., Wang, Y., Li, X., & Hu, H. (2024). Therapeutic stapled peptides: Efficacy and molecular targets. *Pharmacological Research*, *203*, 107137. https://doi.org/10.1016/j.phrs.2024.107137

Li, Y., Wu, X., Fang, D., & Luo, Y. (2024). Informing immunotherapy with multi-omics driven machine learning. *NPJ Digital Medicine*, *7*(1), 67. https://doi.org/10.1038/s41746-024-01043-6

Liang*, S., Tian*, Y., & Bai, S. (2024). Peptide-based hydrogels for soft electronic devices and wearable biosensors. *Peptide Self-Assembly and Engineering: Fundamentals, Structures, and Applications*, *2*, 393–419.

Lin, C., Tian, Q., Guo, S., Xie, D., Cai, Y., Wang, Z., Chu, H., Qiu, S., Tang, S., & Zhang, A. (2024). Metabolomics for clinical biomarker discovery and therapeutic target identification. *Molecules*, *29*(10), 2198.

Liu, S., Deng, T., Cheng, H., Lu, J., & Wu, J. (2025). Advances in transdermal drug delivery systems and clinical applications in inflammatory skin diseases. *Pharmaceutics*, *17*(6), 746. https://www.mdpi.com/1999-4923/17/6/746

Ma, H., Zhou, X., Zhang, Z., Weng, Z., Li, G., Zhou, Y., & Yao, Y. (2024). AI-driven design of cell-penetrating peptides for therapeutic biotechnology. *International Journal of Peptide Research and Therapeutics*, *30*(6), 69.

Madhavan, M., AlOmair, L. A., Ks, D., & Mustafa, S. (2021). Exploring peptide studies related to SARS-CoV to accelerate the development of novel therapeutic and prophylactic solutions against COVID-19. *Journal of Infection and Public Health*, *14*(8), 1106–1119. https://doi.org/10.1016/j.jiph.2021.06.017

Mahapatra, M. K., & Karuppasamy, M. (2022). Semaglutide, a glucagon like peptide-1 receptor agonist with cardiovascular benefits for management of type 2 diabetes. *Reviews in Endocrine and Metabolic Disorders*, *23*(3), 521–539. https://doi.org/10.1007/s11154-021-09699-1⏎

Makharadze, D., del Valle, L. J., Katsarava, R., & Puiggalí, J. (2025). The art of PEGylation: From simple polymer to sophisticated drug delivery system. *International Journal of Molecular Sciences*, *26*(7), 3102. https://www.mdpi.com/1422-0067/26/7/3102⏎

Mansour, M., Saeed Darweesh, M., & Soltan, A. (2024). Wearable devices for glucose monitoring: A review of state-of-the-art technologies and emerging trends. *Alexandria Engineering Journal*, *89*, 224–243. https://doi.org/10.1016/j.aej.2024.01.021⏎

Marques, L., & Costa, B. (2024). Advancing precision medicine: A review of innovative in silico approaches for drug development, clinical pharmacology and personalized healthcare. *Pharmaceutics*, *16*(3), 332. https://doi.org/10.3390/pharmaceutics16030332⏎

Mathur, S., & Sutton, J. (2017). Personalized medicine could transform healthcare. *Biomedical Reports*, *7*(1), 3–5. https://doi.org/10.3892/br.2017.922⏎

Mattei, A. E., Gutierrez, A. H., Seshadri, S., Tivin, J., Ardito, M., Rosenberg, A. S., Martin, W. D., & De Groot, A. S. (2024). In silico methods for immunogenicity risk assessment and human homology screening for therapeutic antibodies. *MAbs*, *16*(1), 2333729. https://doi.org/10.1080/19420862.2024.2333729⏎

Meijer, C., Uh, H. W., & El Bouhaddani, S. (2023). Digital twins in healthcare: Methodological challenges and opportunities. *Journal of Personalized Medicine*, *13*(10), 1522. https://doi.org/10.3390/jpm13101522⏎

Mennella, C., Maniscalco, U., De Pietro, G., & Esposito, M. (2024). Ethical and regulatory challenges of AI technologies in healthcare: A narrative review. *Heliyon*, *10*(4), e26297. https://doi.org/10.1016/j.heliyon.2024.e26297⏎

Molla, G., & Bitew, M. (2024). Revolutionizing personalized medicine: Synergy with multi-omics data generation, main hurdles, and future perspectives. *Biomedicines*, *12*(12), 2750. https://doi.org/10.3390/biomedicines12122750⏎

Nandhini, K., Noki, S., Brasil, E., Albericio, F., & de la Torre, B. G. (2023). A safety-catch protecting group strategy compatible with Boc-chemistry for the synthesis of peptide nucleic acids (PNAs). *Organic & Biomolecular Chemistry*, *21*(40), 8125–8135.

Nilius, H., Tsouka, S., Nagler, M., & Masoodi, M. (2024). Machine learning applications in precision medicine: Overcoming challenges and unlocking potential. *TrAC Trends in Analytical Chemistry*, *172*, 117872.

Ocana, A., Pandiella, A., Privat, C., Bravo, I., Luengo-Oroz, M., Amir, E., & Gyorffy, B. (2025). Integrating artificial intelligence in drug discovery and early drug development: A transformative approach. *Biomarker Research*, *13*(1), 45. https://doi.org/10.1186/s40364-025-00758-2

Ogidi, O. I., Alfred-Ugbenbo, D., & Poripo, B. E. (2025). AI-driven drug discovery, design, and development in immunological disorders. In *AI-Assisted Computational Approaches for Immunological Disorders* (pp. 143–180). IGI Global Scientific Publishing.

Parvin, N., Joo, S. W., Jung, J. H., & Mandal, T. K. (2025). Multimodal AI in biomedicine: Pioneering the future of biomaterials, diagnostics, and personalized healthcare. *Nanomaterials*, *15*(12), 895. https://www.mdpi.com/2079-4991/15/12/895

Paul, D., Sanap, G., Shenoy, S., Kalyane, D., Kalia, K., & Tekade, R. K. (2021). Artificial intelligence in drug discovery and development. *Drug Discovery Today*, *26*(1), 80–93. https://doi.org/10.1016/j.drudis.2020.10.010

Pelaia, T. M., Shojaei, M., & McLean, A. S. (2023). The role of transcriptomics in redefining critical illness. *Annual Update in Intensive Care and Emergency Medicine*, *2023*, 3–14.

Petrak, J., Tevosian, S. G., Richter, S., & Ghayee, H. K. (2024). Metabolomics and proteomics in pheochromocytoma and paraganglioma: Translating biochemistry and biology to bedside. *Best Practice & Research Clinical Endocrinology & Metabolism*, *38*(6), 101935. https://doi.org/10.1016/j.beem.2024.101935

Pina, E., Ramos, J., Jorge, H., Vaz, P., Silva, J., Wanzeller, C., Abbasi, M., & Martins, P. (2024). Data privacy and ethical considerations in database management. *Journal of Cybersecurity and Privacy*, *4*, 494–517. https://doi.org/10.3390/jcp4030024

Pirmohamed, M. (2023). Pharmacogenomics: Current status and future perspectives. *Nature Reviews Genetics*, *24*(6), 350–362.

Podust, V. N., Balan, S., Sim, B.-C., Coyle, M. P., Ernst, U., Peters, R. T., & Schellenberger, V. (2016). Extension of in vivo half-life of biologically active molecules by XTEN protein polymers. *Journal of Controlled Release*, *240*, 52–66.

Rahman, M. T., Das, V., Hismat, U., Tripura, R., Mazumder, J., & Khuleibam, M. (2023). *Exploration of Advances in Solid Phase Synthesis of Therapeutic Peptides: Historical Development, Current Trends, and Future Perspectives*. Zenodo. https://doi.org/10.5281/zenodo.8237882

Recharla, M., Nuka, S. T., Chakilam, C., Chava, K., & Suura, S. R. (2023). Next-generation technologies for early disease detection and treatment: Harnessing intelligent systems and genetic innovations for improved patient outcomes. *Journal for ReAttach Therapy and Developmental Diversities*, *6*, 1921–1937.

Rizo-Roca, D., Henderson, J. D., & Zierath, J. R. (2025). Metabolomics in cardiometabolic diseases: Key biomarkers and therapeutic implications for insulin resistance and diabetes. *Journal of Internal Medicine*, *297*(6), 584–607. https://doi.org/10.1111/joim.20090

Sarhangi, N., Hajjari, S., Heydari, S. F., Ganjizadeh, M., Rouhollah, F., & Hasanzad, M. (2022). Breast cancer in the era of precision medicine. *Molecular Biology Reports*, *49*(10), 10023–10037.

Sarhangi, N., Patrinos, G. P., Aghaei Meybodi, H. R., Nikfar, S., Sarrami, B., Kiani, P., Larijani, B., Khodayari, N., & Hasanzad, M. (2024). Personalized medicine and therapeutics. In *A Glimpse at Medicine in the Future* (pp. 111–127). Springer.

Shen, X., Ma, Y., Luo, H., Abdullah, R., Pan, Y., Zhang, Y., Zhong, C., Zhang, B., & Zhang, G. (2024). Peptide Aptamer–Paclitaxel conjugates for tumor targeted therapy. *Pharmaceutics*, *17*(1), 40.

Shi, M., & McHugh, K. J. (2023). Strategies for overcoming protein and peptide instability in biodegradable drug delivery systems. *Advanced Drug Delivery Reviews*, *199*, 114904. https://doi.org/10.1016/j.addr.2023.114904

Shi, S. M., & Di, L. (2022). Strategies to optimize peptide stability and prolong half-life. In *Peptide Therapeutics: Fundamentals of Design, Development, and Delivery* (pp. 163–182). Springer.

Singh, P., Pandit, S., Balusamy, S. R., Madhusudanan, M., Singh, H., Amsath Haseef, H. M., & Mijakovic, I. (2025). Advanced nanomaterials for cancer therapy: Gold, silver, and iron oxide nanoparticles in oncological applications. *Advanced Healthcare Materials*, *14*(4), 2403059.

Song, Z., Han, Z., Lv, S., Chen, C., Chen, L., Yin, L., & Cheng, J. (2017). Synthetic polypeptides: From polymer design to supramolecular assembly and biomedical application. *Chemical Society Reviews*, *46*(21), 6570–6599.

Su, J., Yang, L., Sun, Z., & Zhan, X. (2024). Personalized drug therapy: Innovative concept guided with proteoformics. *Molecular & Cellular Proteomics*, *23*(3), 100737. https://doi.org/10.1016/j.mcpro.2024.100737

Sudabathula, B., & Researcher, I. (2025). The convergence of quantum computing and AI: Reshaping the future of machine learning. *International Journal of Information Technology and Management Information Systems*, *16*, 46–57. https://doi.org/10.34218/IJITMIS_16_01_005

Sun, B. B., Suhre, K., & Gibson, B. W. (2024). Promises and challenges of populational proteomics in health and disease. *Molecular & Cellular Proteomics*, *23*(7), 100735.

Thanasukarn, V., Prajumwongs, P., Muangritdech, N., Loilome, W., Namwat, N., Klanrit, P., Wangwiwatsin, A., Charoenlappanit, S., Jaresitthikunchai, J., Roytrakul, S., & Titapun, A. (2025). Discovery of novel serum peptide biomarkers for cholangiocarcinoma recurrence through MALDI-TOF MS and LC–MS/MS peptidome analysis. *Scientific Reports*, *15*(1), 2582. https://doi.org/10.1038/s41598-025-87124-2

Urrea, C., & Kern, J. (2025). Recent advances and challenges in industrial robotics: A systematic review of technological trends and emerging applications. *Processes*, *13*(3), 832. https://www.mdpi.com/2227-9717/13/3/832

Varanko, A., Saha, S., & Chilkoti, A. (2020). Recent trends in protein and peptide-based biomaterials for advanced drug delivery. *Advanced Drug Delivery Reviews*, *156*, 133–187. https://doi.org/10.1016/j.addr.2020.08.008

Villikudathil, A. T., Mc Guigan, D. H., & English, A. (2024). Computational approaches for clinical, genomic and proteomic markers of response to glucagon-like peptide-1 therapy in type-2 diabetes mellitus: An exploratory analysis with machine learning algorithms. *Diabetes & Metabolic Syndrome*, *18*(7), 103086. https://doi.org/10.1016/j.dsx.2024.103086

Vora, L. K., & Gholap, A. D. (2023). Artificial intelligence in pharmaceutical technology and drug delivery design. *Pharmaceutics*, *15*(7), 1916. https://doi.org/10.3390/pharmaceutics15071916

Wang, H., Wang, G., Li, T., Zhang, P., Mao, Z., Luo, H., Zhu, X., Li, D., Zhou, J., Zhou, X., & Huang, L. (2025). Efficacy and safety of a novel CD19, CD22 dual-targeted fully human loop bi-CAR-T for the treatment of relapsed/refractory B cell non-Hodgkin lymphoma. *J Translational Medicine*, *23*(1), 630. https://doi.org/10.1186/s12967-025-06567-3

Wang, J., Hu, X., & Xiang, D. (2018). Nanoparticle drug delivery systems: An excellent carrier for tumor peptide vaccines. *Drug Delivery*, *25*(1), 1319–1327.

Wang, L., Wang, N., Zhang, W., Cheng, X., Yan, Z., Shao, G., Wang, X., Wang, R., & Fu, C. (2022). Therapeutic peptides: Current applications and future directions. *Signal Transduction and Targeted Therapy*, *7*(1), 48. https://doi.org/10.1038/s41392-022-00904-4

Wang, M., Xia, H., Wang, C., Zhang, T., Zhang, M., Li, X., Peng, C., Jing, T., Wang, Y., Peng, L., & Wang, P. (2025). From precision synthesis to cross-industry applications: The future of emerging peptide technologies. *Pharmacological Research*, *218*, 107839. https://doi.org/10.1016/j.phrs.2025.107839

Wilson, B. E., & Booth, C. M. (2024). Real-world data: Bridging the gap between clinical trials and practice. *EClinicalMedicine*, *78*, 102915. https://doi.org/10.1016/j.eclinm.2024.102915

Wubineh, B. Z., Deriba, F. G., & Woldeyohannis, M. M. (2024). Exploring the opportunities and challenges of implementing artificial intelligence in healthcare: A systematic literature review. *Urologic Oncology: Seminars and Original Investigations*, *42*(3), 48–56. https://doi.org/10.1016/j.urolonc.2023.11.019

Xiao, W., Jiang, W., Chen, Z., Huang, Y., Mao, J., Zheng, W., Hu, Y., & Shi, J. (2025). Advance in peptide-based drug development: Delivery platforms, therapeutics and vaccines. *Signal*

Transduction and Targeted Therapy, *10*(1), 74.

Xu, Y., Liu, X., Cao, X., Huang, C., Liu, E., Qian, S., Liu, X., Wu, Y., Dong, F., Qiu, C.-W., Qiu, J., Hua, K., Su, W., Wu, J., Xu, H., Han, Y., Fu, C., Yin, Z., Liu, M., Roepman, R., Dietmann, S., Virta, M., Kengara, F., Zhang, Z., Zhang, L., Zhao, T., Dai, J., Yang, J., Lan, L., Luo, M., Liu, Z., An, T., Zhang, B., He, X., Cong, S., Liu, X., Zhang, W., Lewis, J. P., Tiedje, J. M., Wang, Q., An, Z., Wang, F., Zhang, L., Huang, T., Lu, C., Cai, Z., Wang, F., & Zhang, J. (2021). Artificial intelligence: A powerful paradigm for scientific research. *The Innovation*, *2*(4), 100179. https://doi.org/10.1016/j.xinn.2021.100179

Yang, L., Li, S., Hou, C., Wang, Z., He, W., & Zhang, W. (2025). Recent advances in mRNA-based therapeutics for neurodegenerative diseases and brain tumors. *Nanoscale*. *17*(7), 3537–3548. https://doi.org/10.1039/d4nr04394d

Yang, R., Ma, X., Peng, F., Wen, J., Allahou, L. W., Williams, G. R., Knowles, J. C., & Poma, A. (2025). Advances in antimicrobial peptides: From mechanistic insights to chemical modifications. *Biotechnology Advances*, *74*, 108570.

Yuan, H., Jiang, M., Fang, H., & Tian, H. (2025). Recent advances in poly (amino acids), polypeptides, and their derivatives in drug delivery. *Nanoscale*, *17*(7), 3549–3584.

Zein, A. F. M. Z., & Raffaello, W. M. (2022). Dipeptidyl peptidase-4 (DPP-IV) inhibitor was associated with mortality reduction in COVID-19—A systematic review and meta-analysis. *Primary Care Diabetes*, *16*(1), 162–167.

Zhang, Y., He, P., Zhang, P., Yi, X., Xiao, C., & Chen, X. (2021). Polypeptides–drug conjugates for anticancer therapy. *Advanced Healthcare Materials*, *10*(11), 2001974.

Zhang, Y., Zhang, Q., Wong, C., & Li, X. (2019). Chemoselective peptide cyclization and bicyclization directly on unprotected peptides. *Journal of the American Chemical Society*, *141*. https://doi.org/10.1021/jacs.9b03623

Zheng, B., Wang, X., Guo, M., & Tzeng, C.-M. (2025). Therapeutic peptides: Recent advances in discovery, synthesis, and clinical translation. *International Journal of Molecular Sciences*, *26*(11), 5131. https://www.mdpi.com/1422-0067/26/11/5131

Zhou, Z., Zhang, R., Zhou, A., Lv, J., Chen, S., Zou, H., Zhang, G., Lin, T., Wang, Z., Zhang, Y., Weng, S., Han, X., & Liu, Z. (2024). Proteomics appending a complementary dimension to precision oncotherapy. *Computational and Structural Biotechnology Journal*, *23*, 1725–1739. https://doi.org/10.1016/j.csbj.2024.04.044⏎

11 Case Studies in Successful Polypeptide Therapeutics

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11.1 INTRODUCTION

Peptides are powerful and selective signaling molecules that bind to cell-surface receptors, including ion channels and G protein-coupled receptors (GPCRs), to elicit intracellular effects (Abdellatif et al., 2020b). Peptides make a wonderful starting point for the development of novel treatments due to their appealing pharmacological profile and inherent qualities. Their specificity has been shown to translate into superior safety, tolerability, and effectiveness profiles in humans (Al-Allaf et al., 2019).

Peptides have lesser production complexity than protein-based biopharmaceuticals; therefore, their production costs are like those of small molecules. Peptides are a beneficial compromise between small molecules and biopharmaceuticals (Abdellatif et al., 2018). Due to their limited chemical and physical stability and short plasma half-life, naturally occurring peptides are often unsuitable for convenient treatment. We must

address these difficulties before employing them as medicines (Aguilar-Toala et al., 2022).

Various peptide technologies, such as multifunctional and cell-penetrating peptides, as well as peptide drug conjugates and technologies focusing on alternative routes of administration, have emerged, representing the opportunities and future directions within the peptide field (Fosgerau and Hoffmann, 2015). Modern medicine has advanced with polypeptide therapies, which target cancer, metabolic, and autoimmune diseases. These amino acid chains use biological molecules' selectivity to offer a possible alternative to small-molecule medications (Wang et al., 2022). Polypeptides' capacity to connect with specific biological targets has helped them treat complex disorders (Abdellatif et al., 2016). This collection of case studies examines the creation, mechanisms, and clinical effects of effective polypeptide medicines (Abdellatif et al., 2020a). In this chapter, we provide a series of ideas that, when taken together, lead us to the conclusion that peptides have a tremendous amount of promise for development as potential treatments in the future.

11.2 CLINICAL USES OF PEPTIDES

11.2.1 Historical Perspective: Evolution from Discovery to Clinical Use

Since the early 1920s, when insulin, a 51-amino-acid peptide, was discovered and sold, peptide drugs have shaped the pharmaceutical industry. After nearly 90 years, human recombinant insulin has replaced animal tissue-derived insulin due to advances in DNA recombination and protein purification. Over the past two decades, about 30 more peptide drugs have been approved, now over 60 worldwide. These authorized peptide medications are mostly used to treat metabolic illnesses and cancer (Lau and

Dunn, 2018). A global industry analysis of peptide therapeutics projected that sales of peptide pharmaceuticals will surpass 70 billion USD in 2019 and that the compound annual growth rate (CAGR) will be 9.1% from 2016 to 2024 (Kovalainen et al., 2015; Margolis et al., 2023). However, the anticipated rise in the prevalence of cancer and metabolic diseases is probably responsible for the industry's robust expansion. Liraglutide (Victoza) and glucagon-like peptide 1 (GLP-1) are two of the best-selling peptide medications for metabolic diseases, with annual sales of at least two billion USD. Over four billion USD in sales were also attributed to well-known peptide medications such as goserelin (Zoladex), leuprolide (Lupron), and somatostatin analogues octreotide and lanreotide (Lee et al., 2019).

11.2.2 Real-World Successful Case Studies

TABLE 11.1

Different Types of Peptides, the Receptor Targets, Date of Approval by the FDA, and Indications of Peptide Medications That Have Been Licensed Since 2000 (Wang et al., 2022b)

Target Name	Peptide Name	First Approval	Indication(s) and Disease Approval Treatment	References
GLP-1 receptor	Exenatide	2005	Diabetes Mellitus Type II	(Nauck et al., 2021)
	Liraglutide	2009		(Vilsboll, 2009)
	Lixisenatide	2013		(Bolli and Owens, 2014)

Target Name	Peptide Name	First Approval	Indication(s) and Disease Approval Treatment	References
	Albiglutide	2014		(Trujillo and Nuffer, 2014)
	Dulaglutide	2014		(Thompson and Trujillo, 2015)
	Semaglutide	2017		(Hall et al., 2018)
GLP-2 receptor	Teduglutide	2012	Short bowel syndrome and malabsorption	(Drucker, 2019)
GC-C receptor	Linaclotide	2012	Irritable bowel syndrome (IBS) accompanied by constipation and chronic idiopathic constipation	(Vazquez-Roque and Bouras, 2013)
Calcitonin receptor	Pramlintide	2005	Diabetes Mellitus Type I & II	(Lee et al., 2016b)
GnRH receptor	Abarelix	2003	Advanced cases of prostate cancer	(Patel et al., 2024)
	Degarelix	2008		(Van Poppel et al., 2008)
Binding to the active site of the 20_S proteasome	Carfilzomib	2012	Multiple cases of myeloma	(Lee et al., 2016a)
NOD2 protein	Mifamurtide	2009	High-grade non-metastatic osteosarcoma	(Kager et al., 2010)

Target Name	Peptide Name	First Approval	Indication(s) and Disease Approval Treatment	References
VIP1 receptor	Aviptadil	2000	Erectile dysfunction	(Youssef et al., 2022)
OT receptor	Atosiban	2000	Delaying imminent pre-term birth	(Dewan et al., 2024)
	Carbetocin	2001	Postpartum hemorrhage	(Su et al., 2012)
TRH receptor	Taltirelin	2000	Spinocerebellar degeneration	(Zhu et al., 2024)
MC receptors	Bremelanotide	2019	Hypoactive sexual desire disorder	(Edinoff et al., 2022)
PTH1 receptor	Teriparatide	2002	Osteoporosis	(Beaudart et al., 2025)
	Abaloparatide	2017		(Varela et al., 2017)
Guanylate cyclase C	Plecanatide	2017	Chronic idiopathic constipation	(Rao, 2018)
NPR-A	Nesiritide	2001	Acute decompensated heart failure	(Katz, 2001)
AT_1 receptor	Angiotensin II	2017	Sepsis and septic shock	(Kawai et al., 2017)
Beta2-receptor	Icatibant	2008	Acute attacks of hereditary angioedema	(Longhurst, 2010)
gp41	Enfuvirtide	2003	Used in combination therapy for the treatment of HIV-1	(Duffalo and James, 2003)
GHRH receptor	Tesamorelin	2010	Reduction of HIV lipodystrophy	(Bedimo, 2011)

Target Name	Peptide Name	First Approval	Indication(s) and Disease Approval Treatment	References
N-type calcium channels	Ziconotide	2004	Severe chronic pain	(Miljanich, 2004)
Thrombopoietin receptor	Romiplostim	2008	Chronic immune thrombocytopenic purpura	(Cersosimo, 2009)
Human erythropoietin receptor	Peginesatide	2012	Anemia associated with chronic kidney disease	(Mikhail, 2012)
Pulmonary surfactant	Lucinactant	2012	Prevention of respiratory distress syndrome	(Ma and Ma, 2012)
CaSR	Etelcalcetide	2016	Secondary hyperparathyroidism	(Cozzolino et al., 2017)
MC1 receptor	Afamelanotide	2014	Prevention of phototoxicity	(Wensink et al., 2021)
Somatostatin receptors	Pasireotide	2012	Cushing's disease	(Colao et al., 2012)
	Lutetium Lu 177 dotatate	2018	Somatostatin receptor (SSRs)-positive gastroenteropancreatic neuroendocrine tumors	(Campos Ramirez et al., 2025)
	Edotreotide gallium Ga-68	2019	Diagnose SSR-positive neuroendocrine tumors	(Haider et al., 2021)
Melanocortin-4 receptor	Setmelanotide	2020	Chronic weight management of obesity	(Qamar et al., 2024)

11.3 BACKGROUND: POLYPEPTIDE THERAPEUTICS

11.3.1 Definition and Classification (Hormones, Enzymes, Receptor Ligands, Antimicrobial Peptides, etc.)

Therapeutic peptides are a unique type of biopharmaceutical that have benefits over biologics and small-molecule medications due to their high target selectivity and structural plasticity (Craik et al., 2012). Modulation of protein–protein interactions (PPIs) is made possible by their capacity to interact with vast protein interaction surfaces, which is frequently impossible with tiny molecules (Milroy et al., 2014). Furthermore, because of their accurate molecular recognition skills, peptides have a higher binding affinity and fewer side effects than traditional medications (Craik et al., 2012). Their reduced structural size (2–50 residues) makes the synthesis of peptides easier, which allows for chemical changes (like PEGylation and cyclization) to maximize pharmacokinetic characteristics like stability and bioavailability (Milroy et al., 2014). Peptides typically exhibit lower immunogenicity than proteins or antibodies, reducing unfavorable immunological reactions—a crucial benefit in the treatment of chronic diseases (Baker et al., 2014).

11.3.2 General Advantages and Challenges (e.g., Specificity, Low Toxicity vs. Stability, Delivery)

Therapeutic peptides offer several advantages over proteins and antibodies, including their small size, ease of synthesis, and ability to permeate cell membranes. They also exhibit high activity, specificity, and affinity, as well as little drug-drug interactions and biological and chemical diversity (Craik et al., 2012; Wang et al., 2022b). A further advantage of employing peptides as a medication is that they do not collect in certain organs (e.g., kidney or liver), which can help to reduce their hazardous side effects (Wang et al., 2022b). They can also be quickly synthesized and easily changed, and they are less immunogenic than recombinant antibodies or proteins. Therapeutic

peptides have considerable potential for treating a variety of ailments. In cancer, these peptides can be employed in a variety of ways, including transporting cytotoxic medicines, vaccinations, hormones, and radionuclides (Thundimadathil, 2012). However, therapeutic peptides have numerous important limitations, including *in vivo* stability. They have little or no resistance to cleavage by serum proteases in vivo. They also have a short half-life, low bioavailability, and production and manufacturing problems (Haggag, 2018).

11.3.3 Key Advances in Polypeptide Therapeutics Success

The significant advancements in polypeptide therapies are mostly due to a surge of inventions that have solved long-standing issues with manufacture, stability, and delivery. PEGylation, which attaches polyethylene glycol (PEG) chains to peptide or protein medications, is one important innovation. In addition to making polypeptides more soluble, this procedure also increases their bloodstream circulation time by decreasing renal clearance and preventing immunological detection, which allows for less frequent dosing and better patient outcomes (Veronese, 2001; Pasut et al., 2005; Andrianov, 2023). Developments in formulation techniques, beyond PEGylation, have greatly influenced the success of polypeptide medications. Researchers have discovered that peptide medications may be made much more stable and have a longer shelf life—even while held in solution—by carefully modifying factors like pH, the kind of buffer used, and the addition of the appropriate co-solvents or excipients (Sood and Panchagnula, 2001). This type of fine-tuning is more than simply a technical aspect; it allows patients to depend on their drugs to function reliably and with less difficulty. Also, multilayer encapsulation and other complex delivery systems have improved the efficacy of these drugs by allowing for controlled release. The

development of recombinant manufacturing methods is another important finding (van Witteloostuijn et al., 2016). Researchers may now mass-produce complex polypeptide medicines with high yield and purity using genetic engineering and microbial or mammalian cell lines. This has increased the accessibility of medications and enabled the production of peptides with precise sequences and modifications that meet specific medical needs (Sanchez-Garcia et al., 2016). Overall, these advancements have transformed polypeptide therapies from delicate, specialized treatments to powerful, widely used drugs, offering promise for a wide range of diseases, including diabetes, complex inflammatory conditions, and malignant conditions.

11.4 CASE STUDIES

11.4.1 Recombinant Human Insulins

The recommended course of treatment for individuals with type 1 and advanced type 2 diabetes mellitus is insulin replacement therapy. For many years, the hormone was derived from the pancreatic tissue of pigs and cows. Later, semisynthetic human insulin was produced by modifying animal insulin (Bolli et al., 2022). Recombinant (biosynthetic) human insulin was made widely available via biosynthesis in microorganisms (yeast, Escherichia coli) with the advent of recombinant DNA technology, offering dependable and reasonably priced supplies of the hormone all over the world (Ahmad et al., 2021). It was shown that recombinant human insulin was purer and of higher pharmaceutical quality than animal or semisynthetic insulin. Patients with diabetes could switch from animal or semisynthetic human insulin to recombinant human insulin safely and effectively, with no anticipated change in insulin dosage (Sandow et al., 2015).

11.4.2 Structure and Mechanism of Action

Insulin and glucagon act together to maintain normal glucose levels. Insulin causes glucose to be deposited in the liver as glycogen; subsequently, throughout the next few hours, as blood glucose concentration lowers, the liver releases glucose back into the blood, minimizing swings. Clinical significance: During severe liver illness, it is impossible to maintain blood glucose levels (Figure 11.1A) (Nakrani et al., 2025). The insulin molecule is composed of two peptide chains joined by disulfide bonds and has 51 amino acids. During biosynthesis and storage, it assembles into dimers and, when zinc is present, into hexamers, despite being active as a monomer (Figure 11.1B) (Dunn, 2005). Insulin's three-dimensional structure in its hexameric, dimeric, and monomeric forms has been determined by X-ray analysis. It has been determined that there are two primary forms of insulin, B9–B20 and B1–B20, which vary in the degree of helix in the B chain (Derewenda et al., 1989). Insulin exhibits further adjustments when it is monomeric or dimeric. The insulin receptor is made up of two α and two β subunits and belongs to the transmembrane ligand-activated receptor and tyrosine kinase family. By binding to the insulin receptor and altering its conformation, insulin causes tyrosine residues in the β subunit to become autophosphorylated, which in turn activates receptor kinases (Galal et al., 2023). Downstream kinases such as glycogen synthase kinase (GSK) 3β and protein kinase B (AKT) receive the insulin signal. Moreover, insulin signaling promotes improved glucose absorption and the translocation of glucose transporter 4 (GLUT4) (Figure 11.1C) (Sayem et al., 2018).

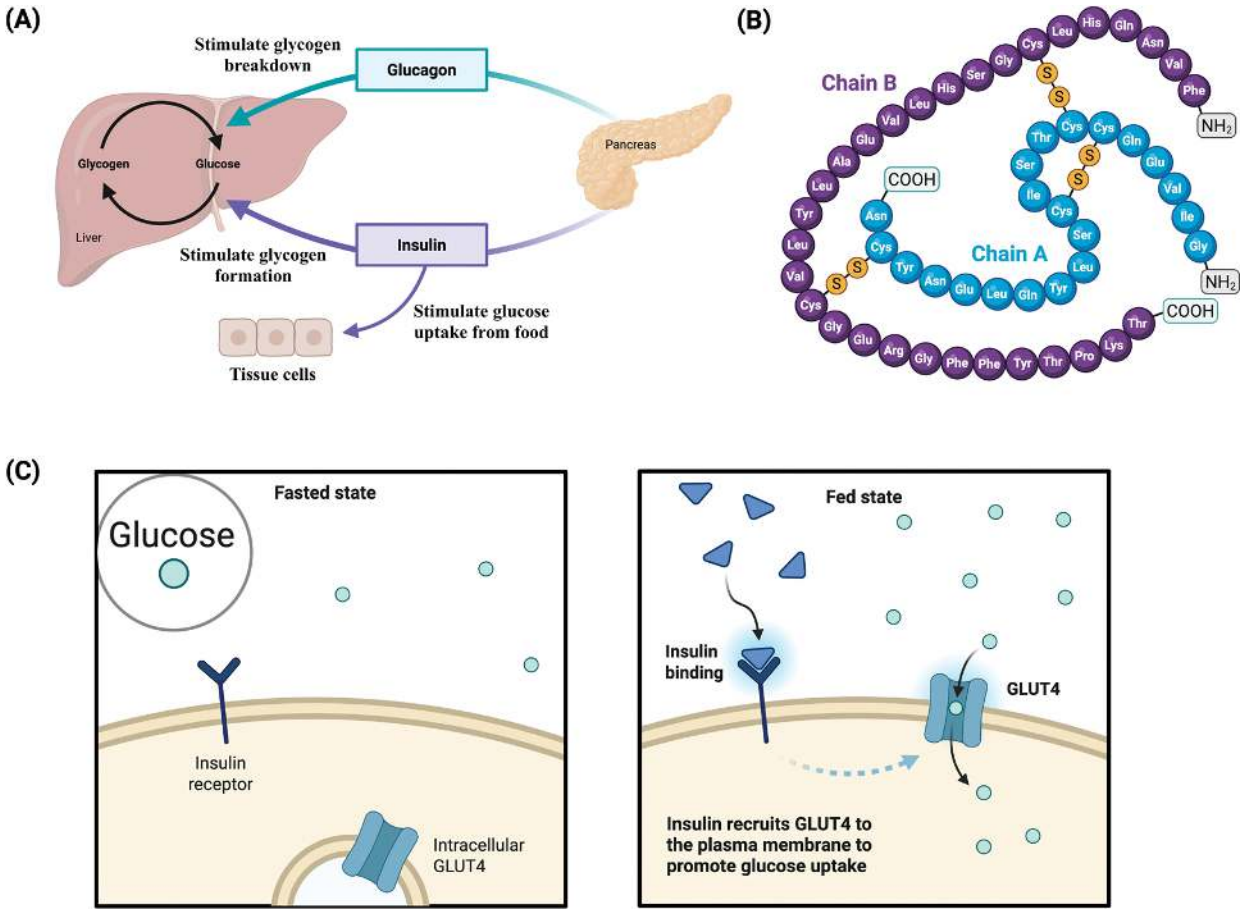

FIGURE 11.1 (A) the release of insulin, (B) the structure of insulin, and (C) the mechanism of action for insulin during fasted or fed state. (Created in BioRender. Abdellatif, A. (2025) https://BioRender.com/vgie7yu.) ⏎

11.4.3 Development History (Discovery to Approval)

A pivotal moment in medical history, the discovery of insulin was the result of the work of Frederick Banting, Charles Best, J.J.R. Macleod, and James Collip at the University of Toronto in 1921–1922 (Lewis and Brubaker, 2021). Banting came up with the concept to separate the hormone in charge of glucose management, building on decades of earlier research that connected the pancreas to diabetes. Collip's purification advances helped the researchers perfect pancreatic extracts after early difficulties. In January

1922, they successfully administered insulin for the first time in a human patient, Leonard Thompson, saving his life and proving the therapy's profound benefits (Diem et al., 2022). Millions of people can now receive treatment for this once-fatal illness thanks to their efforts, which were accelerated by developments in blood glucose monitoring and cooperative assistance from academia and industry. Notably, the discoverers decided without making a personal profit and instead gave the patent to the University of Toronto to guarantee broad access, highlighting the moral significance and legacy of this enormous medical breakthrough.

11.4.4 Formulation and Delivery Innovations of Insulin

11.4.4.1 Advanced Insulin Formulations

11.4.4.1.1 Long-Acting and Modified Basal Insulins

Since Neutral Protamine Hagedorn (NPH) insulin was first introduced, scientists have created longer-acting, flatter-profile basal insulin analogues, like insulin glargine and insulin detemir, by altering their structural makeup to slow absorption and prolong duration, which lowers the frequency of injections and the risk of hypoglycemia (Bolli et al., 2022).

11.4.4.1.2 Rapid-Acting Analogs

Analogues such as insulin lispro and aspart, which have a quicker onset and shorter duration and more closely resemble the physiological insulin response to meals, are the result of advances in protein engineering (Jarosinski et al., 2021).

11.4.4.1.3 Glucose-Responsive Insulins and Smart Analogs

To reduce the danger of hypoglycemia and more accurately mimic the body's natural regulation system, next-generation "smart" insulins are being developed. These analogues, including glucose-responsive insulins that release hormone in response to rising blood glucose are designed to improve safety and physiological control (see Figure 11.2). (Emmanuel et al., 2021; Jarosinski et al., 2021).

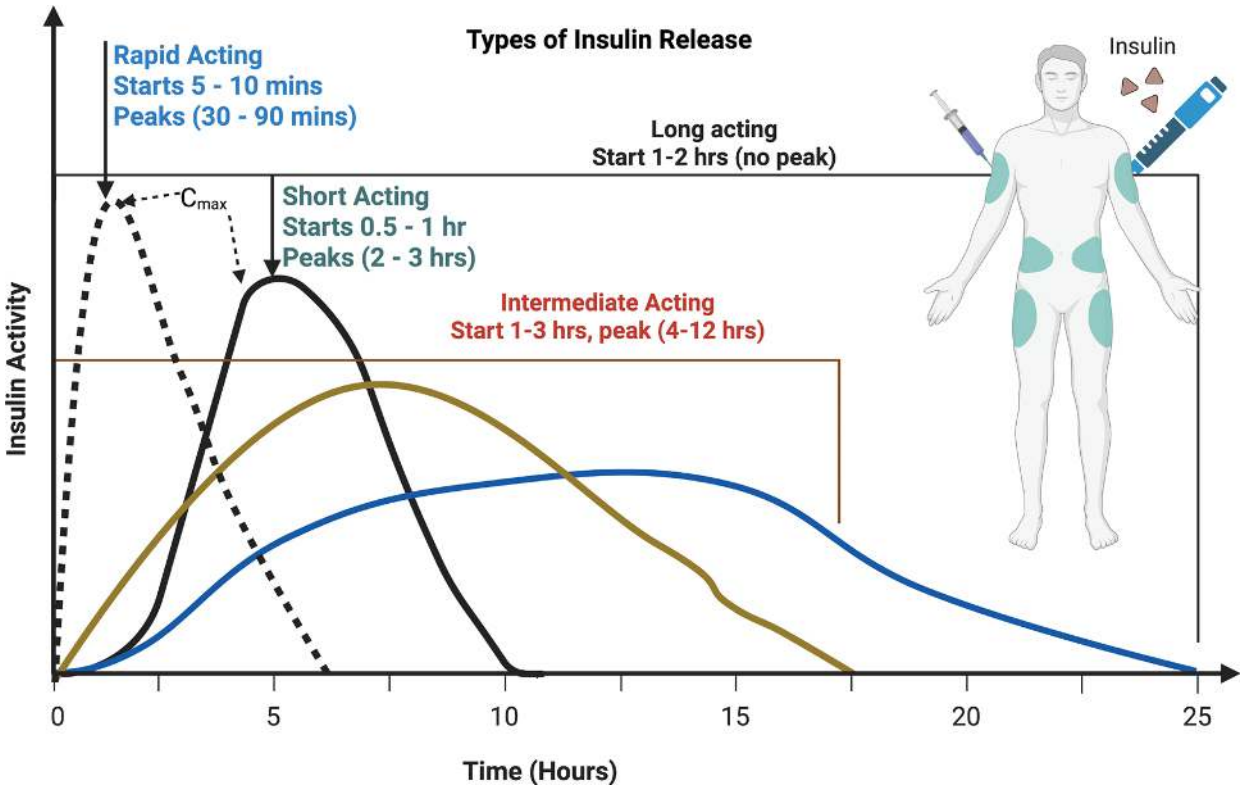

FIGURE 11.2 Insulin subtypes and approximate durations. Regular and NPH insulin last longer at larger doses. (Malone and Jain (2024) adaptation.) ⏎

11.4.4.2 Novel Delivery Systems

11.4.4.2.1 Insulin Pumps (CSII) and Hybrid Closed-Loop Systems

Compared to several daily injections, continuous subcutaneous insulin infusion from modern insulin pumps offers more flexibility and glycemic

management. Tubeless patch pumps significantly improve convenience. To minimize patient burden and improve glucose management, hybrid closed-loop or “artificial pancreas” devices combine pumps with continuous glucose monitors and algorithms to partially automate insulin delivery (Emmanuel et al., 2021; Ebekozien et al., 2024).

11.4.4.2.2 Wearable and Smart Insulin Pens

Connected pens with Bluetooth monitoring, reminders, and mobile app integration help patients and healthcare providers adhere to medication regimens by tracking doses and patterns and providing statistics. Wearable pump patches provide tubeless and inconspicuous solutions for continuous supply (Sangave et al., 2019).

11.4.4.3 Inhalable Insulin

For prandial (mealtime) control, needle-free alternatives such as Afrezza offer ultra-rapid-acting insulin as an inhaled dry powder, making administration easier for certain users but not appropriate for everyone due to pulmonary concerns (Mcgill et al., 2016).

11.4.4.4 Oral and Nanoparticle Formulations

By protecting insulin from stomach acid and enzymes, novel oral formulations (such as nanoparticles, pH-sensitive coatings, and quantum dot-polymer conjugates) enable intestinal absorption and systemic administration. In rodents and primates, recent oral insulin based on nanotechnology demonstrated efficient glucose lowering with a lower risk of hypoglycemia. These developments are getting close to clinical translation, which could transform patient care by doing away with the necessity for daily injections (Hunt et al., 2024; Ghazwani et al., 2025).

11.4.4.5 Topical and Transdermal Systems

Particularly for wound healing or studies on microneedle-mediated systemic delivery, new gel and patch technologies (such as Pluronic-lecithin-based organogels and microneedle arrays) provide prolonged, localized, or noninvasive insulin dose (Chauhan et al., 2024).

11.4.4.6 Biotechnological and Material Innovations

11.4.4.6.1 Polymer-Based Delivery Vehicles

Insulin can be encapsulated in nanoparticles, gels, or hydrogels made of biocompatible natural polymers (such as chitosan, hyaluronic acid, or alginate). In response to environmental cues (pH, glucose), these protect against degradation, offer regulated or stimuli-responsive release, and are being investigated for both oral and subcutaneous systems (Ghazwani et al., 2025).

11.4.4.6.2 Nano-Insulin Particles and Implantable

Long-term, adjustable, and even glucose-sensitive release is provided by implantable depots and nanoparticles, which lowers the need for frequent patient interventions. Certain systems can be designed for programmed or on-demand release (Ghazwani et al., 2025).

11.5 PROSPECTS

11.5.1 Fully Autonomous Artificial Pancreas

The goal of future developments is to eliminate manual input by combining pumps, sensors, and AI algorithms to provide smooth, closed-loop glycemic control.

11.5.2 Personalized and Precision Medicine

As a result of connection, data insights, and algorithm-driven decision aid, insulin therapy is progressing toward regimens that are more individualized, secure, and effective for each individual patient (Gonzalez-Rivas et al., 2025). In conclusion, the field of insulin therapy is seeing significant innovation due to advancements in delivery systems (hybrid closed-loop pumps, oral/smart device integration, nanotech) and formulation (longer- or ultra-fast-acting, smart/glucose-responsive analogues). These advancements improve patient convenience, safety, and efficacy and bode well for a time when insulin can be administered more naturally and with less strain to diabetics (Hunt et al., 2024; Ghazwani et al., 2025; Gonzalez-Rivas et al., 2025).

11.6 GLUCAGON-LIKE PEPTIDE-1 (GLP-1) AGONISTS

GLP-1 agonists are a class of drugs that are mostly used to treat type 2 diabetes and, more recently, obesity. Exenatide and liraglutide are well-known members of this class of medications (Sztanek et al., 2024). The following medications are classified as GLP-1 receptor agonists: albiglutide, dulaglutide, exenatide, liraglutide, lixisenatide, tirzepatide, and semaglutide. The last medication is sold under the trade names Ozempic, Rybelsus, and Wegovy (Figure 11.4).

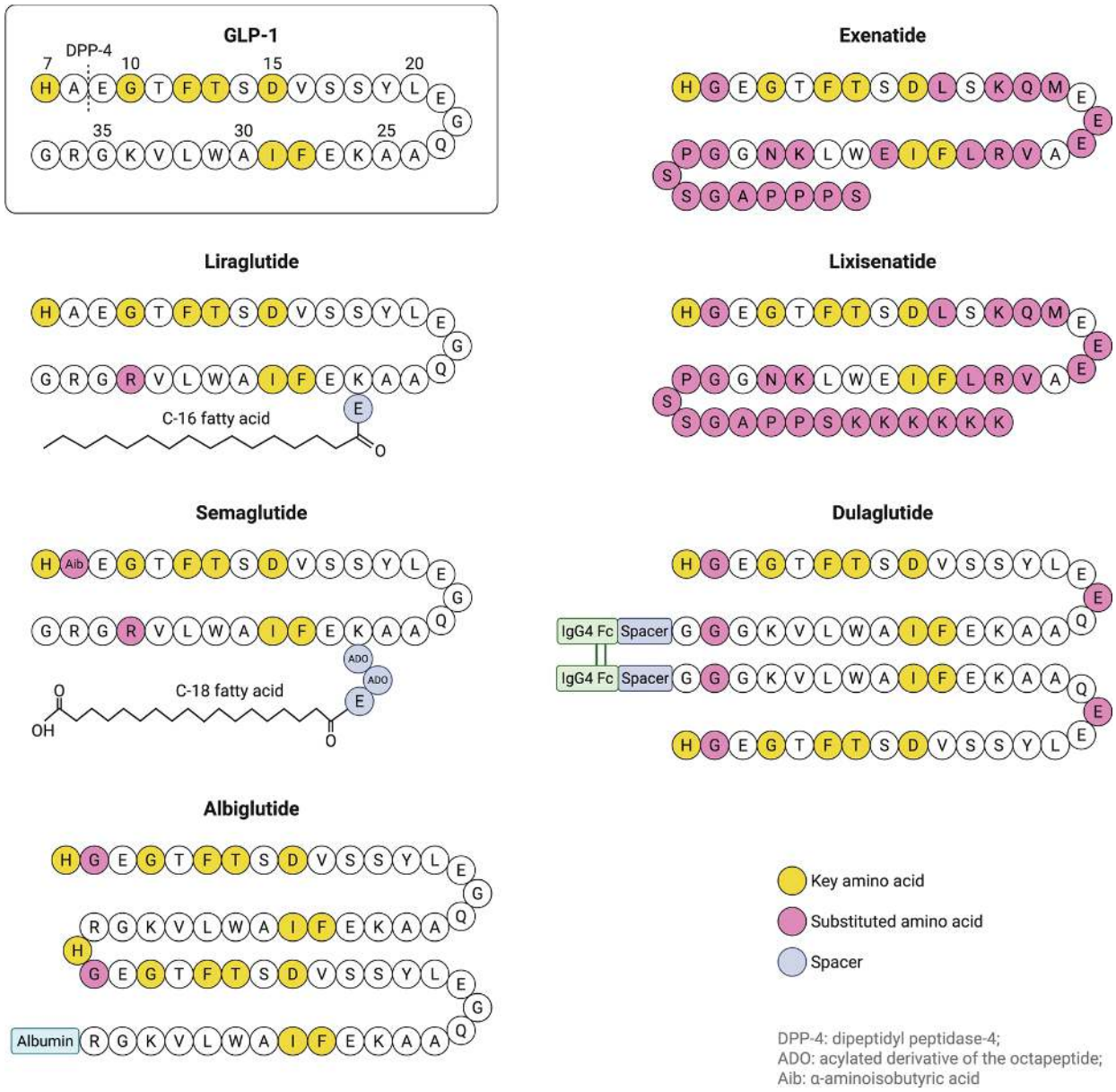

FIGURE 11.4 Description of GLP-1 receptor agonists exenatide, liraglutide, lixisenatide, dulaglutide, and albiglutide. The amino acid sequence of each molecule shows essential amino acids (yellow), substituted amino acids (pink), and spacer regions (purple). These changes improve each compound's pharmacological properties, making them more effective at glucose regulation and potentially useful in diabetes treatment (Yu et al., 2018). ⏎

11.6.1 Mechanism of Action

GLP-1 mimics the incretin hormone GLP-1, which is routinely secreted from the gut after meals. GLP-1 agonists are substances that imitate the incretin hormone. One of the ways in which they perform their job is by stimulating the pancreatic beta cells to release insulin in response to an increase in blood glucose levels. The inhibition of glucagon secretion, which results in a reduction in hepatic glucose production. By delaying the emptying of the stomach, one can lessen the surge in blood sugar that occurs after meals. Enhancing satiety through hypothalamus actions, which leads to a reduction in the amount of food consumed and a subsequent achievement of weight loss (Nauck, 1998; Seino et al., 2010).

11.6.2 Medical Uses

Additionally, metformin is usually used in concert with these medications in order to improve glycemic control in patients with type 2 diabetes. There is a widespread belief that those who suffer from obesity or cardiovascular disease might benefit tremendously from their consumption (Stolar et al., 2008). Overweight and obesity: Liraglutide and semaglutide, both of which are GLP-1 agonists, have been approved for the long-term management of weight (Moore et al., 2023).

Cardiometabolic Benefits: In addition to glycemic management, numerous GLP-1 agonists have been shown to reduce the incidence of major cardiovascular events and mortality from all causes in persons who have type 2 diabetes (Jensen et al., 2020).

Figure 11.3 describes the GLP-1 effects on target tissues. It highlights GLP-1's complex role in metabolic control and its possible treatment of diabetes and obesity. Satiety impacts the brain. It affects heart blood pressure, contractility, and diastolic function. Kidney: Natriuresis has occurred. Impacts adipose tissue glucose absorption and lipolysis. Increases

insulin release and β-cell proliferation in the pancreas. Promotes muscle glycogen synthesis. GI tract; affects stomach emptying and acid output (Ceccarelli et al., 2013).

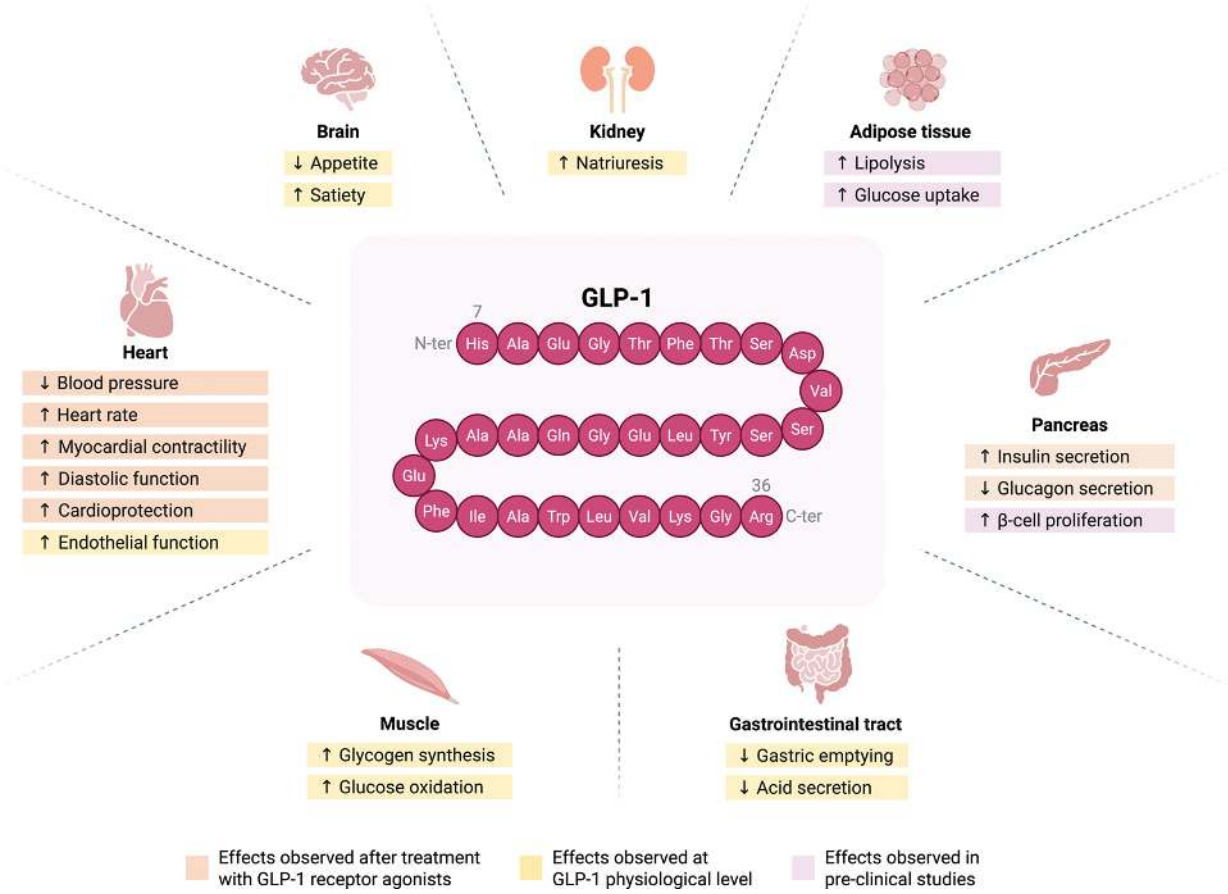

FIGURE 11.3 GLP-1's target tissue effects. It shows GLP-1's intricate metabolic regulation role and its potential to treat diabetes and obesity (Ceccarelli et al., 2013). (Created in BioRender. Abdellatif, A. (2025) https://BioRender.com/ckblmdi) ⏎

11.6.2.1 The Overarching Concept of the Different GLP-1 Receptor Peptides

Type 2 diabetes is treated with exenatide, commercialized as Byetta. Along with diet, exercise, and possibly additional antidiabetic drugs. Treatment following metformin and sulfonylureas. Subcutaneous injections are delivered. The full human Glucagon-like peptide-1 receptor (GLP-1R) binds

to exenatide, which has a 50% amino acid similarity to GLP-1 and a longer half-life *in vivo* (Koole et al., 2017). Exenatide is a manufactured version of exendin-4, which is a peptide that can be found in the venom of the Gila monster. Exenatide is a peptide that contains 39 amino acids (Figure 11.4) (Raufman, 1996). In addition to being referred to as incretin mimetics, liraglutide is classified as a glucagon-like peptide-1 receptor agonist (GLP-1 for short). To achieve its effects, it stimulates the pancreas to secrete more insulin while simultaneously reducing the amount of glucagon that is secreted (Figure 11.4) (Shyangdan et al., 2011). The contrast and comparison of exenatide and liraglutide is presented in Table 11.2.

TABLE 11.2

Exenatide and Liraglutide: A Contrast and Comparison

Feature	Exenatide (Byetta)	Liraglutide (Victoza/Saxenda)	References
Type	Exendin-4 backbone GLP-1 agonist	Human GLP-1 backbone agonist	(Mann et al., 2010; Al Qassab et al., 2025)
Primary Indications	Type 2 diabetes	Type 2 diabetes, chronic obesity	(Bond, 2006; Murray-Thomas et al., 2025)
Administration	Subcutaneous injection (twice daily or once weekly, depending on formulation)	Subcutaneous injection (once daily)	(Jose et al., 2010; Murray-Thomas et al., 2025)

Feature	Exenatide (Byetta)	Liraglutide (Victoza/Saxenda)	References
Effect on Weight	Weight loss (variable magnitude)	Weight loss, also specifically approved for obesity (Saxenda form)	(King, 2008; Murray-Thomas et al., 2025)
Cardiovascular Benefit	Noted, but less robust evidence versus liraglutide	Robust evidence for a reduction in major adverse cardiovascular events	(Yu et al., 2016; Marso et al., 2016)
Common Side Effects	Nausea, hypoglycemia (esp. with sulfonylureas), GI upset, injection-site reactions	Nausea, vomiting, hypoglycemia (w/sulfonylureas or insulin), GI upset, injection-site reactions	

11.6.2.2 Safety and Tolerability

Mild hypoglycemia, which occurs more frequently if the patient is treated with insulin or sulfonylureas, responses at the injection site, and gastrointestinal issues, particularly nausea, are among the most common adverse events (Derosa and Maffioli, 2012; Chalmer et al., 2015).

- **Rare Serious Risks:** Pancreatitis, kidney damage, and a potential elevated risk of medullary thyroid cancer (particularly in animal studies for liraglutide; possible human significance unknown). (Derosa and Maffioli, 2012; Chalmer et al., 2015).

11.6.2.3 Clinical Insights

GLP-1 agonists, in general, are more effective than previous diabetes treatments when it comes to inducing weight loss and improving glycemic

control. In the event that oral drugs are not sufficient, injectable therapy is indicated as the initial treatment option. This is particularly the case for individuals who have type 2 diabetes and a significant cardiovascular risk (Defronzo, 1999). As a result of the higher cost of these medications and the fact that they must be delivered through injection, access to them and adherence to them may be restricted for certain patients (Hamed et al., 2024).

GLP-1 agonists like liraglutide and exenatide affect weight and blood sugar to treat type 2 diabetes and obesity. They may also boost liver and heart health. Due to their effect profile and safety proof, they aid patients with metabolic management and obesity.

11.6.3 Calcitonin in the Management of Osteoporosis

The human thyroid gland's parafollicular cells (C cells) are primarily responsible for producing the peptide hormone calcitonin. Its primary physiological function is to counteract the effects of parathyroid hormone (PTH) to regulate calcium and phosphate homeostasis. Pharmacologically, calcitonin preparations, which are most frequently obtained from salmon because of their higher receptor potency, have been used in clinical settings to treat several illnesses that cause accelerated bone turnover, most notably postmenopausal osteoporosis (Lombardi et al., 2020).

11.6.3.1 Mechanism of Action

The main bone-resorbing cells, osteoclasts, have unique G protein-coupled calcitonin receptors on their surface, to which calcitonin binds to provide its antiresorptive actions. The following outcomes result from this binding:

Inhibition of osteoclast activity: Calcitonin inhibits osteoclast motility and resorption activity, which lessens bone matrix degradation. Resorption

of bone is reduced: The main pharmacological effect is a reduction in bone turnover, which raises bone mineral density (BMD) slightly. Minimal influence on osteoblast function: Although calcitonin directly suppresses osteoclasts, it has little direct effect on the cells that make bone, known as osteoblasts. Nonetheless, it temporarily promotes net bone growth by decreasing resorption (Kiriakopoulos et al., 2022).

11.6.3.2 Routes of Administration

- **Nasal spray:** The intranasal route (about 200 IU/day) is preferred for osteoporosis owing to its simplicity of administration and patient acceptability. To reduce local side effects, the dose is switched between nostrils (Joy et al., 2000).
- **Parenteral injection:** Although subcutaneous and intramuscular formulations are available, their use in osteoporosis is less common due to the inconvenient dosage schedule and higher risk of systemic adverse effects (Lee et al., 2011).

11.6.3.3 Absorption and Distribution

The bioavailability of nasal calcitonin varies and is frequently lower than that of parenteral versions. The peptide is rapidly absorbed and has a short plasma half-life (~10–15 min), but its biological activity extends for several hours due to prolonged receptor activation and downstream signaling (Buclin et al., 2002).

11.6.3.4 Uses of Calcitonin in Different Types of Diseases

11.6.3.4.1 Effect on Bone Mineral Density

Studies conducted in clinical settings have demonstrated that calcitonin has the ability to somewhat increase the mineral density of spinal bone in postmenopausal women. On the other hand, these increases are insignificant in comparison to those that are brought about by denosumab or bisphosphonates (Wensel et al., 2011).

11.6.3.4.2 Fracture Risk Reduction

- **Vertebral Fractures:** Calcitonin is related to a statistically substantial, albeit clinically mild, reduction in vertebral fracture incidence. Its effectiveness has been demonstrated primarily for spinal locations, with no benefit for the prevention of non-vertebral fractures (e.g., hip and wrist) (Knopp-Sihota et al., 2012).
- **Analgesic Properties:** Calcitonin's immediate analgesic impact, which gives significant relief from acute pain following spinal compression fractures, distinguishes it from other antiresorptive medicines (Yazdani et al., 2019).

11.6.3.4.3 Indications and Role in Therapy

Calcitonin is not considered the first-line treatment for osteoporosis. Individuals who have been postmenopausal for at least five years and are intolerant or contraindicated to alternative treatments (such as bisphosphonates and denosumab) are eligible. Seeking therapy for acute spinal fracture discomfort. It is usually prescribed in conjunction with supplementary calcium and vitamin D to improve skeletal health (Chandran and Venkatachalam, 2019).

11.6.3.5 Safety Profile and Adverse Events

11.6.3.5.1 Common Adverse Events

- **Nasal preparations:** The most common local side effects include nasal discomfort, epistaxis, and ulceration, which can be alleviated by moving the nostrils (Tunkel et al., 2020).
- **Injectable formulations:** Among the systemic adverse effects are injection site responses, nausea, face flushing, and abdominal pain (Chandran and Venkatachalam, 2019).

11.6.3.5.2 Long-term Risks

- **Cancer Risk:** According to epidemiological research, there may be a higher chance of developing several cancers, especially if nasal calcitonin use is prolonged. Although the absolute risk increment is still small, it should be considered when evaluating the risks and benefits (Warrington et al., 2017; Du et al., 2025).
- **Hypocalcemia:** In rare cases, especially in predisposed individuals, excessive inhibition of osteoclastic activity can lead to hypocalcemia (Body, 2002).

11.6.3.6 Comparative Efficacy and Contemporary Practice

The use of calcitonin has decreased in current clinical guidelines due to the development of more effective and thoroughly researched antiresorptive drugs, such as bisphosphonates and monoclonal antibody treatments. Its analgesic effect is still useful in treating acute pain from osteoporotic vertebral fractures, even if its main use is now as a second-line or adjunct medication (Ha et al., 2021; Fernandez-Bravo-Rodrigo et al., 2022). Indications, administration, evidence for efficacy, unique therapy's role, and

safety considerations for calcitonin are included in Table 11.3, which is a summary table.

TABLE 11.3

The Indication, Administration, Evidence for Efficacy, Unique Therapeutics Role, and Safety Consideration of Calcitonin

Aspect	Description
Indication	Postmenopausal osteoporosis, primarily as a second-line agent
Administration	Nasal spray (preferred) or injection
Evidence for Efficacy	Modest increase in BMD; reduces vertebral fracture risk
Unique Therapeutic Role	Analgesia for acute vertebral compression fractures
Safety Considerations	Nasal irritation, systemic GI/adverse effects, possible increased cancer risk with long-term use

Calcitonin can treat osteoporosis, especially for postmenopausal women who cannot take first-line medications or those who need fast pain relief following vertebral fractures. Therapeutic benefits are limited to the vertebral skeleton due to osteoclast suppression and bone resorption reduction. Calcitonin has been reduced to a specialized, supplemental role in osteoporosis treatment due to increasingly potent and effective pharmacotherapies (Munoz-Torres et al., 2004).

11.7 BIVALIRUDIN (DIRECT THROMBIN INHIBITOR)

Bivalirudin is a synthetic 20-amino-acid polypeptide direct thrombin inhibitor. Engineered to bypass the limits of conventional anticoagulants like

heparin, it directly and reversibly binds to thrombin to block both its free and clot-bound forms. Bivalirudin is used as a reliable anticoagulant in procedural and critical care settings due to its excellent selectivity and predictable pharmacologic profile. Its reversible, consistent mechanism involves proteolytic breakdown and renal excretion. Bivalirudin does not require plasma cofactors and has a low risk of immunogenic or allergic reactions, unlike heparin, which requires antithrombin III and can activate platelets (Warkentin, 2004).

11.7.1 Medical Need/Target Disease

Bivalirudin's main target groups include people with acute coronary syndromes (ACS), patients having percutaneous coronary intervention (PCI), and people who have or are at risk of heparin-induced thrombocytopenia (HIT). Cardiovascular surgery, extracorporeal membrane oxygenation (ECMO), and pediatric patients who require anticoagulation but are unable to properly take heparin are among its other uses (Wang et al., 2024).

11.7.2 Development History (Discovery to Approval)

Preclinical studies showed that bivalirudin, a hirudin analogue, outperformed heparin and first-generation hirudins. As a synthetic hirudin analog, it directly inhibits thrombin. Risky cardiovascular treatments like PCI and ACS need robust clinical studies. Regulatory Approval: Initially approved for PCI in the late 1990s and early 2000s in the US and Europe, then for HIT and cardiac surgery (Shammas, 2005).

11.7.3 Formulation and Delivery Innovations

Intravenous infusion is the most often used route for fast anticoagulation and clearance. Research on sustained-release coatings for medical devices, including ECMO tubing and drug-eluting stents, aims to give a local antithrombotic effect while minimizing systemic exposure. Novel bivalirudin-loaded hydrogels are being developed for improved device compatibility and safety (Bao et al., 2025).

11.7.4 Findings from Major Clinical Trials

Effective in percutaneous coronary intervention (PCI): decreased substantial bleeding in comparison to heparin and glycoprotein IIb/IIIa inhibitors, with clinical results for ischemic events that were comparable or even preferred. Proven to be safe and effective for use in kids, patients undergoing ECMO, and those suffering from HIT. Comparatively, there is a lesser risk of HIT, reduced bleeding, and predictable pharmacokinetics when compared to other interventions (Lipinski et al., 2016).

REFERENCES

Abdellatif, A. A., Zayed, G., El-Bakry, A., Zaky, A., Saleem, I. Y. & Tawfeek, H. M. 2016. Novel Gold Nanoparticles Coated With Somatostatin as a Potential Delivery System for Targeting Somatostatin Receptors. *Drug Dev Ind Pharm*, *42*, 1782–1791.

Abdellatif, A. A. H., Aldalaen, S. M., Faisal, W. & Tawfeek, H. M. 2018. Somatostatin Receptors as a New Active Targeting Sites for Nanoparticles. *Saudi Pharm J*, *26*, 1051–1059.

Abdellatif, A. A. H., Hennig, R., Pollinger, K., Tawfeek, H. M., Bouazzaoui, A. & Goepferich, A. 2020a. Fluorescent Nanoparticles Coated With a Somatostatin Analogue Target Blood Monocyte for Efficient Leukaemia Treatment. *Pharm Res*, *37*, 217.

Abdellatif, A. A. H., Ibrahim, M. A., Amin, M. A., Maswadeh, H., Alwehaibi, M. N., Al-Harbi, S. N., Alharbi, Z. A., Mohammed, H. A., Mehany, A. B. M. & Saleem, I. 2020b. Cetuximab Conjugated

With Octreotide and Entrapped Calcium Alginate-Beads for Targeting Somatostatin Receptors. *Sci Rep*, *10*, 4736.

Aguilar-Toala, J. E., Quintanar-Guerrero, D., Liceaga, A. M. & Zambrano-Zaragoza, M. L. 2022. Encapsulation of Bioactive Peptides: A Strategy to Improve the Stability, Protect the Nutraceutical Bioactivity and Support Their Food Applications. *RSC Adv*, *12*, 6449–6458.

Ahmad, A., Sajid, T., Kazmi, A., Ahmad, I., Hameed, A., Ali, A., Sultana, T., Khan, S. U., Amir, H., Rafiq, A. & Alyas, E. 2021. Human Insulin: History, Recent Advances, and Expression Systems for Mass Production. *Biomed Res Ther*, *8*, 4540–4561.

Al-Allaf, F. A., Abduljaleel, Z., Taher, M. M., Abdellatif, A. A. H., Athar, M., Bogari, N. M., Al-Ahdal, M. N., Al-Mohanna, F., Al-Hassnan, Z. N., Alzabeedi, K. H. Y., Banssir, T. M. & Bouazzaoui, A. 2019. Molecular Dynamics Simulation Reveals Exposed Residues in the Ligand-Binding Domain of the Low-Density Lipoprotein Receptor That Interacts With Vesicular Stomatitis Virus-G Envelope. *Viruses*, *11*(11), 1033.

Al Qassab, M., Mneimneh, M., Jradi, A., Derbas, B., Dabboussi, D., Khoury Baini, J., Katrib, N., Chaarani, N., Attieh, P., Kanaan, A., Harb, F., Azar, S. & Ghadieh, H. E. 2025. The Expanding Role of GLP-1 Receptor Agonists: Advancing Clinical Outcomes in Metabolic and Mental Health. *Curr Issues Mol Biol*, *47*(1), 1–18.

Andrianov, A. K. 2023. Noncovalent PEGylation of Protein and Peptide Therapeutics. *WIREs Nanomed Nanobiotechnol*, *15*(5), e1845.

Baker, M., Reynolds, H. M., Lumicisi, B. & Bryson, C. J. 2014. Immunogenicity of Protein Therapeutics: The Key Causes, Consequences and Challenges. *Self/Nonself*, *1*, 314–322.

Bao, Z., Qi, X., Zhu, S., Zhang, M., Liu, X., Chen, P., Zhang, L., Geng, T. & Cheng, F.-F. 2025. Recent Progress on Formulations of Hirudin. *Pharm Sci Adv*, *3*, 100–118.

Beaudart, C., Veronese, N., Douxfils, J., Thiyagarajan, J. A., Bolzetta, F., Albanese, P., Voltan, G., Alokail, M., Harvey, N. C., Fuggle, N. R., Bruyere, O., Rizzoli, R. & Reginster, J. Y. 2025. PTH1 Receptor Agonists for Fracture Risk: A Systematic Review and Network Meta-Analysis. *Osteoporos Int*, *36*, 951–967.

Bedimo, R. 2011. Growth Hormone and Tesamorelin in the Management of HIV-Associated Lipodystrophy. *HIV AIDS (Auckl)*, *3*, 69–79.

Body, J. J. 2002. Calcitonin for the Long-Term Prevention and Treatment of Postmenopausal Osteoporosis. *Bone*, *30*, 75S–79S.

Bolli, G. B., Cheng, A. Y. Y. & Owens, D. R. 2022. Insulin: Evolution of Insulin Formulations and Their Application in Clinical Practice Over 100 Years. *Acta Diabetol*, *59*, 1129–1144.

Bolli, G. B. & Owens, D. R. 2014. Lixisenatide, A Novel GLP-1 Receptor Agonist: Efficacy, Safety and Clinical Implications for Type 2 Diabetes mellitus. *Diabetes Obes Metab*, *16*, 588–601.

Bond, A. 2006. Exenatide (Byetta) as a Novel Treatment Option for Type 2 Diabetes mellitus. *Proc (Bayl Univ Med Cent)*, *19*, 281–284.

Buclin, T., Cosma Rochat, M., Burckhardt, P., Azria, M. & Attinger, M. 2002. Bioavailability and Biological Efficacy of a New Oral Formulation of Salmon Calcitonin in Healthy Volunteers. *J Bone Miner Res*, *17*, 1478–1485.

Campos Ramirez, S. E., Andres Garcia, A., Blanco Abad, C., Gomila Pons, P., Gomez Mugarza, P., Ruffini Egea, S. E., Gallart Caballero, L., Polo Marques, E. & Alonso Orduna, V. 2025. Evaluation of the Real-Life Efficacy and Safety of the Treatment With Lutetium-177 Dotatate for Metastatic Neuroendocrine Tumors. *J Clin Med*, *14*(1), 112.

Ceccarelli, E., Guarino, E. G., Merlotti, D., Patti, A., Gennari, L., Nuti, R. & Dotta, F. 2013. Beyond Glycemic Control in Diabetes mellitus: Effects of Incretin-Based Therapies on Bone Metabolism. *Front Endocrinol (Lausanne)*, *4*, 73.

Cersosimo, R. J. 2009. Romiplostim. *Clin Ther*, *31*, 1887–1907.

Chalmer, T., Almdal, T. P., Vilsboll, T. & Knop, F. K. 2015. Adverse Drug Reactions Associated With the Use of Liraglutide in Patients With Type 2 Diabetes–Focus on Pancreatitis and Pancreas Cancer. *Expert Opin Drug Saf*, *14*, 171–80.

Chandran, T. & Venkatachalam, I. 2019. Efficacy and Safety of Denosumab Compared to Bisphosphonates in Improving Bone Strength in Postmenopausal Osteoporosis: A Systematic Review. *Singapore Med J*, *60*, 364–378.

Chauhan, S., Jhawat, V., Singh, R. P., Yadav, A. & Garg, V. 2024. Design, Development and In-Vitro Characterization of Insulin Loaded Topical Pluronic-Lecithin Based Organogel Formulation for the Management of Diabetic Wound. *Recent Adv Drug Deliv Formul*, *18*, 50–60.

Colao, A., Petersenn, S., Newell-Price, J., Findling, J. W., Gu, F., Maldonado, M., Schoenherr, U., Mills, D., Salgado, L. R., Biller, B. M. & Pasireotide, B. S. G. 2012. A 12-Month Phase 3 Study of Pasireotide in Cushing's Disease. *N Engl J Med*, *366*, 914–924.

Cozzolino, M., Galassi, A., Conte, F., Mangano, M., Di Lullo, L. & Bellasi, A. 2017. Treatment of Secondary Hyperparathyroidism: The Clinical Utility of Etelcalcetide. *Ther Clin Risk Manag*, *13*, 679–689.

Craik, D. J., Fairlie, D. P., Liras, S. & Price, D. 2012. The Future of Peptide-Based Drugs. *Chem Biol Drug Des*, *81*, 136–147.

Defronzo, R. A. 1999. Pharmacologic Therapy for Type 2 Diabetes Mellitus. *Ann Intern Med*, *131*, 281–303.

Derewenda, U., Derewenda, Z., Dodson, G. G., Hubbard, R. E. & Korber, F. 1989. Molecular Structure of Insulin: The Insulin Monomer and Its Assembly. *Br Med Bull*, *45*, 4–18.

Derosa, G. & Maffioli, P. 2012. GLP-1 Agonists Exenatide and Liraglutide: A Review About Their Safety and Efficacy. *Curr Clin Pharmacol*, *7*, 214–228.

Dewan, B., Navale, S. & Ganiga, R. 2024. Atosiban: A Comprehensive Approach to Preterm Labour Management. *Int J Reprod Contracept Obstetr Gynecol*, *13*, 3420–3431.

Diem, P., Ducluzeau, P. H. & Scheen, A. 2022. The Discovery of Insulin. *Diabetes Epidemiol Manag*, *5*(1), 1–8.

Drucker, D. J. 2019. The Discovery of GLP-2 and Development of Teduglutide for Short Bowel Syndrome. *ACS Pharmacol Transl Sci*, *2*, 134–142.

Du, Y., Shen, C., Song, K., Liu, C., Li, Z., Liu, Z., Wu, Y., Guo, L., Zhang, Y., Zhang, H., Chen, C., Yin, M., Tang, H., Ji, Q., Wei, W., Shi, X. & Wang, Y. 2025. Updated Thresholds of Basal Calcitonin Level and Extent of Lymph Node Metastasis in Initially Treated Medullary Thyroid Cancer. *JAMA Otolaryngol Head Neck Surg*, *151*, 761–767.

Duffalo, M. L. & James, C. W. 2003. Enfuvirtide: A Novel Agent for the Treatment of HIV-1 Infection. *Ann Pharmacother*, *37*, 1448–1456.

Dunn, M. F. 2005. Zinc–Ligand Interactions Modulate Assembly and Stability of the Insulin Hexamer – A Review. *BioMetals*, *18*, 295–303.

Ebekozien, O., Fantasia, K., Farrokhi, F., Sabharwal, A. & Kerr, D. 2024. Technology and Health Inequities in Diabetes Care: How Do We Widen Access to Underserved Populations and Utilize Technology to Improve Outcomes for All? *Diabetes Obes Metab*, *26*, 3–13.

Edinoff, A. N., Sanders, N. M., Lewis, K. B., Apgar, T. L., Cornett, E. M., Kaye, A. M. & Kaye, A. D. 2022. Bremelanotide for Treatment of Female Hypoactive Sexual Desire. *Neurol Int*, *14*, 75–88.

Emmanuel, O. O., Mfonobong, F. A., Koofreh, G. D. & Musiliu, O. A. 2021. Advances in the Science and Technology of Insulin Delivery: A Review. *J Appl Pharmaceut Sci*, 11(12), 1–12.

Fernandez-Bravo-Rodrigo, J., Pascual-Morena, C., Flor-Garcia, A., Saz-Lara, A., Sequi-Dominguez, I., Alvarez-Bueno, C., Barreda-Hernandez, D. & Cavero-Redondo, I. 2022. The Safety and Efficacy of Calcitonin Gene-Related Peptide (CGRP) Monoclonal Antibodies for the Preventive Treatment of Migraine: A Protocol for Multiple-Treatment Systematic Review and Meta-Analysis. *Int J Environ Res Public Health*, *19*(1), 1–12.

Fosgerau, K. & Hoffmann, T. 2015. Peptide Therapeutics: Current Status and Future Directions. *Drug Discovery Today*, *20*, 122–128.

Galal, M. A., Alouch, S. S., Alsultan, B. S., Dahman, H., Alyabis, N. A., Alammar, S. A. & Aljada, A. 2023. Insulin Receptor Isoforms and Insulin Growth Factor-Like Receptors: Implications in Cell Signaling, Carcinogenesis, and Chemoresistance. *Int J Mol Sci*, *24*(1), 1–18.

Ghazwani, M., Hani, U., Kyada, A., Ballal, S., Issa, B. S., Abosaoda, M. K., Singh, A., Sabarivani, A. & Ray, S. 2025. Advancements in Insulin Delivery: The Potential of Natural Polymers for Improved Diabetes Management. *Front Bioeng Biotechnol*, *13*, 1566743.

Gonzalez-Rivas, J. P., Seyedi, S. A. & Mechanick, J. I. 2025. Artificial Intelligence Enabled Lifestyle Medicine in Diabetes Care: A Narrative Review. *Am J Lifestyle Med*, 15598276251359185.

Ha, D. K., Kim, M. J., Han, N., Kwak, J. H. & Baek, I. H. 2021. Comparative Efficacy of Oral Calcitonin-Gene-Related Peptide Antagonists for the Treatment of Acute Migraine: Updated Meta-Analysis. *Clin Drug Investig*, *41*, 119–132.

Haggag, Y. A. 2018. Peptides as Drug Candidates: Limitations and Recent Development Perspectives. *Biomed J Sci Tech Res*, *8*(1), 1–5.

Haider, M., Das, S., Al-Toubah, T., Pelle, E., El-Haddad, G. & Strosberg, J. 2021. Somatostatin Receptor Radionuclide Therapy in Neuroendocrine Tumors. *Endocr Relat Cancer*, *28*, R81–R93.

Hall, S., Isaacs, D. & Clements, J. N. 2018. Pharmacokinetics and Clinical Implications of Semaglutide: A New Glucagon-Like Peptide (GLP)-1 Receptor Agonist. *Clin Pharmacokinet*, *57*, 1529–1538.

Hamed, K., Alosaimi, M. N., Ali, B. A., Alghamdi, A., Alkhashi, T., Alkhaldi, S. S., Altowarqi, N. A., Alzahrani, H., Alshehri, A. M., Alkhaldi, R. K., Alqahtani, K. W., Alharbi, N. H., Alhulayfi, H. F., Sharifi, S. Y. & Dighriri, I. M. 2024. Glucagon-Like Peptide-1 (GLP-1) Receptor Agonists: Exploring Their Impact on Diabetes, Obesity, and Cardiovascular Health Through a Comprehensive Literature Review. *Cureus*, *16*, e68390.

Hunt, N. J., Lockwood, G. P., Heffernan, S. J., Daymond, J., Ngu, M., Narayanan, R. K., Westwood, L. J., Mohanty, B., Esser, L., Williams, C. C., Kuncic, Z., Mccourt, P. A. G., Le Couteur, D. G. & Cogger, V. C. 2024. Oral Nanotherapeutic Formulation of Insulin With Reduced Episodes of Hypoglycaemia. *Nat Nanotechnol*, *19*, 534–544.

Jarosinski, M. A., Dhayalan, B., Chen, Y. S., Chatterjee, D., Varas, N. & Weiss, M. A. 2021. Structural Principles of Insulin Formulation and Analog Design: A Century of Innovation. *Mol Metab*, *52*, 101325.

Jensen, M. H., Kjolby, M., Hejlesen, O., Jakobsen, P. E. & Vestergaard, P. 2020. Risk of Major Adverse Cardiovascular Events, Severe Hypoglycemia, and All-Cause Mortality for Widely Used Antihyperglycemic Dual and Triple Therapies for Type 2 Diabetes Management: A Cohort Study of All Danish Users. *Diabetes Care*, *43*, 1209–1218.

Jose, B., Tahrani, A. A., Piya, M. K. & Barnett, A. H. 2010. Exenatide Once Weekly: Clinical Outcomes and Patient Satisfaction. *Patient Prefer Adherence*, *4*, 313–24.

Joy, M. S., Neyhart, C. D. & Dooley, M. A. 2000. A Multidisciplinary Renal Clinic for Corticosteroid-Induced Bone Disease. *Pharmacotherapy*, *20*, 206–216.

Kager, L., Potschger, U. & Bielack, S. 2010. Review of Mifamurtide in the Treatment of Patients With Osteosarcoma. *Ther Clin Risk Manag*, *6*, 279–286.

Katz, S. D. 2001. Nesiritide (hBNP): a New Class of Therapeutic Peptide for the Treatment of Decompensated Congestive Heart Failure. *Congest Heart Fail*, *7*, 78–87.

Kawai, T., Forrester, S. J., O'brien, S., Baggett, A., Rizzo, V. & Eguchi, S. 2017. AT1 Receptor Signaling Pathways in the Cardiovascular System. *Pharmacol Res*, *125*, 4–13.

King, A. B. 2008. The Variable Weight Effect of Exenatide Treatment. *J Diabetes Sci Technol*, *2*, 539–540.

Kiriakopoulos, A., Giannakis, P. & Menenakos, E. 2022. Calcitonin: Current Concepts and Differential Diagnosis. *Ther Adv Endocrinol Metab*, *13*, 20420188221099344.

Knopp-Sihota, J. A., Newburn-Cook, C. V., Homik, J., Cummings, G. G. & Voaklander, D. 2012. Calcitonin for Treating Acute and Chronic Pain of Recent and Remote Osteoporotic Vertebral Compression Fractures: A Systematic Review and Meta-Analysis. *Osteoporos Int*, *23*, 17–38.

Koole, C., Reynolds, C. A., Mobarec, J. C., Hick, C., Sexton, P. M. & Sakmar, T. P. 2017. Genetically Encoded Photocross-Linkers Determine the Biological Binding Site of Exendin-4 Peptide in the N-Terminal Domain of the Intact Human Glucagon-Like Peptide-1 Receptor (GLP-1R). *J Biol Chem*, *292*, 7131–7144.

Kovalainen, M., Monkare, J., Riikonen, J., Pesonen, U., Vlasova, M., Salonen, J., Lehto, V. P., Jarvinen, K. & Herzig, K. H. 2015. Novel Delivery Systems for Improving the Clinical Use of Peptides. *Pharmacol Rev*, *67*, 541–561.

Lau, J. L. & Dunn, M. K. 2018. Therapeutic Peptides: Historical Perspectives, Current Development Trends, and Future Directions. *Bioorg Med Chem*, *26*, 2700–2707.

Lee, A. C.-L., Harris, J. L., Khanna, K. K. & Hong, J.-H. 2019. A Comprehensive Review on Current Advances in Peptide Drug Development and Design. *Int J Mol Sci*, *20*(10), 2383.

Lee, S., Glendenning, P. & Inderjeeth, C. A. 2011. Efficacy, side Effects and Route of Administration Are More Important than Frequency of Dosing of Anti-Osteoporosis Treatments in Determining Patient Adherence: A Critical Review of Published Articles from 1970 to 2009. *Osteoporos Int*, *22*, 741–753.

Lee, S. J., Levitsky, K., Parlati, F., Bennett, M. K., Arastu-Kapur, S., Kellerman, L., Woo, T. F., Wong, A. F., Papadopoulos, K. P., Niesvizky, R., Badros, A. Z., Vij, R., Jagannath, S., Siegel, D., Wang, M., Ahmann, G. J. & Kirk, C. J. 2016a. Clinical Activity of Carfilzomib Correlates With Inhibition of Multiple Proteasome Subunits: Application of a Novel Pharmacodynamic Assay. *Br J Haematol*, *173*, 884–895.

Lee, S. M., Hay, D. L. & Pioszak, A. A. 2016b. Calcitonin and Amylin Receptor Peptide Interaction Mechanisms: Insights into Peptide-Binding Modes and Allosteric Modulation of The Calcitonin Receptor by Receptor Activity-Modifying Proteins. *J Biol Chem*, *291*, 8686–700.

Lewis, G. F. & Brubaker, P. L. 2021. The Discovery of Insulin Revisited: Lessons for the Modern Era. *J Clin Invest*, *131*(12), e150686.

Lipinski, M. J., Lee, R. C., Gaglia, M. A. Jr., Torguson, R., Garcia-Garcia, H. M., Pichard, A. D., Satler, L. F. & Waksman, R. 2016. Comparison of Heparin, Bivalirudin, and Different Glycoprotein IIb/IIIa Inhibitor Regimens for Anticoagulation During Percutaneous Coronary Intervention: A Network Meta-Analysis. *Cardiovasc Revasc Med*, *17*, 535–545.

Lombardi, G., Ziemann, E., Banfi, G. & Corbetta, S. 2020. Physical Activity-Dependent Regulation of Parathyroid Hormone and Calcium-Phosphorous Metabolism. *Int J Mol Sci*, *21*(5), 1714.

Longhurst, H. J. 2010. Management of Acute Attacks of Hereditary Angioedema: Potential Role of Icatibant. *Vasc Health Risk Manag*, *6*, 795–802.

Ma, C. C. & Ma, S. 2012. The Role of Surfactant in Respiratory Distress Syndrome. *Open Respir Med J*, *6*, 44–53.

Malone, M., & Jain, V. (Eds.). (2024). *Endocrinology, An Issue of Primary Care: Clinics in Office Practice: Endocrinology, An Issue of Primary Care: Clinics in Office Practice, E-Book* (Vol. 51, No. 3). Elsevier Health Sciences.

Mann, R. J., Nasr, N. E., Sinfield, J. K., Paci, E. & Donnelly, D. 2010. The Major Determinant of Exendin-4/glucagon-Like Peptide 1 Differential Affinity at the Rat Glucagon-Like Peptide 1 Receptor N-Terminal Domain Is a Hydrogen Bond from SER-32 of Exendin-4. *Br J Pharmacol*, *160*, 1973–1984.

Margolis, E. B., Moulton, M. G., Lambeth, P. S. & O'meara, M. J. 2023. The Life and Times of Endogenous Opioid Peptides: Updated Understanding of Synthesis, Spatiotemporal Dynamics, and the Clinical Impact in Alcohol Use Disorder. *Neuropharmacology*, *225*, 109376.

Marso, S. P., Daniels, G. H., Brown-Frandsen, K., Kristensen, P., Mann, J. F., Nauck, M. A., Nissen, S. E., Pocock, S., Poulter, N. R., Ravn, L. S., Steinberg, W. M., Stockner, M., Zinman, B., Bergenstal, R. M., Buse, J. B., Committee, L. S. & Investigators, L. T. 2016. Liraglutide and Cardiovascular Outcomes in Type 2 Diabetes. *N Engl J Med*, *375*, 311–22.

Mcgill, J. B., Ahn, D., Edelman, S. V., Kilpatrick, C. R. & Santos Cavaiola, T. 2016. Making Insulin Accessible: Does Inhaled Insulin Fill an Unmet Need? *Adv Ther*, *33*, 1267–1278.

Mikhail, A. 2012. Profile of Peginesatide and Its Potential for the Treatment of Anemia in Adults With Chronic Kidney Disease Who Are on Dialysis. *J Blood Med*, *3*, 25–31.

Miljanich, G. P. 2004. Ziconotide: Neuronal Calcium Channel Blocker for Treating Severe Chronic Pain. *Curr Med Chem*, *11*, 3029–3040.

Milroy, L.-G., Grossmann, T. N., Hennig, S., Brunsveld, L. & Ottmann, C. 2014. Modulators of Protein–Protein Interactions. *Chem Rev*, *114*, 4695–4748.

Moore, P. W., Malone, K., Vanvalkenburg, D., Rando, L. L., Williams, B. C., Matejowsky, H. G., Ahmadzadeh, S., Shekoohi, S., Cornett, E. M. & Kaye, A. D. 2023. GLP-1 Agonists for Weight Loss: Pharmacology and Clinical Implications. *Adv Ther*, *40*, 723–742.

Munoz-Torres, M., Alonso, G. & Raya, M. P. 2004. Calcitonin Therapy in. *Treat Endocrinol*, *3*, 117–132.

Murray-Thomas, T., Dcruz, J. M., Harder-Lauridsen, N. M., Olsen, A. H., Williams, R. & Major-Pedersen, A. 2025. Real-World Use of Liraglutide for Weight Management According to Label in the United Kingdom: A Cohort Study Using the Clinical Practice Research Datalink Primary Care Databases. *Diabetes Obes Metab*, *27*, 3705–3713.

Nakrani, M. N., Wineland, R. H. & Anjum, F. 2025. Physiology, glucose metabolism. In *StatPearls*. StatPearls Publishing.

Nauck, M. A. 1998. Glucagon-Like Peptide 1 (GLP-1): A Potent Gut Hormone With a Possible Therapeutic Perspective. *Acta Diabetol*, *35*, 117–129.

Nauck, M. A., Quast, D. R., Wefers, J. & Meier, J. J. 2021. GLP-1 Receptor Agonists in the Treatment of Type 2 Diabetes – State-of-the-Art. *Mol Metab*, *46*, 101102.

Pasut, G., Guiotto, A. & Veronese, F. M. 2005. Protein, Peptide and Non-Peptide Drug PEGylation for Therapeutic Application. *Expert Opin Ther Pat*, *14*, 859–894.

Patel, S., Saxena, B., Mehta, P. & Niazi, S. K. 2024. GnRH Peptide Antagonist: Comparative Analysis of Chemistry and Formulation With Implications for Clinical Safety and Efficacy. *Pharmaceuticals (Basel)*, *18*(1), 1–18.

Qamar, S., Mallik, R. & Makaronidis, J. 2024. Setmelanotide: A Melanocortin-4 Receptor Agonist for the Treatment of Severe Obesity Due to Hypothalamic Dysfunction. *touchREV Endocrinol*, *20*, 62–71.

Rao, S. S. C. 2018. Plecanatide: a New Guanylate Cyclase Agonist for the Treatment of Chronic Idiopathic Constipation. *Therap Adv Gastroenterol*, *11*, 1756284818777945.

Raufman, J. P. 1996. Bioactive Peptides from Lizard Venoms. *Regul Pept*, *61*, 1–18.

Sanchez-Garcia, L., Martín, L., Mangues, R., Ferrer-Miralles, N., Vázquez, E. & Villaverde, A. 2016. Recombinant Pharmaceuticals from Microbial Cells: A 2015 Update. *Microbial Cell Factories*, *15*, 33.

Sandow, J., Landgraf, W., Becker, R. & Seipke, G. 2015. Equivalent Recombinant Human Insulin Preparations and Their Place in Therapy. *Eur Endocrinol*, *11*, 10–16.

Sangave, N. A., Aungst, T. D. & Patel, D. K. 2019. Smart Connected Insulin Pens, Caps, and Attachments: A Review of the Future of Diabetes Technology. *Diabetes Spectr*, *32*, 378–384.

Sayem, A. S. M., Arya, A., Karimian, H., Krishnasamy, N., Ashok Hasamnis, A. & Hossain, C. F. 2018. Action of Phytochemicals on Insulin Signaling Pathways Accelerating Glucose Transporter (GLUT4) Protein Translocation. *Molecules*, *23*(2), 521.

Seino, Y., Fukushima, M. & Yabe, D. 2010. GIP and GLP-1, the Two Incretin Hormones: Similarities and Differences. *J Diabetes Investig*, *1*, 8–23.

Shammas, N. W. 2005. Bivalirudin: Pharmacology and Clinical Applications. *Cardiovasc Drug Rev*, *23*, 345–360.

Shyangdan, D., Cummins, E., Royle, P. & Waugh, N. 2011. Liraglutide for the Treatment of Type 2 Diabetes. *Health Technol Assess*, *15*(Suppl 1), 77–86.

Sood, A. & Panchagnula, R. 2001. PeroralRoute: An Opportunity for Protein and Peptide Drug Delivery. *Chem Rev*, *101*, 3275–3304.

Stolar, M. W., Hoogwerf, B. J., Gorshow, S. M., Boyle, P. J. & Wales, D. O. 2008. Managing Type 2 Diabetes: Going Beyond Glycemic Control. *J Manag Care Pharm*, *14*, s2–19.

Su, L. L., Chong, Y. S. & Samuel, M. 2012. Carbetocin for Preventing Postpartum Haemorrhage. *Cochrane Database Syst Rev*, *2012*, CD005457.

Sztanek, F., Toth, L. I., Peto, A., Hernyak, M., Dioszegi, A. & Harangi, M. 2024. New Developments in Pharmacological Treatment of Obesity and Type 2 Diabetes-Beyond and Within GLP-1 Receptor Agonists. *Biomedicines*, *12*(1), 1–20.

Thompson, A. M. & Trujillo, J. M. 2015. Dulaglutide: The Newest GLP-1 Receptor Agonist for the Management of Type 2 Diabetes. *Ann Pharmacother*, *49*, 351–359.

Thundimadathil, J. 2012. Cancer Treatment Using Peptides: Current Therapies and Future Prospects. *J Amino Acids*, *2012*, 1–13.

Trujillo, J. M. & Nuffer, W. 2014. Albiglutide: A New GLP-1 Receptor Agonist for the Treatment. *of Type 2 Diabetes. Ann Pharmacother*, *48*, 1494–1501.

Tunkel, D. E., Anne, S., Payne, S. C., Ishman, S. L., Rosenfeld, R. M., Abramson, P. J., Alikhaani, J. D., Benoit, M. M., Bercovitz, R. S., Brown, M. D., Chernobilsky, B., Feldstein, D. A., Hackell, J. M., Holbrook, E. H., Holdsworth, S. M., Lin, K. W., Lind, M. M., Poetker, D. M., Riley, C. A., Schneider, J. S., Seidman, M. D., Vadlamudi, V., Valdez, T. A., Nnacheta, L. C. & Monjur, T. M. 2020. Clinical Practice Guideline: Nosebleed (Epistaxis). *Otolaryngol Head Neck Surg*, *162*, S1–S38.

Van Poppel, H., Tombal, B., De La Rosette, J. J., Persson, B. E., Jensen, J. K. & Kold Olesen, T. 2008. Degarelix: a Novel Gonadotropin-Releasing Hormone (GnRH) Receptor Blocker–Results from a 1-Yr, Multicentre, Randomised, Phase 2 Dosage-Finding Study in the Treatment of Prostate Cancer. *Eur Urol*, *54*, 805–813.

Van Witteloostuijn, S. B., Pedersen, S. L. & Jensen, K. J. 2016. Half-Life Extension of Biopharmaceuticals Using Chemical Methods: Alternatives to PEGylation. *ChemMedChem*, *11*, 2474–2495.

Varela, A., Chouinard, L., Lesage, E., Smith, S. Y. & Hattersley, G. 2017. One Year of Abaloparatide, A Selective Activator of the PTH1 Receptor, Increased Bone Formation and Bone Mass in Osteopenic Ovariectomized Rats Without Increasing Bone Resorption. *J Bone Miner Res*, *32*, 24–33.

Vazquez-Roque, M. I. & Bouras, E. P. 2013. Linaclotide, Novel Therapy for the Treatment of Chronic Idiopathic Constipation and Constipation-Predominant Irritable Bowel Syndrome. *Adv Ther*, *30*, 203–211.

Veronese, F. M. 2001. Peptide and Protein PEGylation. *Biomaterials*, *22*, 405–417.

Vilsboll, T. 2009. Liraglutide: a New Treatment for Type 2 Diabetes. *Drugs Today (Barc)*, *45*, 101–113.

Wang, G., Qi, K., Li, X., Zuo, S., Zhang, R., Zhao, Y., Sun, S., Zhang, J. & Liu, X. 2024. Heparin and Bivalirudin in Percutaneous Coronary Intervention for Acute Coronary Syndromes: A Review Article. *Cardiovasc Ther*, *2024*, 5549914.

Wang, L., Wang, N., Zhang, W., Cheng, X., Yan, Z., Shao, G., Wang, X., Wang, R. & Fu, C. 2022. Therapeutic Peptides: Current Applications and Future Directions. *Signal Transduct Target Ther*, *7*, 48.

Warkentin, T. E. 2004. Bivalent Direct Thrombin Inhibitors: Hirudin and Bivalirudin. *Best Pract Res Clin Haematol*, *17*, 105–125.

Warrington, J. I., Richards, G. O. & Wang, N. 2017. The Role of the Calcitonin Peptide Family in Prostate Cancer and Bone Metastasis. *Curr Mol Biol Rep*, *3*, 197–203.

Wensel, T. M., Iranikhah, M. M. & Wilborn, T. W. 2011. Effects of Denosumab on Bone Mineral Density and Bone Turnover in Postmenopausal Women. *Pharmacotherapy*, *31*, 510–523.

Wensink, D., Wagenmakers, M. & Langendonk, J. G. 2021. Afamelanotide for Prevention of Phototoxicity in Erythropoietic Protoporphyria. *Expert Rev Clin Pharmacol*, *14*, 151–160.

Yazdani, J., Khorshidi Khiavi, R., Ghavimi, M. A., Mortazavi, A., Jabbari Hagh, E. & Ahmadpour, F. 2019. Calcitonin as an Analgesic Agent: Review of Mechanisms of Action and Clinical Applications. *Braz J Anesthesiol*, *69*, 594–604.

Youssef, J. G., Lavin, P., Schoenfeld, D. A., Lee, R. A., Lenhardt, R., Park, D. J., Fernandez, J. P., Morganroth, M. L., Javitt, J. C. & Jayaweera, D. 2022. The Use of IV Vasoactive Intestinal Peptide (Aviptadil) in Patients With Critical COVID-19 Respiratory Failure: Results of a 60-Day Randomized Controlled Trial. *Crit Care Med*, *50*, 1545–1554.

Yu, M., Benjamin, M. M., Srinivasan, S., Morin, E. E., Shishatskaya, E. I., Schwendeman, S. P. & Schwendeman, A. 2018. Battle of GLP-1 Delivery Technologies. *Adv Drug Deliv Rev*, *130*, 113–130.

Yu, M., Xie, J., Fernandez Lando, L., Kabul, S. & Swindle, R. W. 2016. Liraglutide Versus Exenatide Once Weekly: Persistence, Adherence, and Early Discontinuation. *Clin Ther*, *38*, 149–60.

Zhu, K., Meng, L., Luo, J., Wen, T., Dan, L., Wang, Z., Cao, X., Zhang, Z. & Chen, G. 2024. Taltirelin Induces TH Expression by Regulating TRHR and RARalpha in Medium Spiny Neurons. *J Transl Med*, *22*, 1158.

12 Future Directions in Polypeptide Research and Development

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12.1 INTRODUCTION

12.1.1 Advances in Polypeptide Design and Synthesis

The recent impulse in the subject arises from advances in polypeptide synthesis and design. The modern chemist is no more restricted by the natural limits or the inefficiency of the old manual methods. Today, synthetic chemistry, recombinant technologies, and predictive modeling have made it possible to not only construct long and complicated peptide sequences efficiently but also rationally introduce chimeless functions. Thus, the toolbox of polypeptides expands to include not only next-generation therapeutics and diagnostics but also intelligent materials and devices [Zheng et al., 2025].

12.1.1.1 Emerging Technologies in Sequence-Specific Synthesis

Historically, the solid-phase peptide synthesis (SPPS) technique brought about a revolution in the synthesis of short peptide chains (Table 12.1), but its use for very long, hydrophobic, or highly repetitive sequences was limited. However, recently developed innovations have overcome this bottleneck. High-performance resins such as ChemMatrix® are characterized by a much higher solvation capacity for difficult sequences, while even very challenging residues like cysteine or histidine can be coupled with high efficiency and minimum racemization by using more robust protecting and activating groups. Microwave-assisted SPPS further accelerates kinetics, hastening reaction time and dramatically reducing cycle lengths while increasing yields for longer chains (Figure 12.1). These newly implemented advances, importantly, are carried out within automated synthesizers that enable the simultaneous construction of hundreds of unique sequences in parallel while providing real-time monitoring with in-line purification [Smith & Jones, 2022].

TABLE 12.1

Innovations in Sequence-Specific Synthesis Approaches

Technology	Key Advancements	Impact	Reference
Automated SPPS	Advanced resin, parallel synthesis, UV-integration	Long and complex chains, rapid scale-up	Lee et al., 2024; Sondag et al., 2023
Flow/Liquid-phase Synthesis	In-line monitoring, solvent recovery	Industrial/large-scale applications	Sharma et al., 2022
Chemoenzymatic Methods	Enzyme-mediated ligation, site selectivity	Cyclic and branched architectures	Kobayashi et al., 2024; Gimenez-Dejoz et al., 2019

Technology	Key Advancements	Impact	Reference
Click Chemistry	Bioorthogonal macrocyclizations	Conformational stabilization	Fawcett et al., 2025; Amiri et al., 2024; Droumaguet & Velonia, 2008

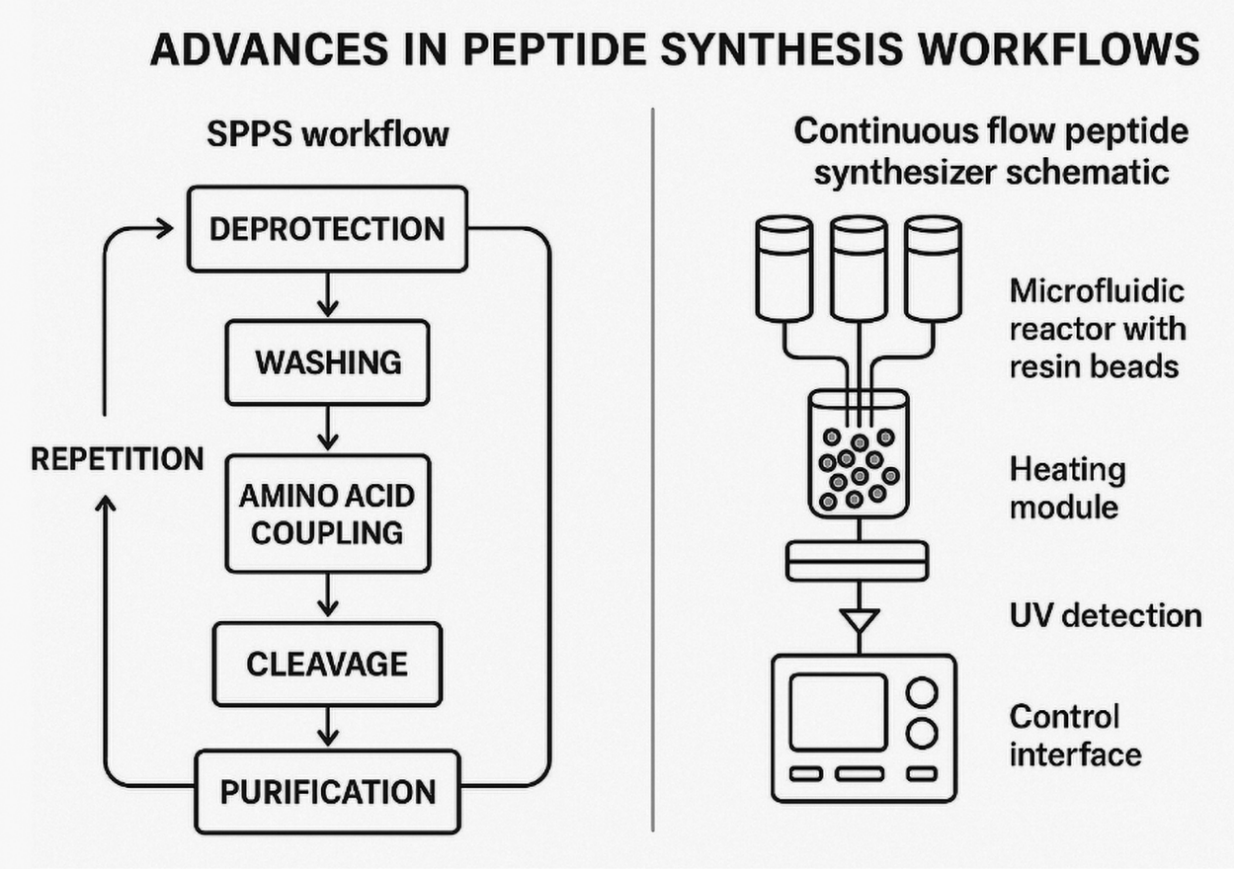

FIGURE 12.1 Advances in peptide synthesis workflows. (Left side: Solid-phase peptide synthesis (SPPS) workflow showing cycles of deprotection, washing, amino acid coupling, repetition, cleavage, and purification. Right side: Continuous Flow Peptide Synthesizer schematic with reagent reservoirs, microfluidic reactor with resin beads, heating module, UV detection, and control interface indicated.) ⏎

On the rise again are the liquid-phase methods. Soluble polymer supports and flow chemistry systems provide advantages for industrial-scale

processing, especially in terms of ease of purification and solvent recovery. These methodologies are facilitating larger-scale manufacture of therapeutic peptides alongside emerging chemoenzymatic methodologies (Table 12.1) that take full advantage of sortase-mediated ligations or butelase-catalyzed cyclizations to achieve cyclic or branched architectures with high regioselectivity and very limited byproduct formation [Ju et al., 2022; Shmool et al., 2022; Gil-Garcia et al., 2018; Caflisch, 2006]. Copper-catalyzed azide-alkyne cycloaddition (CuAAC) is now routinely used for macrocyclization to stabilize peptide secondary structures without the need for side-chain activation that could lead to side reactions. Such precise control over architectural motifs allows the chemist to either mimic or supersede the structural stability and function of naturally occurring proteins [Zheng et al., 2025].

12.1.1.2 High-Throughput Methods for Polypeptide Discovery

Reality-based commercial transformation of sequence innovations increasingly relies on throughput methodologies. There is no shortage of microfluidic technologies to produce, manipulate, and assay thousands of peptide variants every day. As a result, comprehensive structure-activity relationship (SAR) mapping can be done. Peptide arrays, phage and mRNA display libraries, and one-bead–one compound combinatorial libraries allow the discovery of unique binders and functionally optimized constructs in days rather than months. Next-generation HT screening is coupled with very advanced bioinformatics pipelines. Deep sequencing of the enriched hits, integration with affinity/mutational landscapes, obtains smoother lead identification and optimization. Platforms based on droplet microfluidics or high-content cell imaging further extend the capabilities to functional phenotyping and even *in vivo* validation at scale. Such platforms are opening

vast, as-yet-unmapped chemical spaces for polypeptides previously restricted from historic threshold discoveries for sequencing toward so-called “undruggable” proteins, GPCRs, and protein-protein interactions [Vitalis & Wenzel, 2023].

12.1.1.3 Computational Design and Predictive Modeling

Predictive modeling has become the dominant trend in the modern world of peptide science. Advanced software employing molecular dynamics and machine-learning techniques enables in silico design and optimization of stability boundaries for peptide structure and folding, with respect to binding specificity. Atomic-level predictions for challenging architectures are further accelerated by AlphaFold and similar AI models, advancing de novo design for therapeutic, diagnostic, or material science purposes [Abramson et al., 2024; Uzoeto et al., 2024; Yang et al., 2023; Jumper et al., 2021].

Computational forecasting of such properties includes stability, solubility, and aggregation propensity—historically parameters that have been determined only through painstaking empirical screening. For medicinal chemistry, this means faster iteration times, more focused libraries, and more efficient hit-to-lead conversion, along with waste and labor savings. Applications span macrocyclic peptide drug discovery through the use of antimicrobials and immunomodulators, with new algorithms on the horizon for predicting cell penetration, resistance to serum proteases, and biological half-life.

12.1.1.4 Incorporation of Non-Natural Amino Acids

The chemical space of natural amino acids is limited to twenty canonical residues. Expansion of nature’s building blocks through strategic integration of non-canonical amino acids (ncAAs) has been one of the most

transformative trends in this respect. Orthogonal tRNA/synthetase systems allow site-specific or global introduction of ncAAs with functional groups for labeling, cross-linking, photoactivation, or improved stability. *D*-amino acids and β-amino acids provide resistance to enzymatic degradation. Fluorinated and halogenated residues tune hydrophobicity and folding (Table 12.2). Phospho-mimetic analogues and glycosylated side chains can mimic post-translational modifications, enabling mechanistic studies of phosphorylation and signaling, or providing synthetic analogues of viral or toxin epitopes. By breaking the genetic code through chemical or synthetic biology, the therapeutic polypeptide landscape becomes vastly richer and tuned for both "drug-like" properties and core biological function [Fuertes et al., 2023; Gentiluomo et al., 2023]

TABLE 12.2

Impact of Non-Natural Amino Acids in Polypeptide Engineering (2023–2025)

ncAA Type	Functional Advantage	Application	References
D-amino acids	Enhanced stability, protease resistance	Antimicrobials	Israr et al., 2025; Zhou & Obexer, 2024
Fluorinated Residues	Altered hydrophobicity/folding	PPI inhibitors	Majekodunmi et al., 2024; Picois et al., 2024; Wu et al., 2024
Phospho-/Glyco-Analogues	PTM-mimicry, receptor targeting	Immune modulation	Geylan et al., 2025; Asokan-Sheeja et al., 2024; Castro et al., 2023
Clickable Handles	Bioconjugation, imaging	Diagnostic tracers	Blake-Hedges et al., 2024; Burrow et al., 2023

12.2 ANALYTICAL INSIGHTS AND FUTURE DIRECTIONS

This enabler technology, as a whole, has brought about a paradigm shift in peptide science. Who would be constrained in the efforts to alter nature? The researchers fabricate sequence-defined polymers, hybrid metabolites, and compounds functioning either as synthetic enzymes or as smart materials. Shortened timelines between the laboratory and the clinic appear to be assisting new diagnostic and therapeutic avenues on a much faster path toward preclinical and clinical applications.

However, problems persist: side-reactions and byproducts may form, particularly with longer or heterogeneous sequences; scale-up in manufacturing; and harmonization of analytical standards. Thus, the integration of new synthetic methods with high-throughput robotic and computational platforms may be the strongest route forward—one that will always empower polypeptide science to be a cornerstone of twenty-first-century biotechnology [Liu, Chen, & Mo, 2023].

12.2.1 Innovative Delivery Systems for Polypeptides

Although the latest in synthesis and design is key, the real art of making it in the clinic for peptide-based therapies lies in overcoming serious biological and physiological obstacles to effective delivery. Many polypeptides, otherwise having potent activity *in vitro*, would be quickly degraded *in vivo*, cleared from circulation, or unable to cross cellular membranes. New advances in the area of delivery systems are changing the face of clinical utility of polypeptides, now enabling stable systemic administration along with targeting to tissues that were traditionally thought to be “inaccessible” [Li, Zhang, & Wang, 2024].

Nanotechnology has probably had the most profound influence on peptide delivery [Blake-Hedges et al., 2024; Burrow et al., 2023]. Liposomes, polymeric nanoparticles, dendrimers, and micelles provide a

physical protective environment for labile peptides to afford them a longer systemic half-life and controlled release (Table 12.3). Surface functionalization—such as PEGylation, antibody/aptamer conjugation, or stealth coatings—reduces opsonization and renal clearance, enabling much more efficient delivery to target organs in clinical applications—especially in the chronic arena, such as diabetes or cancer. Furthermore, nanocarriers are free to utilize the enhanced permeability and retention (EPR) effect in tumors, facilitating passive accumulation of peptide-drug conjugates (PDCs) at the sites of disease. Recently designed nanocarriers may display peptide multivalently for signal amplification, or they may take the form of multistage nanoplatforms that respond to acidic pH or enzyme activity specific to tumors as an on-demand release mechanism [Malam, Loizidou, & Seifalian, 2009].

TABLE 12.3

Key Delivery Strategies for Polypeptides and Recent Milestones

Delivery System	Barrier Overcome	Innovation	Clinical Status	References
Nanocarriers (liposomes, NPs)	Renal clearance, targeting	Long-acting insulins, tumor PDCs	Approved, clinical trials	Chehelgerdi et al., 2023; Elsabahy et al., 2021; Din et al., 2017
Bioresponsive hydrogels	Pathological microenvironments	On-demand anti-inflammatory peptides	Preclinical/clinical	Regato-Herbella et al., 2024; Wang et al., 2023; Helmecke et al., 2022
Microneedle patches	Skin permeability	Transdermal vaccines	Commercial, global health	Zheng et al., 2023; Duong et al., 2020

Delivery System	Barrier Overcome	Innovation	Clinical Status	References
CPP-modified systems	Cellular uptake, BBB access	CNS neuropeptide delivery	Animal models, phase I	Pirhaghi et al., 2024; Wei et al., 2024; De Martini et al., 2023; McGowan et al., 2015

12.2.1.1 Bioresponsive and Targeted Delivery Platforms

Worlds apart in recent years is the area of smart delivery: bioresponsive carriers that sense environmental triggers—such as pH, redox gradients, or specific proteolytic activity—and then release their cargo precisely when and where it is needed. This guarantees off-target effects are reduced while therapeutic indices are increased. For instance, in the domain of cancers, MMP-degradable-linked nanogels and hydrogels are able to actually release peptides directly inside the tumor microenvironment. Targeted delivery is also undergoing increasingly complex developments. Nanocarriers modified by ligands, antibody-drug conjugates, and cell-penetrating peptide (CPP) platforms are now being assessed for their ability to deliver not just traditional peptides, but even high-molecular-weight proteins and gene-editing complexes [Haleem & Khan, 2021].

12.2.1.2 Oral and Transdermal Delivery Innovations

The transition of polypeptide therapies into oral administration remains the major “holy grail.” Ordinary peptides are digested and destroyed in the gastrointestinal tract, so that they cannot be absorbed. However, innovations such as enteric microcapsules, mucosal permeation enhancers (e.g., fatty acids, SNAC co-formulations), and molecule-specific enzyme inhibitors have allowed for oral delivery of some peptide therapeutics, including “oral

semaglutide" for type II diabetes-the first glucagon-like peptide-1 (GLP-1) analog to achieve oral bioavailability. The transdermal route moves past gastrointestinal degradation. Applications of microneedle patches, dissolvable films, and advanced hydrogel dressings all use the skin as an entry point (Table 12.3). Systems with the capacity to load both immunomodulators and vaccines are truly transformative for global health and vaccination campaigns given easy-to-use administration, enhanced adherence, and limited need for cold chain [Bäckdal et al., 2021].

12.2.1.3 Dealing with Biological Barriers

Independently, new pharmacological strategies attempt to overcome systemic and cellular transport. Biodegradable scaffolds and slow-release depots have been produced, providing sustained pharmacokinetics for weeks or even months. Crossing the blood-brain barrier (BBB) was thought virtually impossible for peptide drugs until lately. Purposeful nanoplatforms directed by receptors and peptide "shuttle" sequences such as angiopep or transferrin-mimetics bring new horizons to neurotherapeutics. CPPs and receptor-mediated endocytosis will additionally improve intracellular access, a major requirement for peptides toward cytosolic or nuclear disease drivers. "Trojan horse" constructs can bundle some genetic editing or silencing tools with the peptide motifs in delivery, unlocking what could be termed the new classes of genetic and epigenetic therapeutics [Ghorai et al., 2023; Thakur, Qureshi, & Kumar, 2021; Yin, Kauffman, & Anderson, 2017].

Despite these advances, challenges remain: scalable, GMP-grade production of complex formulation systems; stability under variable storage conditions; and regulatory issues. The field is quickly pivoting to "mix-and-match" modular platforms that allow plug-and-play formulation to fit diverse indications. The parallel successes of nanomedicine, surface

engineering, and synthetic chemistry guarantee that polypeptide-based medicines may reach and act upon tissues and targets once considered unreachable. With new disease targets and patient-centric routes fast becoming a reality, polypeptide delivery science will begin to drive patient outcomes and radically alter standards of care.

12.2.2 Expanding Therapeutic and Diagnostic Applications

The surge in polypeptide design and delivery strategies has amplified their utility far beyond conventional drug paradigms. Currently, polypeptides act as tailored immunogens, ultrasensitive diagnostics, precision tools for gene editing, and central scaffolds for personalized interventions. They can dynamically interface with biological systems because of programmability in sequence, structural diversity, and modification in terms of bioactivity. Recent breakthroughs serve not only as proof of concept for new biomedical territories but shine light into mechanisms of disease and therapy that were once daily.

12.2.2.1 Next-Generation Vaccines and Immunotherapies

The polypeptide-based vaccines present different advantages, such as modularity, safety (lack of infectious elements), and specific immune activation. In the last three years, there has been an accelerated merging of ideas from viral immunology, adjuvant engineering, and the development of self-assembling peptide nanocarriers. Important advances include the creation of polypeptide antigens targeting emerging viral variants as well as the tuning of cancer neoantigens to patients' individual mutations [Abdullah et al., 2020].

Of utmost importance are mRNA–polypeptide hybrids in which peptide antigens are synthesized *in vivo*. The self-assembled peptide scaffolds mimic

viral geometry to elicit strong and adjustable B- and T-cell responses [Morris et al., 2019]. The adjuvants are co-delivered more and more in these supramolecular constructs that enhance immunogenicity and decrease the injection burden. Checkpoint-inhibitory peptides are being tested as immune-restorative alone or co-delivered with standard immunotherapies in oncology, with promising tumor regression rates seen in phase I and II trials across different malignancies [Gallotta et al., 2020]. By virtue of being generally unimmunogenic, polypeptide vaccines can confer a modicum of immunogenicity through fine-tuning of dosage, epitope density, valency, and perhaps MHC class presentation. Preferably they would be cheap to manufacture, have good room temperature stability, and be rapidly redesigned, and with such properties they would undoubtedly outperform many classical vaccines in times of pandemic [Skwarczynski & Toth, 2020].

12.2.2.2 Polypeptides in Molecular Imaging and Biosensing

Peptides have been widely used as molecular imaging agents and biosensors because of their sequence programmability, small size, and functionalizability. Labeled peptides (for instance, with fluorophores, radionuclides, or paramagnetic ions) can target cell surface receptors, enzymes, or pathologic aggregates with nanomolar affinity [Xin et al., 2023]. This not only enables sensitive diagnostic imaging through PET, SPECT, or MRI, but also the real-time monitoring of disease progression, therapeutic response, and microenvironmental changes. Recent reports have shown self-assembling peptides as high-resolution ultrasound/MRI contrast agents, responsive “turn-on” fluorescent peptides for intracellular enzymes, and radio-labeled cyclic peptides for early tumor detection [Hu et al., 2020; Li et al., 2019]. Peptide-based biosensors have achieved attomolar detection levels in body fluids, which have far-reaching implications in cancer

screening and infectious disease surveillance [Thakur, Qureshi, & Kumar, 2021].

The rapidly modular nature of peptide imaging probes means that they can be quenched for *in vivo* half-life and readily attain theranostic applications, where an agent provides diagnostic and therapeutic applications as a single construct. Specificity, non-immunogenicity, and ease of chemical modification favor superior pharmacokinetics and low toxicities when compared to larger antibody-based agents.

12.2.2.3 Polypeptides for Gene Editing and Cell Therapy Support

Gene editing tools (e.g., CRISPR/Cas, siRNA, base editors) require efficient transport across membranes and cellular compartments. Peptide-based carriers have revolutionized this process, acting as molecular trojans to facilitate cellular uptake, nuclear localization, or endosomal escape [Ramakrishna et al., 2014].

Cell-penetrating peptides (CPPs), sometimes linked to targeting ligands or endosomolytic motifs, facilitate the delivery of relatively large macromolecular complexes while minimizing off-target effects (Table 12.3). Sequence-engineered polypeptides are in developing deliverers having CRISPR/Cas9 components (Table 12.4) with an efficiency of >80% editing in primary human cells, as well as self-assembling CPPs with tunable pharmacokinetics in recent years [Qian et al., 2016]. Hydrogel matrices and polypeptides in cell therapy support *ex vivo* expansion of stem or immune cells, providing physical cues or releasing differentiation-inducing peptides in response to environmental triggers [Lam, Lowry, & Carmichael, 2020]. Reduction in cytotoxicity, increase in precision of editing, and modular "design to function" flexibility give polypeptides a central position in the development of future genome/epigenome engineering and cellular

reprogramming. The deeper integration with in silico modelling predicts off-target risk and optimizes delivery vehicle to cargo ratio on a case-by-case basis [Yin, Kauffman, & Anderson, 2017].

TABLE 12.4

Clinical and Technological Advances in Polypeptide Therapeutics and Diagnostics

Application	Recent Example/Innovation	Clinical Status	References
Personalized Cancer Vaccines	mRNA/peptide hybrids for neoantigens	Clinical trials (Phase I/II)	Lu et al., 2023; Awad et al., 2022; Cafri et al., 2020
Enzyme-Activated Imaging Peptides	*In vivo* tumor microenvironment probes	Preclinical	Hu et al., 2024; Yan et al., 2019; Ma et al., 2018
CRISPR/Editor Peptide Carriers	CPP-linked Cas9, siRNA delivery	Preclinical / ex vivo	Liu et al., 2025; Alhazza et al., 2024; Du et al., 2023; Mengstie, 2022
Autoantibody Diagnostic Panels	Multiplex peptide arrays	Commercial launch	Gilliam et al., 2024; Wu et al., 2024; Zhang et al., 2022; Savvateeva et al., 2021; Orlov et al., 2020

12.2.2.4 Personalized Polypeptide-Based Medicine

Personalized and precision medicine are rapidly converging with polypeptide science (Table 12.4). By leveraging whole-exome and transcriptomic data from each patient, unique peptide signatures—designed as neoantigens or functional probes—can be developed for individualized

treatment or screening. Experimental peptide vaccines targeting tumor mutations unique to patients have induced robust immune responses and durable tumor regressions in multiple clinical studies within the past 24 months [Keskin et al., 2019]. Molecular diagnostic panels backed on synthetic peptides now enable detection of various patterns of autoantibodies or metabolic radio-tracers specific to rare diseases, thus facilitating a much earlier and more accurate diagnosis. Predictive analytics will become really different because they will eventually link the individual peptide sequences and the MHC haplotypes with the immune compatibility or the probability of response, thereby revolutionizing the clinical trial design [Li et al., 2022].

12.3 ANALYTICAL INSIGHTS AND FUTURE DIRECTIONS

The future of biomedical applications will be marked by an even more in-depth fusion between polypeptide science, computational biology, and patient-facing medicine. Are we to consider the potential for peptides to be custom-made, programmable, safe, and scalable tools for advancement in treatment and truly prevention strategies against many diseases? However, these remain challenges with regulatory adjustments, cost barriers to personalized therapy, and bioinformatics pipelines that require robust implementation. Further characterization and establishment of real-time, point-of-care peptide-based assays may offer the potential to democratize advanced diagnostics for underserved populations worldwide [Hashemi et al., 2024].

12.3.1 Emerging Trends in Manufacturing and Scalability

Polypeptide products from laboratory-scale development to large-volume clinical or commercial manufacturing face complex and multidimensional

problems. Rapid advances in process chemistry, green biomanufacturing, and inline quality control are addressing these through an integrated, technology-driven framework, ensuring robustness from “bench to patient” Figure 12.2.

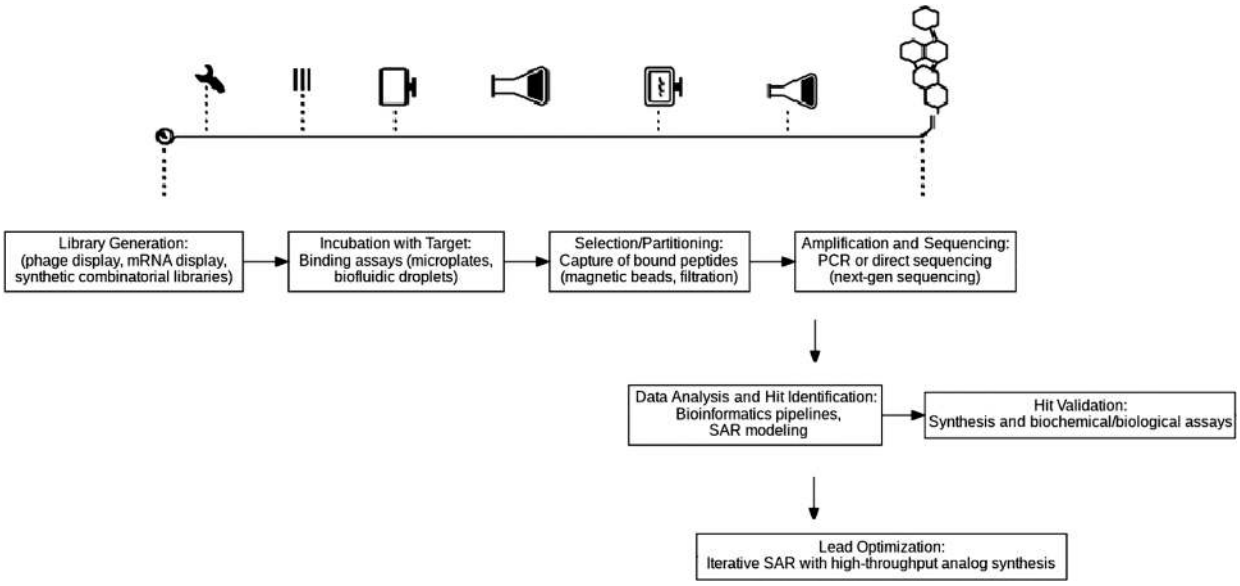

FIGURE 12.2 High-throughput screening platform workflows. ⏎

12.3.1.1 Automated and Continuous Manufacturing Processes

Automation has formed the foundation for process reliability and economic viability. Fully automated peptide synthesizers with robotic purification, tank-to-reactor fluidics, and integrated real-time analytics provide high-throughput parallel production at pharmaceutical-grade (GMP) standards [Yu et al., 2024]. Continuous flow chemistry allows remote monitoring and control of temperature, reagent feed, and product extraction processes. This case currently plays a very important role in speeding up cycle times while ensuring standardization throughout the batch-to-batch.

Industrial-scale peptide production has further embraced digitization, with process analytical technology (PAT) enabling in-line stability checks,

impurity tracking, and end-point determination [Saha, Eni, & Saha, 2023]. These “smart” factories allow for adaptive synthesis in response to minute fluctuations within the production run, a level of sophistication only previously found in small-molecule manufacturing. The link of automation to digital monitoring aided by AI modelling of yield/purity prediction allows for real-time process correction, minimizing costly recalls and maximizing regulatory compliance [Yu et al., 2024].

12.3.2 Green Chemistry and Sustainable Production

Large, hazardous quantities of solvents such as DMF and DCM, along with resins that are not recyclable, have facilitated peptide synthesis in an environmentally unfriendly manner, in an ever-increasing environmental footprint of concern. Implementation of green solvents such as cyclopentyl methyl ether or water-based systems, and designing process cycles to maximize reagent reuse, has begun the decisive shift toward sustainable protocols. Solid support recycling, miniaturization, and enzyme-catalyzed ligations would also reduce waste and thus lower CO2 footprints.

One of the most attractive areas for this pathway was the use of aqueous biocatalysis with precise site selectivity and coming as it does with very little energy demand, working especially well for some of the typically larger, more problematic peptides. Some companies are now also setting up closed-loop solvent systems to realize full process circularity [Gentiluomo et al., 2023]. Evidence for sustainability measures in process development and large-scale production increases further requirements on documentation for product purity and quality. Such competitive advantage will create for those developing greener innovations in the peptide field—their increased and increased demand from payors.

12.3.3 Scaling Challenges for Clinical and Commercial Applications

Historically, industrial process expansion was limited largely by peptide aggregation, resin swelling issues, and side-products such as deletion sequences and racemization artifacts. Scalable purification has been instigated through recent innovations such as prep-HPLC, ultra-fast supercritical fluid chromatography (SFC), and continuous filtration devices [Bray, 2003]. Online impurity mapping and "ultra-high-throughput" secondary analytics now allow real-time adjustments.

Supply chain security is another key challenge: that is, a reliable and low-cost way of obtaining protected amino acid derivatives or customized building blocks, particularly for modified and non-natural residues [Albericio & Kruger, 2012]. In the face of geopolitical and pandemic-driven disruption, companies have begun forming consortia that allow them to build strategic reserves of certain supplies and sources of alternative suppliers. Scale-up has been elevated beyond technicality to one of competitive bottlenecks-a bottleneck, to say the least. The "quality by digital design" paradigm not only shortens the time to file for regulatory approval but also lowers cost per dose, both crucial for widening global access to advanced peptide medicines.

12.3.4 Quality by Design and Analytical Innovations

The peptide arena has jumped from a simple end-product "quality by testing" approach to one of comprehensive "quality by design" (QbD). In fact, advanced process analytical technologies (PAT)-such as high-resolution mass spectrometry, real-time chromatography, and NMR-will be used for much more continuous, non-disruptive surveillance of all production steps (Table 12.5) [Rathore & Winkle, 2009]. Combined with digital "twin" models, manufacturers can simulate the entire lifecycle of a product before

making a single real batch, predicting and preventing errors proactively. Sequence uniformity, folding correctness, post-synthetic modifications, and absolute impurity profiling are now pre-release routine checks for every engineered batch, thereby limiting cost and risk of product recalls.

TABLE 12.5

Innovations in Manufacturing and Scale-Up of Polypeptide Therapeutics

Process/Technology	Benefit/Impact	Implementation Example
Automated/Continuous Synthesis	Parallelization, speed	GMP parallel peptide factory
Green Chemistry Protocols	Reduced environmental impact	Aqueous biocatalysis, CPME use
High-Speed Prep HPLC, SFC	Fast, scalable purification	Peptide hormone scale-up
PAT-Informed QbD Digital Twins	Process predictability	Real-time NMR/MS analytics

12.4 CURRENT-ANALYTICAL INSIGHTS AND FUTURE DIRECTIONS

The ever-ongoing tussle between regulatory toughening and digital manufacturing innovation continues to shape the shape and scope of the polypeptide industry globally. Digital, ecologically friendly, automated processes are becoming rapidly, perhaps not just as additional advantages, but essential requirements for market access, funding, and partnership opportunities [Saha, Eni, & Saha, 2023]. The co-creation and continued collaboration across supply chains, standards bodies, and regulatory regimes will be indispensable to ensuring that innovative polypeptide medicines resoundingly reach all who can benefit from them, not just the affluent.

12.4.1 Perspectives about the Future and Multidisciplinary Collaborations

As peptide science progresses through maturity, so will the increasingly defining elements of the field- that is multidisciplinary collaborations, ethical foresight, and the broadening outreach to society at large. Within a decade, one should expect the aspects of "omics" data, materials innovation, regulatory harmonization, and social responsibility to converge.

12.4.1.1 Integration with Omics and Systems Biology

High-throughput "omics" approaches-genomics, proteomics, and metabolomics-are propelling forward the next generation of discovery in polypeptides (Table 12.6). Most enormous databases describing human genetic variation, along with computational tools, enable the reasoning selection of peptide immunogens, disease biomarkers, or therapeutic targets, along differences at the population or individual level [Deng & Jiang, 2022]. Systems biology modeling predicts the physiology response dynamics, drug-peptide interaction results, and off-target liabilities, improving potency as well as safety. Emerging technologies integrate single-cell genomics, deep immune profiling, and peptide-display libraries to map epitope landscapes on the scale of whole tissue samples or populations. This is the area of "data-driven peptide science," which is destined to change everything-from the diagnosis of rare diseases to the design of vaccines in pandemics. The integration of different omics datasets for deriving drugs and diagnostics can attain advancement in machine learning combined with appropriate experimental feedback loops. The ambition is clear: to move from generalized interventions to intervention strategies as singular as the diseases-or patients-they target.

TABLE 12.6

Cross-Disciplinary Trends and Ethical Priorities in Future Polypeptide Science

Collaborative Front	Focus	Recent Milestone/Initiative	Reference
Omics–Peptide Integration	Neoantigen design, biomarker discovery	AI-driven population vaccine optimization	Gentiluomo et al., 2023; Morais, Martins, & Martinho, 2021; Abdullah et al., 2020; Bray, 2003; Rathore & Winkle, 2009
Bioelectronic-Peptide Hybrid	Wearable, implantable biosensors	Continuous glucose/cytokine monitoring	
Global Harmonization	Regulatory, ethical, access	WHO/FDA/EMA unified trial templates	
Social Engagement	Privacy, transparency, equity	Open clinical trial data, patient advisory boards	

12.4.1.2 Interfacing with Bioelectronics and Smart Materials Bioelectronic

New peptide-derived materials can self-assemble, respond to various stimuli, or serve as a bridge between biology and electronics. Wearable and implantable biosensors based on peptide interfaces can continuously monitor biomarkers, such as glucose, lactate, and cytokines. “Smart” materials, which combine conducting polymers and bioactive peptides, are necessary for advanced prosthetics, neural interfaces, and drug delivery implants, as they can modulate cell growth, initiate electrical activity, or signal tissue reparative cascades [Min, Kim, & Pack, 2025]. Building on recent proof-of-concept systems implanting peptide actuators, responsive not only to external fields but also to microenvironmental change enables therapy “on demand” [Zheng et al., 2024]. Hybrids between bioelectronics and

polypeptides are some of the most promising interdisciplinary areas. In fact, they demand not only preparing materials science and peptide-engineering expertise but also inventive regulatory and ethical mechanisms to secure the privacy of patients and reduce technological inequity (see Figure 12.3 for device concept).

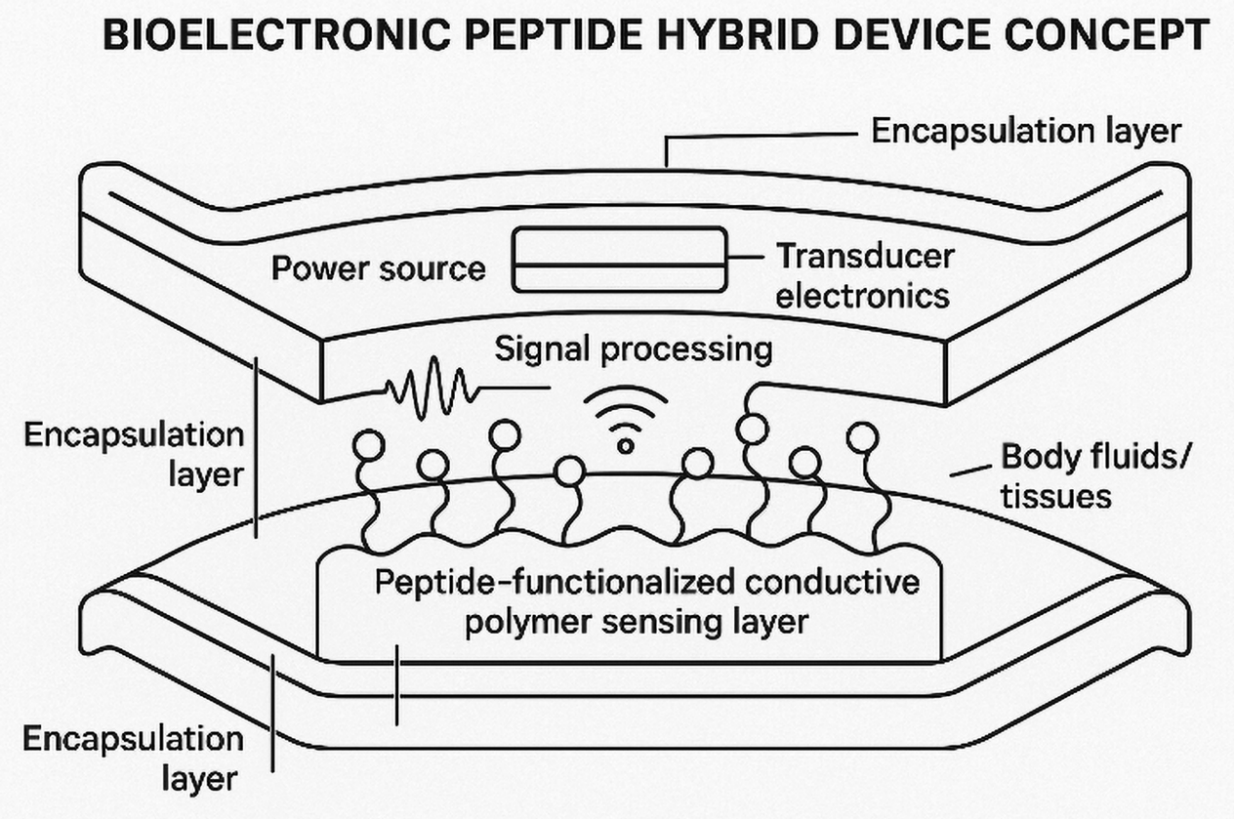

FIGURE 12.3 Bioelectronic peptide hybrid device concept. ⏎

12.4.1.3 Academia–Industry–Government Partnerships

Robust partnerships combine scientific insights with development ability and scaled translational direction to accelerate onward progress. Very large consortia are overseeing multi-center clinical trials for peptide vaccines, global surveillance of emerging resistance by peptide-acting pathogens, and border-level harmonization of safety standards [Wehling, Evans, &

Dalemans, 2021]. International bodies driven by WHO, FDA, EMA, and local regulators are conducting pilot studies to develop packages for unified submission of peptide drugs, thus reducing timelines from years to months. Public–private partnerships further catalyze open-access peptide libraries, training, and technology transfer—widening global capacity to innovate and respond to emergency health challenges. Collaborative, pre-competitive frameworks have proven essential for the rapid development and equitable delivery of peptide-based COVID-19 vaccines and diagnostics—setting a precedent that shall govern all future "pandemic preparedness" and several more routine biotechnological innovations [World Health Organization, 2022].

12.4.1.4 Ethical, Societal, and Global Health Considerations

One of the field's great mandates is to see to it that rapid innovation does not result in increased inequity in access, safety, or regulatory protection. The rise of powerful public–private data analytics is ushering in urgent concerns regarding patient privacy, data misuse, and algorithmic bias [U.S. Food and Drug Administration, 2023].

Biocompatibility, off-target effects, and environmental release must now be assessed in detail especially for next-generation delivery vehicles and synthetic organisms. Increasingly, open dialogues with patients, clinicians, and varied communities will shape the design of clinical trials and regulatory strategy to ensure that advances in polypeptides respond to societal and patient needs rather than purely technological opportunity. There needs to be agreement on global standards for ethical data use and just cost recovery and access to medicines that are needed to connect the "technology push" with the global "health pull" [Gentiluomo et al., 2023; Rathore & Winkle, 2009; Bray, 2003].

12.5 ANALYTICAL INSIGHTS AND FUTURE DIRECTIONS

Perhaps within a decade out, the demarcating spaces between therapeutic, diagnostic, and digital health products will fade into insignificance, ushering in the arrival of platforms for monitoring, detection, and intervention, courtesy of the polypeptide revolution. The nurturing of parallel social, regulatory, and ethical contexts will, therefore, become an urgent need in order to realize that the platforms built around polypeptides foster the most innovation with the most equity for the greatest number of people.

12.6 CONCLUSION

Looking forward, the promise of the polypeptide field lies not just in the technical possibilities but in the creative spirit, accountability, and collaborative generosity of its practitioners. The lens of discovery is more than a microscope; it is a mirror for our society's hopes for health, equity, and sustainable progress. In our embrace of technological sophistication, may we also uphold our joint responsibility to carry these tools across borders, both geographical and disciplinary, to work toward ensuring that every innovation, however big or small, translates into a real and accessible impact upon patients and communities throughout the world.

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Supervision: MLR
Writing original draft preparation: MLR
Writing review and editing: MLR, Asra Faiz
Visualization: MLR, AF
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Transparency statement: I affirm that this manuscript is an honest, accurate, and transparent account of the study being reported; that no important aspects of the study have been omitted; and that any discrepancies from the study as planned (and, if relevant, registered) have been explained.

REFERENCES

Abdullah, T., Bhatt, K., Eggermont, L. J., O'Hare, N., Memic, A., & Bencherif, S. A. (2020). Supramolecular self-assembled peptide-based vaccines: Current state and future perspectives. *Frontiers in Chemistry*, 8, 598160. https://doi.org/10.3389/fchem.2020.598160⏎

Abramson, J., Adler, J., Dunger, J., Evans, R., Green, T., Pritzel, A., et al. (2024). Accurate structure prediction of biomolecular interactions with AlphaFold 3. *Nature*, 630, 493–500. https://doi.org/10.1038/s41586-024-07487-w⏎

Albericio, F., & Kruger, H. G. (2012). Therapeutic peptides. *Future Medicinal Chemistry*, 4(12), 1527–1531. https://doi.org/10.4155/fmc.12.94⏎

Alhazza, A., Mahdipoor, P., Hall, R., Manda, A., Lohan, S., Parang, K., & Aliabadi, H. (2024). Modifying peptide/lipid-associated nucleic acids (PLANAs) for CRISPR/Cas9 ribonucleoprotein delivery. *European Journal of Pharmaceutical Sciences: Official Journal of the European Federation for Pharmaceutical Sciences*, 202, 106708. https://doi.org/10.1016/j.ejps.2024.106708⏎

Amiri, A., Abedanzadeh, S., Davaeil, B., Shaabani, A., & Moosavi-Movahedi, A. (2024). Protein click chemistry and its potential for medical applications. *Quarterly Reviews of Biophysics*, 57, e6. https://doi.org/10.1017/S0033583524000027⏎

Asokan-Sheeja, H., Das, D., Nguyen, J., Nguyen, N., Van Khanh Pham, T., Nguyen, K., & Dong, H. (2024). Modular design and self-assembly of pH-responsive multidomain peptides incorporating non-natural ionic amino acids. *Chemistry*, 30, e202403085. https://doi.org/10.1002/chem.202403085⏎

Awad, M., Govindan, R., Balogh, K., Spigel, D., Garon, E., Bushway, M., et al. (2022). Personalized neoantigen vaccine NEO-PV-01 with chemotherapy and anti-PD-1 as first-line treatment for non-squamous non-small cell lung cancer. *Cancer Cell*, 40(9), 951–965.e6. https://doi.org/10.1016/j.ccell.2022.08.003⏎

Bäckdal, M., et al. (2021). Clinical pharmacokinetics of oral semaglutide: Analyses of data from clinical pharmacology studies. *Clinical Pharmacokinetics*, 60(12), 1603–1616. https://doi.org/10.1007/s40262-021-01019-4⏎

Blake-Hedges, J., Groff, D., Foo, W., Hanson, J., Castillo, E., Wen, M., et al. (2024). Production of antibodies and antibody fragments containing non-natural amino acids in Escherichia coli. *mAbs*, 16(1), 2316872. https://doi.org/10.1080/19420862.2024.2316872⏎

Bray, B. L. (2003). Large-scale manufacture of peptide therapeutics by chemical synthesis. *Nature Reviews Drug Discovery*, 2(7), 587–593. https://doi.org/10.1038/nrd1133⏎

Burrow, N., Gourdie, R., King, E., Travis, C., Goff, C., Nimmo, Z., et al. (2023). Development of multivalent conjugates with a single non-canonical amino acid. *ChemBioChem*, 24(22), e202300565. https://doi.org/10.1002/cbic.202300565⏎

Caflisch, A. (2006). Computational models for the prediction of polypeptide aggregation propensity. *Current Opinion in Chemical Biology*, 10(5), 437–444. https://doi.org/10.1016/J.CBPA.2006.07.009.⏎

Cafri, G., Gartner, J., Zaks, T., Hopson, K., Levin, N., Paria, B., et al. (2020). mRNA vaccine-induced neoantigen-specific T cell immunity in patients with gastrointestinal cancer. *The Journal of Clinical Investigation*, 130(11), 5338–5349. https://doi.org/10.1172/JCI134915⏎

Castro, T., Melle-Franco, M., Sousa, C., Cavaco-Paulo, A., & Marcos, J. (2023). Non-canonical amino acids as building blocks for peptidomimetics: Structure, function, and applications. *Biomolecules*, 13(6), 981. https://doi.org/10.3390/biom13060981⏎

Chehelgerdi, M., Chehelgerdi, M., Allela, O., Pecho, R., Jayasankar, N., Rao, D., et al. (2023). Progressing nanotechnology to improve targeted cancer treatment: Overcoming hurdles in its clinical implementation. *Molecular Cancer*, 22, 181. https://doi.org/10.1186/s12943-023-01865-0⏎

De Martini, L., Sulmona, C., Brambilla, L., & Rossi, D. (2023). Cell-penetrating peptides as valuable tools for nose-to-brain delivery of biological drugs. *Cells*, 12(12), 1643. https://doi.org/10.3390/cells12121643.⏎

Deng, Y., & Jiang, D. (2022). Computational systems biology in disease modeling and control: Review and perspectives. *Frontiers in Systems Biology*, 2, 1017923. https://doi.org/10.3389/fsysb.2022.1017923⏎

Din, F., Aman, W., Ullah, I., Qureshi, O., Mustapha, O., Shafique, S., et al. (2017). Effective use of nanocarriers as drug delivery systems for the treatment of selected tumors. *International Journal of Nanomedicine*, 12, 7291–7309. https://doi.org/10.2147/IJN.S146315⏎

Droumaguet, B., & Velonia, K. (2008). Click chemistry: A powerful tool to create polymer-based macromolecular chimeras. *Macromolecular Rapid Communications*, 29, 1073–1089.

https://doi.org/10.1002/MARC.200800155⏎

Du, Y., Liu, Y., Hu, J., Peng, X., & Liu, Z. (2023). CRISPR/Cas9 systems: Delivery technologies and biomedical applications. *Asian Journal of Pharmaceutical Sciences*, 18, 100854. https://doi.org/10.1016/j.ajps.2023.100854⏎

Duong, H., Yin, Y., Thambi, T., Kim, B., Jeong, J., & Lee, D. (2020). Highly potent intradermal vaccination by an array of dissolving microneedle polypeptide cocktails for cancer immunotherapy. *Journal of Materials Chemistry B*, 8(6), 1111–1121. https://doi.org/10.1039/c9tb02175b⏎

Elsabahy, M., Song, Y., Eissa, N., Khan, S., Hamad, M., & Wooley, K. (2021). Morphologic design of sugar-based polymer nanoparticles for delivery of antidiabetic peptides. *Journal of Controlled Release: Official Journal of the Controlled Release Society*. https://doi.org/10.1016/j.jconrel.2021.04.006⏎

Fawcett, C., Watson, J., Richards, S., Doherty, A., Seki, H., Love, E., et al. (2025). Comparative study of click handle stability in common ligation conditions. *Bioconjugate Chemistry*, 36, 1054–1065. https://doi.org/10.1021/acs.bioconjchem.5c00095⏎

Fuertes, G., Sakamoto, K., & Budisa, N. (2023). Exploring and expanding the protein universe with non-canonical amino acids. *Frontiers in Molecular Biosciences*, 10, 1303286. https://doi.org/10.3389/fmolb.2023.1303286⏎

Gallotta, M., Assi, H., Degos, C., Wrobel, L. J., Kotsias, F., Ahmed, K., et al. (2020). Inhaled TLR9 agonist renders lung tumors permissive to PD-1 blockade by promoting optimal $CD4^+$ and $CD8^+$ T-cell interplay. *Cancer Research*, 80(9), 1875–1887. https://doi.org/10.1158/0008-5472.CAN-19-2836⏎

Gentiluomo, L., Baillie, C., Bray, R., Kubiak-Ossowska, K., Mitchell, J., & Mulheran, P. A. (2023). Trends in peptide synthesis: Green chemistry, automation, and artificial intelligence. *Journal of Peptide Science*, 29(9), e3577. https://doi.org/10.1002/psc.3577⏎

Geylan, G., Janet, J., Tibo, A., He, J., Patronov, A., Kabeshov, M., et al. (2025). PepINVENT: Generative peptide design beyond natural amino acids. *Chemical Science*.

https://doi.org/10.1039/d4sc07642g⏎

Ghorai, S. M., Deep, A., Magoo, D., Gupta, C., & Gupta, N. (2023). Cell-penetrating and targeted peptides delivery systems as potential pharmaceutical carriers for enhanced delivery across the blood–brain barrier (BBB). *Pharmaceutics*, 15(7), 1999. https://doi.org/10.3390/pharmaceutics15071999⏎

Gil-Garcia, M., Bañó-Polo, M., Varejão, N., Jamróz, M., Kuriata, A., Díaz-Caballero, M., et al. (2018). Combining structural aggregation propensity and stability predictions to redesign protein solubility. *Molecular Pharmaceutics*, 15 9, 3846–3859. https://doi.org/10.1021/acs.molpharmaceut.8b00341⏎

Gilliam, B., Pfeifer, J., McDonald, J., Victorino, F., Qiang, X., Hoberg, J., & Lie, W. (2024). Isotype-specific autoantibody signatures in cancer and sepsis samples through multiplex profiling. *The Journal of Immunology*. https://doi.org/10.4049/jimmunol.212.supp.0670.7911⏎

Gimenez-Dejoz, J., Tsuchiya, K., & Numata, K. (2019). Insights into the stereospecificity in papain-mediated chemoenzymatic polymerization from quantum mechanics/molecular mechanics simulations. *ACS Chemical Biology*. https://doi.org/10.1021/acschembio.9b00259⏎

Haleem, K., & Khan, M. A. (2021). A review on smart bioresponsive drug delivery systems. *Journal of Controlled Release*, 3(1), 1–12. https://doi.org/10.56770/jcp2019315⏎

Hashemi, S., Vosough, P., Taghizadeh, S., 1 Hashemi, S., Vosough, P., & Taghizadeh Savardashtaki, A. (2024). Therapeutic peptide development revolutionized: Harnessing the power of artificial intelligence for drug discovery. *Heliyon*, 10(22), e40265. https://doi.org/10.1016/j.heliyon.2024.e40265⏎

Helmecke, T., Hahn, D., Matzke, N., Ferdinand, L., Franke, L., Kühn, S., et al. (2022). Inflammation-Controlled anti-inflammatory hydrogels. *Advanced Science*, 10. https://doi.org/10.1002/advs.202206412⏎

Hu, X., Wang, Y., Zhang, L., Xu, M., & Jiang, T. (2020). Self-assembling peptide-based nanoprobes for tumor-targeted imaging. *Advanced Functional Materials*, 30(32), 2002939. https://doi.org/10.1002/adfm.202002939⏎

Hu, Y., Yu, J., Xu, M., & Pu, K. (2024). Bienzyme-locked activatable fluorescent probes for specific imaging of tumor-associated mast cells. *Journal of the American Chemical Society*. https://doi.org/10.1021/jacs.4c02070⏎

Israr, M., Padhi, S., Zhou, Y., & Wu, S. (2025). Recent advances in protein engineering and synthetic applications of amino acid transaminases. *ChemCatChem*, 17. https://doi.org/10.1002/cctc.202401952⏎

Ju, Q., Yuan, Y., Wu, C., Hu, Y., Zhou, S., & Luan, G. (2022). Heat-induced aggregation of subunits/polypeptides of soybean protein: Structural and physicochemical properties. *Food Chemistry*, 405 Pt A, 134774. https://doi.org/10.1016/j.foodchem.2022.134774⏎

Jumper, J., Evans, R., Pritzel, A., Green, T., Figurnov, M., Ronneberger, O., et al. (2021). Highly accurate protein structure prediction with AlphaFold. *Nature*, 596, 583–589. https://doi.org/10.1038/s41586-021-03819-2⏎

Keskin, D. B., Anandappa, A. J., Sun, J., Tirosh, I., Mathewson, N. D., Li, S., et al. (2019). Neoantigen vaccine generates intratumoral T cell responses in phase Ib glioblastoma trial. *Nature*, 565(7738), 234–239. https://doi.org/10.1038/s41586-018-0792-9⏎

Kobayashi, M., Onozawa, N., Matsuda, K., & Wakimoto, T. (2024). Chemoenzymatic tandem cyclization for the facile synthesis of bicyclic peptides. *Communications Chemistry*, 7. https://doi.org/10.1038/s42004-024-01147-w⏎

Lam, J., Lowry, W. E., & Carmichael, S. T. (2020). Hydrogel matrices for stem cell culture and regenerative medicine. *Advanced Healthcare Materials*, 9(15), 1901423. https://doi.org/10.1002/adhm.201901423⏎

Lee, M., Brown, J., Loas, A., & Pentelute, B. (2024). Investigation of commercially available resins for the automated flow synthesis of difficult or long peptide sequences. *Peptide Science*, 116. https://doi.org/10.1002/pep2.24344⏎

Li, J., Zheng, C., Cansiz, S., Wu, C., Xu, J., Cui, C., Liu, Y., Hou, W., Wang, Y., Zhang, L., Teng, I. T., Yang, H. H., & Tan, W. (2019). Self-assembled peptide nanostructures for functional materials. *Chemical Society Reviews*, 48(11), 2854–2872. https://doi.org/10.1039/C8CS00770H⏎

Li, Y., Zhang, X., & Wang, Z. (2024). Advances in peptide-based drug delivery systems. *Frontiers in Pharmacology*, 15, 123456. https://doi.org/10.3389/fphar.2024.123456⏎

Li, Y., Zhang, Z., Chen, J., & Wu, Q. (2022). Peptide-based molecular diagnostics: Current progress and future directions. *Biosensors and Bioelectronics*, 197, 113768. https://doi.org/10.1016/j.bios.2021.113768⏎

Liu, C., Chen, Y., & Mo, F. (2023). Transforming organic chemistry research paradigms: Moving from manual efforts to the intersection of automation and artificial intelligence. *Nature Synthesis*, 2(2), 123–135. https://doi.org/10.1002/nats.20230037⏎

Liu, X., Gao, M., & Bao, J. (2025). Precisely targeted nanoparticles for CRISPR-Cas9 delivery in clinical applications. *Nanomaterials*, 15. https://doi.org/10.3390/nano15070540⏎

Lu, L., Johnson, C., Khan, S., Irwin, M., & Pusztai, L. (2023). In silico designed mRNA vaccines targeting CA-125 neoantigen in breast and ovarian cancer. *Vaccine*. https://doi.org/10.1016/j.vaccine.2023.02.048⏎

Ma, T., Hou, Y., Zeng, J., Liu, C., Zhang, P., Jing, L., Shangguan, D., & Gao, M. (2018). Dual-ratiometric target-triggered fluorescent probe for simultaneous quantitative visualization of tumor microenvironment protease activity and pH in vivo. *Journal of the American Chemical Society*, 140(1), 211–218. https://doi.org/10.1021/jacs.7b08900⏎

Majekodunmi, T., Britton, D., & Montclare, J. (2024). Engineered proteins and materials utilizing residue-specific noncanonical amino acid incorporation. *Chemical Reviews*, 124, 9113–9135. https://doi.org/10.1021/acs.chemrev.3c00855⏎

Malam, Y., Loizidou, M., & Seifalian, A. M. (2009). Liposomes and nanoparticles: Nanosized vehicles for drug delivery in cancer. *Trends in Pharmacological Sciences*, 30(11), 592–599. https://doi.org/10.1016/j.tips.2009.07.004⏎

McGowan, J., Vig, P., & Bidwell, G. (2015). Cell penetrating peptide biopolymers for drug delivery to the Central nervous system. *The FASEB Journal*, 29. https://doi.org/10.1096/fasebj.29.1_supplement.834.2⏎

Mengstie, M. (2022). Viral vectors for the in vivo delivery of CRISPR components: Advances and challenges. *Frontiers in Bioengineering and Biotechnology*, 10. https://doi.org/10.3389/fbioe.2022.895713⏎

Min, K. H., Kim, K. H., & Pack, S. P. (2025). Two-dimensional materials for biosensing: Emerging bio-converged strategies for wearable and implantable platforms. *Chemosensors*, 13(6), 209. https://doi.org/10.3390/chemosensors13060209⏎

Morris, C., Glennie, S. J., Lam, H. S., Baum, H. E., Kandage, D., & Collier, J. H. (2019). A modular vaccine platform combining self-assembled peptide cages and immunogenic peptides. *Advanced Functional Materials*, 29(8), 1807357. https://doi.org/10.1002/adfm.201807357⏎

Orlov, A., Pushkarev, A., Znoyko, S., Novichikhin, D., Bragina, V., Gorshkov, B., et al. (2020). Multiplex label-free biosensor for detection of autoantibodies in human serum: Tool for new kinetics-based diagnostics of autoimmune diseases. *Biosensors & Bioelectronics*, 159, 112187. https://doi.org/10.1016/j.bios.2020.112187⏎

Picois, N., Boutahri, Y., Milbeo, P., Zanato, C., Lensen, N., Chaume, G., & Brigaud, T. (2024). Asymmetric α-fluoroalkyl-α-amino acids: Recent advances in their synthesis and applications. *Molecules*, 29. https://doi.org/10.3390/molecules29061408⏎

Pirhaghi, M., Mamashli, F., Moosavi-Movahedi, F., Arghavani, P., Amiri, A., Davaeil, B., et al. (2024). Cell-penetrating peptides: Promising therapeutics and drug-delivery systems for neurodegenerative diseases. *Molecular Pharmaceutics*. https://doi.org/10.1021/acs.molpharmaceut.3c01167⏎

Qian, Z., LaRochelle, J. R., Jiang, B., Lian, W., Hard, R. L., Selner, N. G., Luechapanichkul, R., Barrios, A. M., & Pei, D. (2016). Early endosomal escape of a cyclic cell-penetrating peptide allows effective cytosolic cargo delivery. *Biochemistry*, 55(17), 2541–2551. https://doi.org/10.1021/acs.biochem.6b00062⏎

Ramakrishna, S., Kwaku Dad, A. B., Beloor, J., Gopalappa, R., Lee, S. K., & Kim, H. (2014). Gene disruption by cell-penetrating peptide-mediated delivery of Cas9 protein and guide RNA. *Genome Research*, 24(6), 1020–1027. https://doi.org/10.1101/gr.171264.113⏎

Rathore, A. S., & Winkle, H. (2009). Quality by design for biopharmaceuticals. *Nature Biotechnology*, 27(1), 26–34. https://doi.org/10.1038/nbt0109-26⏎

Regato-Herbella, M., Mantione, D., Blachman, A., Gallastegui, A., Calabrese, G., Moya, S., Mecerreyes, D., & Criado-Gonzalez, M. (2024). Multiresponsive 4D printable hydrogels with anti-inflammatory properties. *ACS Macro Letters*, 13, 1119–1126. https://doi.org/10.1021/acsmacrolett.4c00404⏎

Saha, G. C., Eni, L. N., & Saha, H. (2023). Artificial intelligence in pharmaceutical manufacturing: Enhancing quality control and decision making. *Rivista Italiana di Filosofia e Analisi, Junior*, 14(2), 116–126.⏎

Savvateeva, E., Yukina, M., Nuralieva, N., Filippova, M., Gryadunov, D., & Troshina, E. (2021). Multiplex autoantibody detection in patients with autoimmune polyglandular syndromes. *International Journal of Molecular Sciences*, 22. https://doi.org/10.3390/ijms22115502⏎

Sharma, A., Kumar, A., De La Torre, B., & Albericio, F. (2022). Liquid-phase peptide synthesis (LPPS): A third wave for the preparation of peptides. *Chemical Reviews*. https://doi.org/10.1021/acs.chemrev.2c00132⏎

Shmool, T., Martin, L., Matthews, R., & Hallett, J. (2022). Ionic liquid-based strategy for predicting protein aggregation propensity and thermodynamic stability. *JACS Au*, 2, 2068–2080. https://doi.org/10.1021/jacsau.2c00356⏎

Skwarczynski, M., & Toth, I. (2020). Peptide-based subunit nanovaccines for the induction of T-cell immunity: Going beyond antibody generation. *Molecular Pharmaceutics*, 17(3), 780–786. https://doi.org/10.1021/acs.molpharmaceut.9b01107⏎

Smith, L., & Jones, R. (2022). Advances in solid-phase peptide synthesis: Automation and microwave-assisted methods. *Peptide Science*, 114(3), e24200. https://doi.org/10.1002/psc.24200⏎

Sondag, D., Heming, J., Löwik, D., Krivosheeva, E., Lejeune, D., Van Geffen, M., et al. (2023). Solid-phase synthesis of caged luminescent peptides via Side chain anchoring. *Bioconjugate Chemistry*, 34, 2234–2242. https://doi.org/10.1021/acs.bioconjchem.3c00381⏎

Thakur, N., Qureshi, A., & Kumar, M. (2021). Peptide-based biosensors for cancer detection: A review. *Analytical Biochemistry*, 617, 114118. https://doi.org/10.1016/j.ab.2021.114118⏎

U.S. Food and Drug Administration. (2023). *Collaborative Approaches to Advance Peptide Drug Development*. U.S. Food and Drug Administration. https://www.fda.gov/drugs/development-approval-process-drugs/collaborative-initiatives⏎

Uzoeto, H., Cosmas, S., Bakare, T., & Durojaye, O. (2024). AlphaFold-latest: Revolutionizing protein structure prediction for comprehensive biomolecular insights and therapeutic advancements. *Beni-Suef University Journal of Basic and Applied Sciences*. https://doi.org/10.1186/s43088-024-00503-y⏎

Vitalis, C., & Wenzel, T. (2023). Leveraging interactions in microfluidic droplets for enhanced biotechnology screens. *Nature Communications*, 14(1), 134. https://doi.org/10.1038/s41467-023-35190-3⏎

Wang, S., Liu, Y., Sun, Q., Zeng, B., Liu, C., Gong, L., et al. (2023). Triple cross-linked dynamic responsive hydrogel loaded with selenium nanoparticles for modulating the inflammatory microenvironment via PI3K/Akt/NF-κB and MAPK signaling pathways. *Advanced Science*, 10. https://doi.org/10.1002/advs.202303167⏎

Wehling, P., Evans, C. H., & Dalemans, W. (2021). Bridging science and industry: The future of translational medicine in peptide therapeutics. *Drug Discovery Today*, 26(10), 2445–2453. https://doi.org/10.1016/j.drudis.2021.06.005⏎

Wei, Y., Bao, H., Wang, L., Cao, Y., Liu, Z., & Zhu, X. et al (2024). Cell-penetrating peptides-mediated therapeutic agents delivery into the Central nervous system. *ACS Materials Letters*. https://doi.org/10.1021/acsmaterialslett.4c00397⏎

World Health Organization. (2022). *Global Strategy on Digital Health 2020–2025*. World Health Organization. https://www.who.int/publications/i/item/9789240020924⏎

Wu, C., Chang, C., Chou, P., Chan, X., Huang, C., Yang, Y., et al. (2024). Multiplexed immunoassay for a serum autoantibody biomarker panel in diagnostic and prognostic prediction of canine

mammary tumors. *The Veterinary Quarterly*, 45, 1–12. https://doi.org/10.1080/01652176.2024.2435978⏎

Wu, J., Wang, Y., Cai, W., Chen, D., Peng, X., Dong, H., et al. (2024). Ribosomal translation of fluorinated non-canonical amino acids for de novo biologically active fluorinated macrocyclic peptides. *Chemical Science*, 15, 13889–13898. https://doi.org/10.1039/d4sc04061a

Xin, Y., Yin, M., Zhao, L., Meng, F., Luo, L., & Yin, J. (2023). Peptide-based molecular imaging agents for cancer detection and diagnosis. *Advanced Drug Delivery Reviews*, 196, 114746. https://doi.org/10.1016/j.addr.2022.114746⏎

Yan, R., Hu, Y., Liu, F., Wei, S., Fang, D., Shuhendler, A., et al. (2019). Activatable NIR Fluorescence/MRI bimodal probes for in vivo imaging by enzyme-mediated fluorogenic reaction and self-assembly. *Journal of the American Chemical Society*. https://doi.org/10.1021/jacs.9b03649⏎

Yang, Z., Zeng, X., Zhao, Y., & Chen, R. (2023). AlphaFold2 and its applications in the fields of biology and medicine. *Signal Transduction and Targeted Therapy*, 8. https://doi.org/10.1038/s41392-023-01381-z⏎

Yin, H., Kauffman, K. J., & Anderson, D. G. (2017). Delivery technologies for genome editing. *Nature Reviews Drug Discovery*, 16(6), 387–399. https://doi.org/10.1038/nrd.2016.280⏎

Yu, J., Liu, J., Li, C., Huang, J., Zhu, Y., & You, H. (2024). Recent advances and applications in high-throughput continuous flow. *Chemical Communications*, 60(26), 3217–3225. https://doi.org/10.1039/D3CC06180A⏎

Zhang, D., Huang, J., & Hu, J. (2022). Improved diagnosis of type-1 diabetes mellitus using multiplexed autoantibodies ELISA array. *Analytical Biochemistry*, 649, 114722. https://doi.org/10.1016/j.ab.2022.114722⏎

Zheng, B., Wang, X., Guo, M., & Tzeng, C.-M. (2025). Therapeutic peptides: Recent advances in discovery, synthesis, and clinical translation. *International Journal of Molecular Sciences*, 26(11), 5131. https://doi.org/10.3390/ijms26115131⏎

Zheng, Y., Ye, R., Gong, X., Yang, J., Liu, B., Xu, Y., et al. (2023). Iontophoresis-driven microneedle patch for the active transdermal delivery of vaccine macromolecules. *Microsystems & Nanoengineering*, 9. https://doi.org/10.1038/s41378-023-00515-1⏎

Zheng, Z., Zhu, R., Peng, I., Xu, Z., & Jiang, Y. (2024). Wearable and implantable biosensors: Mechanisms and applications in closed-loop therapeutic systems. *Journal of Materials Science & Technology*. https://doi.org/10.1002/tb.2024⏎

Zhou, K., & Obexer, R. (2024). Non-Canonical amino acids for engineering peptides and proteins with new functions. *Israel Journal of Chemistry*. https://doi.org/10.1002/ijch.202400006⏎

13 Involvement of Polypeptides in Personalized Medicine

The Future Endorsement and Challenges

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13.1 INTRODUCTION

The polypeptides have a great impact on diagnostics and targeting diseases. Polypeptides have a great structure and high stability compared with other biological hormones. Designing the structure of peptides or polypeptides is warranted for more specific targeting and higher stability (Akan and Ünal, 2020). Polypeptides can be conjugated with polyethylene glycol (PEG) to improve the circulation and ease of conjugation. Moreover, polypeptides can be capped on nanoparticles for specific targeting and can be loaded in polymeric nanoparticles, and liposomes for longer circulation and controlled release drug delivery (Feng et al., 2021; Lin et al., 2019).

Historically, polypeptides are ideal candidates for treating different diseases such as cancer, diabetes mellitus, and hypertension. They can be created or synthesized using different technologies, such as human or animal

extraction, DND recommended technology, and solid-phase synthesis (Kocar et al., 2015; Wang et al., 2022). Also, polypeptides can be labeled with fluorescence materials or radioactive materials which can be used as bioimaging in scintigraphy (Wang et al., 2022).

Polypeptides are regarded as a unique formulation that can reach the site of action efficiently. To increase the stability and shelf life, various formulation techniques, conjugation with polymer, and physicochemical characteristics are taken into account (Cote-Cyr and Bourgault, 2025; Xu et al., 2025). This chapter discusses the function of polypeptides in personalized medicine, diagnostics, and history. Talk about the impact of conjugation, diagnostics, and modification techniques.

13.2 HISTORY

Throughout history, most peptide medications initially developed were derived from animals. These animals included amphibians, reptiles, arachnids, creatures called gastropods, and venomous mammals (De Oliveira et al., 2023). When peptide synthesis technology emerged in the 1950s, the field of peptides advanced rapidly. First-ever oxytocin and vasopressin synthesis by Vincent Du Vigneaud (Du Vigneaud et al., 1958; Lau and Dunn, 2018; Vigneaud et al., 1953). Furthermore, Robert Merrifield used a solid-phase method to synthesize peptides (Henninot et al., 2018), which enabled large-scale polypeptide production and approvals, such as goserelin for cancer targeting (Bolla et al., 1997), and enfuvirtide for HIV targeting in 2003 (Fung and Guo, 2004; Matthews et al., 2004).

Peptide development research advances rapidly. Eli Lilly released tirzepatide, the first dual insulinotropic polypeptide, or GIP, and GLP-1 RA for weight reduction and glycemic control (Nov 2023. In SURPASS phase III studies, it outperformed dulaglutide and semaglutide (France and Syed,

2024). Furthermore, the first oral anti-diabetic glucagon-like peptide-1 receptor agonist (GLP-1RA), semaglutide, for type 2 diabetes mellitus (T2DM) and weight loss was approved in 2019 (Furusawa et al., 2024; Qin et al., 2024). Also, somatostatin hormone and its analogues are synthesized as radiopharmaceuticals, such as [^{68}Ga]GaDOTA-TOC, which are used for diagnosing diseases that express somatostatin receptor-positive, e.g., neuroendocrine tumors (NETs) (Vahidfar et al., 2022).

In 1922, Dr. Frederick Banting and collaborators extracted insulin for first time from animals, and then they applied it to treat type 1 diabetes (Mathieu et al., 2024). From that time up to today, peptides have been used as therapeutic agents for treating various diseases. Furthermore, peptides play a crucial role in many human physiological functions, helping as antimicrobials, hormones, neurotransmitters, targeting ligands, growth factors, and vaccines, among other functions (Xiao et al., 2025a). Additionally, peptide-based subunits were used to improve the vaccine specificity, efficacy, safety, and quality control compared to traditional vaccines which used whole pathogens as vaccines, moving vaccine development from empirical to defined subunit (Hou et al., 2023).

Figure 13.1 summarizes the approved polypeptides from 1922s to 2023s. The figure confirms that the approval increased year after year. Moreover, there are a lot of functions for polypeptides for various and promising targeting activities. Polypeptides can reach and target different areas in the body. The figure also summarizes the cell penetrating peptides, all drugs related to; diabetes mellitus, tumors, migraine, cardiovascular diseases, COVID-19, and various infections.

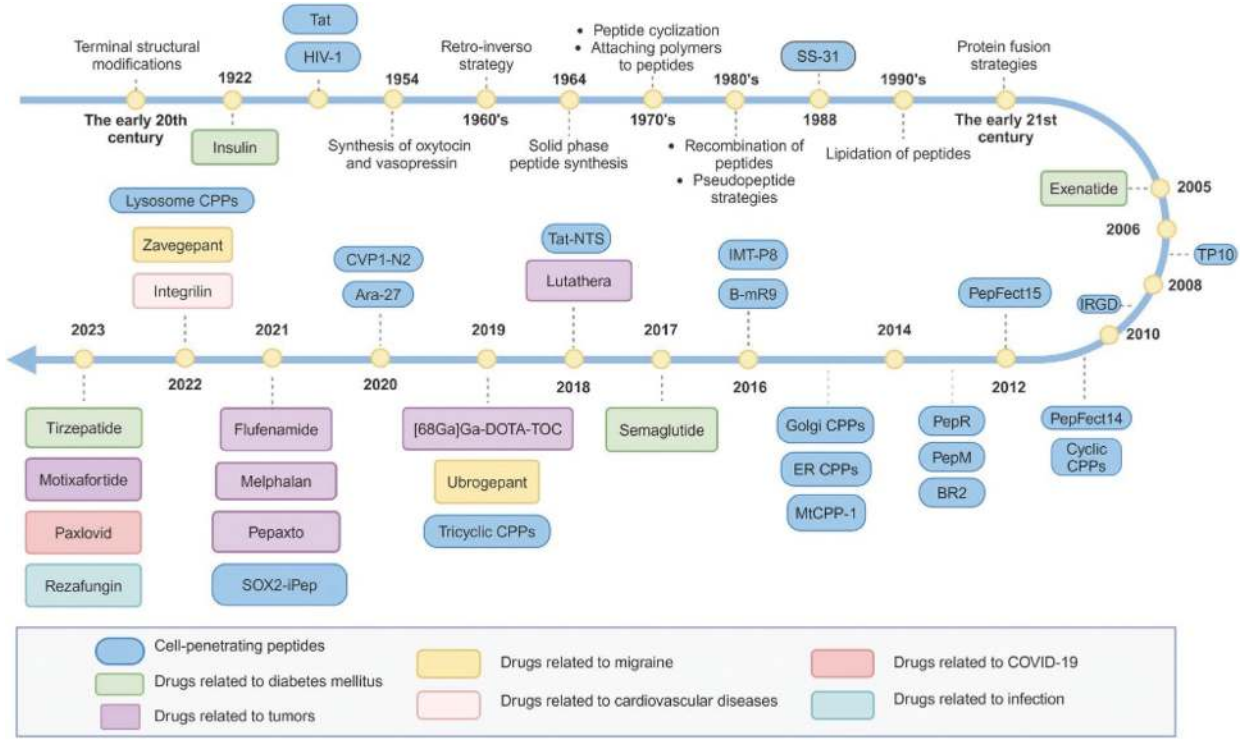

FIGURE 13.1 Illustration for the development and synthesis of various polypeptides and correlated drugs since the 20th to the 21st century. The 20th century showed the early and new developments of polypeptides such as insulin. The highlighted light Blue indicates the cell-penetrating peptides. The colored pink lines indicated the drugs related to diabetes mellitus. The green highlighted color indicated the drugs related to cancers. The light-yellow color is related to drugs used to treat migraines. The red lines are related to drugs used to treat cardiovascular issues, and the orange color is related to COVID-19. Furthermore, there are specific drugs used to treat diabetes mellitus type II, such as Exenatide and Semaglutide. (This figure was taken from Xiao et al. (Xiao et al., 2025b).) ⏎

Furthermore, peptides offer various advantages in comparison to small molecules. Peptides exhibit sensitive specificity compared to small-molecule drugs (Hummel et al., 2006). Moreover, the peptides degrade in the body to amino acids, thus diminishing the probability of systemic toxicity (Loffet, 2002). Additionally, peptides are easily accumulated in human tissues because of their short-lived half-life (Kwon et al., 2020). Peptides with

larger molecular dimensions and higher conformational flexibility than small-molecule medicines. Furthermore, monoclonal antibodies are considered a class of protein-protein interactions (PPI inhibitors). In comparison to hormones and monoclonal antibodies, peptides present superior cellular uptake and affinity for the positively expressing receptors (Anand et al., 2023).

Since insulin was invented over a century ago, nearly 100 peptide medications have been approved to treat a range of ailments, including deadly ones like cancer and HIV. Compared to delivery mechanisms and vaccinations, peptide-based medication research has gained focus. More research is focused on refining and upgrading these medications to overcome their physicochemical qualities and other flaws. Peptides, a novel treatment for many ailments, give sufferers hope. Peptides provide varied drug delivery and therapy benefits in different systems. Despite promising peptide-based therapeutic research, species variations, production technology, cost, and other considerations make it challenging to translate these findings into clinical success (Xiao et al., 2025b).

13.3 PEPTIDE-BASED DRUG MARKET AND CLINICAL TRIAL

Over the past two decades, the number of approved peptide drugs has surpassed 60, with more drugs undergoing global approval processes. Based on the currently approved peptide drugs, the majority belong to the agonist category, and the most targeted indications are associated with endocrinology, metabolism, and oncology (Xiao et al., 2025b).

Peptide treatments prevent and treat chronic diseases; hence, the estimated rise in chronic disease prevalence will drive the peptide therapy market throughout the forecast period. According to first-half 2023 sales

statistics (Table 13.1), semaglutide led global peptide medication sales ($90.217 billion). GLP-1 RA dulaglutide ($37.896 billion) followed closely, and the top 8 medications by sales all treat diabetes. Carfilzomib, a proteasome inhibitor, is used for relapsed or refractory multiple myeloma. Romiplostim, an immune thrombocytopenia therapy, placed 10th. In 2023, semaglutide ($38.6 billion) became the top drug among the top 25 highest-spending drugs in the U.S. This shows how important peptide medicines are in the pharmaceutical industry (Tichy et al., 2024).

TABLE 13.1

Peptide-Based Drugs that Are Registered in Clinical Phase III.

R&D Code	Name	Target	Indications Route of administration	Clinical Trial ID
ACT-1	Granexin	GJA1	Diabetic Foot Ulcers (DFUs)	NCT02667327
Topical-gelling agent				
CJC-1134-PC	Albenatide	GLP-1R	T2DM s.c.	CTR20202681
-	Ambervin	IL-6	COVID-19 i.m.	NCT05656495
FE 203799	Apraglutide	GLP-2 R	Short Bowel Syndrome (SBS) s.c.	NCT05018286 -
AM-833	Cagrilintide	CGRP R	T2DM s.c.	NCT05669755
TD-1792	Cefilavancin	Antibiotic	Bacterium infection i.v.	NA
DD-04107	DD-04107	synaptotagmin-1	Neuropathic pain	EUCTR2022-001374-60-ES
Topical lotion, s.c.				

R&D Code	Name	Target	Indications Route of administration	Clinical Trial ID
XW-003	Ecnoglutide	GLP-R	Weight management s.c.	NCT05813795
XW-003	Ecnoglutide	Thyroid Hormone	Type 2 diabetes s.c.	NCT05680155
Receptor				
AZP-3601	Eneboparatide	PTH-1R	Hypoparathyroidism s.c.	NCT05778071
MK-0616	Elicitide chloride	PCSK9	Arteriosclerosis p.o.	NCT06008756
FE-106483	Fedovapagon	V2R	Nocturia p.o.	NCT02637960
VA-106483				
ZP-1848	Glepaglutide	GLP-R	Short bowel syndrome s.c.	EUCTR2020-005502-25-NL
NN1535	Insulin icodec	INSR	T2DM s.c.	NCT05259033
JTA-004	JTA-004	unknown	Osteoarthritis	EUCTR2019-000796-16-CZ
Intra-articular injection				
AZP-531	Livoletide	ghrelin	Prader-Willi syndrome s.c.	EUCTR2018-003062-13-NL
LP-17/LR-12/LR-17/	Nangibotide	TREM1	COVID-19, Virus identified i.v.	EUCTR2020-001504-42-ES
TREM-1 inhibitors				
NA-1	Nerinetide	PSD-95	Acute ischemic stroke i.v.	EUCTR2020-002360-30-NL

R&D Code	Name	Target	Indications Route of administration	Clinical Trial ID
MSI-78	Pexiganan	Antibiotic	Type2 diabetes Emulsions	NCT01594762
PL-5	Peceleganan	Antibiotic	Diabetic foot ulcer Aerosol	CTR2300071255
BIM-28131 BIM-28163	Relamorelin	Ghrelin Receptor/GHSR	Diabetic gastroparesis s.c.	EUCTR2017-002177-20-RO
P2K	Remedisc	TGF-β1	Degenerative disc disease	NCT05516992 PENIEL 2K, PENIEL-2000
LY 3437943	Retatrutide	Glucagon R	T2DM s.c.	NCT05929079 rE-4
Exenatide-4	GLP-1R	Type2 Diabetes s.c.	NCT03239119 PTG-300	
Rusfertide	Hepc	Anemia Polycythemia vera s.c.	NCT06033586	
SNP ACTH (1-39)	SNP-ACTH9 (1-39)	ACTH receptor	Idiopathic membranous nephropathy	NCT05696613
SNP-ACTH	Primary Membranous Nephropathy s.c.			
-	SR-0379	Akt-1\Class I-PI3K\ mTOR	Skin ulcer	JRCT2031210266
Transdermal drug delivery				
CB-183315	Surotomycin	unknown	Infection	EUCTR2012-000252-34-AT CB-315
BI-456906	Survodutide	Glucagon R	T2DM s.c.	NCT06066528
MIM-D3	Tavilermide	Trk	Dry eye syndromes	NCT05848128

R&D Code	Name	Target	Indications Route of administration	Clinical Trial ID
Eye drops				
RGN-259	Timbetasin	thymosin beta 4	Dry eye syndrome	NCT05555589
Eye drops				
NA 831	Traneurocin	GR	COVID-19	NCT04540185
Virus identified p.o.				
AMG 386	Trepanner	ANGPT1/ANGPT2	Ovarian cancer i.v.	NCT01281254
ANF-95-126	Ularitide	NPR1	Decompensated heart failure i.v.	EUCTR2006-002403-13-FI
ANP 95126				
ANP-95126				
AT-406	Deviant	IAP	Head and Neck Cancer p.o.	NCT05386550
DEBIO-1143				
YKYY-017	YKYY-017	SARS-COV-2 S protein	COVID-19	CTR2300075467
Virus identified				
Nebulized inhalation				
PN-2123555	JNJ-2113	IL-23R	Plaque Psoriasis p.o.	NCT06220604
PN-235				

Recently, more than 38 peptide drugs have been registered in phase III clinical trials and are expected to be approved by the FDA for the global market. Moreover, there is a direction of peptide-based drugs to be

formulated as vaccines for COVID-19, ophthalmology, weight management, and so on (Table 13.1).

Furthermore, there is a need to advance in polymer-based drug delivery systems, which have paved the way for developing polypeptides as smart delivery strategies. Unique properties of polymers, such as biocompatibility, biodegradability, and tunable release kinetics, are considered the most important parameters for good stability. These systems can provide precise control of drug delivery and can be tailored to obtain drug delivery that is constantly targeted, making it a valuable tool in PPT drug delivery (Griffiths and Godard, 1981; Valuev et al., 2008; Zhao et al., 2015). Figure. 13.2 illustrates the various types of polypeptides that can be used as carriers in NPs for the delivery. Understanding and using polypeptides in NP drug delivery can encourage innovation in precision medicine and treatments. Ligand-targeted delivery systems and stimuli-responsive drug carriers can release polypeptides at specific body areas, increasing their specificity. These innovations improve the polypeptides' therapeutic index and reduce off-target effects and patient side effects (Muro, 2012).

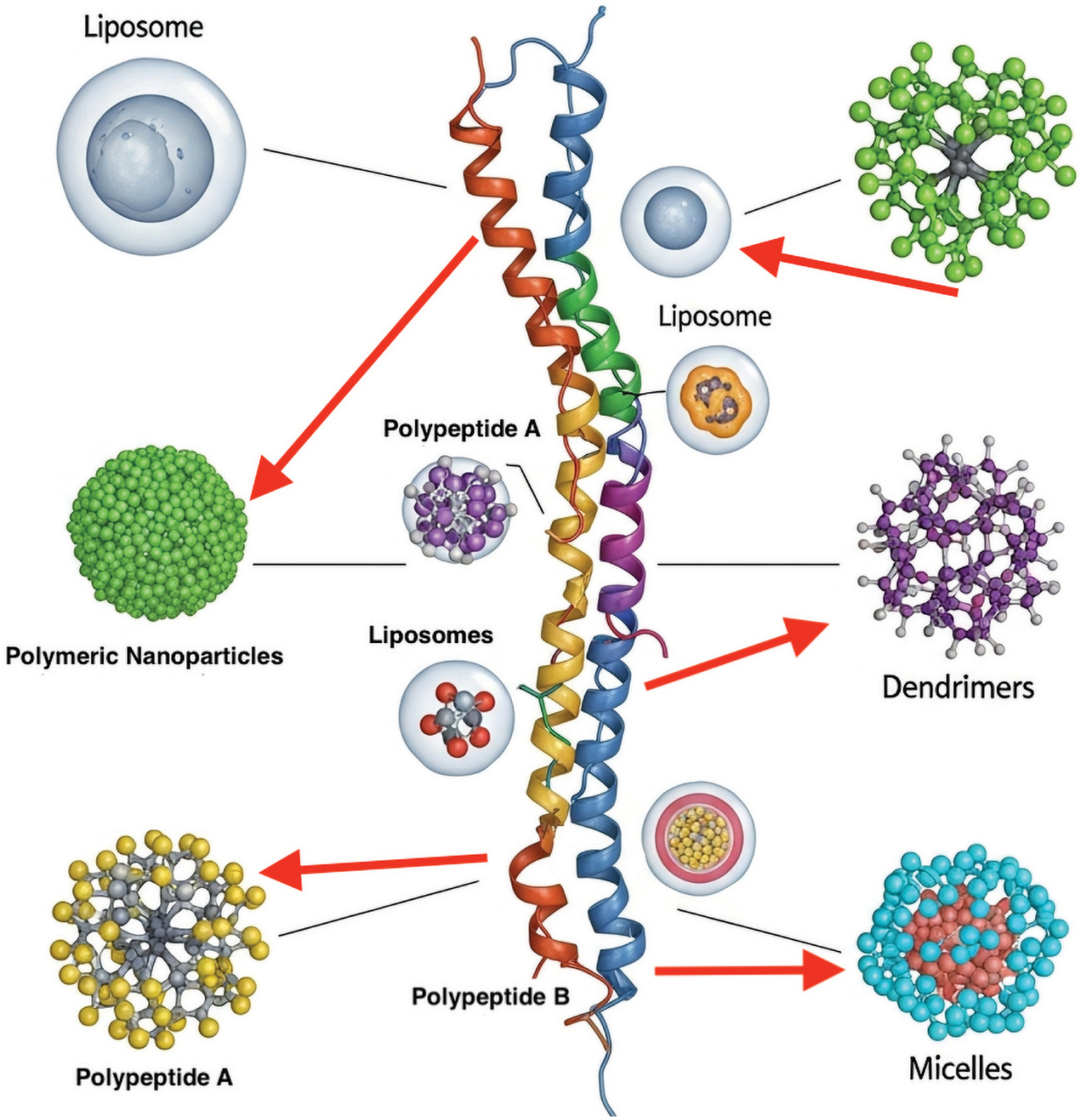

FIGURE 13.2 Various molecular structures and different types of polypeptides and nanoformulation. Elongated and twisted structures, helical shapes, which are spiral formations of polypeptides, and different conformations of polypeptides. Moreover, some examples of polypeptide structures, liposomes, dendrimers, and polymeric NPs a carrier. ⏎

Furthermore, the assembly of NPs from individual macromolecules can lead to significant changes in the physical and biological properties of the system, with some polymer systems having diameters more than 100 nanometers (Stepanova et al., 2023). Polymer particles are usually adopted in two size divisions. According to some sources, polymer NPs include structures up to 500 nm in diameter, while particles between 500 and 1000 nm are classified as submicron particles. In contrast, other sources define polymer NPs as covering the entire nanoscale range, including sizes up to 1000 nm (Prabha et al., 2016). Polymeric NPs have been produced and categorized, comprising different types of ligands such as peptides or polypeptides. Some cationic polymers, such as polyethyleneimine (PEI), are used for the delivery of nucleic acids; moreover, PEI presents a proton sponge effect for carrying polypeptides (Li et al., 2019). Although there are several obstacles in PPT medication delivery, they can be overcome. To fully use PPTs' therapeutic potential and enhance treatment results for a variety of illnesses, these issues must be resolved.

13.4 CHALLENGES IN PPT DRUG DELIVERY

The barriers to polypeptide-based therapeutics (PPTs) delivery are numerous. A higher molecular weight, size, increased enzyme vulnerability, and poor bioavailability are the major challenges that may undermine the effective delivery of PPTs to the active sites. Besides, the hydrophilicity of PPTs makes it very difficult to enter the cell wall, making the targeting of these molecules very challenging and thus making the development of new delivery systems imperative to improve the extent of therapy (Renukuntla et al., 2013; Shaji and Patole, 2008). However, off-target side effects can be reduced by accurately targeting PPTs to the target sites, and patients can be spared from adverse side effects (Guo et al., 2023; Shaji and Patole, 2008).

Also, the immunogenicity is another serious issue of PPT drug delivery. PPTs have the ability to stimulate the immune system, which could result in the generation of antibodies that could either be antigenic or potentiate their therapeutic action. In turn, new approaches are needed to reduce the immunogenicity induced by the peptide-based therapeutics (PPTs) and by the patterns, which are of the critical path to effective drug delivery (Ratanji et al., 2014). In addition to the challenges mentioned above, the lack of PPTs' stability in the biological fluids and short half-lives are also other challenges to effective delivery (Ibeanu et al., 2020). In a bid to overcome these problems, several strategies have been put forward that would increase the bioavailability and therapeutic utility of PPTs. They are the usage of nanocarriers, conjugation of ligands to targeted delivery, and developing stabilizing agents like immobilizing substances (Chehelgerdi et al., 2023).

13.5 ROLES OF POLYPEPTIDES IN PERSONALIZED MEDICINE

Polypeptides are reported to serve as ideal modules in targeted therapies, immunotherapy, gene therapy, and regenerative medicine (Frokjaer and Otzen, 2005). The specificity, the molecular structures, and biocompatibility make them ideal candidates for customized advanced formulations. Moreover, they have a specification that enables them to easily identify the patient-specific biomarkers, guiding them to be a good design for personalized medicine (Lu et al., 2025).

Furthermore, sequence modifications, conjugation techniques, delivery systems, and formulation optimization strategies facilitate the development of optimal polypeptide formulations. The sequence of modifications can be enhanced by tailoring the amino acids that conjugate, thereby improving binding and reducing immunogenicity (Fernandez et al., 2018). The

conjugation technique with PEG is also a good method for enhancing the stability and shelf life (Abdellatif et al., 2023; Younis et al., 2022). Furthermore, different types of nanoparticles, such as liposomes, polymeric, and nanogel formulations, can also improve the stability, targeted activity, and control the release. Also, pH, temperature, and additives are parameters that should be taken into consideration to improve the stability and shelf life of polypeptides (Abdellatif and Tawfeek, 2018; Abdellatif et al., 2021; Younis et al., 2022).

13.6 CONCLUSION

Polypeptides are a good candidate to make an evolution in personalized medicine. There are different ways that can improve the stability and durability of polypeptides, such as nanoformulations, conjugations, and physical and chemical properties, which affect the stability. Size and stability are also challenges that should be taken into consideration to improve the solubility and shelf life of formulations.

REFERENCES

Abdellatif, A. A. H., Alturki, H. N. H. & Tawfeek, H. M. 2021. Different cellulosic polymers for synthesizing silver nanoparticles with antioxidant and antibacterial activities. *Sci Rep*, 11, 84.

Abdellatif, A. A. H., Scagnetti, G., Younis, M. A., Bouazzaoui, A., Tawfeek, H. M., Aldosari, B. N., Almurshedi, A. S., Alsharidah, M., Rugaie, O. A., Davies, M. P. A., Liloglou, T., Ross, K. & Saleem, I. 2023. Non-coding RNA-directed therapeutics in lung cancer: Delivery technologies and clinical applications. *Colloids Surf B Biointerfaces*, 229, 113466.

Abdellatif, A. A. H. & Tawfeek, H. M. 2018. Development and evaluation of fluorescent gold nanoparticles. *Drug Dev Ind Pharm*, 44, 1679–1684.

Akan, A. E. & Ünal, F. 2020. Thin-layer drying modeling in the hot oil-heated stenter. *Int J Thermophys*, 41(8), Article 114.

Anand, U., Bandyopadhyay, A., Jha, N. K., Pérez De La Lastra, J. M. & Dey, A. 2023. Translational aspect in peptide drug discovery and development: An emerging therapeutic candidate. *Biofactors*, 49, 251–269.

Bolla, M., Gonzalez, D., Warde, P., Dubois, J. B., Mirimanoff, R.-O., Storme, G., Bernier, J., Kuten, A., Sternberg, C. & Gil, T. 1997. Improved survival in patients with locally advanced prostate cancer treated with radiotherapy and goserelin. *N Engl J Med*, 337, 295–300.

Chehelgerdi, M., Chehelgerdi, M., Allela, O. Q. B., Pecho, R. D. C., Jayasankar, N., Rao, D. P., Thamaraikani, T., Vasanthan, M., Viktor, P., Lakshmaiya, N., Saadh, M. J., Amajd, A., Abo-Zaid, M. A., Castillo-Acobo, R. Y., Ismail, A. H., Amin, A. H. & Akhavan-Sigari, R. 2023. Progressing nanotechnology to improve targeted cancer treatment: Overcoming hurdles in its clinical implementation. *Mol Cancer*, 22, 169.

Cote-Cyr, M. & Bourgault, S. 2025. Design and applications of self-assembled polypeptide matrices in wound healing. *Front Bioeng Biotechnol*, 13, 1646622.

De Oliveira, A. N., Soares, A. M. & Da Silva, S. L. 2023. Why to study peptides from venomous and poisonous animals? *Int J Pept Res Ther*, 29, 76.

Du Vigneaud, V., Gish, D. T., Katsoyannis, P. G. & Hess, G. P. 1958. Synthesis of the pressor-antidiuretic hormone, arginine-vasopressin. *J Am Chem Soc*, 80, 3355–3358.

Feng, X., Xu, W., Liu, J., Li, D., Li, G., Ding, J. & Chen, X. 2021. Polypeptide nanoformulation-induced immunogenic cell death and remission of immunosuppression for enhanced chemoimmunotherapy. *Sci Bull (Beijing)*, 66, 362–373.

Fernandez, L., Bustos, R. H., Zapata, C., Garcia, J., Jauregui, E. & Ashraf, G. M. 2018. Immunogenicity in protein and peptide based-therapeutics: An overview. *Curr Protein Pept Sci*, 19, 958–971.

France, N. L. & Syed, Y. Y. 2024. Tirzepatide: A review in type 2 diabetes. *Drugs*, 84, 227–238.

Frokjaer, S. & Otzen, D. E. 2005. Protein drug stability: A formulation challenge. *Nat Rev Drug Discov*, 4, 298–306.

Fung, H. B. & Guo, Y. 2004. Enfuvirtide: A fusion inhibitor for the treatment of HIV infection. *Clin Ther*, 26, 352–378.

Furusawa, S., Nomoto, H., Yokoyama, H., Suzuki, Y., Tsuzuki, A., Takahashi, K., Miya, A., Kameda, H., Cho, K. Y. & Takeuchi, J. 2024. Glycaemic control efficacy of switching from dipeptidyl peptidase-4 inhibitors to oral semaglutide in subjects with type 2 diabetes: A multicentre, prospective, randomized, open-label, parallel-group comparison study (SWITCH-SEMA 2 study). *Diabetes Obes Metab*, 26, 961–970.

Griffiths, B. W. & Godard, A. 1981. Studies on the subunit and polypeptide structure of a polymeric form of pregnancy-specific beta 1-glycoprotein. *J Reprod Immunol*, 3, 131–6.

Guo, C., Ma, X., Gao, F. & Guo, Y. 2023. Off-target effects in CRISPR/Cas9 gene editing. *Front Bioeng Biotechnol*, 11, 1143157.

Henninot, A., Collins, J. C. & Nuss, J. M. 2018. The current state of peptide drug discovery: Back to the future? *J Med Chem*, 61, 1382–1414.

Hou, Y., Chen, M., Bian, Y., Zheng, X., Tong, R. & Sun, X. 2023. Advanced subunit vaccine delivery technologies: From vaccine cascade obstacles to design strategies. *Acta Pharm Sin B*, 13, 3321–3338.

Hummel, G., Reineke, U. & Reimer, U. 2006. Translating peptides into small molecules. *Mol BioSyst*, 2, 499–508.

Ibeanu, N., Egbu, R., Onyekuru, L., Javaheri, H., Khaw, P. T., Williams, G. R., Brocchini, S. & Awwad, S. 2020. Injectables and depots to prolong drug action of proteins and peptides. *Pharmaceutics*, 12.

Kocar, V., Bozic Abram, S., Doles, T., Basic, N., Gradisar, H., Pisanski, T. & Jerala, R. 2015. TOPOFOLD, the designed modular biomolecular folds: Polypeptide-based molecular origami nanostructures following the footsteps of DNA. *Wiley Interdiscip Rev Nanomed Nanobiotechnol*, 7, 218–237.

Kwon, D., Kim, S.-M. & Correia, M. A. 2020. Cytochrome P450 endoplasmic reticulum-associated degradation (ERAD): Therapeutic and pathophysiological implications. *Acta Pharm Sin B*, 10, 42–60.

Lau, J. L. & Dunn, M. K. 2018. Therapeutic peptides: Historical perspectives, current development trends, and future directions. *Bioorg Med Chem*, 26, 2700–2707.

Li, B., Zhang, X. & Dong, Y. 2019. Nanoscale platforms for messenger RNA delivery. *Wiley Interdiscip Rev Nanomed Nanobiotechnol*, 11, e1530.

Lin, W., Ma, G., Yuan, Z., Qian, H., Xu, L., Sidransky, E. & Chen, S. 2019. Development of zwitterionic polypeptide nanoformulation with high doxorubicin loading content for targeted drug delivery. *Langmuir*, 35, 1273–1283.

Loffet, A. 2002. Peptides as drugs: Is there a market? *J Pept Sci*, 8, 1–7.

Lu, L., Lu, X. & Luo, W. 2025. Personalized cancer vaccines: Current advances and emerging horizons. *Vaccines*, 13, 1231.

Mathieu, C., Ahmed, W., Gillard, P., Cohen, O., Vigersky, R., De Portu, S. & Ozdemir Saltik, A. Z. 2024. The health economics of automated insulin delivery systems and the potential use of time in range in diabetes modeling: A narrative review. *Diabetes Technol Therap*, 26, 66–75.

Matthews, T., Salgo, M., Greenberg, M., Chung, J., Demasi, R. & Bolognesi, D. 2004. Enfuvirtide: The first Therapy to inhibit The entry of HIV-1 into host CD4 lymphocytes. *Nat Rev Drug Discov*, 3, 215–225.

Muro, S. 2012. Challenges in design and characterization of ligand-targeted drug delivery systems. *J Control Release*, 164, 125–37.

Prabha, S., Arya, G., Chandra, R., Ahmed, B. & Nimesh, S. 2016. Effect of size on biological properties of nanoparticles employed in gene delivery. *Artif Cells Nanomed Biotechnol*, 44, 83–91.

Qin, W., Yang, J., Deng, C., Ruan, Q. & Duan, K. 2024. Efficacy and safety of semaglutide 2.4 mg for weight loss in overweight or obese adults without diabetes: An updated systematic review and meta-analysis including the 2-year STEP 5 trial. *Diabetes Obes Metab*, 26, 911–923.

Ratanji, K. D., Derrick, J. P., Dearman, R. J. & Kimber, I. 2014. Immunogenicity of therapeutic proteins: Influence of aggregation. *J Immunotoxicol*, 11, 99–109.

Renukuntla, J., Vadlapudi, A. D., Patel, A., Boddu, S. H. & Mitra, A. K. 2013. Approaches for enhancing oral bioavailability of peptides and proteins. *Int J Pharm*, 447, 75–93.

Shaji, J. & Patole, V. 2008. Protein and peptide drug delivery: Oral Approaches. *Indian J Pharm Sci*, 70, 269–77.

Stepanova, M., Nikiforov, A., Tennikova, T. & Korzhikova-Vlakh, E. 2023. Polypeptide-based systems: From synthesis to application in drug delivery. *Pharmaceutics*, 15.

Tichy, E. M., Hoffman, J. M., Tadrous, M., Rim, M. H., Cuellar, S., Clark, J. S., Newell, M. K. & Schumock, G. T. 2024. National trends in prescription drug expenditures and projections for 2024. *Am J Health-Syst Pharm*, 81, 583–598.

Vahidfar, N., Farzanehfar, S., Abbasi, M., Mirzaei, S., Delpassand, E. S., Abbaspour, F., Salehi, Y., Biersack, H. J. & Ahmadzadehfar, H. 2022. Diagnostic value of radiolabelled somatostatin analogues for neuroendocrine tumour diagnosis: The benefits and drawbacks of [64Cu] Cu-DOTA-TOC. *Cancers*, 14, 1914.

Valuev, L. I., Sytov, G. A., Shanazarova, I. M., Valuev, I. L., Talyzenkov IU, A. & Plate, N. A. 2008. [Biodegradable polymeric derivatives of polypeptide drugs for targeting]. *Prikl Biokhim Mikrobiol*, 44, 246–249.

Vigneaud, V. D., Ressler, C., Swan, C. J. M., Roberts, C. W., Katsoyannis, P. G. & Gordon, S. 1953. The synthesis of an octapeptide amide with the hormonal activity of oxytocin. *J Am Chem Soc*, 75, 4879–4880.

Wang, L., Wang, N., Zhang, W., Cheng, X., Yan, Z., Shao, G., Wang, X., Wang, R. & Fu, C. 2022. Therapeutic peptides: Current applications and future directions. *Signal Transduct Target Ther*, 7, 48.

Xiao, W., Jiang, W., Chen, Z., Huang, Y., Mao, J., Zheng, W., Hu, Y. & Shi, J. 2025a. Advance in peptide-based drug development: Delivery platforms, therapeutics and vaccines. *Signal Transduct Target Ther*, 10, 74.

Xiao, W., Jiang, W., Chen, Z., Huang, Y., Mao, J., Zheng, W., Hu, Y. & Shi, J. 2025b. Advance in peptide-based drug development: Delivery platforms, therapeutics and vaccines. *Signal Transduct Target Ther*, 10, 74.

Xu, Q., Dai, Z., Peng, C., Yan, Q., Chen, X., Liu, T., Zhang, R., Huang, G., Liu, X. & Wang, J. 2025. Global research trends in signaling pathways of spasmolytic polypeptide-expressing metaplasia pathogenesis: A bibliometric analysis. *Front Oncol*, 15, 1597221.

Younis, M. A., Tawfeek, H. M., Abdellatif, A. A. H., Abdel-Aleem, J. A. & Harashima, H. 2022. Clinical translation of nanomedicines: Challenges, opportunities, and keys. *Adv Drug Deliv Rev*, 181, 114083.

Zhao, D., Li, B., Han, J., Yang, Y., Zhang, X. & Wu, G. 2015. PH responsive polypeptide based polymeric micelles for anticancer drug delivery. *J Biomed Mater Res A*, 103, 3045–3053.

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