A Review Article on Cell-Penetrating Peptides as Nanocarriers for Intracellular Drug Delivery

By

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A thesis submitted to the School of Pharmacy in partial fulfillment of the requirements for the degree of Bachelor of Pharmacy (B. Pharm.)

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Declaration

It is hereby declared that

1. The thesis submitted is my own original work while completing degree at Brac University.
2. The thesis does not contain material previously published or written by a third party, except where this is appropriately cited through full and accurate referencing.
3. The thesis does not contain material which has been accepted, or submitted, for any other degree or diploma at a university or other institution.
4. I have acknowledged all main sources of help.

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Approval

The thesis titled "A Review Article on Cell-Penetrating Peptides as Nanocarriers for Intracellular Drug Delivery" submitted by Name (ID) of Spring 2025 has been accepted as satisfactory in partial fulfillment of the requirement for the degree of Bachelor of Pharmacy.

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Ethics Statement

This project does not involve any kind of animal or human trial.

Abstract

Cell-penetrating peptides (CPPs) have emerged as a highly promising class of nanocarriers capable of overcoming cellular barriers to deliver therapeutic molecules intracellularly. CPPs, characterized by their cationic, amphipathic, or hydrophobic nature, facilitate efficient translocation across lipid bilayers, transporting diverse cargoes such as nucleic acids, proteins, and nanoparticles directly into cells. This review systematically explores the classification, structural characteristics, and uptake mechanisms of CPPs, emphasizing their conjugation with nanocarriers to enhance therapeutic efficacy. Significant applications of CPP-based systems across various biomedical fields, including cancer therapy, neurological disorders, and genetic diseases, are discussed. Despite their substantial potential, CPPs face critical challenges such as non-specific cellular uptake, endosomal entrapment, proteolytic instability, and potential cytotoxicity. Recent advancements addressing these issues through targeted design, stimuli-responsive constructs, and computational modeling demonstrate promising progress towards clinical translation. The review concludes with future perspectives on leveraging CPPs in RNA therapeutics, CRISPR gene editing, theranostics, and precision medicine.

Keywords: Cell-Penetrating Peptides, Nanocarriers, Intracellular Delivery, Endosomal Escape, Therapeutic Applications, Targeted Therapy, Nanomedicine, Drug Delivery Systems, CPP Stability, Clinical Translation.

Dedication

"To my beloved parents, thank you for your endless love, constant support, and the sacrifices you have made to shape my path. You deserve this accomplishment just as much as I do."

Acknowledgement

I am extremely grateful to Dr. Sabrina Sharmin for her invaluable guidance and ongoing support throughout this project. Her professionalism, constructive feedback, and ongoing support have helped to guide and influence the direction of this project. Her patience and understanding gave me the motivation to address some of the challenges I encountered during the course of my thesis.

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List of Acronyms

CPP – Cell-Penetrating Peptide

BBB – Blood-Brain Barrier

siRNA – Small Interfering RNA

mRNA – Messenger RNA

PEG – Polyethylene Glycol

CRISPR – Clustered Regularly Interspaced Short Palindromic Repeats

Cas9 – CRISPR-associated protein 9

HA2 – Hemagglutinin 2

ATP – Adenosine Triphosphate

CME – Clathrin-Mediated Endocytosis

PLGA – Poly-(lactic-co-glycolic acid)

MAP – Model Amphipathic Peptide

MRI – Magnetic Resonance Imaging

RGD – Arginine-Glycine-Aspartic Acid

AMPs – Antimicrobial Peptides

PCI – Photochemical Internalization

AI – Artificial Intelligence

ML – Machine Learning

Chapter 1

Introduction

Advanced drug delivery systems have a fundamental dilemma in the successful delivery of therapeutic molecules to intracellular targets. The selective permeability of cell membrane has posed a major challenge to most of the conventional therapeutic agents, which have to contend with great challenges in the effort to gain access to their targets in the intracellular compartments. Most hydrophilic drugs, big molecules including nucleic acids and proteins as well as several other synthetic compounds are in general incapable of crossing lipid bilayers on their own (Torchilin, 2006). This shortcoming seriously lowers the effectiveness of most potentially effective therapeutics, notably those that deal with intracellular receptors, enzymes, or genetic material. Therefore, the invention of powerful intracellular delivery processes has represented one of the main objectives of research in pharmaceutical sciences, nanotechnology, and molecular medicine.

Cell-penetrating peptides (CPPs) are one of those various strategies under exploration and have recently received significant attention as a type of delivery vector that can translocate through cellular membranes and deliver diverse bioactive cargoes internally into the cells. The cationic, amphipathic, or hydrophobic nature of CPPs usually short in length (5-30 amino acids) is what allows these elements to interact with lipid membranes, thereby enhancing access to the intracellular environment (Guidotti et al., 2017). CPPs deliver in cells easily and without the requirement of any receptors or energization-dependent present in the pathway, although endocytotic translocation and direct translocation pathways play a part, they are a versatile means of intracellular delivery.

This knowledge of CPPs originates back to the late 1980s and early 1990s when scientists discovered that outer membrane proteins (human immunodeficiency virus transactivator of

transcription protein (Tat)) and the Drosophila homeodomain protein Antennapedia (Antp) can carry cell-penetrating cargoes into cells in a way that is independent of endocytosis (Frankel & Pabo, 1988; Derossi et al., 1994). These discoveries provided the foundation upon which a larger category of peptides could be discovered and developed that could mediate transmembrane transport. Since that time, a wide range of CPPs have been characterized such as naturally occurring and synthetic and chimeric, with differing structural properties and cellular uptake efficiencies (Bechara & Sagan, 2013).

The fundamental process that CPPs mediate the entry process in the cell is not completely elucidated yet and the subject of current study. In a general manner, two substantial routes are used in CPPs to penetrate into the cell (direct penetration and endocytosis). Direct penetration refers to energy-dependent mechanisms like inverted micelle formation, pore formation or carpet like mechanisms but endocytic ways are their clathrin mediated endocytosis, caveolae mediated and macropinocytosis (Zorko & Langel, 2005). This pathway may vary depending on some factors such as the physicochemical characteristics of the peptide, structure and charge of the addition cargo, concentration of the peptide, and the nature of targets cells.

A cargo versatility is one of the most outstanding benefits of CPPs. CPPs have found application in the delivery of numerous therapeutics, including small molecule drugs and plasmid DNA, as well as siRNA, mRNA, CRISPRCas9 components, proteins, and imaging agents (Langel, 2019). Nanocarriers The CPPS may also be conjugated to nanoparticles like liposomes, dendrimers, polymeric nanoparticles, micelles, and metallic nanoparticles that greatly improve the internalization of a target cell by the system. In this perspective, CPPs are ligands or surface modifications that impart nanocarrier with the capability of targeting or translocating cell membranes and enhance cellular uptake as well as the bioavailability of therapeutics that are encapsulated or bound on the surface of nanocarriers (Trabulo et al., 2010).

Penetration peptide (CPP)-mediated delivery systems described within the context of modern therapeutic research proved to be rather effective in regard to treating a wide range of disease states. In oncology, e.g. CPPs have been used in these ways: as delivery vectors to target tumors with cytotoxic agents or gene-silencing moieties, targeting tumor cells and bypassing multidrug resistance or enabling tumor cells to scavenge the non-specific routes of absorption of many classical compounds. In neurosciences similar peptides have been evaluated, in crossing the blood-brain barrier (BBB) which became a century old stumbling block in drug delivery to the central nervous system. Similarly, CPPs form a potential avenue of intracellular correction of enzyme deficiencies or a gene-editing tool in view of genetic-metabolic diseases.

In spite of the propitious perspectives of cytotropic penetrating peptides (CPPs) to realize the targeted delivery therapy, a number of impediments still limit their clinical translation. Of primary importance is a lack of cellular specificity. Despite the high efficacy in intracellular delivery CPPs do not favor a particular subset of diseased cells or cell type, and have therefore a large distribution systemically and can have off-target toxicities, which is a particular concern in cancer therapy where healthy cell uptake leads to a negative result. Solutions to this limitation have been addressed by continuous research on stimulus directed CPPs, dual-targeting, and cleavable links which would realize the release of CPPs when triggered by PH change, redox signaling, or signaling pathways which are uniquely elevated in diseased tissue (Desale et al., 2015).

Intracellular trafficking and endosomal retention restrict transport of CPP-cargo complexes across biological membranes in an efficient transportation. Once endocytosed CPPs and their cargos can be trapped in endosomes, and subsequently broken down in lysosomes, thus becoming bio-inactive. In order to improve endosomal escape, various strategies have been embraced by the researchers and amongst them include: (1) fusion-promoting elements (i.e., fusogenic sequences favoring membrane fusion); (2) pH-sensitive domains destabilizing the

endosomal membrane; and (3) chemical modifications destabilizing endosomal membrane integrity (Liu et al., 2021).

Biological activity Cell-penetrating peptides (CPPs) operate on the certain capacity of cellular entry to transport intracellular cargo through the plasma membrane. Therefore, their in vivo stability and PK should be well determined. It is known through a large amount of data that most of the CPPs are highly leached by serum- and tissue-related proteases, something that limits their use in the body. In order to increase circulating lifetime, researchers took advantage of cyclization approach, replacement of D-amino acid and the use of PEGylation, with all three increasing stability and circulation time.

Close attention should also be accorded to immunogenic and toxicological considerations. In spite of the general view of CPPs to be non-immunogenic, their long-term effects and their interaction with the immune system remain uncomprehended in their entirety. Detailed preclinical and clinical trials are still necessary in order to gauge the suitability in terms of safety as well as offering improved designs in the application on human beings.

Peptide chemistry, molecular modeling, and high- throughput screening advances during the recent past have identified ways to rationally design future CPP that are more stable, selective and which exhibit better intracellular trafficking properties. Incorporation of such methods, with the new technologies of nanomedicine, such as RNA therapeutics, CRISPR, and customizing medication delivery, are being used in an effort to build on targeted therapy. CPPs thus are presently in a central platform in the innovation of new strategies of transition of extra cellular-therapeutic agents into intra cellular systems.

Cell-penetrating peptides (CPPs) have been one of the most diverse kinds of nanocarriers that hold the potential of revolutionizing intracellular drug delivery. These, in combination with other nanotechnological platforms, open the possibility of developing new therapeutic modes

applicable to providing better remedies to problems that have hitherto been thought to be intractable. The current review is an organized evaluation of the structural design of CPPs, the cellular entry mechanism employed by them and their medical implications as nanocarriers. At the same time, it outlines the dilemmas that prevail today with regard to clinical translation and presents future hopes.

Chapter 2

Methodology

An overall search was done using the following databases over the literature: PubMed, Google Scholar, ScienceDirect, and scopus. The key included papers published since 2010 up to 2024 were found by search terms as follows: cell-penetrating peptides, CPP-based nanocarriers, mechanisms of intracellular delivery, therapeutic usage of CPPs. The search criteria were relevant to the classification into CPPs, cellular uptake mechanisms, conjugating to nanocarriers, their therapeutic uses, and new technological development. A systematic review of articles was also carried out by all the titles and abstracts, followed by a full-text analysis to include in the review. Data extracted were synthesized to be thematic so that there is clarity and flow in discussing concerning advances in CPP, challenges and future perspectives. Furthermore, the research articles that present the computational modeling, new CPP designs, preclinical and clinical studies were rated critically in order to view rigorous picture of CPP-based designed delivery systems and their trans-latent potential.

Chapter 3

Classification and Structural Characteristics of Cell-Penetrating Peptides (CPPs)

Cell-penetrating peptides (CPPs) represent a non-homogenous group of short polypeptides which vary in sequence, origin of biosynthesis, physicochemical properties, and the format of not only their structures but also that of their conformations. It is necessary to have a systematic understanding of their typologies and structural characteristics so as to undertake a rational engineering and optimization of such scaffolds as platforms of drug delivery. The peptides can be systematically classified on the basis of their biological sources; on their physicochemical properties (like hydrophobicity, the charge and amphiphilicity); as well as on their propensity to adopt a certain secondary structure (viz. the 1-helix, the 8- strand, or no structure at all).

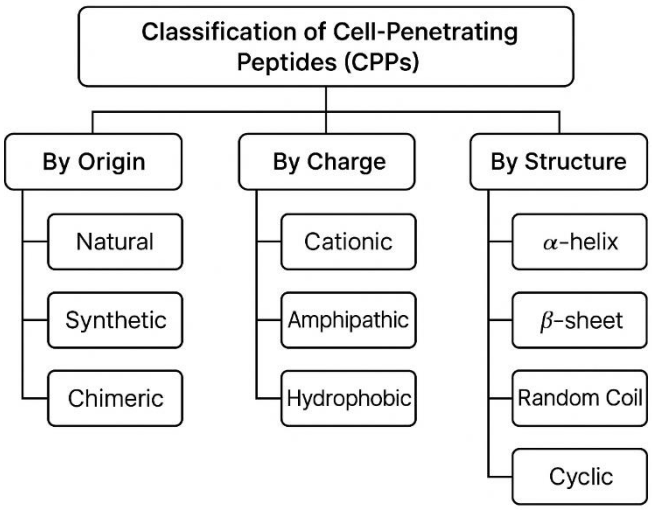

Fig 1: *Classification of Cell-penetrating Peptides (CPPs)*

3.1 Classification Based on Origin

3.1.1 Socio Economic and Financial Strain

One of the most ancient cationic candidate peptides that has been used is the one that is based on naturally occurring proteins that bear their own intrinsic translocation features. These include:

- Tat peptide of trans-activator of transcription (Tat) of HIV-1.
- Penetratin, derived from the Antennapedia homeodomain of Drosophila melanogaster.
- VP22, derived from herpes simplex virus.

The basic fact that certain peptides also harbor inherent membrane-translocating properties had been known to scientists long before and it was on the basis of this fact that the following cell-penetrating peptide family was discovered. The most prominent works that are dedicated to this field are the researches by Frankel and Pabo (1988) and Derossi et al. (1994).

3.1.2. Synthetic CPPs

Artificial cell-penetrating peptides (CPPs) are designed to have improved stability, lowered immunogenicity or an optimised cellular internalisation activity. Among the synthetic CPPs, it is possible to note:

- Polyarginines (e.g., R8, R9)
- Transportan and its derivatives

Their synthetic nature allows for greater control over charge density, amphipathicity, and functional conjugation (Futaki et al., 2001).

3.1.3. Chimeric and Engineered CPPs

In the scientific community, it is common to obtain sequence-engineered peptides that are constituted of specific functional domains of two or more cell-penetrating peptides (CPPs) or having extra modules attached to them, like nuclear localization signal or endocytic escape

motif to increase their targeting specificity as well as versatility of functionality. Examples include:

- Transportan 10: a shorter and more efficient version of Transportan
- PepFect and NickFect series: tailored for nucleic acid delivery (EL Andaloussi et al., 2007)

3.2. Classification Based on Physicochemical Properties

CPPs are also categorized by their overall charge and amphipathicity, which significantly influence their uptake mechanism and cargo compatibility.

3.2.1. Cationic CPPs

These are the most common and include sequences rich in basic amino acids, particularly arginine and lysine. Their positive charge facilitates electrostatic interaction with the negatively charged cell membranes and glycosaminoglycans (Bechara & Sagan, 2013). Examples:

- Tat (YGRKKRRQRRR)
- R8 or R9 (oligoarginines)
- Penetratin (RQIKIWFQNRRMKWKK)

3.2.2. Amphipathic CPPs

Amphipathic CPPs contain both hydrophilic (often charged) and hydrophobic segments. This duality allows them to insert into lipid bilayers more efficiently. They can be:

- Primary amphipathic: amphipathicity arises from the linear sequence (e.g., Model amphipathic peptide - MAP)

- Secondary amphipathic: form amphipathic helices upon interacting with membranes (e.g., Transportan)

3.2.3. Hydrophobic CPPs

These peptides contain nonpolar amino acids and rely on hydrophobic interactions for membrane passage. Although less commonly used, they show potential for low-toxicity delivery (Trabulo et al., 2010).

3.3. Structural Motifs and Conformations

CPPs may adopt different secondary structures, which influence their membrane interactions and internalization mechanisms.

3.3.1. α-Helices

Many amphipathic CPPs adopt an α-helical conformation upon membrane interaction. This structure enhances their ability to insert into lipid bilayers and disrupt membrane integrity for translocation (Deshayes et al., 2005). Examples:

- Transportan
- MPG (a fusion of HIV gp41 and SV40 NLS)

3.3.2. β-Sheets and Random Coils

Some CPPs like Penetratin adopt β-sheet conformations, while others like Tat remain in random coil structures due to high arginine/lysine content (Wender et al., 2000).

3.3.3. Cyclic and Stapled Peptides

To improve proteolytic stability and enhance cellular uptake, cyclic or stapled CPPs have been developed. These structures increase rigidity and bioavailability in vivo (Fonseca et al., 2009).

3.4. Key Structural Features Affecting Function

Several recurring features contribute to CPP functionality:

- Arginine-rich domains enhance internalization via guanidinium-phosphate interactions with membranes.
- Hydrophobic residues facilitate bilayer interaction and insertion.
- Sequence length influences uptake efficiency; typically, CPPs range from 8 to 30 amino acids.
- Terminal modifications, such as acetylation or amidation, can improve stability and reduce immunogenicity (Milletti, 2012).

Chapter 4

Mechanisms of Cellular Uptake

The cell-penetrating peptides (CPPs) transportation through cell membranes by rigorously clarifying the mechanism needs to be done to maximize the usefulness of the CPPs as drugs-delivery vehicles. In spite of their propensity to universally penetrate membranes, CPPs do not intake a single venerable mechanism; activity is affected by parameters, which enlist the peptide sequence and the conformation, the concentration concentration, the cargo mix, the membrane composition, and the physiological micro-environment (Bechara & Sagan, 2013). At large, CPPs have two major entry pathways, which include: direct translocation pathway which uses very little or no energy and the endocytic pathway involving ATP. The post-internalization of the peptide, especially, the ability of the CPP to avoid the endocytic pathways, defines their final bioactivity and efficacy.

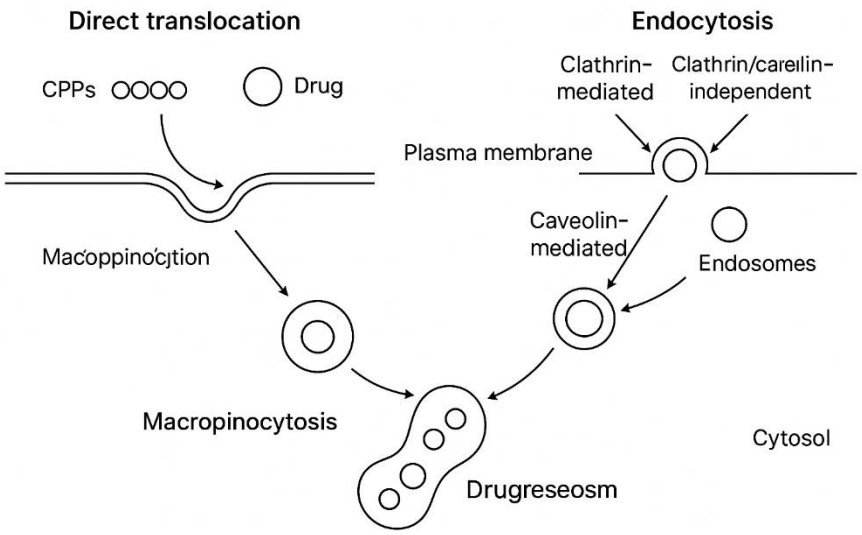

Fig 2: *Mechanisms of CPP-Mediated Cellular Uptake*

4.1. Direct Translocation (Energy-Independent Pathways)

Direct penetration refers to the non-endocytic uptake of CPPs, which occurs rapidly and does not require metabolic energy. This process is often observed at low temperatures or in ATP-depleted conditions, indicating independence from traditional endocytic machinery (Zorko & Langel, 2005).

4.1.1. Pore Formation Model

In this model, CPPs insert themselves into the membrane, forming transient pores through which cargo can pass. Arginine-rich peptides like oligoarginines can induce such structures due to the high positive charge density interacting with phospholipid head groups (Herce & García, 2007).

4.1.2. Carpet Model

Here, CPPs accumulate on the membrane surface in a detergent-like fashion until a threshold concentration is reached. At that point, they disrupt membrane integrity, allowing entry via diffusion. This mechanism is proposed for amphipathic peptides that can align parallel to the bilayer (Deshayes et al., 2005).

4.1.3. Inverted Micelle Model

CPPs and cargoes interact with lipids to form inverted micelles, encapsulating the complex and translocating it across the bilayer. This mechanism is less common but has been proposed for hydrophobic CPPs and hydrophobic cargoes (Madani et al., 2011).

Despite early support for direct translocation, many researchers now believe it is more prevalent under artificial conditions (e.g., peptide alone, high concentrations, absence of serum), and less significant for physiological delivery of large or hydrophilic cargoes (Nakase et al., 2012).

4.2. Endocytosis (Energy-Dependent Pathways)

For most CPP-cargo complexes, especially those involving large molecules or nanocarriers, endocytosis is the predominant route of internalization. This is an active, energy-requiring process and occurs via several distinct subtypes.

4.2.1. Clathrin-Mediated Endocytosis (CME)

CME is clear biological process in which the trans-Golgi clathrin-coated pits are generated and transport them to cytoplasm through which they internalize plasma membrane through endocytosis. When internalized, the cargo proteins housed in these endocytic vesicles are directed to early endosomes which when the escape mechanisms are not up to the mark, there is a possibility of routing to lysosomes (wise, 2014). It has been established that Tat and R9, derived by HIV-1 can be collected intracellularly through CME in a given circumstance (Futaki et al., 2001).

4.2.2. Caveolae-Mediated Endocytosis

The involved pathway is the one that concerns the flask-shaped membrane invaginations that are cholesterol and caveolin protein-enriched. Ordinarily this pathway plays an imperative role in non-degradative trafficking, does not go through the lysosomes and thus makes this pathway very beneficial when it comes to drug delivery. It is through this pathway that transportan and a few chimeric cationic peptides (CPPs) exploit the route (Ziegler, 2008).

4.2.3. Macropinocytosis

Macropinocytosis is an alternative form of endocytosis that is non-specific and involves ruffling of the membrane and also formation of large vesicles known as macropinosomes. It is most in the arginine-rich CPPs like Tat and R8 (Nakase et al., 2004). The process is an effective process of delivery of the cargo as it has the tendency to internalise large molecular complexes such as nanoparticles and proteins.

4.2.4. Other Pathways

The clathrin mediated and the caveolae mediated pathways have been well recognised yet little is understood about alternative, clathrin and caveolae independent means. It has been shown that these pathways can be promoted with the help of the lipid raft, the flotillin complex, and other process involving microdomains (Howl and Jones 2010).

4.3. Factors Determining Uptake Pathway

The pathway used by a CPP can vary depending on multiple variables:

- Peptide concentration: High levels of peptide often influence direct translocation, while low concentrations prefer endocytosis.
- Cargo size and type: Large cargoes or nanocarriers tend to use endocytic routes.

- Cell type and membrane composition: Different cells exhibit varying expression of endocytic receptors and lipid profiles.
- Presence of serum: Serum proteins can inhibit direct translocation, promoting receptor-mediated uptake (Trabulo et al., 2010).

Thus, uptake is often multimodal, with several pathways simultaneously contributing to internalization.

4.4. Endosomal Escape: An Important Step

Once inside the cell via endocytosis, CPPs and their cargoes must escape the endosome to exert therapeutic effects. Otherwise, they risk lysosomal degradation.

4.4.1. Fusogenic Peptides and Domains

Some CPPs are engineered with pH-sensitive fusogenic sequences (e.g., derived from viral peptides like HA2 from influenza) that destabilize endosomal membranes in acidic conditions (Akita et al., 2010).

4.4.2. Proton Sponge Effect

Polymers or peptide modifications (e.g., histidine-rich sequences) can buffer endosomal pH, inducing osmotic swelling and rupture of the vesicle—facilitating cytoplasmic release (Liu et al., 2021).

4.4.3. Redox-Sensitive or Enzyme-Cleavable Linkers

Disulfide bonds and other cleavable bonds enable cargo release in response to intracellular reductive environments or specific enzyme activity (Desale et al., 2015).

Improving endosomal escape remains a major challenge for CPP-based systems and is a key focus of ongoing research.

Chapter 5
CPP-Conjugated Nanocarrier Systems

CPPs contain the innate ability to self-insert in biological barrier and do it spontaneously; however, when integrated with nanocarrier structures, the therapeutic performance of CPPs is significantly promoted. Introduction of the CPP functionalities within nanoparticles imparts superior stability of structures besides providing a spatiotemporal control on the release of a wide range of payloads, where in addition to small-molecule therapeutics, proteins, nucleic acids and imaging probes (Trabulo et al., 2010). These hybrid conjugates overcome the drawbacks of either of its parts when those are used separately, i.e., low target specificity or poor membrane permeability. CPP-modified nanocarriers have therefore presented since the most crucial approach in development of next-generation drug-carriers.

5.1. Types of Nanocarriers Used with CPPs

CPPs have been conjugated to or encapsulated within various nanocarrier systems. Each type offers unique physicochemical advantages and compatibility with different therapeutic applications.

5.1.1. Liposomes

Liposomes are spherical vesicles composed of phospholipid bilayers. Their biocompatibility, cargo versatility, and capacity for surface modification make them a popular nanocarrier choice. When conjugated with CPPs, liposomes exhibit enhanced cellular uptake and improved endosomal escape (Torchilin, 2006). For example, CPP-modified PEGylated liposomes have been used to deliver doxorubicin and paclitaxel with increased tumor penetration (Torchilin et al., 2001).

5.1.2. Polymeric Nanoparticles

Biodegradable polymers such as PLGA (poly-(lactic-co-glycolic acid)), PEG, and chitosan are widely used in nanoparticle formulations. CPPs can be adsorbed or covalently linked to the surface, enabling efficient delivery of hydrophobic or hydrophilic drugs. In one study, Tat-conjugated PLGA nanoparticles significantly enhanced brain delivery of encapsulated drugs (Liu et al., 2008).

5.1.3. Dendrimers

Dendrimers are highly branched, nanoscale macromolecules with modifiable surface groups. CPP-dendrimer conjugates enable multivalent display of both therapeutic agents and targeting ligands. Their uniform size and high loading capacity make them attractive for gene and protein delivery (Kesharwani et al., 2015).

5.1.4. Micelles

Micelles are self-assembled structures formed by amphiphilic block copolymers. CPPs can be incorporated into their hydrophilic corona or conjugated on the surface. This system is particularly useful for poorly water-soluble drugs, offering both solubilization and membrane transport capabilities (Desale et al., 2015).

5.1.5. Inorganic Nanoparticles

Metallic and silica-based nanoparticles (e.g., gold, iron oxide, mesoporous silica) are valued for their imaging and theranostic potential. CPPs enhance cellular uptake of these particles for cancer imaging and drug delivery. CPP-modified gold nanoparticles have shown effective delivery of antisense oligonucleotides and siRNA (Rosi et al., 2006).

5.2. Surface Modification Strategies

Transformation of CPPs into nanocarrier systems could be carried out by various modes of chemical and physical strategies with respect to the aim of job launch, targeting, and consistency.

5.2.1. Covalent Conjugation

This is due to the fact that polycationic peptides can easily be immobilized via covalent binding on the surfaces of nanoparticles via maleimide, carbodiimide or clicks chemistry. Such conjugation makes possible high stability, inhibition of premature discharge of peptides, and installation of specific spatial orientation (Fonseca et al., 2009). What should not be tampered with in the process is the structural part as well as the functional part of the peptide in the membrane.

5.2.2. Electrostatic Complexation

The assembly of CPP and the negatively charged nanocarrier can be done simply, and reversibly, when the CPP is charged and the particle is negatively charged, e.g. when it is siRNA-loaded particle. This is especially common in the formulation of gene delivery complexes (Futaki et al., 2001). Nevertheless, such complexes may face aggregation or instability in serum unless further stabilized.

5.2.3. Physical Adsorption or Fusion Peptides

CPPs may be adsorbed onto the carrier surface via hydrophobic interactions or integrated as part of a fusion peptide. For example, peptides combining CPP sequences with targeting or endosomal escape domains can be genetically fused to therapeutic proteins or embedded within liposomal bilayers (Akita et al., 2010).

5.2.4. PEGylation and Multifunctional Coatings

Polyethylene glycol (PEG) is frequently used to prolong circulation time and reduce immune recognition. CPPs can be tethered to the end of PEG chains, or attached via cleavable linkers that release CPPs in the target environment (e.g., low pH or high glutathione levels), combining stealth properties with activatable delivery (Liu et al., 2021).

5.3. Benefits of CPP-Functionalized Nanocarriers

Combination of carrier systems with cell-penetrating peptides (CPPs) has already shown obvious benefits in terms of bringing the carrier to cells and efficacy:

- Increased cellular uptake: CPPs can enhance the translocation of nanocarriers across these plasma membranes and thus increase the delivery of the nanocarriers to non-phagocytic cells.
- Endosomal escape: some CPPs facilitate rupture of endosomal membranes, therefore, delivering cargo to the cytosol.
- Cargo flexibility: A variety of payloads of such systems comprise nucleic acids, proteins, and chemotherapeutics.
- Sustained/stimuli responsive release: Nanocarrier also provides sustained release or stimuli responsive release, reducing to a minimum the burst effects and thereby the side effects.
- Correction in the CPPs tissue penetration and biodistribution: CPPs promote an improvement of the tissue penetration of the target tissue, e.g., tumor penetration or the passage of blood-brain barrier, which is why the coverage of carriers can be made to inaccessible locations.
- Multifunctionality: Modular characteristics of nanocarriers allow loading imaging agents and drugs together and, therefore, enable theranostic purposes.

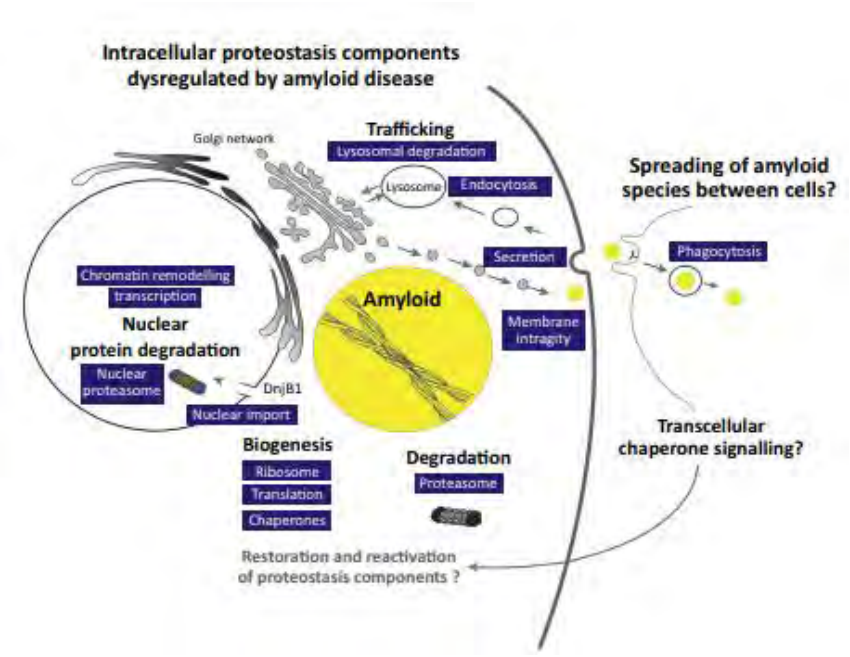

Fig 3: *Modes of CPP-cargo internalization in relation to peptide structure and nanoparticle configuration. Adapted from Kauffman et al. (2015).*

5.4. Limitations and Considerations

Despite their potential, CPP-nanocarrier systems also present challenges:

- Non-specific uptake: CPPs often lack selectivity, leading to accumulation in non-target tissues.
- Proteolytic degradation: CPPs are susceptible to enzymatic breakdown in vivo, limiting systemic application.
- Batch reproducibility and scale-up: Conjugation techniques must be optimized for manufacturing consistency.
- Potential cytotoxicity: Cationic CPPs may disrupt cell membranes at high concentrations, requiring dose optimization (Milletti, 2012).

Chapter 6

Applications in Therapeutics

Cell-penetrating peptides (CPPs), due to their remarkable ability to translocate across biological membranes and deliver a diverse range of cargoes, have found widespread applications in various therapeutic domains. Their use has been extensively explored in cancer treatment, neurological disorders, genetic diseases, and antimicrobial therapy. In each case, CPPs either serve as direct delivery enhancers or as functional components of nanocarrier systems, thereby improving cellular uptake, bioavailability, and therapeutic efficacy (Guidotti et al., 2017).

6.1. Cancer Therapy

Cancer is still regarded as one of the most active and sophisticated diseases to combats basically due to the fact that neoplasms are highly heterogeneous and most likely to develop drug resistance or due to the fact that there is minimal penetrability of therapeutic components to the tumor tissues. Therefore, a pursuit of improved intracellular delivery of anticancer drugs has turned out to be a hotbed of research. Which in this respect cell-penetrating peptides (CPPs) have shown much potential.

6.1.1. Chemotherapeutic Delivery

The carrier proteins (CPPs) have been used in the enhancement of traditional chemotherapeutic regimens that entail used of doxorubicin, paclitaxel, and cisplatin. CPPs allow intracellular retention of these agents and this is especially true in multidrug-resistant tumour cells and thus, overcome the extracellular pumping out of P-glycoprotein (Torchilin, 2006). As an example,

the Tat-treated liposomes that were filled with doxorubicin exhibited significantly stronger cytotoxicity on MCF-7 breast cancer cells in comparison with the uncomplexed counterpart (Sawant & Torchilin, 2010).

6.1.2. Nucleic Acid and Gene Therapy

Current uses of cationic polymers (CPPs) as transfection agents of siRNA, miRNA, and plasmid DNA may be used in silencing gene goals as well as gene replacement treatment. Nanocarriers in combination with CPPs provide the system responsible to perform effective cytoplasm delivery and escape in endosomes of nucleic acids (Desale et al., 2015). This type of hybrid design is imperative in silencing of an oncogene e.g. KRAS and MYC or replacing the tumor suppressor e.g. p53.

6.1.3. Tumor Targeting and Penetration

CPPs can be conjugated to tumor-homing ligands (e.g., RGD peptides or antibodies) to achieve selective accumulation in tumor tissue. Some CPPs, such as iRGD, exhibit tumor-penetrating abilities by interacting with neuropilin-1, facilitating deeper tissue penetration beyond the tumor vasculature (Sugahara et al., 2009).

6.2. Neurological Disorders

One of the most immeasurable hurdles towards drug administration to CNS is the blood-brain barrier (BBB). On their part the utilisation of CPPs has shown good prospects in drug, peptide, protein transport across the BBB, which may lead to developing the new avenues of treatment of neurodegenerative diseases, brain tumors, as well as neuroinflammation.

6.2.1. Brain Delivery

Tat, SynB1, and Penetratin CPPs have been identified to have the ability to penetrated the BBB and deliver cargo to neurons and glial cells (Lindgren et al., 2000). In models of Parkinson disease, brain distributions of dopallelel nanoparticle-dopamine or nanoparticle-siRNA bearing Tat modifications have been found to be enhanced.

6.2.2. Alzheimer's and Parkinson's Disease

β-secretase inhibitors, anti-amyloid antibodies, and neurotrophic factors are some of the drugs that are being investigated to be delivered using Ps. This confers their effectiveness to induce cellular uptake of proteins or CRISPR/Cas9 extracts thus making it viable in genetic forms of neurodegeneration (Kristensen et al., 2016).

6.3. Genetic and Metabolic Disorders

CPPs have now been shown to be able to deliver functional proteins, enzymes, and gene-editing tools in congenital disorders resulting due to neither genetic nor metabolic enzyme deficiencies.

6.3.1. Protein Replacement Therapy

In the same respect, CPPs can be used to deliver functional or recombinant proteins into the cells afflicted by lysosomal storage diseases or cystic fibrosis. One prominent example has been the use of Penetratin-conjugated hinged SOD1 (SOD1) that has been used to deliver SOD1 to oxidative stress in vivo in the protection of ischemia-reperfusion injury (Zhou et al., 2011).

6.3.2. Gene Editing

CPPs are a form of molecule that aid in delivering CRISPR/Cas9 complexes to the inside of cells and enables in correcting the gene at a definite place with a reductive possibility of off-target effects, compared to viral vectors (Mout et al., 2017). They have the potential to be used in the ex vivo cell therapy particularly in the treatment of hematologic or inherited conditions.

6.4. Antimicrobial and Antiviral Applications

A few CPPs are known to possess intrinsic antimicrobial activity, while some other CPPs function as delivery modifiers for antibiotics, peptides, or vaccines.

6.4.1. Antimicrobial Peptide Delivery

Recent studies have revealed that it is possible to impart the hybrid molecules with an added stability and, at the same time, enhancement of the penetrating ability of their membranes to an antimicrobial peptide (CPP), by conjugating it with a conventional peptide (AMP) like the LL-37 or magainin (Splith & Neundorf, 2011).

6.4.2. Antiviral Therapeutics

Teams are presently examining CPPs as a conceivable conveyance medium to antiviral siRNAs, peptides, and meeting obstructers used to focus on viruses such as HIV, HCV, and SARS-CoV-2. Interests in developing Tat- or Penetratin-based conjugates are already demonstrated to have a significant reduction in collaboration with the viral replication in vitro (Ezzat et al., 2015).

6.5. Inflammation and Autoimmune Diseases

This can be used to create targeted anti-inflammatory activity as noted by researchers who have been able to conjugate anti-inflammatory moieties to certain CPPs to preserve conveyed toxicity to the system but under relatively less levels and with specifically extended tissue targeting. This is depicted by a couple of studies. To start with, in laboratory specimens, CPP-

linked NF-kB preventatives and corticosteroids diminished the course of inflammatory cytokine creation by macrophage cells. Second, administration of CPP that can carry small-molecule inhibitors or siRNA against TNF-alpha or JAK-STAT pathway can be promising in such complicated diseases as rheumatoid arthritis (Ghosh et al., 2017).

6.6. Vaccine and Immunotherapy Applications

The cationic peptide carriers (CPPs) are being employed more as cargo with the ability of delivering antigens and adjuvant by endosomal and cell barriers decompartmentalization and hence boosting antigen delivery and priming of the immunogenic response. On top of this CPPs conjugated to vaccine antigens (HPV, HBV peptide etc) induce excellent CD8 + and CD4 + T-cell immunity. CPPs are currently examined in the realm of cancer immunotherapy in the management of tumor-associated antigens to dendritic cells and to intracellular delivery of checkpoint blockade (Kim et al., 2020).

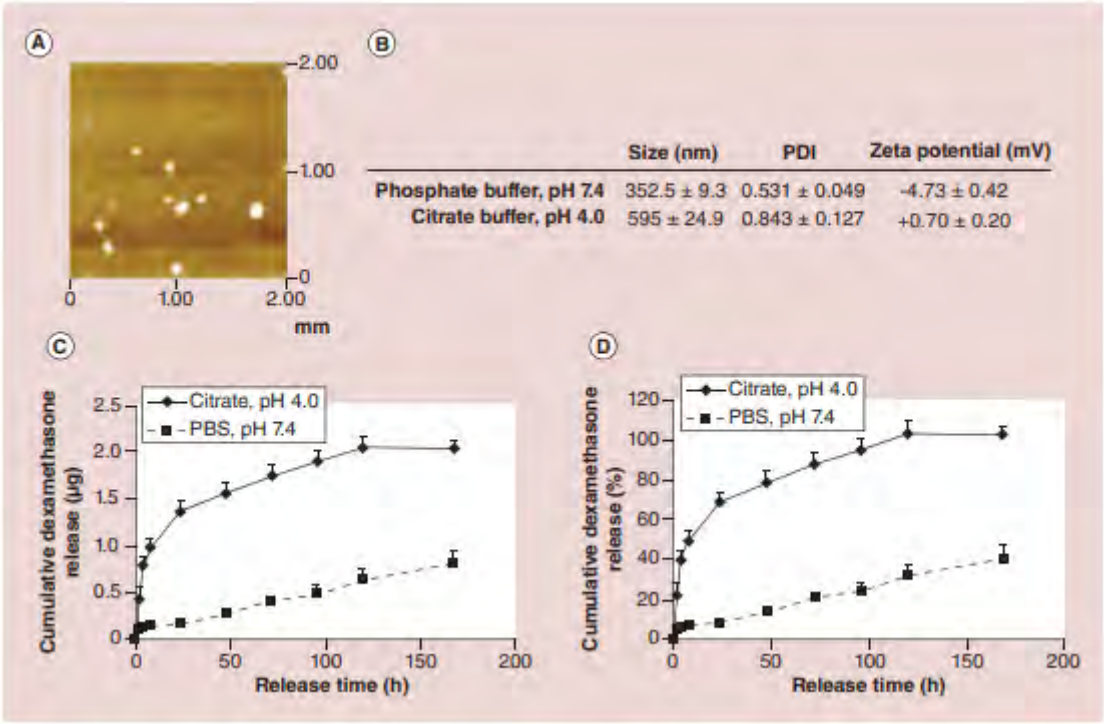

Fig 4: *Animal and clinical assets of the drug delivery of peptide-protein using CPPs preclinical and clinical applications. Kristensen. (2016).*

Chapter 7

Limitations and Challenges

CPPs are the attractive applicants in the use of an intracellular drug delivery due to their ability in translocation across the plasma membrane. However, there are a number of weaknesses that have limited their potential clinical application. Although CPPs have been shown to be membrane-permeable, versatile cargo, and low immunogenicity, they still have low cell-type specificity, fast trapping in endosomal caves, low stability in vivo, and even related issues of toxicity. These barriers should be overcome as it is essential to improve the potential of CPP-based delivery systems in terms of therapeutic efficiency and translational prospect.

7.1. Lack of Tissue and Cell Specificity

By far, one of the major drawbacks of cell-penetrating peptides (CPPs) is that the uptake of these derivatives is non-cell specific. These molecules or rather enter the wide spectrum of cell population and tissues regardless of whether there is any disease or the location of the tissue. In as much as such extensive internalization is beneficial in terms of systemic use, it imposes unwanted bio distribution in healthy organs, lowers the therapeutic index and increases the systemic toxicity. Their selectivity as well can thus be further enhanced, therefore this is an area under active research. Strategies being studied consist of CPPs conjugated to homing-targeting ligands, which homing ligands may be peptides, antibodies and aptamers, as well as stimuli-responsive CPPs, which are active only in a microenvironment characteristic of a disease, such as the acidic tumor microenvironment (Langel, 2019).

7.2. Endosomal Entrapment

Although CPPs can successfully enter cells, many are internalized via endocytosis, resulting in their sequestration within endosomes or lysosomes. Without efficient escape mechanisms, the therapeutic cargo may be:

- Degraded in lysosomes
- Recycled out of the cell
- Unable to reach intracellular targets

This endosomal barrier significantly reduces the biological activity of nucleic acids, proteins, or organelle-targeted drugs (Liu et al., 2021). Several strategies are being explored to improve endosomal escape:

- Fusogenic peptides (e.g., HA2) that disrupt endosomal membranes
- pH-sensitive polymers that swell and rupture under acidic conditions
- Histidine-rich sequences that trigger the "proton sponge effect" Despite these efforts, reliable and efficient endosomal escape remains a major bottleneck.

7.3. Proteolytic Degradation and In Vivo Instability

CPPs are oligopeptide units made of only naturally present amino acids thus may be digested by enzymes through serum protease as well as peptidase intracellularly. This instability significantly reduces the in vivo circulating half-life, bioavailability and delivery.

To reduce proteolytic degradation a number of stabilization methods are used:

- peptide cyclization to limit access by the enzyme,
- D-amino acid substitution or retro-inverso, and
- covalent coupling to PEG or PEG entrapment with nanocarriers.

Such strategies increase structural integrity, although they can reduce at the same time the membrane-interaction behavior; therefore, a wise balance between conserving the native structure and maximizing membrane behavior continues to be the most important.

7.4. Potential Toxicity and Immunogenicity

Although CPPs are generally considered low immunogenic, their repetitive use or structural modifications (e.g., addition of targeting moieties or polymers) can provoke immune responses or complement activation (Kristensen et al., 2016).

Additionally, cationic CPPs, especially those rich in arginine or lysine residues, can disrupt cell membranes at high concentrations, leading to:

- Haemolysis
- Mitochondrial dysfunction
- Inflammatory responses

Careful dose optimization, cytotoxicity screening, and immune profiling are necessary before progressing to in vivo or clinical applications.

7.5. Manufacturing and Scale-Up Challenges

Peptide synthesis, especially when involving non-natural residues, cleavable linkers, or complex conjugation steps, can be costly and technically demanding. CPP-based nanocarriers must be manufactured with:

- Batch-to-batch consistency
- Defined purity and stability
- Regulatory compliance

For clinical translation, the scalability and reproducibility of these systems remain major industrial hurdles. In addition, sterility, storage stability, and lyophilization compatibility must be established (Fonseca et al., 2009).

7.6. Limited Clinical Translation

Although numerous CPP-based systems have demonstrated success in in vitro and animal models, very few have reached clinical trials. Factors contributing to this gap include:

- Poor understanding of in vivo pharmacokinetics and biodistribution
- Insufficient data on long-term safety
- Regulatory uncertainty due to complex multi-component systems

Further toxicological studies, standardized testing protocols, and cross-disciplinary collaboration are needed to bridge the gap between laboratory innovation and clinical application.

Chapter 8

Strategies to Overcome Current Barriers

Specificity, fast proteolytic degradation, lysosome trap in endosomes, and hipotoxicity are limitations of cell-penetrating peptides (CPPs) therapeutic potentialities. A continuum of engineering and formulation strategies has therefore been developed to overcome these shortfalls. Such interventions provide simultaneous increases in targeting efficiency, structural stability, intracellular trafficking mechanisms, and reduces the level of toxicity, making CPP-based systems more permissible to clinical translation.

8.1. Targeting Specificity Enhancements

A major issue with CPPs is their non-selective uptake. Enhancing tissue and cell specificity is critical for reducing off-target effects and improving therapeutic indices.

8.1.1. Ligand-Targeted CPPs

CPPs can be conjugated with targeting ligands such as:

- Folic acid (for cancer cells overexpressing folate receptors)
- RGD peptides (for integrin-expressing tumor vasculature)
- Antibodies or antibody fragments (for high-affinity receptor binding)

These dual-functional CPPs provide both penetration and selectivity, facilitating receptor-mediated uptake at disease sites (Lindgren et al., 2000).

8.1.2. Activatable CPPs

These CPPs remain inactive in circulation but become activated in disease-specific environments, such as:

- Acidic pH (tumor microenvironment, endosomes)
- High glutathione (cytosol of cancer cells)
- Matrix metalloproteinase (MMP) activity (tumor invasion zones)

They are often constructed with cleavable linkers or pH-sensitive masking groups that reveal the CPP moiety only in target sites (Desale et al., 2015).

8.2. Strategies for Endosomal Escape

To avoid lysosomal degradation after endocytic uptake, various modifications aim to enhance cytosolic delivery of CPP-cargo complexes.

8.2.1. Fusogenic Peptides

Incorporating viral-derived peptides (e.g., HA2 from influenza hemagglutinin) into CPP conjugates helps disrupt endosomal membranes in acidic environments (Akita et al., 2010).

8.2.2. Proton Sponge Effect

Peptides or carriers rich in histidine or polyamines cause osmotic swelling and rupture of endosomes by buffering the acidic pH, thus promoting escape into the cytosol (Liu et al., 2021).

8.2.3. Photochemical Internalization (PCI)

Light-sensitive compounds can be co-delivered with CPPs to induce localized endosomal rupture upon photoactivation, ensuring spatiotemporal control over cytosolic delivery (Berg et al., 2011).

8.3. Proteolytic Stability Improvements

Improving resistance to enzymatic degradation is crucial for maintaining bioactivity in vivo.

8.3.1. Peptide Cyclization

Head-to-tail or side-chain cyclization shields the peptide backbone from proteases while often improving membrane interaction (Fonseca et al., 2009).

8.3.2. D-Amino Acid Substitution and Retro-Inverso Peptides

Replacing L-amino acids with D-enantiomers or designing retro-inverso analogs increases proteolytic resistance without severely affecting functionality (Milletti, 2012).

8.3.3. PEGylation and Polymer Conjugation

Attaching hydrophilic polymers like PEG improves serum stability, reduces immunogenicity, and prolongs circulation time, while also offering a platform for multivalent ligand presentation (Sawant & Torchilin, 2010).

8.4. Stimuli-Responsive and Smart Delivery Systems

Advancements in nanotechnology allow CPPs to be incorporated into intelligent delivery platforms that respond to internal or external stimuli.

8.4.1. pH/Redox-Responsive Systems

Nanocarriers or linkers that respond to acidic pH (in tumors/endosomes) or high intracellular glutathione enable site-specific release of the CPP or cargo (Ruan et al., 2019).

8.4.2. Enzyme-Cleavable Systems

Linkers sensitive to MMPs or cathepsins allow CPP activation or drug release specifically at diseased tissues, minimizing systemic exposure.

8.4.3. Light-, Heat-, or Ultrasound-Triggered Systems

External stimuli can be used to trigger membrane permeabilization, CPP activation, or cargo release, offering precision in drug delivery timing and location (Kim et al., 2020).

8.5. Rational Design and Computational Modelling

With the recent advances in machine learning and computational biology these offerings are being used to optimise the sequences of cationic peptides in order to improve therapeutic functionality.

- The silicon-based peptide design platforms is able to predict the membrane affinity, secondary structure, and the capability to internalise inside the cell.
- High-throughput screening along with deep learning algorithms allows finding new peptides with cationicity and slight levels of cytotoxicity and perfect uptake routes (Kauffman et al., 2015).

Such strategies facilitate a faster process of discovery of the next-generation cationic peptides with desired therapeutic needs.

8.6. Modular Nanocarrier Systems

Combining CPPs with multi-functional nanocarriers allows integration of:

- CPPs for membrane penetration
- Targeting ligands for selectivity

- Imaging agents for diagnostics (theranostics)
- Responsive elements for controlled release

This modular architecture provides customizable systems suitable for precision medicine, particularly in cancer and gene therapy (Ramasamy et al., 2014).

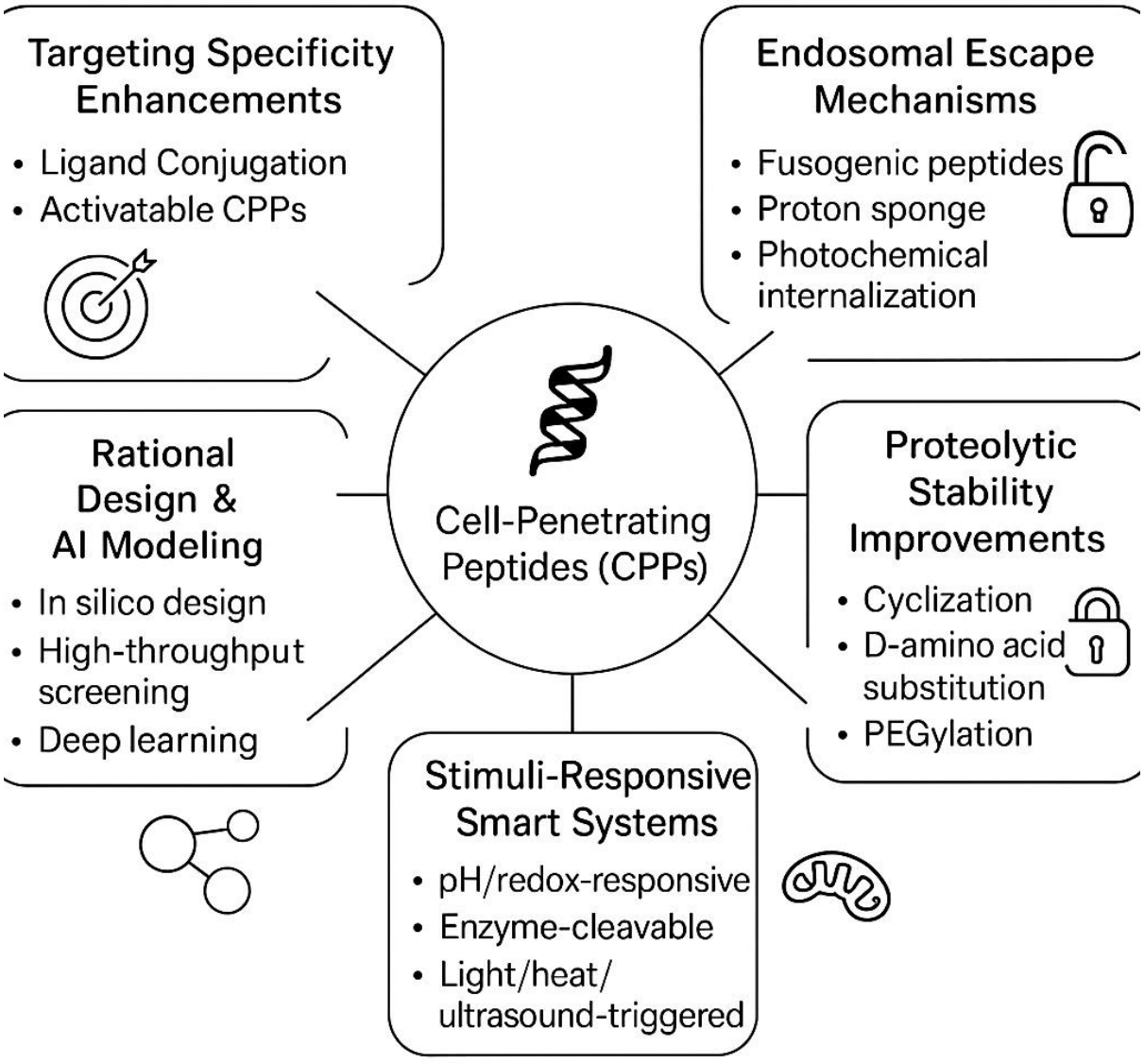

Fig 5: *Schematic representation of key strategies used to overcome the limitations of cell-penetrating peptide-based therapeutic systems. Adapted and synthesized from multiple sources: Lindgren et al. (2000); Desale et al. (2015); Akita et al. (2010); Fonseca et al. (2009); Ruan et al. (2019); Kauffman et al. (2015); Ramasamy et al. (2014).*

Chapter 9

Emerging Trends and Future Perspectives

Cell-penetrating peptides (CPPs) are being transformed paradigmatically in the modern age by the advances in fields of nanotechnology, synthetic biology, computational design and personalized medicine. With an increasing pressure on therapeutic demand in targeted and highly efficient intracellular delivery CPPs are undergoing a re-engineering process to a higher level of precision, a reduction in toxicity and multifunctionality. The current discussion outlines the notable trends and analyzes the prospect of applying CPPs to the future treatment platforms.

9.1. CPPs in RNA and CRISPR-Based Therapeutics

One of the most promising frontiers is the use of CPPs in delivering RNA-based therapies—including siRNA, mRNA, and antisense oligonucleotides—as well as CRISPR-Cas9 gene editing components. The challenge of cytosolic and nuclear delivery of such fragile biomolecules necessitates carriers with efficient membrane translocation, protection from degradation, and precise endosomal escape—functions ideally suited to next-generation CPPs (Mout et al., 2017).

CPPs have been successfully used to:

- Deliver Cas9-sgRNA ribonucleoproteins for ex vivo gene editing
- Facilitate systemic siRNA delivery to silence oncogenes or inflammatory cytokines
- Transport synthetic mRNA for protein replacement or vaccine development, including COVID-19-related applications (Ezzat et al., 2015)

These applications align CPPs with the future of precision gene and RNA therapy.

9.2. Theranostics: Combining Therapy and Diagnostics

CPPs are being integrated into theranostic platforms, which combine therapeutic delivery with diagnostic imaging or real-time monitoring. CPP-conjugated nanoparticles can be engineered to:

- Deliver drugs and simultaneously carry fluorophores, MRI contrast agents, or radioisotopes
- Target disease-specific biomarkers for real-time imaging of therapeutic efficacy

Such multifunctional systems have great potential in cancer imaging, early diagnosis, and personalized treatment planning (Torchilin, 2006).

9.3. CPPs in Immunotherapy and Vaccinology

CPPs are increasingly explored in cancer immunotherapy and vaccine delivery due to their ability to deliver antigens and adjuvants across cellular and endosomal membranes, facilitating antigen presentation and T cell activation.

Future developments may include:

- CPP-based delivery of immune checkpoint inhibitors (e.g., anti-PD-1 peptides)
- Conjugates for mRNA or peptide vaccines that target intracellular pathogens or tumor antigens
- Use in tolerogenic vaccines for autoimmune disease treatment

These advances may significantly influence the next generation of immunotherapeutics and vaccines, particularly in the context of emerging infectious diseases and personalized oncology (Kim et al., 2020).

9.4. Peptide Libraries and AI-Driven Design

With the advent of machine learning (ML) and artificial intelligence (AI), the identification and optimization of novel CPPs have become faster and more accurate.

Key developments include:

- In silico screening of peptide libraries for cell penetration, solubility, and toxicity
- AI models predicting CPP-cargo compatibility, uptake pathways, and organ distribution
- Evolutionary algorithms and deep learning used to design peptides with enhanced functional properties (Kauffman et al., 2015)

These tools dramatically reduce experimental costs and accelerate rational design of peptide therapeutics.

9.5. CPPs in Organelle-Targeted Delivery

Recent research is exploring the capacity of CPPs to deliver therapeutics to specific intracellular organelles, such as:

- Nucleus (e.g., for gene editing or transcription factor delivery)
- Mitochondria (e.g., for oxidative stress or apoptosis regulation)

- Lysosomes or endoplasmic reticulum (e.g., enzyme replacement therapy)

This level of precision opens up new possibilities in treating organelle-associated diseases, such as mitochondrial disorders or lysosomal storage diseases (Kristensen et al., 2016).

9.6. Integration with Smart and Responsive Materials

CPPs are increasingly being embedded in stimuli-responsive platforms that react to:

- pH gradients (e.g., tumor or endosomal acidity)
- Redox potential (e.g., high glutathione levels in cancer cells)
- Enzyme expression (e.g., matrix metalloproteinases)

These smart systems enable controlled activation, targeted release, and reduced systemic toxicity. Combining CPPs with hydrogels, microneedles, and 3D-printed drug delivery devices is also being investigated for local and sustained therapeutic administration (Ramasamy et al., 2014).

9.7. Clinical Translation and Regulatory Advances

Despite limited clinical approvals to date, several CPP-based systems are advancing through preclinical and early clinical trials. With better characterization of pharmacokinetics, immune interactions, and toxicological profiles, regulatory frameworks are expected to become more supportive of peptide-based nanocarriers.

In the future, regulatory focus may prioritize:

- Multifunctional delivery platforms (example: CPP + targeting ligand + diagnostic agent)
- Patient-specific therapies, where peptide vectors are tailored to individual genomes or tumor profiles
- Off-the-shelf therapeutic kits, enabling clinical use of CPP-based cargo systems in outpatient settings

Chapter 10

Conclusion

Cell-penetrating peptides (CPPs) are a type of short molecular objects that have the capability to enter the cells and transport the various cargo molecules into them intracellularly without employing the endocytic pathways. Such a multifunctional attribute makes CPPs priceless in drug delivery, gene therapy, molecular imaging, and other applications that are related.

This review has highlighted the structural and functional diversity of CPPs, along with their uptake mechanisms, classification, and conjugation with various nanocarriers. Such hybrid systems enable not only improved delivery efficiency but also greater control over release profiles, tissue penetration, and subcellular targeting. CPP-conjugated nanocarriers have demonstrated substantial potential in areas such as cancer therapy, central nervous system drug delivery, genetic disease correction, and immunomodulation.

Recent developments in the field of cell-penetrating peptides (CPPs) are recorded in the current review, proving that the latter can still be viewed as a suitable means toward successful delivery of therapeutic macromolecules to the intracellular space. In spite of multiple preclinical studies confirming the efficacy of CPP, there is still a barrier to translating such findings into clinical practice and this can be best described as the difficulty in overcoming these barriers of nonspecific cellular uptake endosomal entrapment, proteolytic instability, cytotoxicity and limited scalability of large-scale manufacturing. These limitations are slowly being mitigated by recent advances, i.e., the application of targeted CPP design approaches, stimuli-responsive CPP constructs, and smart nanocarrier conjugation, and in so doing, the research atmosphere is closing the gap between testing and clinical application.

Moving ahead, it is expected that the evolving CPPs would overlap with various new technologies such as RNA therapeutics, CRISPR gene editing, delivery to organelles, and

theranostics. As current advances in precision medicine, nanotechnology, and synthetic biology continue, CPPs will become the foundation of the future of targeted drug delivery devices and systems, and thus safer, smarter, and more effective interventions in a broad variety of disease indications.

In conclusion, cell-penetrating peptides, especially when employed in synergy with advanced nanocarriers, offer a powerful and adaptable platform for intracellular drug delivery. With continued research and clinical validation, CPPs have the potential to transform the therapeutic landscape and address unmet medical needs across diverse biomedical domains.

References

Akita, H., Harashima, H., & Kogure, K. (2010). Nonviral gene delivery using the combination of cell-penetrating peptides with liposomes. *Molecular Pharmaceutics*, 7(4), 1196–1204. https://doi.org/10.1021/mp100001u

Bechara, C., & Sagan, S. (2013). Cell-penetrating peptides: 20 years later, where do we stand? *FEBS Letters*, 587(12), 1693–1702. https://doi.org/10.1016/j.febslet.2013.04.031

Berg, K., Folini, M., Prasmickaite, L., Selbo, P. K., Bonsted, A., Engesaeter, B. O., ... & Weyergang, A. (2011). Photochemical internalization: A novel technology for delivery of macromolecules into cytosol. *Cancer Research*, 71(7), 2452–2460. https://doi.org/10.1158/0008-5472.CAN-10-3227

Derossi, D., Joliot, A. H., Chassaing, G., & Prochiantz, A. (1994). The third helix of the Antennapedia homeodomain translocates through biological membranes. *Journal of Biological Chemistry*, 269(14), 10444–10450.

Desale, K., Prabhakar, B., & Park, S. Y. (2015). Targeted delivery of doxorubicin using pH-responsive peptide micelles for anticancer therapy. *Nanomedicine*, 10(12), 2069–2083. https://doi.org/10.2217/nnm.15.11

EL Andaloussi, S., Lehto, T., Mäger, I., & Langel, Ü. (2007). Cell-penetrating peptides: A tool for delivery of therapeutics. *Expert Opinion on Drug Delivery*, 4(5), 519–530. https://doi.org/10.1517/17425247.4.5.519

Ezzat, K., Helmfors, H., Tudoran, O., Juks, C., Lindberg, S., Padari, K., ... & Langel, Ü. (2015). Scavenger receptor-mediated uptake of cell-penetrating peptide nanocomplexes: A key mechanism for oligonucleotide delivery. *Journal of Biological Chemistry*, 290(50), 29988–29997. https://doi.org/10.1074/jbc.M115.670919

Fonseca, S. B., Pereira, M. P., & Kelley, S. O. (2009). Recent advances in the use of cell-penetrating peptides for medical and biological applications. *Advanced Drug Delivery Reviews*, 61(11), 953–964. https://doi.org/10.1016/j.addr.2009.06.001

Frankel, A. D., & Pabo, C. O. (1988). Cellular uptake of the Tat protein from human immunodeficiency virus. *Cell*, 55(6), 1189–1193. https://doi.org/10.1016/0092-8674(88)90263-2

Futaki, S., Suzuki, T., Ohashi, W., Yagami, T., Tanaka, S., Ueda, K., & Sugiura, Y. (2001). Arginine-rich peptides: An abundant source of membrane-permeable peptides having potential as carriers for intracellular protein delivery. *Journal of Biological Chemistry*, 276(8), 5836–5840. https://doi.org/10.1074/jbc.M007540200

Ghosh, D., Kohli, A. G., Moser, F., Endy, D., & Belcher, A. M. (2017). Reprogramming cells through genetically encoded synthetic biomaterials. *ACS Synthetic Biology*, 6(9), 1644–1650. https://doi.org/10.1021/acssynbio.6b00263

Guidotti, G., Brambilla, L., & Rossi, D. (2017). Cell-penetrating peptides: From basic research to clinics. *Trends in Pharmacological Sciences*, 38(4), 406–424. https://doi.org/10.1016/j.tips.2017.01.003

Herce, H. D., & García, A. E. (2007). Molecular dynamics simulations suggest a mechanism for translocation of the HIV-1 TAT peptide across lipid membranes. *Proceedings of the National Academy of Sciences*, 104(52), 20805–20810. https://doi.org/10.1073/pnas.0706574105

Howl, J., & Jones, S. (2010). Bioportides: A new class of multifunctional membrane-transfer peptides. *Biochemical Society Transactions*, 38(2), 605–609. https://doi.org/10.1042/BST0380605

Kauffman, W. B., Fuselier, T., He, J., & Wimley, W. C. (2015). Mechanism matters: A taxonomy of cell penetrating peptides. *Trends in Biochemical Sciences*, 40(12), 749–764. https://doi.org/10.1016/j.tibs.2015.10.002

Kesharwani, P., Jain, K., & Jain, N. K. (2015). Dendrimer as nanocarrier for drug delivery. *Progress in Polymer Science*, 39(2), 268–307. https://doi.org/10.1016/j.progpolymsci.2013.07.005

Kim, Y., Park, J. H., & Park, J. H. (2020). Recent progress of cell-penetrating peptides as new carriers for intracellular delivery of biologics. *Biomolecules & Therapeutics*, 28(3), 220–234. https://doi.org/10.4062/biomolther.2020.015

Kristensen, M., Birch, D., & Nielsen, H. M. (2016). Applications and challenges for use of cell-penetrating peptides as delivery vectors for peptide and protein cargos. *International Journal of Molecular Sciences*, 17(2), 185. https://doi.org/10.3390/ijms17020185

Kristensen, M., Birch, D., & Nielsen, H. M. (2016). Applications and challenges for use of cell-penetrating peptides as delivery vectors for peptide and protein cargos. *International Journal of Molecular Sciences*, 17(2), 185. https://doi.org/10.3390/ijms17020185

Langel, Ü. (Ed.). (2019). *Cell-penetrating peptides: Methods and protocols*. Humana Press.

Lindgren, M., Hallbrink, M., Prochiantz, A., & Langel, Ü. (2000). Cell-penetrating peptides. *Trends in Pharmacological Sciences*, 21(3), 99–103. https://doi.org/10.1016/S0165-6147(00)01447-6

Liu, X., Liu, C., Zhou, J., Chen, C., & Qu, Y. (2021). Strategies for improving the efficiency of endosomal escape in nanoparticles delivery. *Asian Journal of Pharmaceutical Sciences*, 16(6), 768–784. https://doi.org/10.1016/j.ajps.2021.01.003

Liu, Y., Guo, Q., Yang, X., Deng, Q., & Shi, Y. (2008). Brain-targeting gene delivery using Tat-modified poly(lactic-co-glycolic acid) nanoparticles. *Nanotechnology*, 19(49), 495103. https://doi.org/10.1088/0957-4484/19/49/495103

Madani, F., Lindberg, S., Langel, Ü., Futaki, S., & Gräslund, A. (2011). Mechanisms of cellular uptake of cell-penetrating peptides. *Journal of Biophysics*, 2011, Article 414729. https://doi.org/10.1155/2011/414729

Milletti, F. (2012). Cell-penetrating peptides: Classes, origin, and current landscape. *Drug Discovery Today*, 17(15–16), 850–860. https://doi.org/10.1016/j.drudis.2012.03.002

Mout, R., Ray, M., Yesilbag Tonga, G., Lee, Y. W., Tay, T., Sasaki, K., & Rotello, V. M. (2017). Direct cytosolic delivery of CRISPR/Cas9–ribonucleoprotein for efficient gene editing. *ACS Nano*, 11(3), 2452–2458. https://doi.org/10.1021/acsnano.6b07600

Nakase, I., Akita, H., Kogure, K., Gräslund, A., Langel, Ü., Harashima, H., & Futaki, S. (2012). Efficient intracellular delivery of nucleic acid pharmaceuticals using cell-penetrating peptides. *Accounts of Chemical Research*, 45(7), 1132–1139. https://doi.org/10.1021/ar200219c

Ramasamy, T., Ruttala, H. B., Chitrapriya, N., Poudel, B. K., & Kim, J. O. (2014). Smart nanocarriers for the targeted delivery of therapeutic agents: Recent advances. *Journal of Drug Targeting*, 22(9), 801–815. https://doi.org/10.3109/1061186X.2014.931372

Rosi, N. L., Giljohann, D. A., Thaxton, C. S., Lytton-Jean, A. K. R., Han, M. S., & Mirkin, C. A. (2006). Oligonucleotide-modified gold nanoparticles for intracellular gene regulation. *Science*, 312(5776), 1027–1030. https://doi.org/10.1126/science.1125559

Ruan, S., He, Q., Gao, H., & Gao, H. (2019). Dual-targeting delivery of drugs to tumors using multifunctional nanoparticles. *Biomaterials Science*, 7(2), 489–499. https://doi.org/10.1039/C8BM01150E

Sawant, R. R., & Torchilin, V. P. (2010). Intracellular delivery of chemotherapy using actively targeted nanocarriers. *Advanced Drug Delivery Reviews*, 62(13), 1139–1150. https://doi.org/10.1016/j.addr.2010.07.011

Splith, K., & Neundorf, I. (2011). Antimicrobial peptides with cell-penetrating peptide properties and vice versa. *European Biophysics Journal*, 40(4), 387–397. https://doi.org/10.1007/s00249-011-0667-1

Sugahara, K. N., Teesalu, T., Karmali, P. P., Kotamraju, V. R., Agemy, L., Girard, O. M., ... & Ruoslahti, E. (2009). Tissue-penetrating delivery of compounds and nanoparticles into tumors. *Cancer Cell*, 16(6), 510–520. https://doi.org/10.1016/j.ccr.2009.10.013

Torchilin, V. P. (2006). Recent approaches to intracellular delivery of drugs and DNA and organelle targeting. *Annual Review of Biomedical Engineering*, 8, 343–375. https://doi.org/10.1146/annurev.bioeng.8.061505.095735

Torchilin, V. P., Levchenko, T. S., Rammohan, R., Volodina, N., Papahadjopoulos-Sternberg, B., & D'Souza, G. G. M. (2001). Cell transfection in vitro and in vivo with nontoxic TAT peptide–lipid conjugates. *Proceedings of the National Academy of Sciences*, 98(15), 8786–8791. https://doi.org/10.1073/pnas.151184498

Trabulo, S., Cardoso, A. L., Mano, M., & De Lima, M. C. P. (2010). Cell-penetrating peptides—Mechanisms of cellular uptake and generation of delivery systems. *Pharmaceuticals*, 3(4), 961–993. https://doi.org/10.3390/ph3040961

Wender, P. A., Mitchell, D. J., Pattabiraman, K., Pelkey, E. T., Steinman, L., & Rothbard, J. B. (2000). The design, synthesis, and evaluation of molecules that enable or enhance cellular uptake: Peptoid molecular transporters. *Proceedings of the National Academy of Sciences*, 97(24), 13003–13008. https://doi.org/10.1073/pnas.97.24.13003

Zhou, J., Li, M., Hou, Y., Luo, Y., & Sun, X. (2011). Intracellular delivery of superoxide dismutase using cell-penetrating peptide against ischemia-reperfusion injury. *Drug Delivery*, 18(4), 225–231. https://doi.org/10.3109/10717544.2010.545511

Ziegler, A. (2008). Thermodynamic studies and binding mechanisms of cell-penetrating peptides with lipids and glycosaminoglycans. *Advanced Drug Delivery Reviews*, 60(4–5), 580–597. https://doi.org/10.1016/j.addr.2007.10.010

Zorko, M., & Langel, Ü. (2005). Cell-penetrating peptides: Mechanism and kinetics of cargo delivery. *Advanced Drug Delivery Reviews*, 57(4), 529–545. https://doi.org/10.1016/j.addr.2004.10.005