

Therapeutic Strategies and Clinical Significance of Cell Penetrating Peptides

White Paper



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Introduction

Cytoplasm, the interior of all living cells, is surrounded by a thin molecular layer called plasma membrane. This membrane serves to separate the cell from its surrounding environment to protect the cell from chemical and physical changes and to regulate the traffic of molecules into and out of the cell. This flexible and exceedingly thin (~ 6 nm) plasma membrane is ironically very strong. It is mostly made from a bilayer of phospholipid molecules, acting as a wall with a hydrophobic exterior that excludes water and hydrophilic molecules. The hydrophobic core of the phospholipid bilayer forms an extremely effective seal and only very few molecules can pass directly through the lipid bilayer to go from one side of the membrane to the other. Many substances that the cell needs for survival cannot cross the lipid bilayer on their own, including glucose (a sugar that cells burn for energy), amino acids (the building blocks of proteins) and ions such as sodium and potassium. There is a variety of protein molecules embedded within the membrane and act as channels and pumps to move these vital molecules into and out of the cell. Therefore, it is known that the cell is selective permeable, regulating what enters and exits the cell, which facilitates the transport of materials needed for survival ^[1].

Living organisms have developed different strategies to overcome this membrane barrier for different purposes. For example, some viruses, such as the HIV virus, use their own host cell receptors to insert into the cell membrane and use the structure of the host cell for replication. Antimicrobial peptides (APs) are used by organisms to destroy bacteria by forming pores that permeabilize the plasma membrane and destroy the vital chemical gradients across the cell. These APs are composed of short sequences of a combination of hydrophilic and hydrophobic amino acids. It is believed that these peptides are able to interact favorably with the plasma membrane with their high content of hydrophobic amino acids. It has been observed that these peptides are even able to cross the bacterial membrane on their own after forming the transient pores ^[2].

A family of short peptides with a highly hydrophilic property has been discovered, which also seems to be able to cross the plasma membrane on their own. These peptides are called Cell Penetrating Peptides (CPPs) or a more pictorial name Trojan Peptides. The first discovered CPP was the HIV-1 TAT peptide in 1988^[3, 4]. Over more than twenty years since then, many other CPPs have been discovered and rationally designed. According to their sequential properties, CPPs are generally categorized into three types: cationic, amphipathic and hydrophobic. The first two types of CPPs contain a relatively high abundance of positively charged amino acids such as arginine and lysine ^[5], or have sequences that contain alternating patterns of polar/charged amino acids and non-polar/hydrophobic amino acids. The hydrophobic CPPs contain polar residues with a low net charge or hydrophobic amino acid groups that are crucial for cellular uptake ^[15].

CPPs facilitate cellular uptake of various molecules. The cargo, such as RNA/DNA, peptide, PNA, radioisotope, liposomes, nanoparticles and even large protein, can link to the CPP either in a covalent form or through non-covalent interactions. Representative of most commonly used CPPs with their origins and classes are summarized in **Table 1**.

Table 1. Representative CPPs with their origins and classes.

CPP	Sequence	Origin	Class
HIV-1 TAT protein, TAT ₄₈₋₆₀	GRKKRRQRRRPPQ	HIV-1 TAT protein	Cationic
HIV-1 TAT protein, TAT ₄₉₋₅₇	RKKRRQRRR	HIV-1 TAT protein	Cationic
Penetratin, pAntp(43-58)	RQIKIWFQNRRMKWKK	Antennapedia Drosophila melanogaster	Cationic
Polyarginines	Rn	Chemically synthesized	Cationic
DPV ₁₀₄₇	VKRGLKLRHVRPRVTRMDV	Chemically synthesized	Cationic
MPG	GALFLGFLGAAGSTMGAWSQPKKKRKV	HIV glycoprotein 41/ SV40 T antigen NLS	Amphipathic
Pep-1	KETWWETWWTEWSQPKKKRKV	Tryptophan-rich cluster/SV40 T antigen NLS	Amphipathic
pVEC	LLIILRRRIRKQAHASHK	Vascular endothelial Cadherin	Amphipathic
ARF(1-22)	MVRRFLVTLRIRACGPPRVRV	p14ARF protein	Amphipathic
BPrPr(1-28)	MVKSIGSWILVLFVAMWSDVGLCKRP	N terminus of unprocessed bovine prion protein	Amphipathic
MAP	KLALKLALKALKAAKLA	Chemically synthesized	Amphipathic
Transportan	GWTLSAGYLLGKINKALAALAKKIL	Chimeric galanin–Mastoparan	Amphipathic
p28	LSTAADMQGVVTDGMASGLDKDYLPDD	Azurin	Amphipathic
VT5	DPKGDPKGVTVTVTVTVTGKDPKPD	Chemically synthesized	Amphipathic
Bac 7 (Bac 1-24)	RRIRPRPPRLPRPRPLPFPRPG	Bactenecin family of antimicrobial peptides	Amphipathic
C105Y	CSIPPEVKFNKPFVYLI	a1-Antitrypsin	Hydrophobic
PFVYLI	PFVYLI	Derived from synthetic C105Y	Hydrophobic
Pep-7	SDLWEMMMVSLACQY	CHL8 peptide phage Clone	Hydrophobic

Mechanisms of CPP Cellular Uptake

The mechanisms used by CPPs to cross into virtually any living cell in a receptor and energy independent manner have been a matter of debate for many years. The core of the puzzle lies in the fact that these peptides are highly cationic and they lack any hydrophobic component that would allow them to get inserted efficiently in the hydrophobic core of the plasma membrane ^[6]. One solution was raised in 2014 with the essential role of guanidinium groups and two universal cell components: fatty acids and cell membrane pH gradient.

It was discovered that the pathway employed by CPPs for direct insertion consists the following steps (**Fig. 1**):

1. Higher extracellular pH deprotonates fatty acids and attracts arginine rich peptides.
2. The positively charged guanidinium groups bind strongly to the deprotonated carboxyl groups of the fatty acids.
3. The positive charge of the peptides is now screened by the fatty acids. This allows the efficient insertion of the peptides into the core of the plasma membrane. This insertion destabilizes the plasma membrane, nucleating a transient toroidal pore.
4. The peptides diffuse through this pore towards the interior of the cell while the lower pH in the cytosol protonates the fatty acids and releases the peptides.
5. The peptides are released into the cell and the transient pore becomes unstable and closes.
6. The protonated and neutral fatty acids rapidly flip-flop across the plasma membrane and eventually become in contact with the extracellular media at a higher pH where they become negatively charged, and then the process starts over again.

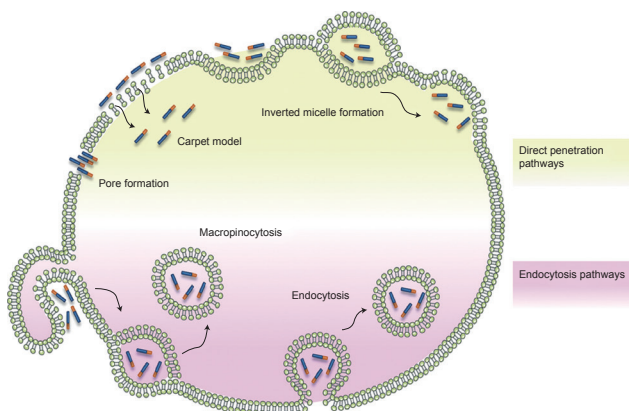


Figure 1. Schematic representation of proposed mechanisms for CPP internalization. The diagram illustrates that the involved pathways can be divided into two groups: direct penetration of plasma membrane (yellow) and endocytic pathways (purple). Direct penetration of plasma membrane involves several energy-independent models including membrane insertion of CPPs through pore formation and membrane destabilization through the carpet-like model or inverted micelle formation. Endocytic internalization of CPPs is an energy-dependent process that comprises macropinocytosis and endocytosis. Picture generated from

DOI: [10.1016/j.tips.2017.01.003](https://doi.org/10.1016/j.tips.2017.01.003)

Evidence pointed out that CPPs are capable of spontaneously crossing the plasma membrane. This has been shown in numerous ways such as in giant unilamellar vesicles (GUVs) composed of cell membrane components from pure phospholipids to actual cell membranes ^[7]. It has been shown experimentally that CPPs form small transient pores across the plasma membrane, which allows the transport of other cargos that are covalently attached to them. It has also been suggested that CPPs might be able to combine with the negatively charged groups at the plasma membrane and move through the membrane without the involvement of a pore. However, this still cannot explain how other cargos could diffuse with the peptides across the membrane to the interior of the cell. Another issue is that these conclusions were made based on experiments that looked at the partition of the peptides between octanol and a water solution after adding detergent that is obviously not present in cells and would destroy the plasma membrane.

Despite the ample evidence showing CPPs' ability of directly crossing the plasma membrane without any intermediate step, several researchers still claim that endocytosis is an intermediate step required for peptides' entry into the cells. This presumption was based on the observations of fluorescently labeled CPPs in fixed cells. It was found that the fixation of cells could lead to artificial redistribution of CPPs inside the cells. Endocytosis includes micropinocytosis, clathrin- and/or caveolin-dependent endocytosis, clathrin- and/or caveolin-independent endocytosis, as well as dynamin-dependent and/or dynamin-independent endocytosis. CPPs can utilize more endocytic pathways during internalization, and thus increase their uptake into the cells. Macropinocytosis results in the formation of vesicles called macropinosomes that are formed during inward folding of the plasma membrane. Clathrin and caveolin are proteins in the intracellular part of the cell membrane during endocytosis; they are required for the invagination of the membrane and the formation of vesicles that are coated with these proteins. The clathrin-coated vesicles are a few hundred nanometers in diameter, while the caveolin-coated vesicles have a diameter below one hundred nanometers. Dynamin is a protein involved in the invagination of the cell membrane and is necessary for the formation of these vesicles [1, 2].

Endocytosis is an energy-dependent mechanism (**Fig. 1**). A number of studies have shown the contribution of endocytosis to different CPP internalization mechanisms and their cargo molecules. This might be taken as a clear suggestion that CPP uptake must be mediated by an initial endosomal uptake. The problem with this assumption is that peptides that are rich in arginine and lysine amino acids usually have a strong affinity for the cell plasma membrane and the cell is continuously endocytosing for several other reasons that are not related to the CPPs, such as recycling the plasma membrane or absorbing extracellular fluids through macropinocytosis. Therefore, any peptide or molecule with affinity for the plasma membrane will eventually be internalized by endocytosis. However, this does not necessarily imply that these molecules will be found freely diffusing into the cytoplasm and the nucleus as CPPs [1, 2, 15, 16, 17].

Design of CPP-Cargo Conjugations

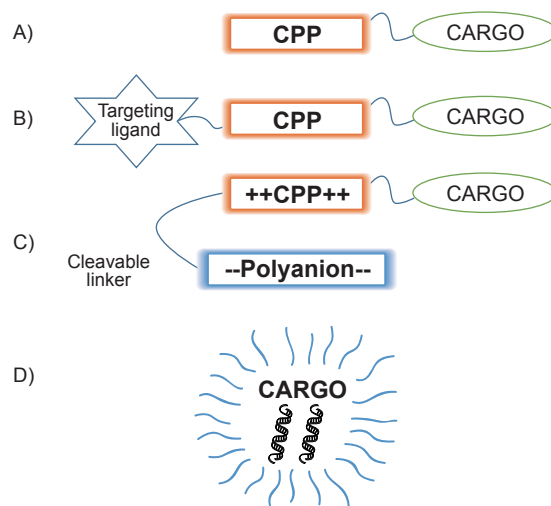


Figure 2. Schematic representation of CPP-cargo conjugation methods. (A) The most common method is covalent conjugation between cargo and CPP. (B) Ligand-CPP-cargo for improved targeting. (C) CPP construct consisting a peptide, cargo and protecting polyanion with a target specific cleavable linker. Cleavage of the linker makes the peptide dissociate from the polyanion and become an active CPP. (D) non-covalent CPP-cargo conjugates through electrostatic or hydrophobic interactions, commonly for RNA or DNA wrapping.

Various methods have been developed and optimized to deliver cargos into the cell. Generally, the approaches can be categorized into simple covalent CPP-cargo conjugations (**Fig. 2A**), additional ligand added onto the CPP-cargo complex for improved targeting efficiency (**Fig. 2B**), exquisite cleavage site between the CPP and cargo (**Fig. 2C**), and non-covalent CPP-cargo conjugates through electrostatic or hydrophobic interactions (**Fig. 2D**).

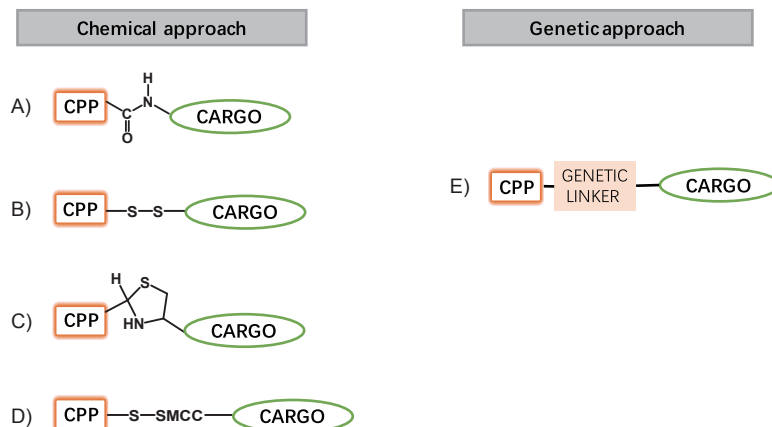


Figure 3. Covalent conjugations by chemical or genetic methods. (A) The most common method is covalent conjugation between the cargo and CPP. (B) Ligand-CPP-cargo for improved targeting. (C) Stable carbon-heteroatom bond for CPP-cargo complex. (D) Bi-functional crosslinker are induced between CPP and cargo. (E) Expressed CPP-cargo complex through genetically encoded approach.

CPPs are usually connected via a covalent linkage to the cargo molecules (**Fig. 3**). The most common way is to conjugate CPP with a cargo directly through carboxyl group and amide group reactions (**Fig. 3A**). Another common approach is the use of suitable amino acid side chains such as the thiol group of Cys or the amino group of Lys. Disulfide bonds are also commonly added to benefit rapid reductions in the intracellular environment, enabling release of the cargo (**Fig. 3B**). Other stable covalent linkages that have been employed include thioether, thiolmalmeide, thiazolidine, oximine and hydrazine linkages (**Fig. 3C**), as well as bi-functional cross-linkers such as SMCC (**Fig. 3D**). If the cargo is a peptide or protein, the two can be produced as a single chimeric protein in bacteria (**Fig. 3E**).

To improve the targeting efficiency of CPP-cargo complex, extra target ligands can be modified (**Fig. 2B**). Because of the biodistribution of CPP-conjugated drugs can lead to a reduction of drug efficiency due to a lower local concentration. Cost must also be considered, as well as risks of off-target effects. Hence, maximizing local concentration of CPP-cargo is crucial, especially for tumoral cell.

Moreover, to obtain a selective CPP that can target tumor tissues without involving normal cells is the insertion of particular cleavage sites to the sequence of CPP (**Fig. 2C**). These can be cleaved by metalloproteinases such as MMP-2/-9, which play an important role in angiogenesis and metastasis of tumors and are frequently over-expressed in cancer tissues. For instance, an MMP-2 cleavage site was introduced by Li *et al.* between a CPP and a polyanionic peptide in order to block the penetration in normal tissue, building an activatable pro-form. The ACPPE was conjugated to protoporphyrin IX, a light-sensitive molecule, and was therefore utilized as therapy against different forms of cancer by photodynamic therapies [7]. After cleavage and activation of the CPP in cancer tissue, this photosensitizer could be introduced into the cells generated by irradiation reactive oxygen species.

A stable covalent linkage between the cargo and CPP is not always necessary for translocation as simple mixing

of the two has been shown to be efficient. The synthetic covalent bond between CPP and nucleic acid may alter the biological activity of the latter (**Fig. 2D**). In 1997, the first non-covalent CPP for delivery of nucleic acids, MPG (see **Table 1**), was designed by the group of Heitz and Divita, which was closely followed by the development of Pep-1 for non-covalent cellular delivery of proteins and peptides by Morris *et al.* in 2001. The groups of Wender and Futaki demonstrated that oligoarginine sequences (Arg8) were sufficient to drive molecules into cells and proposed that their uptake mechanism involved a bidentate hydrogen bond between thguanidinium moieties of the arginine residues and phosphate groups in the membrane ^[5,6]. Therefore, a new non-covalent strategy requiring no chemical modification with short amphipathic CPPs such as MPG and Pep-1 as carriers has been successfully applied for cargo deliveries. These non-covalent conjugates are formed through either electrostatic or hydrophobic interactions. With this method, cargos such as nucleic acids and proteins could be efficiently delivered while maintaining full biological activities. MPG forms highly stable complexes with siRNA with a low degradation rate and can be easily functionalized for specific targeting, becoming major advantages compared to the covalent CPP technology ^[8].

CPP-based Strategies for Delivery of Therapeutic Molecules

1. Protein Delivery

Therapeutic proteins can be delivered enterally or parenterally through intravenous, subcutaneous or inhaled routes of entry. Proteins must traverse mucosal barriers in some cases and travel through the extracellular space into the bloodstream before entering the target cells. Conjugation of CPPs to biotherapeutics or co-administration of CPPscan resolve some of the commonly encountered problems with systemic routes by increasing cellular absorption and ensuring bioavailability to otherwise impenetrable organs such as the brain ^[18].

The use of proteins as therapeutic agents constitutes a very promising approach for the treatments of various diseases. An effective delivery of protein by CPP is from 30 kDa to 120-150 kDa. In one study, the author performed a comparison study of CPP-conjugated protein delivery system using seven arginine and Streptolysin O (SLO)-mediated systems. To compare CPP and SLO mediated protein delivery systems, they used GFP and ESRRB protein known to regulate pluripotency-related genes for delivery into human bone marrow stromal cells (hBMSCs) and human testicular stromal cells (hTSCs). It was found that CPP-conjugated protein delivery was more efficient and had lower cytotoxicity and higher biological activities than SLO mediated protein delivery system. These results suggest that delivery of CPP-conjugated proteins is an efficient tool for introducing biologically active proteins into cells and may have important implications in clinical cell-based therapies ^[9].

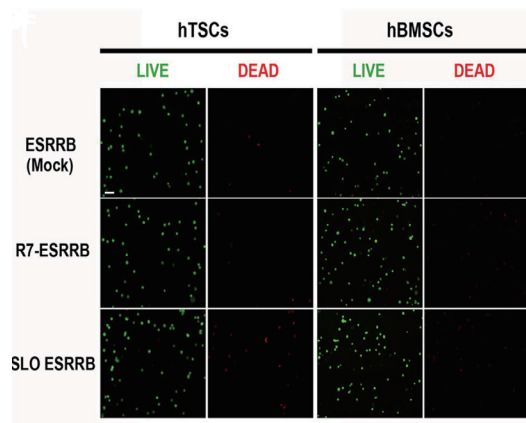


Figure 4. CPP-conjugated proteins are efficiently transduced inside of human mesenchymal stromal cells. Transduction of GFP and R7-GFP were detected by confocal microscopy. GFP or R7-GFP were visualized in green. Nuclei were counterstained with DAPI and the images were merged. Scale bars represent 20 μ m. Picture generated from DOI: [10.1038/srep04378](https://doi.org/10.1038/srep04378)

2. siRNA Delivery

RNA interference (RNAi) has become an indispensable tool for studying gene functions and constitutes an attractive approach for the development of novel therapeutic strategies for pathological disorders. CPPs have been used for delivery of siRNAs either by covalent or non-covalent approaches. Efficient delivery of siRNAs has been reported by their covalent association with transportin, Tat and penetratin. However, a non-covalent pattern is more popular due to stable complexation, such as the MPG peptide. The preparation of non-covalent complexes between siRNAs and the CPPs is through aggregates or nanoparticles with a net positive charge [19].

Ji and his group designed a cyclic peptide named RMP, which can self-assemble into nanoparticle and to deliver siRNA in both *in vitro* and *in vivo* settings. Delivery of the RPM/VEGFR2 (zebrafish)-siRNA into zebrafish embryos resulted in inhibition of neovascularization. Administration of RPM/VEGFR2 (mouse)-siRNA to tumor-bearing nude mice led to a significant inhibition of tumor growth, a marked reduction of vessels and a downregulation of VEGFR2 (messenger RNA and protein) in tumor tissue [10] (**Fig. 5**).

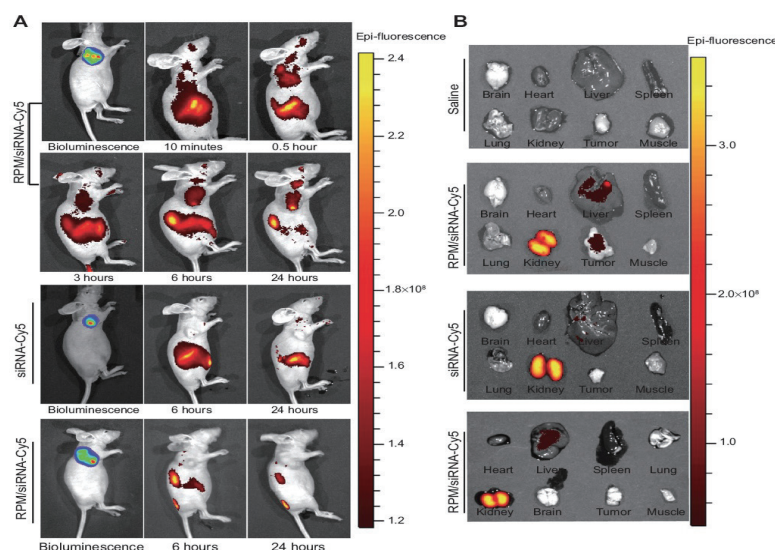


Figure 5. Intravenous injection of RPM/siRNA (siVEGFR2) results in reduced tumor growth in mice. Picture generated from DOI: [10.2147/IJN.S63717](https://doi.org/10.2147/IJN.S63717)

3. Antisense Oligonucleotide Delivery

Antisense technology to target desired genes and modulate a variety of cellular functions attracts tremendous pharmacological interests. Specific oligonucleotides (ONs), once inside the cells, can hybridize with complementary mRNA strands, which causes translational arrest or mRNA degradation through activation of the cellular enzymes of the RNaseH family and consequently blockage of gene expression. Chemical modification of ONs can drastically improve their biocompatibility, selectivity and stability in the biological environment.

Promising results of the *in vivo* use of ONs conjugated with CPPs were obtained in an animal model of Duchenne muscular dystrophy [12]. A good selection of CPPs to ONs included (R_xR)₄, TP10 and several NLS peptides. A review article published in 2019 showed that Wood and his coworkers chose a series of Arg-rich CPPs called Pip to enhance the delivery of attached steric-blocking ONs, making them become a potentially widespread use as neuromuscular and neurodegenerative drugs. The authors focused on CPP-ONs developments toward the treatment of neuromuscular diseases, Duchenne muscular dystrophy and spinal muscular atrophy. CPP conjugates with Steric-blocking Oligonucleotides successfully altered the dystrophin production, and cationic residues were the determinant of splicing activity [11] (**Fig. 6**).

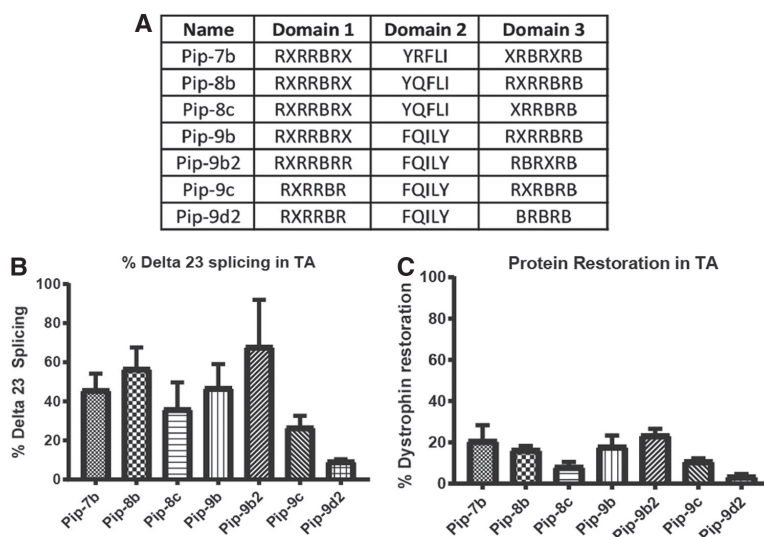


Figure 6. CPP conjugates of Steric-blocking Oligonucleotides to alter the dystrophin production. (A) Table of exemplar CPP sequences of Pip 7, 8 and 9 series of CPP-PMOs. (B, C) Splice-switching activity in tibialis anterior of mdx mice following a single 12.5 mg/kg intravenous administration as measured by (B) quantitative RT-PCR of exon 23 skipping levels and (C) dystrophin protein restoration as assessed by western blot. R—arginine; X—aminohexanoic acid; B—beta-alanine.

Picture generated from DOI: [10.1089/nat.2018.0747](https://doi.org/10.1089/nat.2018.0747)

4. Gene Delivery

One important potential of CPP application is gene therapy, which delivers therapeutic genes into the nucleus of target cells to achieve expression of a deficient or incorrectly expressed gene product [20]. The use of cationic peptides for gene delivery is particularly interesting because they are able to efficiently condense DNA due to electrostatic interaction, to attach to liposomes or polymers allowing for efficient targeting, to improve cellular internalization and promote endosomal escape, and to provide nuclear localization of condensates when short NLS peptides are used.

Langel and his coworkers modified CPPs with an optimal combination of overall charge and hydrophobicity in the peptide backbone, which successfully augmented the delivery of gene *in vivo*. Luciferase activity assay was used to estimate the efficiency [12] (**Fig. 7**).

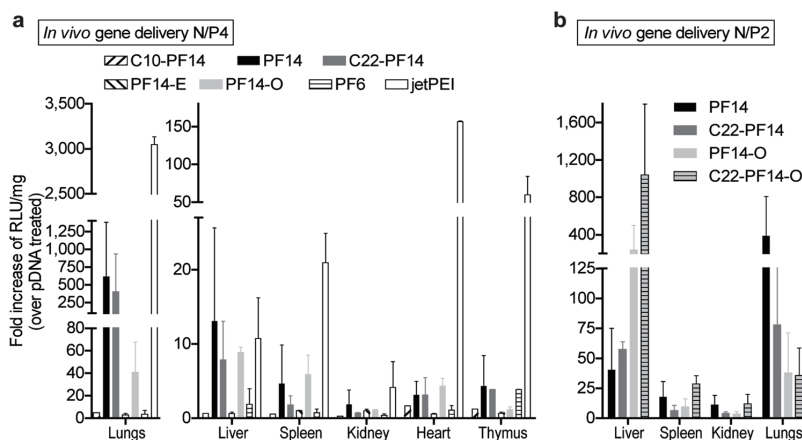


Figure 7. The PF14 analogue C22-PF14-O provides efficient and safe gene delivery *in vivo*. (a) Systemic *in vivo* gene delivery efficacies of CPP/pDNA at N/P4, 20 µg pDNA dose. (b) The effect of increasing the pDNA dose on *in vivo* gene induction at N/P2 and pDNA 50 µg. Picture generated from DOI: [10.1038/s41598-017-17316-y](https://doi.org/10.1038/s41598-017-17316-y)

5. Other Molecule Deliveries

CPPs have been successfully used for the intracellular delivery of different cargos, including nanoparticles, PNA, liposomes and nucleic acids, both *in vitro* and *in vivo*, which results in successful transduction in plant cells and animal tissues including the brain.

CPP-derived Therapeutics in Pre-clinical and Clinical Trials

Several preclinical studies have been performed on experimental animals to search for an effective model to test various therapeutic uses. These include cerebral ischemia, amyotrophic lateral sclerosis (ALS), myocardial injury, cancer, muscular dystrophy, cardiology, anti-prion treatment, as well as viral and bacterial infections (**Fig. 8** and **Table 2**). Many of these pre-clinical CPP therapeutic conjugates behave well in Phase I and Phase II clinical trials.

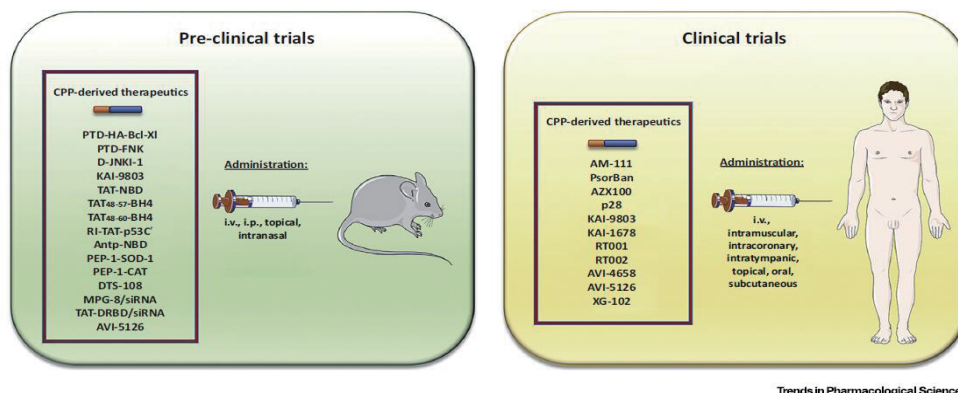


Figure 8. Schematic representation of pre-clinical and clinical evaluations of selected CPP-derived therapeutics. Numerous studies have been performed to investigate the therapeutic applications of various CPPs in both animal models and humans. Administration of CPP-derived therapeutics can be undertaken through relatively noninvasive administration routes, such as intravenous (I.V.), intraperitoneal (I.P.), intranasal, topical, intramuscular, *per os*, intracoronary, intratympanic and subcutaneous. Picture generated from DOI: [10.1016/j.tips.2017.01.003](https://doi.org/10.1016/j.tips.2017.01.003)

Table 2. Examples of CPP-Conjugated Therapeutics under Clinical Development.

Pharmaceutical organization	Compound	CPP-cargo	Therapeutic use
Auris Medical	AM-111	TAT-JBD20 (D-JNKI-1)	Hearing loss
CellGate, Inc.	PsorBan	R7-cyclosporin A	Psoriasis
Capstone Therapeutics	AZX100	PTD4-HSP20 <i>Phosphopeptide</i>	Scar prevention/reduction
CDG Therapeutics, Inc.	p28	p28	Cancer
KAI Pharmaceuticals	KAI-9803	TAT- δ PKC <i>inhibitor</i>	Myocardial infarction
KAI Pharmaceuticals	KAI-1678	TAT- ϵ PKC <i>inhibitor</i>	Pain: postherpetic neuralgia, spinal cord injury, postoperative
Revanche Therapeutics, Inc.	RT001	MTS-botulinum toxin A	Lateral canthal lines Crow's feet Facial wrinkles
Revanche Therapeutics, Inc.	RT002	TransMTS1-botulinum toxin A	Glabellar lines
Sarepta Therapeutics	AVI-4658	N/A	Duchenne muscular dystrophy
Sarepta Therapeutics	AVI-5126	(R-Ahx-R) ₄ -PMO	Cardiovascular disease Coronary artery bypass
Xigen SA	XG-102	TAT-JBD20 (D-JNKI-1)	Inflammation Intraocular inflammation Pain

Revanche Therapeutics, Inc. developed a compound named RT002 as a topical treatment for lateral canthal lines. This compound conjugates with the optimized CPP of TAT 49–57 domains to enable large molecules to cross the skin. The clinical development program for RT002 also includes a currently ongoing Phase II study of the cervical dystonia treatment.

In the context of pain and inflammation, Xigen SA Company moved into clinical development of XG-102 (previously known as D-JNKI-1), a 31-D-amino-acid peptide inhibitor for JNK pathway activation, which has been shown to have potential for the treatment of intraocular inflammation in rat models of uveitis. In this first clinical trial using XG-102, it was shown that it is safe and well-tolerated to administer as a single subconjunctival injection as adjunct therapy in patients with recent post-surgery or post-trauma intraocular inflammation.

A few clinical evaluations of CPP-derived therapeutics were developed for the treatment of dermatological diseases. The first compound that entered a clinical trial was a cyclosporine polyarginine conjugate (PsorBan1; CellGate, Inc.) as a topical treatment of psoriasis by transdermal delivery of cyclosporine A (CsA). The compound was then entered into Phase IIa, showing potential benefit in patients with mild-to-moderate psoriasis without the adverse effects associated with systemic administration of cyclosporine.

Clinical trials of CPP-derived therapeutics have also been conducted on neuromuscular degenerative diseases. Sarepta Therapeutics, for example, has developed a set of arginine-rich CPPs conjugated with PMOs, some of which were tested in Duchenne Muscular Dystrophy (DMD) ^[13]. Progressive muscle degeneration in DMD is due to frame-shift mutations disrupting the open reading frame of the gene encoding dystrophin, which compromises its expression. Chemically modified oligonucleotides such as PMOs have been used to modify splicing and induce exon skipping, thus restoring the open reading frame and, consequently, the production of the functional protein. And the CPP-PMO, which is AVI-4658 made by Kinali and coworkers, designed to skip exon 51 in the dystrophin mRNA in DMD patients.

Clinical evaluations of CPP-derived cancer therapeutics were also developed. CDG Therapeutics isolated p28, a 28-amino-acid peptide derived from the bacterial protein azurin. After penetration of cancer cells, this peptide enters the nucleus where it binds to a region within the DNA-binding domain of the tumor suppressor protein p53, inhibiting its degradation ^[14]. This causes an intracellular increase of p53 that leads to inhibition of the cell cycle, thereby preventing cancer cell proliferation.

Conclusion

With a diverse sequence variety and a wide range of physical and chemical properties, CPPs can be linear, cyclical, cationic, anionic, hydrophobic, hydrophilic, amphipathic, non-amphipathic, random coiled, α -helical or β -sheets. However, CPPs differ from most other peptides with respect to specific features that reflect various mechanisms used to enter the cell.

The diversity of CPPs is advantageous for drug discovery. Because many filters are applied in each step of the drug discovery process, starting with an arsenal of diverse CPPs increases the chances that at least one will progress. CPPs, like small molecules, must undergo a large panel of assays to assess toxicity, tissue distribution, cell selectivity, solubility, plasma stability, among others. By mapping how different classes of CPPs behave relatively to these parameters, it is crucial to determine whether a problem is systemic to a given class of CPPs or whether it can be overcome through chemical modifications that do not alter the uptake ^[20, 21].

Multiple examples, including data from laboratories as well as pre-clinical and clinical trials, have clearly demonstrated the ability of CPPs to transport various types of cargo molecules across cell and tissue barriers, thereby allowing them to reach their targets. CPP techniques have greatly improved and the development of CPP-conjugated therapeutics in human therapies appears increasingly feasible and clinically significant ^[22, 23].

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