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Cell Penetrating Peptides

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Most prospective therapeutic and diagnostic agents have very poor cell permeability and low bioavailability. Cell penetrating peptides (CPPs), also known as protein transduction domains have the ability to translocate through the cell membranes. As such, they have received formidable attention in the current advances in drug delivery as promising tools to overcome drug delivery problems. These peptides have been used to deliver drugs, imaging agents, and other therapeutic biomolecules across the cell membrane into the cytoplasm.

Although the mechanism of their intracellular translocation is not clear, the amino acid composition which gives them a net positive charge seems to play a key role in this process.[1] Different studies have hypothesized that internalization occurs via endocytosis, direct transport through the cell membrane or both.

The primary structure of CPPs is generally composed of cationic residues such as arginines and lysines.

Several naturally occurring and synthetic CPPs have been investigated in delivery of various cargo such as nucleic acids, proteins, quantum dots, contrast agents and small organic molecules.[2] In all of these studies, CPPs exhibited minimal toxicity in biological systems, suggesting their potential as drug delivery vehicles. The table below highlights some of the most common naturally occurring and synthetic cell penetrating peptides.

In this issue, we will be discussing the various CPPs and their advances in therapeutic applications.

References

1. Richard *et al.*, J. Biol. Chem. **2003**, 585-59
2. Fonseca *et al.* Advanced Drug Delivery Reviews, **2009**, 61, 953-964

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Commonly Used CPPs

Peptide	Sequence
HIV-1 TAT 48-60	GRKKRRQRRPPQ
Antennapedia 43-58 (Penetratin)	RQIKIWFQNRRMKWKK
Transportan	GWTLNSAGGYLLGKINLKALAALAKKIL
Polyarginine	RRRRRRRRR
Pep1	KETWWETWWTWWSQPKKKRKV
BMV Gag- (7-25)	KMTRAQRRAAARRNRRWTAR

Table 1. Sequences of commonly used cell penetrating peptides

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HIV TAT 48-60

Constrained Peptide Designs
- >Head to tail backbone cyclization
- >Side chain to side chain cyclization
- >Side Chain to backbone cyclization

Chemistries Used in Synthesis of Constrained Peptides
- >Copper Alkyne-Azide assisted Cycloaddition (CuAAC)
- >Hydrocarbon stapling
- >Lactam bridge formation
- >Disulfide bonds

The HIV–1 TAT$_{48–60}$ peptides are derived from an 86–amino acid TAT protein involved in replication of HIV–1. Studies have shown that the helical domain of TAT protein contains clusters of basic amino acids and plays a crucial role in translocation of TAT peptides into the cells.[1] This domain contains multiple arginines which plays a vital role in the intracellular translocation capability of TAT peptides. When one arginine residue is deleted, TAT peptides cell permeability is decreased by half.[2]

HIV–1 TAT peptides have been used to deliver a variety biological molecules including large proteins such as RNase A, β–galactosidase among other proteins.[4,5] Other biomolecules that have successfully been transported into the cells by linking them to TAT peptides include; liposomes,[6] nanoparticles,[4] DNA, siRNA[7] and small molecules.

References

1. Vives *et al*. J. Biol. Chem. **1997**, 272, 16010-16017
2. Tung and Weissleder, Adv. Drug Del. Rev. **2003**, 55, 281-29
3. Berry *et al*., Nanomedicine, **2008**, 3, 357-365
4. Torchilin *et al*., Drug Discovery Today: Technologies, **2008**, 5, e95-e103
5. Zhao *et al*., Med Res Rev, **2004**, 24, 1-12
6. Torchilin *et al*., PNAS, **2001**, 98, 8786-8791
7. Astriab-Fisher *et al*., Pharm. Res., **2002** , 19, 744-754

Peptide	Sequence
TAT 47 - 57	YGRKKRRQRRR
TAT 47 - 57 Dye - labeled	Dye-YGRKKRRQRRR
Custom TAT derivatives	Cargo-K(Dye)-YGRKKRRQRRR

Antennapedia 43-58 (Penetratin)

Antennapedia 43–58, a 16 amino acid fragment from the third helix of Drosophila antennapedia protein, was shown to have the capability of translocating through cell membranes.[1] Similar to TAT peptides, antennapedia has been used as a delivery system of various cargo through the cell membrane into the cytoplasm. Villa et. al. studies have shown that penetratin constructs effectively translocates into melanoma cells.[2] More studies by Avignolo et al. have used antennapedia to transport monoclonal antibodies into the colorectal carcinoma cell lines (HCT116).[3]

References

1. Derossi *et al*., J. Biol. Chem. **1994**, 269, 10444-10450
2. Villa *et al*., FEBS letters, **2000**, 473, 241-248
3. Avignolo *et al*., The FASEB Journal, **2008**, 22, 1237-1245

Peptide	Sequence
Antennapedia 43-58	RQIKIWFQNRRMKWKK
Antennapedia 43-58 Dye - labeled	Dye-RQIKIWFQNRRMKWKK
Custom Antennapedia 43-58 derivatives	Cargo-K(Dye)-RQIKIWFQNRRMKWKK

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Custom Oligonucleotides Synthesis:
- >DNA and RNA Triphosphates
- >DNA up to 190 base pairs
- >Long RNA

Polyarginines

Oligoarginines of 6–20 residues have been studied extensively for their ability to penetrate into cytoplasm through the cell membrane. It was found that optimal cell membrane permeation is achieved by oligoarginines residues between 5 and 15.[1] In particular, nona–arginine peptides were shown to have improved cell penetration efficiency compared to TAT peptides.[2] Thus, most studies have utilized octa– and nona–arginine peptides as delivery medium for most biological molecules including siRNA, anticancer drugs, small molecules, proteins, peptides, and oligonucleotides.[2,3]

Since oligoarginine peptides are the most commonly used CPPs, their optimization to reduce cell toxicity and improve protease stability has been investigated. Replacement of L–arginines with D–amino acids resulted to protease resistant polyarginines with better intracellular translocation compared to the L–oligoarginines peptides.[4]

In addition, fatty acids have been incorporated to generate more active peptides with low toxicity. Lee et. al. incorporated C14 fatty acid chains. The resulting lipo–oligoarginines peptides had increased cell permeation, improved metabolic stability and minimal cytotoxicity.[5]

Oligoarginine peptides with minimal cell adsorption and uptake have also been designed by incorporating a polyglutamic chain as a counter ion domain. The protease labile linker between the polyglutamic and polyarginine domains releases the polyarginine domain for intracellular translocation.[6]

It has been portrayed that the guanidino functional group plays a critical role in the intracellular translocation of oligoarginines peptides. Hence, several other guanidine containing molecules have been discovered. Wender et al., designed a polyguanidine peptoid derivative with improved cellular uptake compared to the TAT peptides and nona–arginine peptides containing D–amino acids.[4] This derivatization enhanced protease stability while maintaining cell permeation capability.

References

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3. Wu *et al*., Nucleic Acids Research, 2007, 35, 5182-5191
4. Wender et al., Proc. Natl. Acad. Sci. USA, **2000**, 97, 13003-13008
5. Lee et al. Mol. Biosyst., **2010**, 6, 2049-

Peptide	Sequence
(Arg)9	RRRRRRRRR
(D-Arg)9	rrrrrrrr
(Arg)9 Dye - labeled	Dye-RRRRRRRRR-
Custom (Arg)9 derivatives	Cargo-K(Dye)-RRRRRRRRR

Transportan

Transportan is a 21–mer non–arginine chimera of N–terminal neuropeptide galanin and venom peptide mastoparan.[1] Transportan has been used to deliver peptides, proteins, peptide nucleic acids and small molecules into various cell lines.[2]

Several transportan analogs such as transportan 10, TP10, have been investigated. In TP10, the six N–terminal amino acid residues have been truncated, yet this peptide retains the cell translocating capabilities of the original peptide due to its amphiphatic features.[3]

References

1. Langel et al., Regul. Pept. **1996**, 62, 47-52
2. Pooga *et al*., FASEB J. **2001**, 15, 1451-1453
3. Lindgren et al., The Biochemical Journal, **2004**, 377, 69-76

Peptide	Sequence
Transportan	GWTLNSAGGYLLGKINLKALAALAKKIL
Transportan TP10	Dye-RQIKIWFQNRRMKWKK
Transportan Dye - labeled	AGGYLLGKINLKALAALAKKIL
Custom Transportan derivatives	Cargo-K(Dye)-GWTLNSAGGYLLGKINLKALAALAKKIL

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Other Drug Delivery Techniques

Dendrimers: These molecules have unique features such as high loading capacities for bioconjugations and uniformity, Thus, they have received considerable attention in biomedical field as drug delivery systems for drugs and imaging agents.[1] Dendrimers with guanidine modifications have been investigated as delivery systems for DNA, RNA, nanopar–ticles, proteins and small molecules such as dyes, in different cell lines. Dendrimer cargo can be incorporated either in the cavities or covalently bound to the dendrimers using different chemistries.

References

1. Bonduelle *et al*., Pharmaceuticals. **2010**, 3, 636-666

Bio–Synthesis is a leading global manufacturer of high quality custom peptides and oligonucleotides. Our experts have enabled us to provide clients with the ability to design and manufacture advanced glycosylated and carbohydrate modified peptides for biopharmaceutical, diagnos–tic, and research applications. We continue to maintain integrity and professionalism as we serve our clients in areas spanning from proteomics to cell biology.

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