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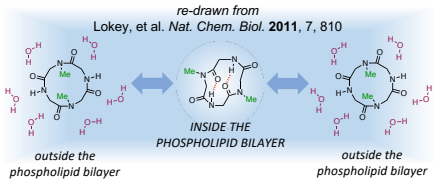
Peptide Cell Permeability

Bowers Lab

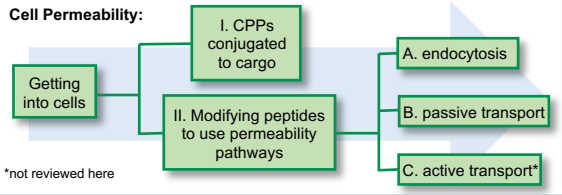
Macrocyclic Peptide Cell Permeability – the ability of a macrocyclic peptide to pass through the cell membrane and reside in the cell's cytoplasm. There are two main mechanisms that allow peptides to enter the cell – passive permeability and active transport such as endocytosis. Passive permeability is the movement of the peptide directly through the cell membrane into the cytoplasm. Active transport requires the peptide to interact with a cell surface protein or with specific parts of the cell membrane itself to enter the cell.

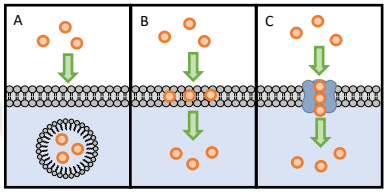


Useful Reviews:

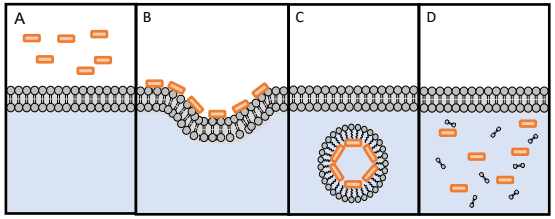
- Matsson, P.; Doak, B. C.; Over, B.; Kihlberg, J. Cell permeability beyond the rule of 5. *Advanced Drug Delivery Reviews*. **2016,** *101,* 42-61.
- Walport, L. J.; Obexer, R.; Suga, H. Strategies for transitioning macrocyclic peptides to cell-permeable drug leads. *Current Opinion in Biotechnology*. **2017,** *48,* 242-250.
- Guidotti, G.; Brambilla L.; Rossi, D. Cell-penetrating peptides: from basic research to clinics. *Trends in Pharmacological Sciences*. **2017,** *38 (4),* 406-424.
- Peraro, L., Kritzer, J. A. Emerging methods and design principles for cell-penetrant peptides. *Angew. Chem. Int. Ed.* **2018,** *57,* 11868.

Cell Permeability:





Endocytosis Permeability Pathway General Mechanism:



a) extracellular peptide; b) aggregation to cell wall; c) endocytosis; d) endosomal escape

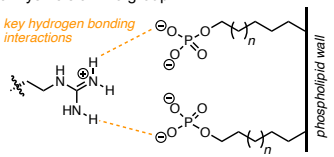
Cell Penetrating Peptides (CPP):

Name	Sequence	Class	MW	Charge
Tat	YGRKKRRQRRR	C	1558.9	+9
R9	RRRRRRRRR	C	1422.7	+10
Penetratin	RQIKIWFQNRRMKWKK	C	2245.8	+8
DPV3	RKKRRRESRKKRRRES	C	2211.6	+11
pVEC	LLILRRRIRKQAHAHSK	A	2208.7	+7.2
TP10	AGYLLGKINLKALAALAKKIL	A	2181.8	+5
Pep-1	KETWWETWWTEWSQPKKRV	A	2847.2	+4
MAP	KLALKLALKALKAALKLA	A	1876.5	+6
p28	LSTAADMQGVVTDGMASGLDKDYLKPDD	A	2913.2	-3
Transportan	GWTLNSAGYLLGKINLKALAALAKKIL	A	2840.5	+5
TP2	PLIYLRLLRGQF	H	1487.8	+3
C105Y	CSIPPEVKFNKPFVYLI	H	1993.4	+1.9
KFGF	AAVLLPVLLAAP	H	1146.5	+1
Pep-7	SDLWEMMMVSLACQY	H	1806.2	-1.1

re-drawn from Peraro, et al. *Angew. Chem. Int. Ed.* **2018**, 57, 11868
C = cationic; A = amphipathic; H = hydrophobic

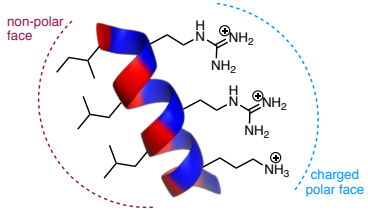
Guanidinium-Based Patterning (Cationic):

Guanidinium head groups such as those on arginine, form bi-valent H-bonds with negatively charged groups on the cell surface. This targets the peptide to the cell membrane and facilitates entry into the cell. Arginines with the guanidinium group are more favorable for cell permeability than lysine's amino group.



Amphipathic Patterning:

The charged, polar side of the molecule forms interactions with the negatively charged groups on the cell surface. The hydrophobic face of the helix then associates with the lipids in the cell membrane to help the molecule enter the cell.



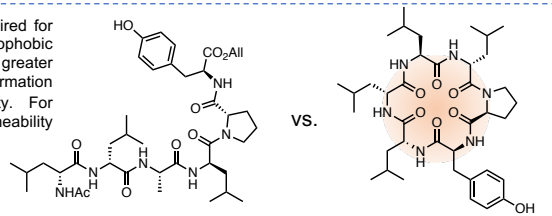
CPPs tend to use endocytotic pathways to enter the cell. The cargo the CPP is bound to is capable of affecting the mechanism of uptake and of endosomal escape. Different CPPs may need to be tested before finding one that works best for getting the desired peptide into cells.

Passive Cell Permeability:

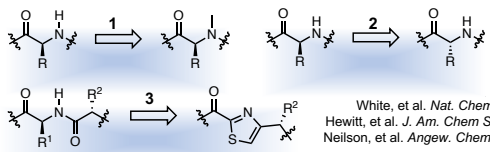
Many groups have studied how to make peptides passively permeable. The following factors have been shown to be the most important ones and examples of how they can be altered to improve passive permeability are included.

Flexibility – some flexibility in the backbone is required for switching between hydrophilic and hydrophobic conformations, too much flexibility results in a greater entropic penalty for switching to permeable conformation and weakens IMHB necessary for permeability. For example, the structure on the right gains gains permeability through decreasing flexibility by macrocyclization.

Rezai, et al. *J. Am. Chem. Soc.* **2006,** 128, 2510



H-bond donors/acceptors – H-bond donors and acceptors should be limited, especially donors. N-methylation (**1**), shielding through changing stereochemistry of lipophilic side chains (**2**), and heterocycles (**3**) all limit interaction between H-bonding residues and the solvent.



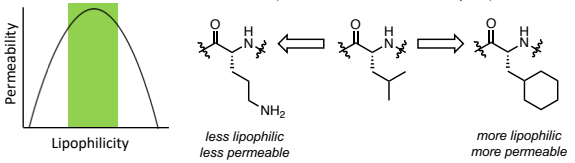
White, et al. *Nat. Chem.* Bio. **2011,** 7, 810
Hewitt, et al. *J. Am. Chem Soc.* **2015,** 137, 715
Neilson, et al. *Angew. Chem.* **2014**, 126, 12255

Size/Volume – The larger the cyclic peptide, as measured by ring size, molecular weight, 2D polar surface area, and 3D polar surface area, the lower the permeability. This trend holds even for molecules with similar lipophilicities. Papers cite 1000 Da as the maximum M.W. for passive permeability.

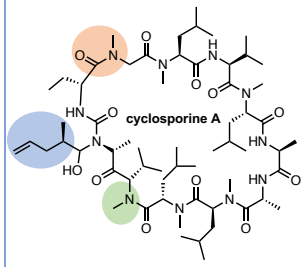
Pye, et al. *J. Med. Chem.* **2017,** 60, 1665.

Lipophilicity – There is a lipophilicity range that gives maximum permeability that tends to center around ALogP ~4. Below this and the peptide lacks hydrophobic groups to interact with the membrane. Above this and the fight between permeability and solubility/aggregation takes over. There are permeable peptides outside of this range, but typically peptides in the range show maximum permeability.

Hewitt, et al. *J. Am. Chem. Soc.* **2015,** 137, 715



Why should we care about cell permeability? Why is it difficult for peptides to be cell permeable?



Guidelines for bRo5

MW	<1000 Da*
cLogP	−2 to 10
HBD	< 6
HBA	< 15
PSA	< 250 A^2

*Exceptions exist Includes: Peptides, macrocycles target flat areas and grooves in proteins, protein-protein interactions.

Most of the targets for peptide therapeutics reside on the interior of the cell. Thus, in order to get the desired effect from the therapeutic, we need to ensure that the peptide can enter the cell to interact with its target.

Peptides are notoriously difficult to get into cells. Two of the biggest issues are size and the polar backbone. Often times alterations to the backbone, as well as side chains, are necessary for getting peptides into cells. Modifying them to be cell permeable is also difficult as they are outside Lipinski's Rule of 5, unlike small molecules. Cyclosporine A (left) is a natural, orally bioavailable, macrocyclic peptide that is often studied to determine how to make cyclic peptides permeable. Following the beyond Rule of 5 guidelines can aid in making peptides cell permeable.

Decreases flexibility through macrocyclization

Decreases number of H-bond donors through N-methylation

Increases overall lipophilicity of the molecule using unnatural amino acids

Matsson, P. et al. *Adv. Drug Delivery Rev.* **2016**, 101, 42

Conclusion: Cell permeability of cyclic peptides is difficult to predict. Libraries can be designed to be more likely to be cell permeable by limiting size, and including unnatural and N-methylated amino acids to favor potentially cell-permeable peptides. All of these studies, however, have been done with model peptides and while we can learn a lot, there's something to be said for having a therapeutically relevant peptide where certain residues cannot be altered.