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Amphipathic Proline-Rich Cell Penetrating Peptides for Mitochondria Targeting

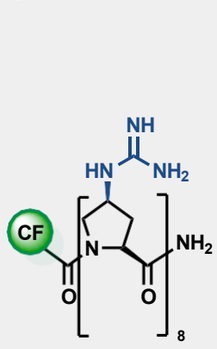
Adeline M. Schmitt and Helma Wennemers*
Laboratory of Organic Chemistry, D-CHAB, ETH Zürich, Switzerland

Introduction

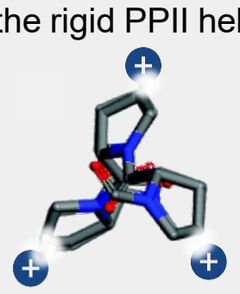
Cell penetrating peptides (CPPs) cross the cellular membrane and serve as delivery vectors to translocate cargo into cells.[1] They can also be useful for target-specific delivery, for example, of bioactive molecules to a specific cellular organelle. Here, targeting mitochondria constitutes an important goal since mitochondria dysfunction is associated with many diseases, including neurodegenerative and auto-immune diseases, diabetes, and cancer.[2] Selective delivery of bioactive compounds to mitochondria is challenging due to the dense and hydrophobic double layered-membrane.[3]

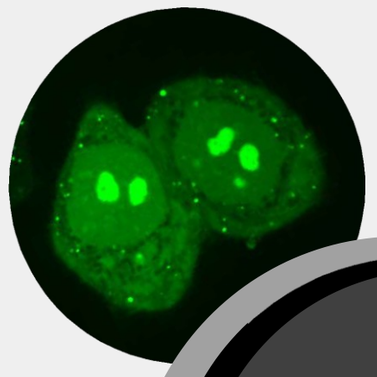
Background

Our group developed an oligoproline-based CPP (Z_8) that localizes in the cytoplasm and the nucleus. This CPP exhibits higher cellular uptake in comparison to more flexible peptides (e.g octaarginine) thanks to the preorganization of the cationic charges in defined positions along the rigid PPII helix. [4]



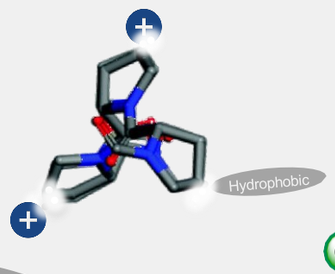
Z_8 peptide

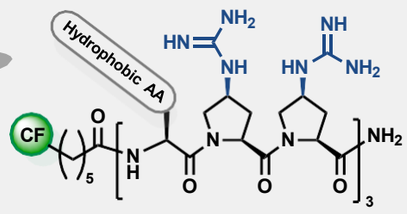




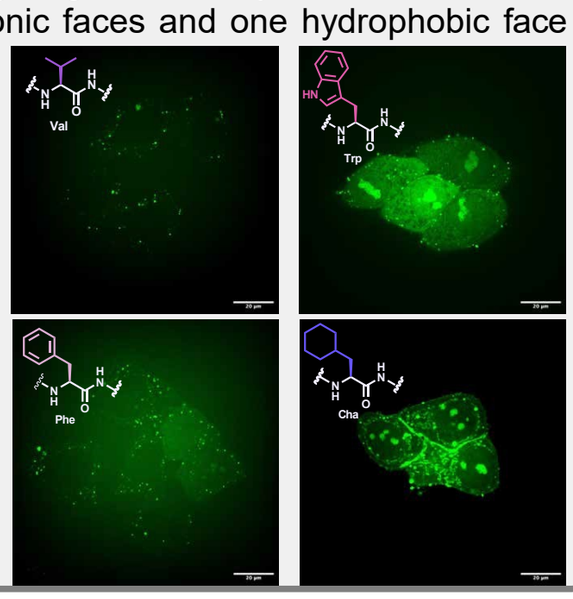
Concept

We envisioned that oligoproline peptides with hydrophobic amino acids installed at every third position allow for mitochondria targeting.[5] Selectivity would be achieved by the PPII helical conformation with two cationic faces and one hydrophobic face enabling the crossing of the mitochondria membrane.



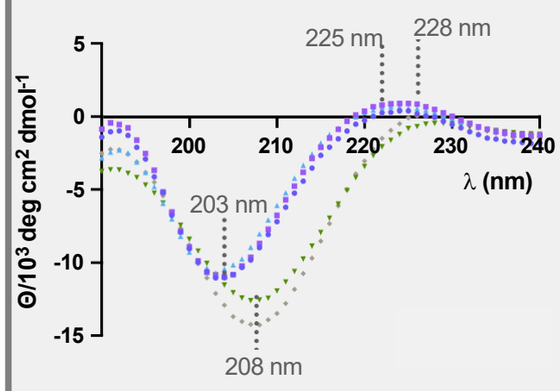


A cyclohexyl side chain enables the mitochondria targeting.

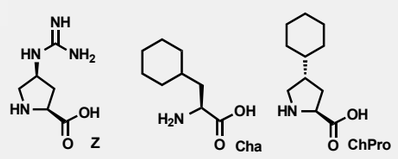


Circular Dichroism

All peptides are PPII helical

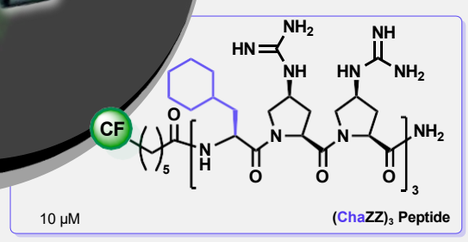


- $(ChaZZ)_3$
- $(ChaZZ)_3$-Cha
- $(ChaZZ)_2$-Cha
- $(ChProZZ)_2$-ChPro
- $(ChProZZ)_3$



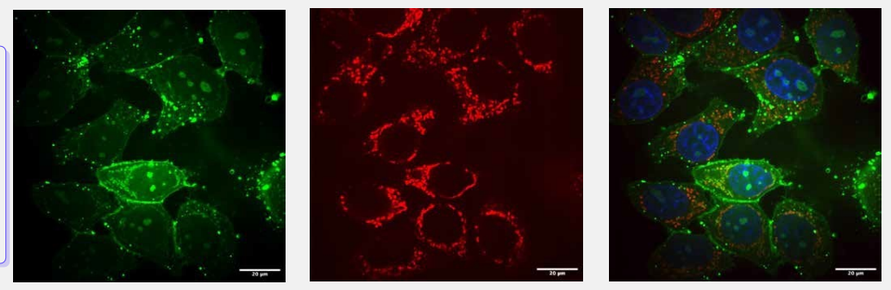
50 µM, H_2O

Colocalization Studies in Live Cells

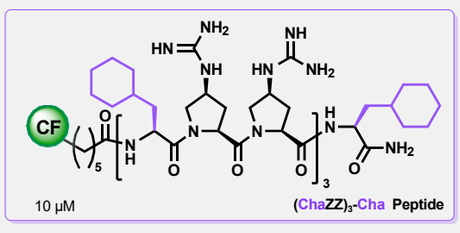


10 µM — $(ChaZZ)_3$ Peptide

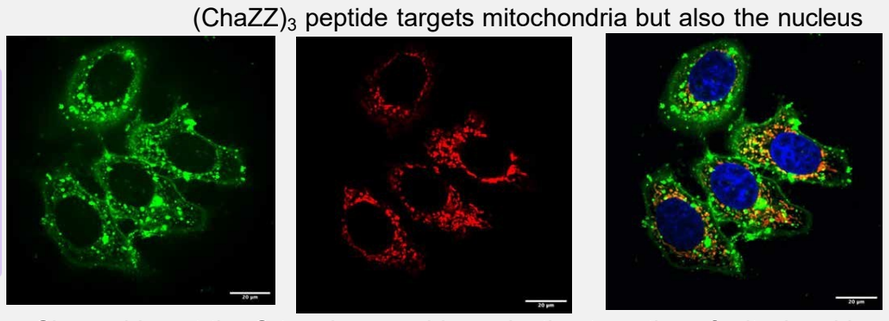
Peptide Channel	Mitochondria Stain	Merge



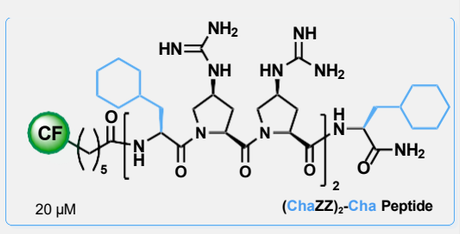
$(ChaZZ)_3$ peptide targets mitochondria but also the nucleus



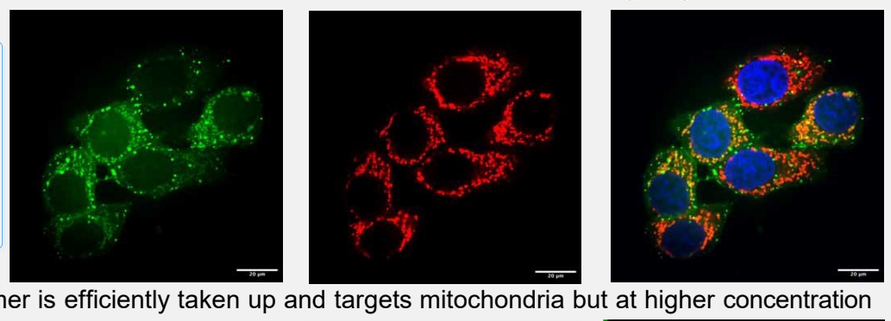
10 µM — $(ChaZZ)_3$-Cha Peptide



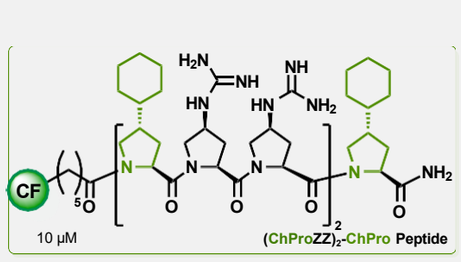
Adding one Cha residue at the C-terminus enables selective targeting of mitochondria



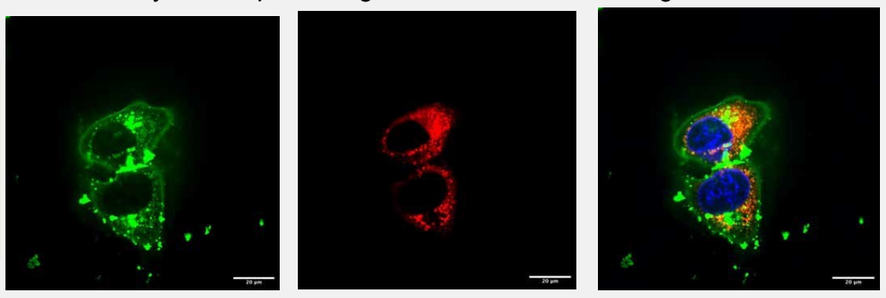
20 µM — $(ChaZZ)_2$-Cha Peptide



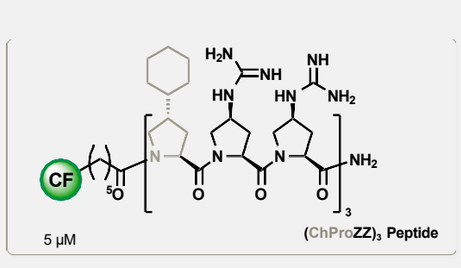
Also the shorter 7-mer is efficiently taken up and targets mitochondria but at higher concentration



10 µM — $(ChProZZ)_2$-ChPro Peptide



Implementing ChPro instead of Cha increases the cellular uptake

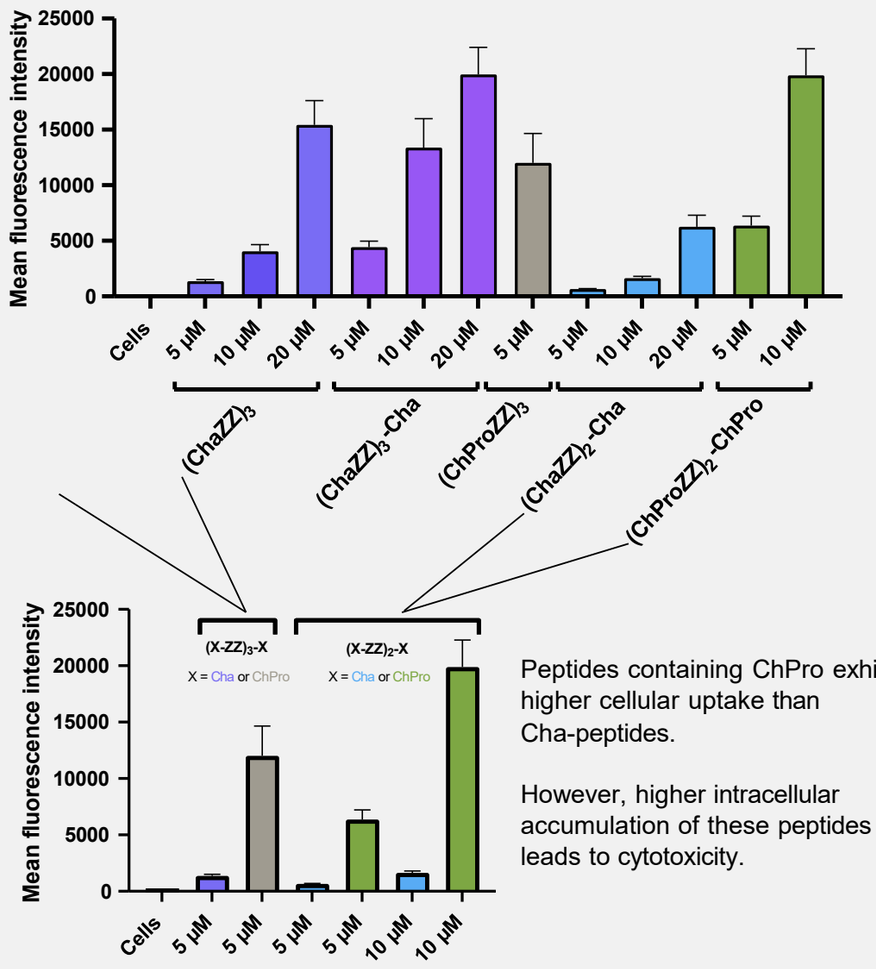


5 µM — $(ChProZZ)_3$ Peptide

The 9-mer ChPro analogue targets mitochondria at lower (5µM) concentration selectively

Confocal microscopy, MCF-7 cells, 1h incubation at 37 °C

FACS Analysis



Peptides containing ChPro exhibit higher cellular uptake than Cha-peptides.

However, higher intracellular accumulation of these peptides leads to cytotoxicity.

Fluorescence-Activated Cell Sorting, MCF-7 cells, 1h incubation at 37 °C

Let's keep in touch!

References

1 Langel, U., Cell-Penetrating Peptides*, Methods and Protocols,* **2011,** Humana, Totowa, NJ.
2 Heitz, F.; Morris, M. C.; Divita, G., *Br. J. Pharmacol.* **2009**, *157*, 195–206.
3 Bakovic, M.; Schenkel, L. C., *Int. J. Cell Biol.* **2014**, *2014*, 1–13.
4 Nagel, Y. A.; Raschle, P. S.; Wennemers, H.; *Angew. Chem. Int. Ed.* **2017**, *56*, 122–126.

For previous work on mitochondria targeting peptides, see
[5] [a] Jean, S. R.; Ahmed, M.; Lei, E. K.; Wisnovsky, S. P; Kelley, S. O.; *Acc. of Chem. Res.*, **2016,** *49*, 1893-1902
[b] Kalafut, D.; Anderson, T. N.; Chmielewski, J.; *Bioorg Med Chem Lett.* **2012,** *22*, 561-3.