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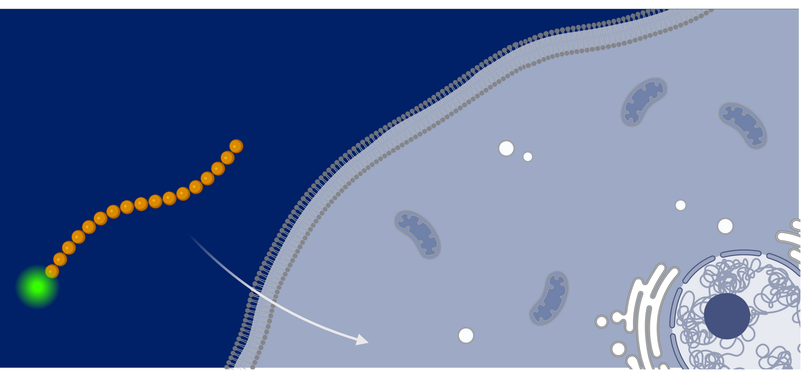
pH-Dependent Cellular Uptake of CPPs – Guanidinium versus Oxyguanidinium Proline

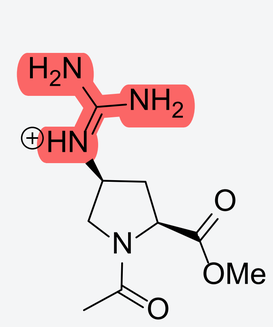
Rahel Heeb, Laura Massaad, Helma Wennemers*
Laboratory of Organic Chemistry, ETH Zurich



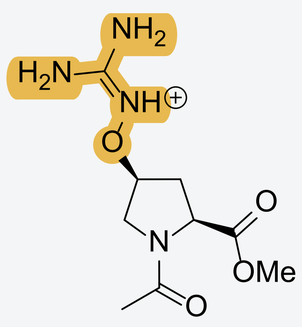
Introduction

The delivery of bioactive molecules across cellular membranes represents a significant obstacle to developing protein and nucleic acid-based therapies. Cell penetrating peptides (CPPs) are attractive tools to transport such cargo across the cellular membrane.[1] Our group showed that rigid cationic oligo guanidinium proline (Gup_8) peptides exhibit higher cellular uptake in comparison to previously established flexible peptides (e.g., octa arginine).[2] Key to their high cellular uptake is their rigidity and charge arrangement in distances of ~9 Å along the PPII helical backbone, which enables tight ionic interactions between the cationic guanidinium prolines and anionic cell surface glycans. Building on this work, we developed a CPP analogue in which the guanidinium prolines were replaced with oxyguanidinium prolines (Gop).[3] We studied the conformational properties of Gop derivatives and the cellular uptake in normal versus acidic cancer cell microenvironments.[4]

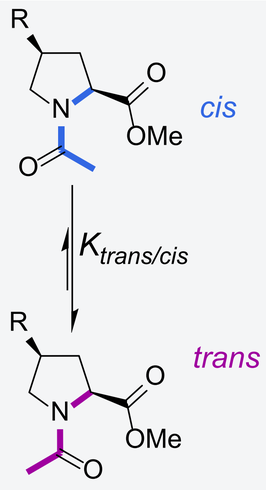
Guanidinium versus Oxyguanidinium Proline

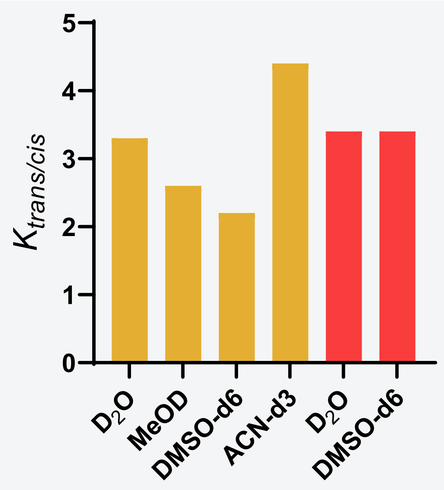


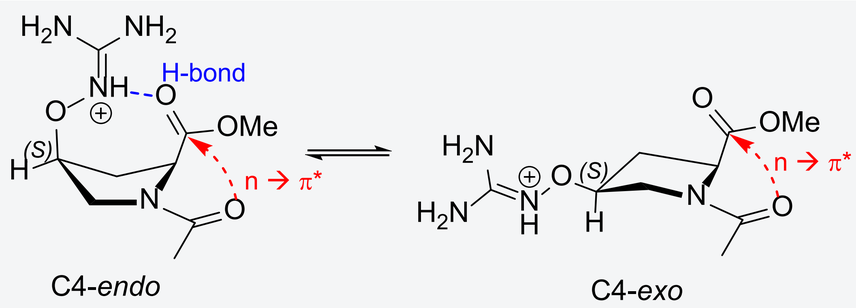
Ac-Gup-OMe
p*Ka* > 12



Ac-Gop-OMe
p*Ka* ~ 6.8

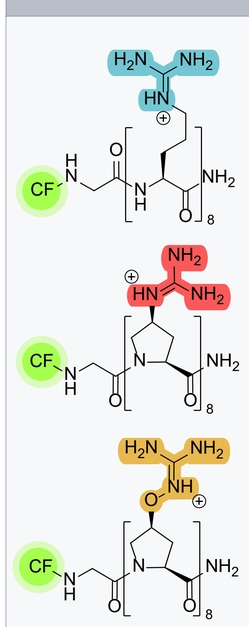




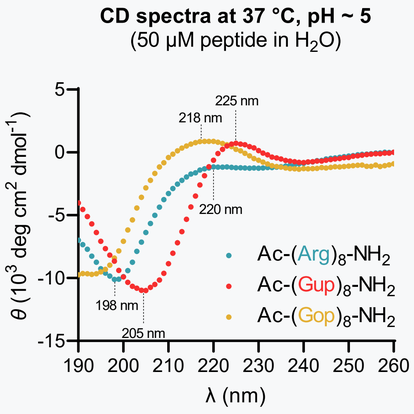


	acidic pH	basic pH
Ac-Gop-OMe	C4-*endo*	C4-*exo*
Ac-Gup-OMe	C4-*endo*	C4-*endo*

Structural Analysis of Octamers

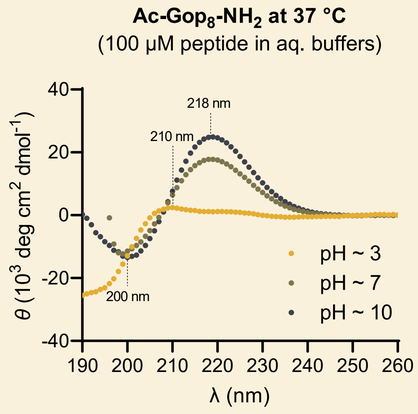


CD spectra at 37 °C, pH ~ 5
(50 µM peptide in H_2O)



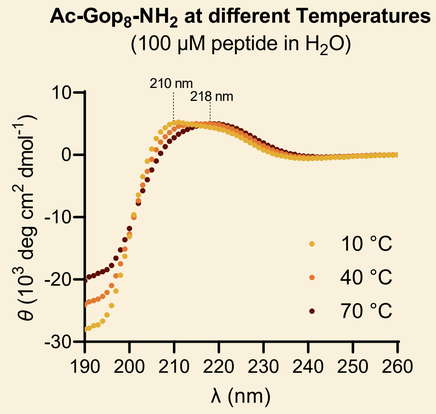
Ac-$(Arg)_8$-NH_2: "Random Coil"
Ac-$(Gup)_8$-NH_2: PPII helix
Ac-$(Gop)_8$-NH_2: PPI & shifted PPII helix

Ac-Gop_8-NH_2 at 37 °C
(100 µM peptide in aq. buffers)



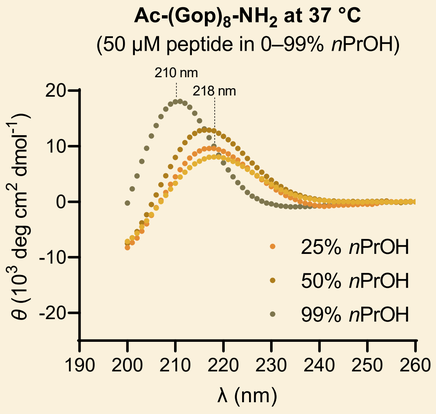
Acidic pH: Mixture of PPI and PPII helix
Basic pH: *Exo*-pucker and PPII helix with substituents pointing to the outside

Ac-Gop_8-NH_2 at different Temperatures
(100 µM peptide in H_2O)



Higher temperature favours the PPII over the PPI helix

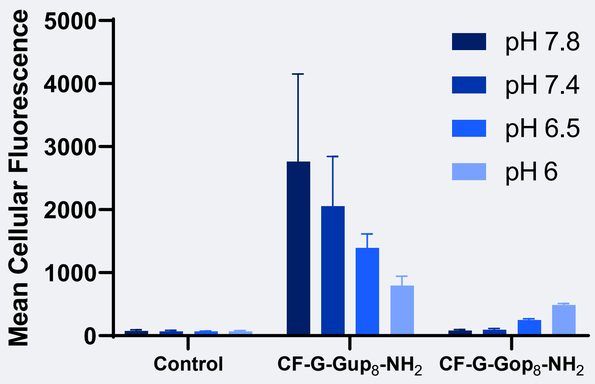
Ac-$(Gop)_8$-NH_2 at 37 °C
(50 µM peptide in 0–99% *n*PrOH)



*n*PrOH favours the PPI helical conformation

Cellular Uptake Studies

Flow Cytometry

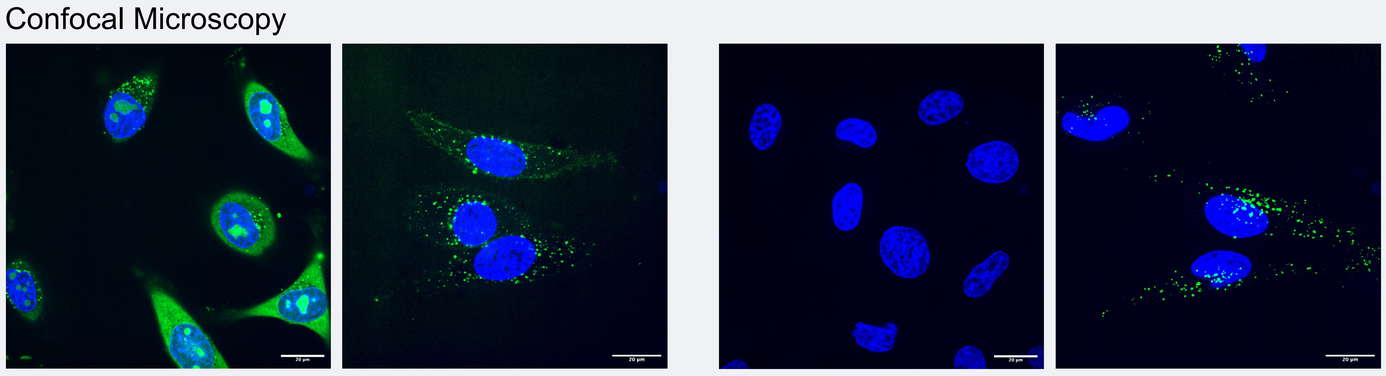


HeLa cells, 1 h, 37 °C, 5% CO_2, 10 µM

Ac-$(Gup)_8$-NH_2: lower uptake upon lowering the pH – cell surface charge change or inhibition of uptake mechanism

Ac-$(Gop)_8$-NH_2: higher uptake upon lowering the pH – protonation state of the peptide

Confocal Microscopy



CF-G-Gup_8-NH_2 pH 7.5 | CF-G-Gup_8-NH_2 pH 6 | CF-G-Gop_8-NH_2 pH 7.5 | CF-G-Gop_8-NH_2 pH 6

HeLa cells, 1 h, 37 °C, 5% CO_2, live cells, 10 µM; green channel = CF-peptide, blue channel = Hoechst 33342 nucleus stain

Conclusion and Outlook

- Oxyguanidinium proline was introduced as an analogue of guanidinium proline
- The p*Ka* of Gop is 6.8, making it more than 10^5-fold more acidic than Gup
- The pucker of Gop depends on the pH
- The conformation of the Gop_8 depends on the pH, temperature and solvent
- Our findings render Gop-peptides promising as a tumour tissue specific delivery system

References

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[2] Nagel, Y. A.; Raschle, P. S.; Wennemers, H.; *Angew. Chem. Int. Ed.* **2017**, *56*, 122–126
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