Constant-pH MD simulations as a tool to unveil the pH effects on biomolecules

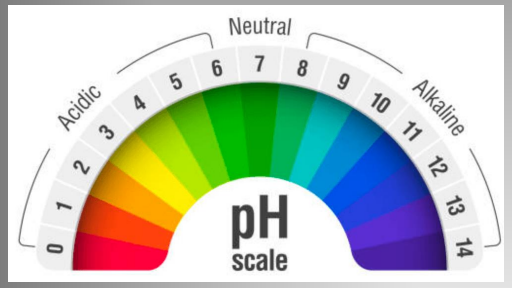
Miguel Machuqueiro

Ciências ULisboa

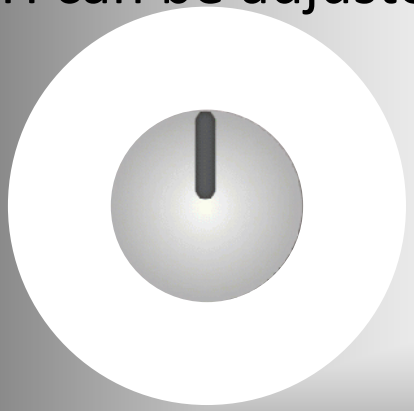
BioISI

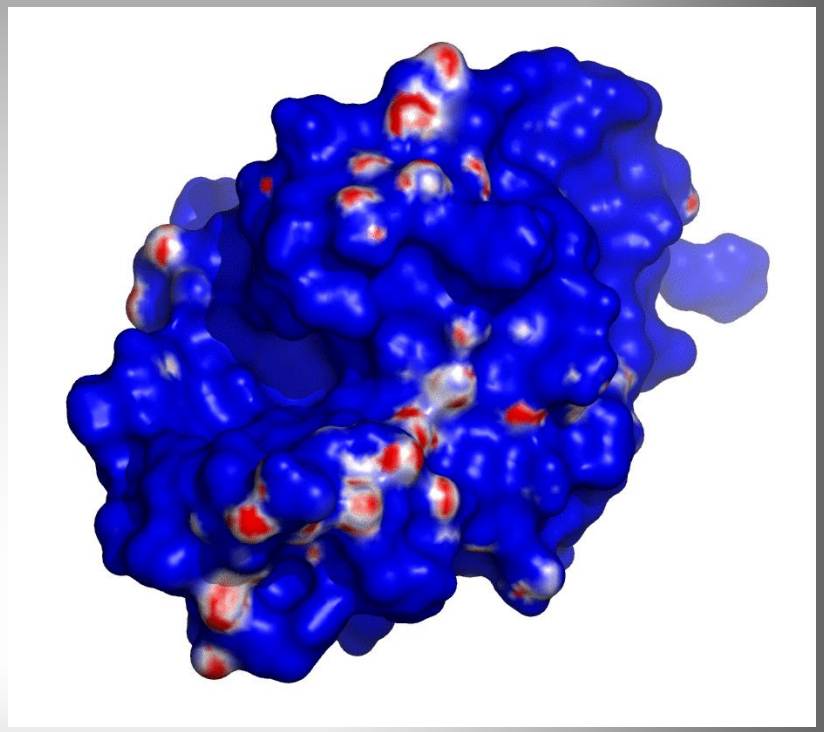
S. Paulo, Oct 9, 2024

⌑ The Impact of pH in Biomolecules



pH can be adjusted



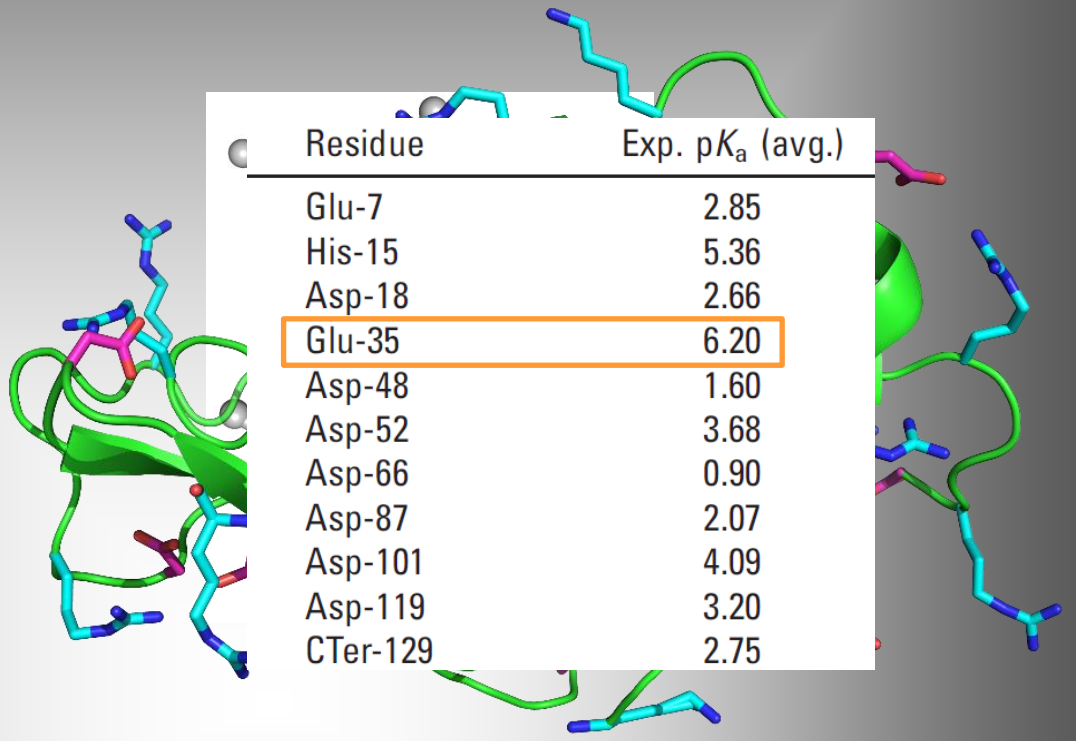


Hen Egg White Lysozyme (PDB ID: 4LZT)

⌑ The Impact of pH in Biomolecules

Pentapeptides:
Ac-Ala-Ala-X-Ala-Ala-NH_2

X Residue	pK_a *
CTr	3.67
Asp	3.94
Glu	4.25
His	6.54
NTr	8.00
Cys	8.55
Tyr	9.84
Lys	10.40



Residue	Exp. pK_a (avg.)
Glu-7	2.85
His-15	5.36
Asp-18	2.66
Glu-35	6.20
Asp-48	1.60
Asp-52	3.68
Asp-66	0.90
Asp-87	2.07
Asp-101	4.09
Asp-119	3.20
CTer-129	2.75

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* Grimsley, Scholtz, Pace, *Protein Sci.*, **2009**, 18, 247

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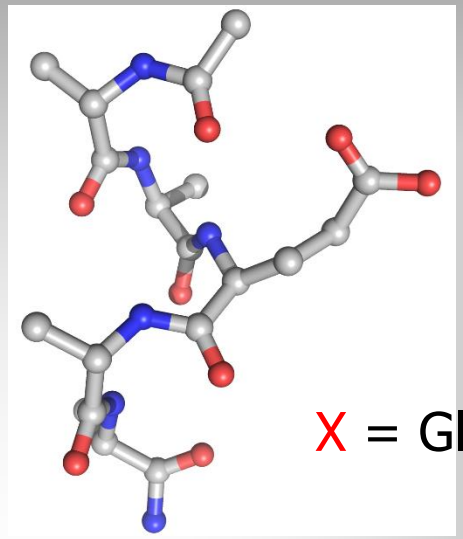

X = Glu

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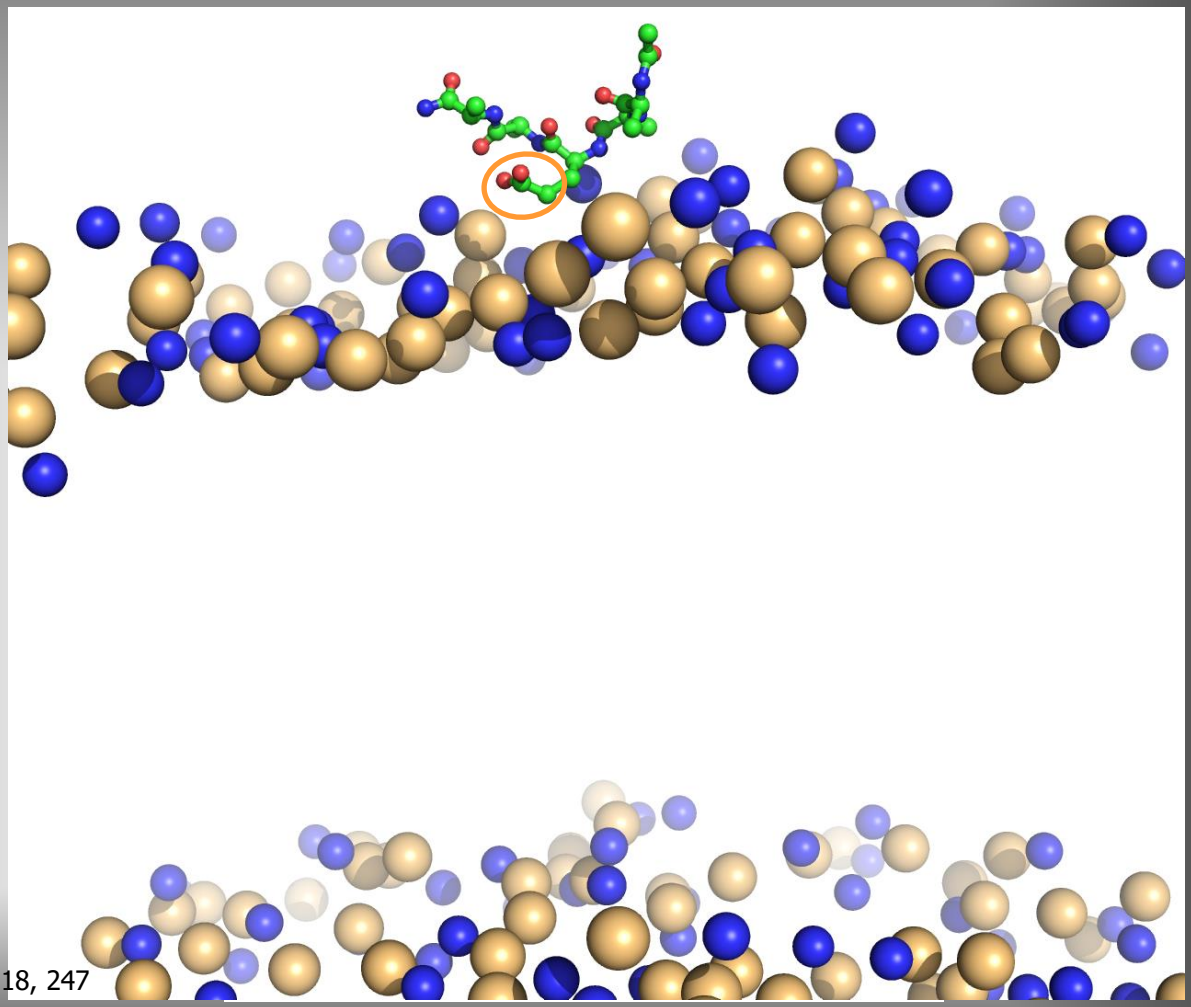

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Teixeira, Vila-Viçosa, Reis, Machuqueiro, *J. Chem. Theory Comput.*, **2016**, 12, 930

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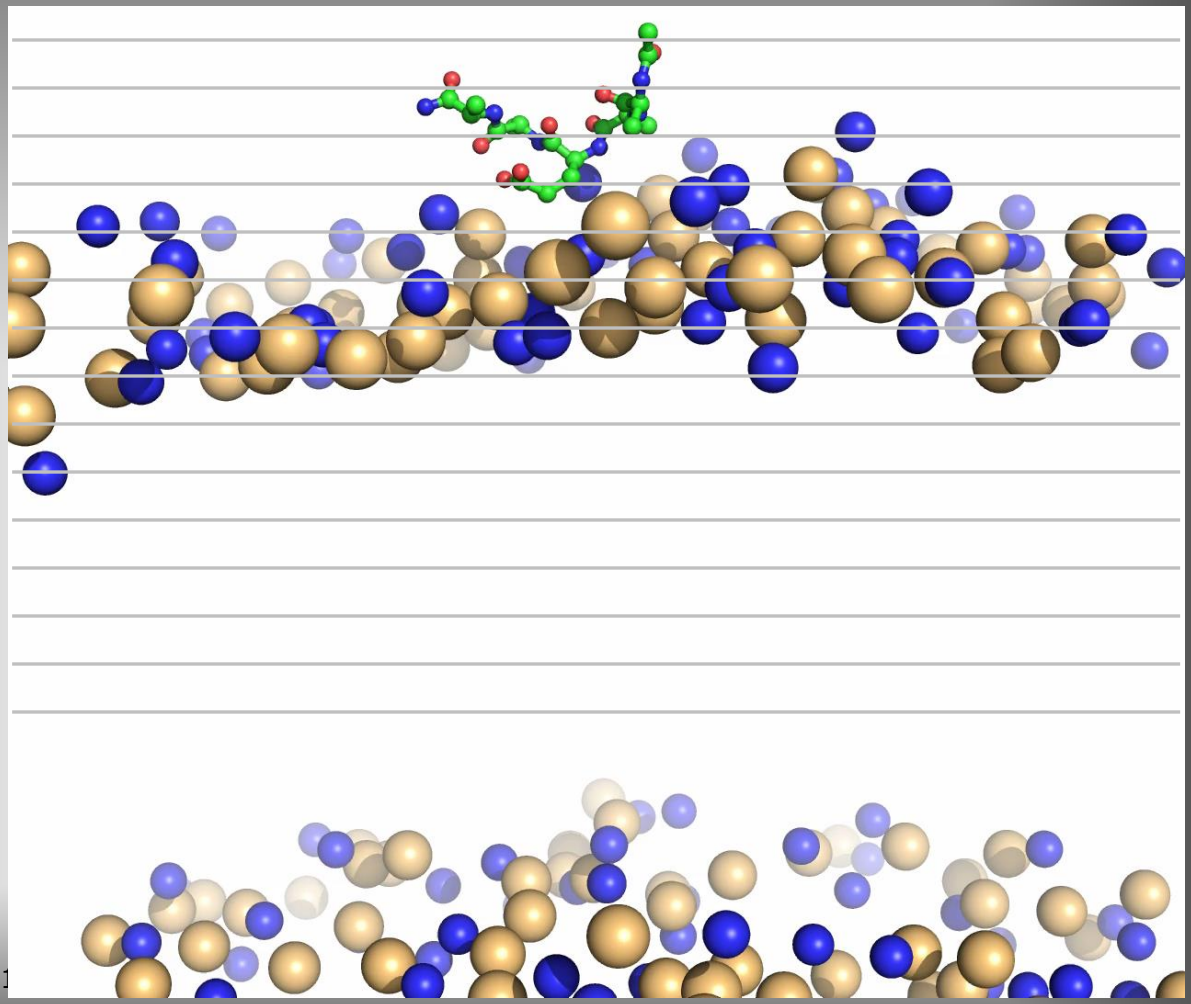

* Grimsley, Scholtz, Pace, *Protein Sci.*, **2009**, 1

Teixeira, Vila-Viçosa, Reis, Machuqueiro, *J. Chem. Theory Comput.*, **2016**, 12, 930

⌑ pK_a values @ water/membrane interface

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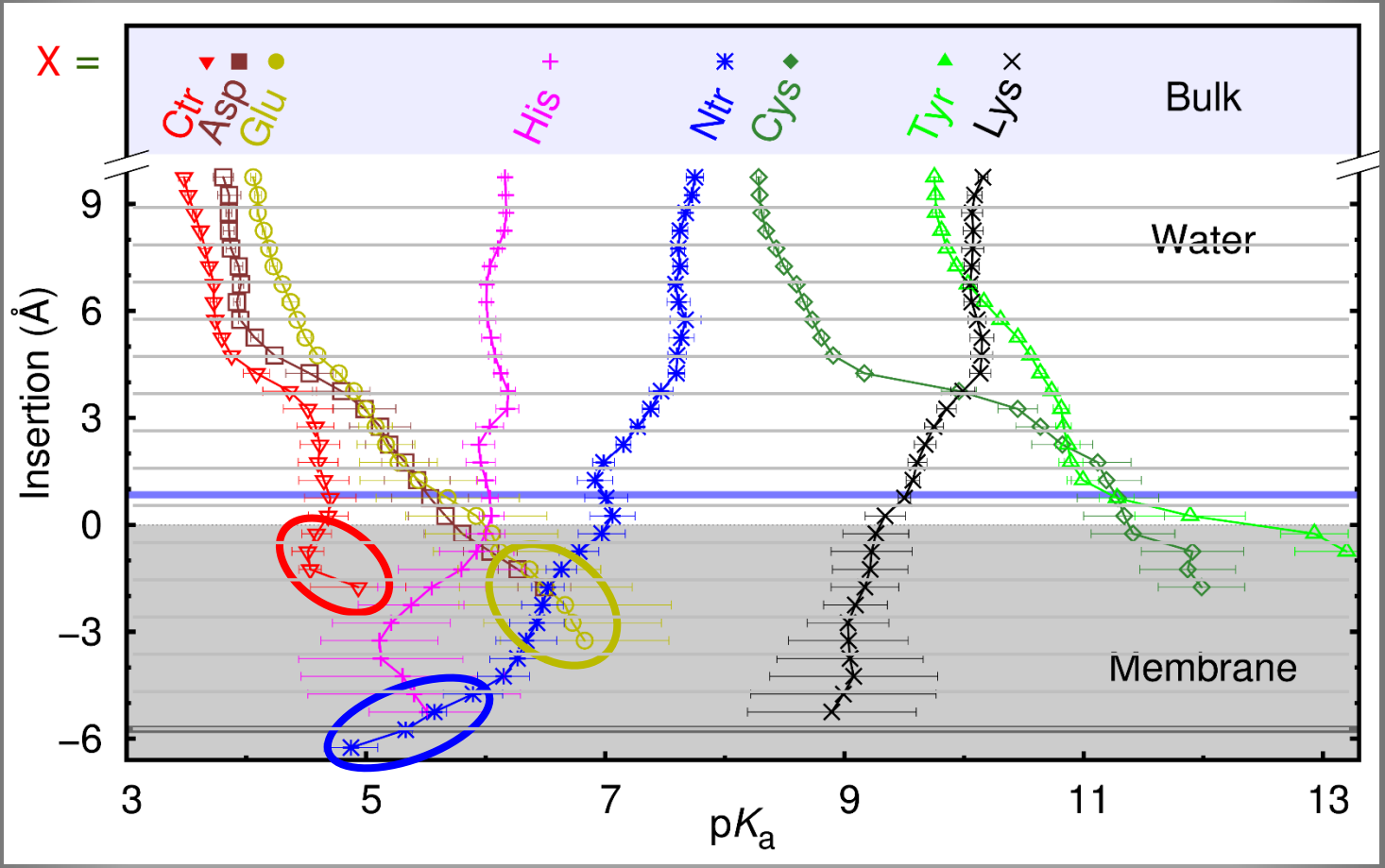
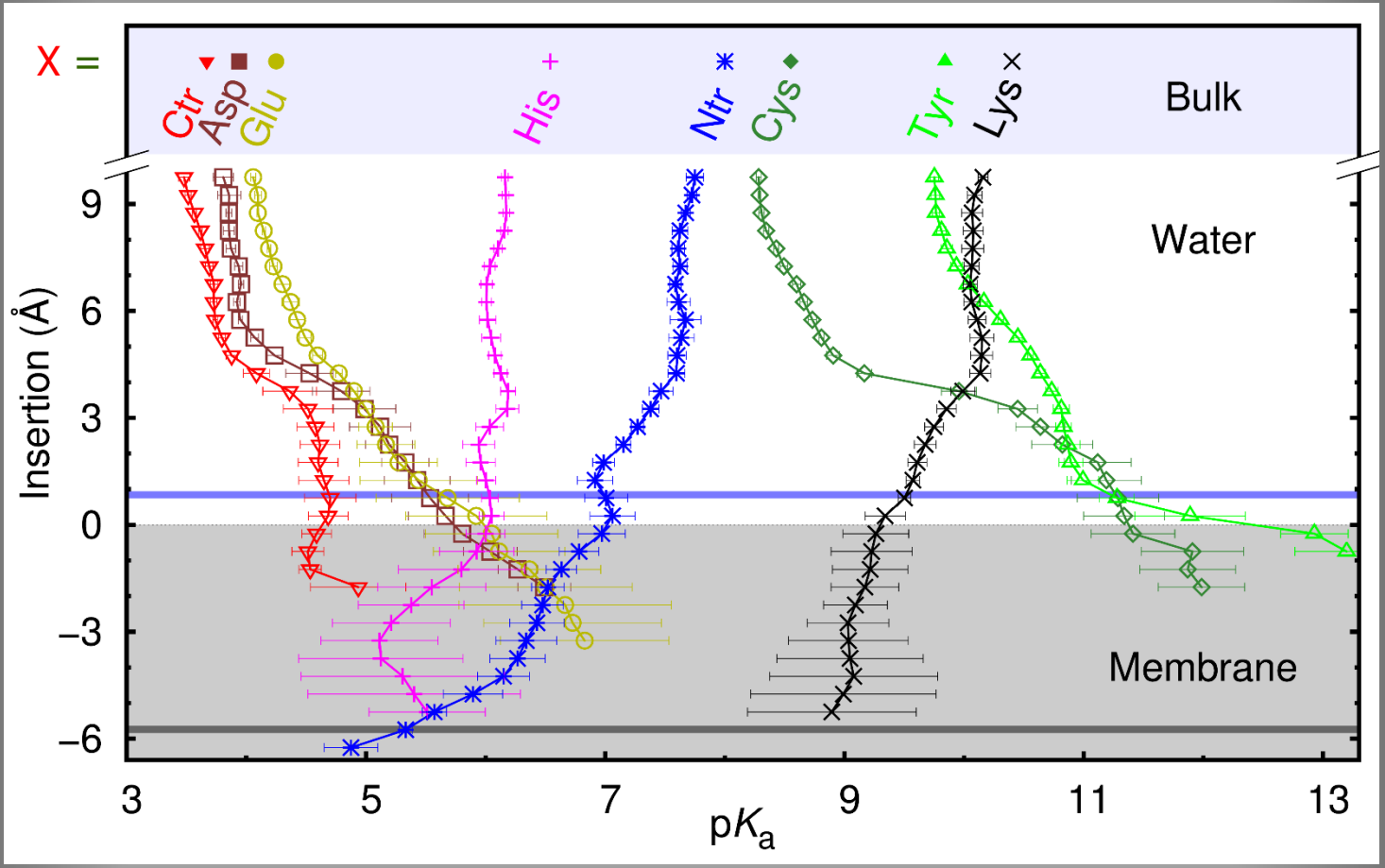


Teixeira, Vila-Viçosa, Reis, Machuqueiro, *J. Chem. Theory Comput.*, **2016**, 12, 930

¤ pK_a values @ water/membrane interface

But, how did we perform these calculations?



⌑ How to deal with pH effects in MD

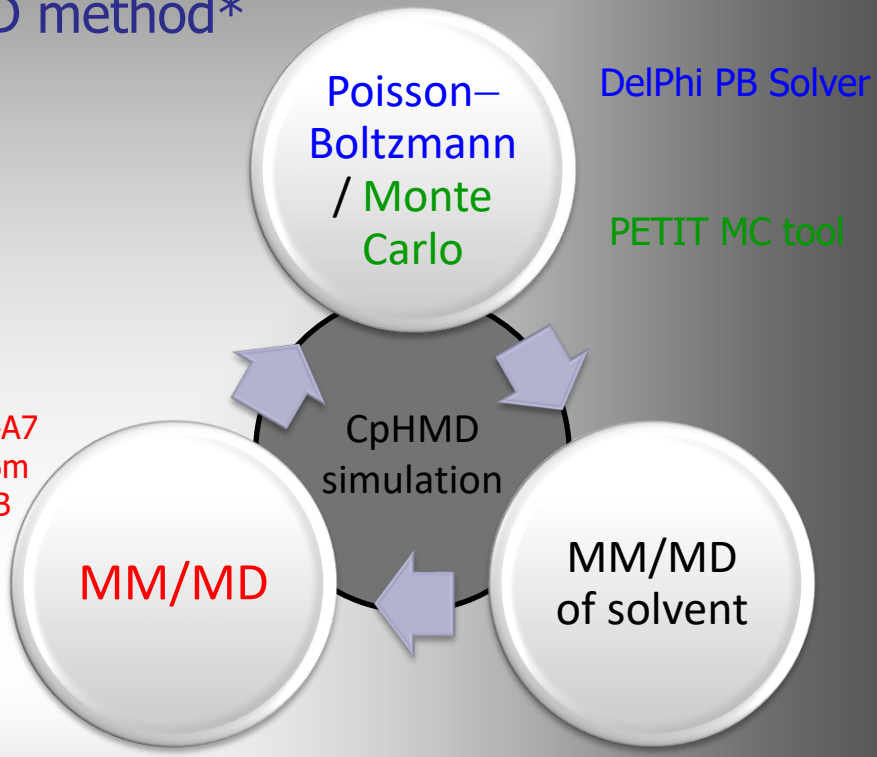
But, how did we perform these calculations?

Stochastic Titration constant-pH MD method*

Protonation/Conformation coupling

- PB/MC samples protonation states
- MM/MD samples solute conformations

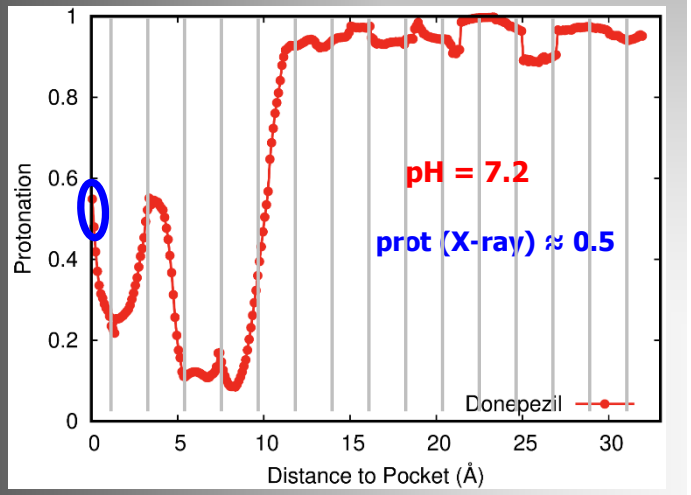
GROMACS:
- GROMOS 54A7
- CHARMM 36m
- AMBER 14SB

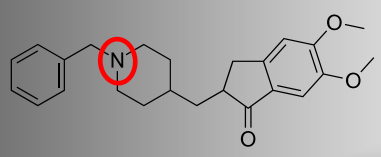


* Baptista, Teixeira & Soares, *J. Chem. Phys.*, **2002**, 177:4184; Machuqueiro & Baptista, *J. Phys. Chem. B*, **2006**, 110:2927
Machuqueiro & Baptista, *J. Am. Chem. Soc.*, **2009**, 131, 1258; Teixeira et al. & Machuqueiro, *J. Chem. Theory Comput.*, **2016**, 12, 930
Vila-Vicosa et al. & Machuqueiro, *J. Chem. Theory Comput.*, ***2018***, 14, 3289; Vila-Vicosa et al. & Machuqueiro, *J. Chem. Theory Comput.*, ***2019***, 15, 3108

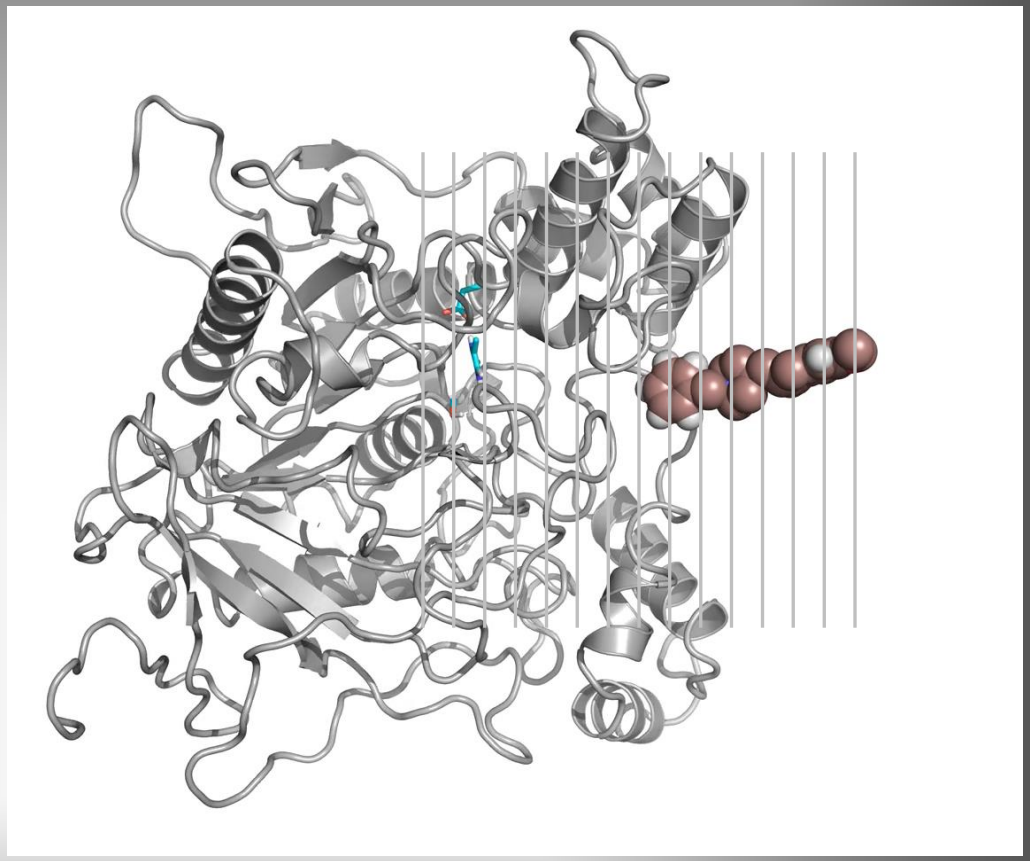
⌑ Examples of CpHMD simulations

- Drug/Protein binding processes





$pK_a \approx 8.5$

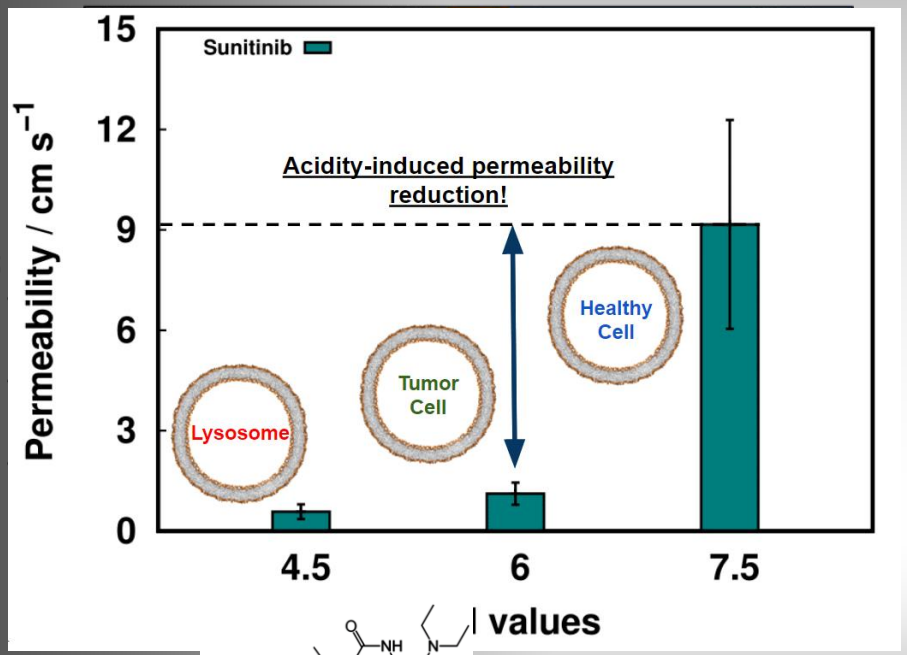


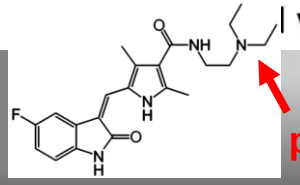
pH titration of donepezil binding to AChE

Yousef, Oliveira, (…) & Machuqueiro, **2024**, *Submitted*

⌑ Examples of CpHMD simulations

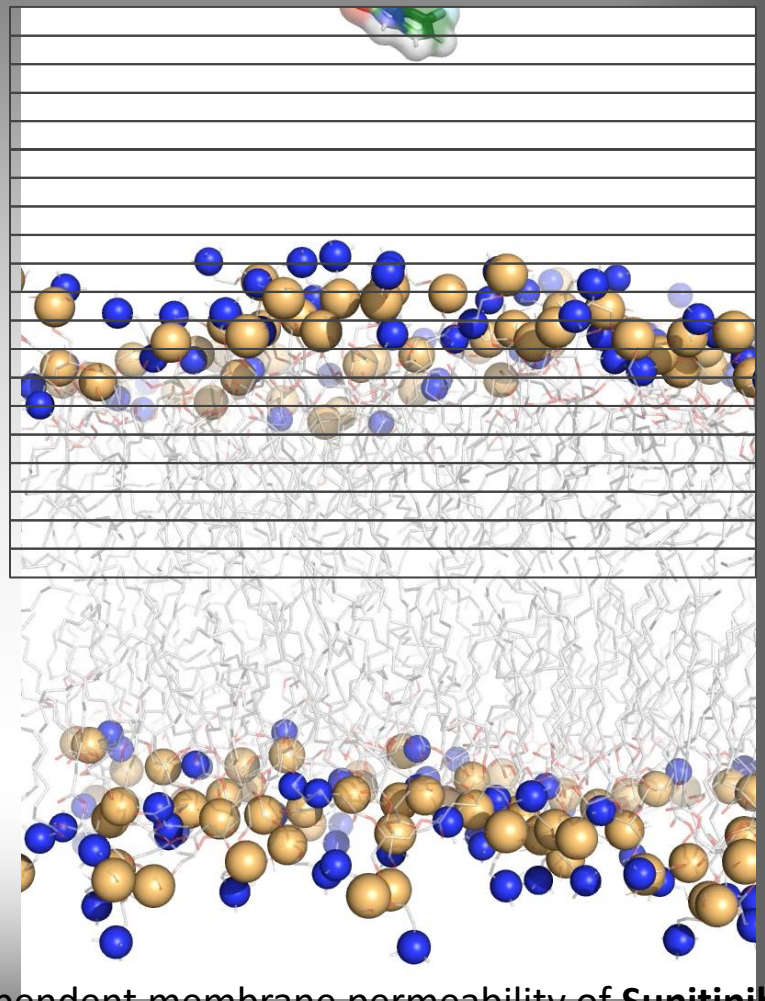
- Drug/membrane permeability





Sunitinib

pK_a = 8.5



pH-dependent membrane permeability of **Sunitinib**

⌑ The case of peptide dendrimers

Polyamidoamine (PAMAM)

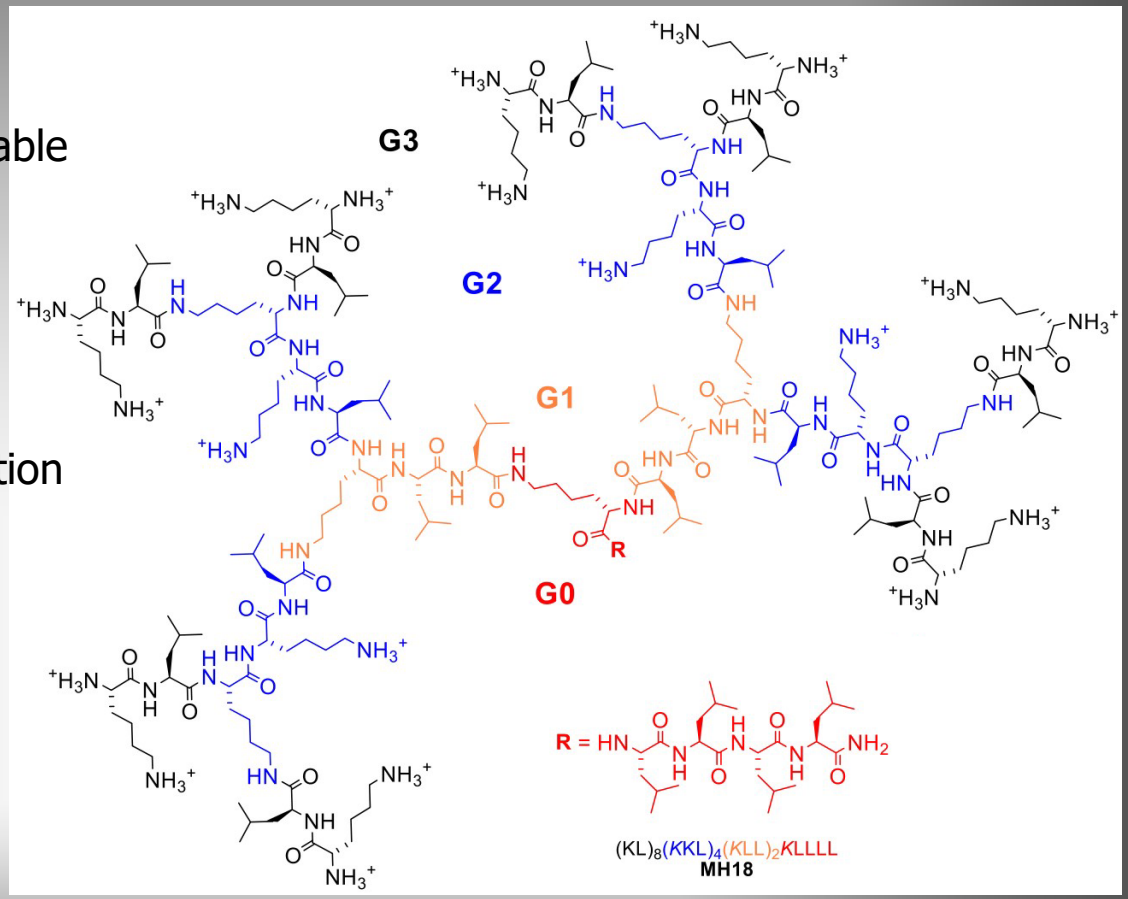
Dendrimers: tree-like molecules

Multivalency and high functionalizable globular-like structures

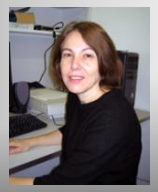
Peptide Dendrimers: built from amino acids

Carriers for nucleic acids: transfection

React to pH: important for internalization via endosome and escape from late endosome



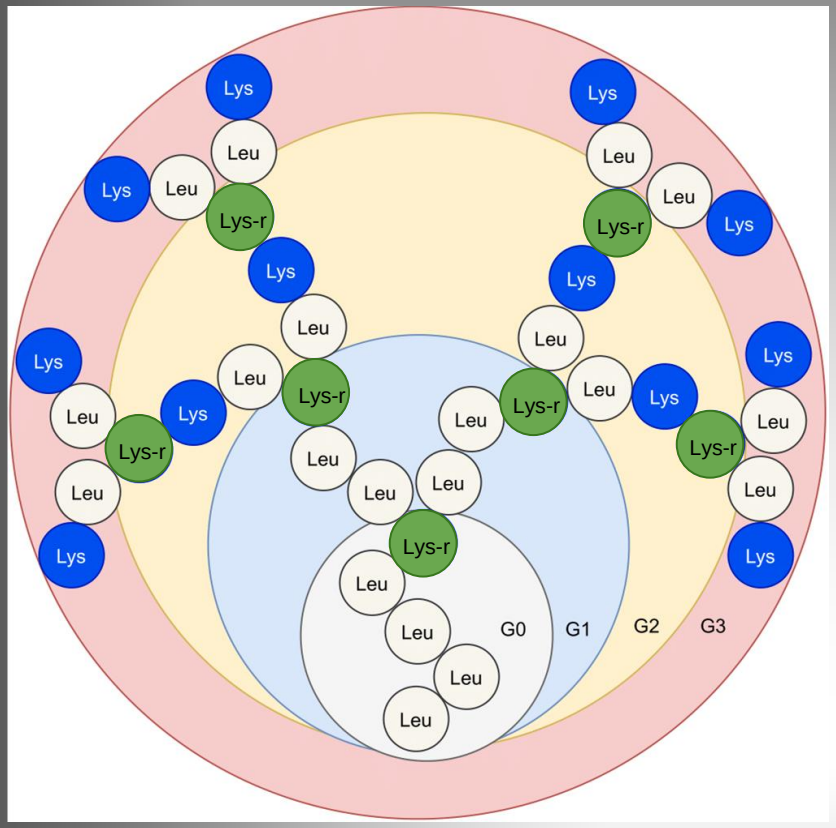
Filipe Rodrigues



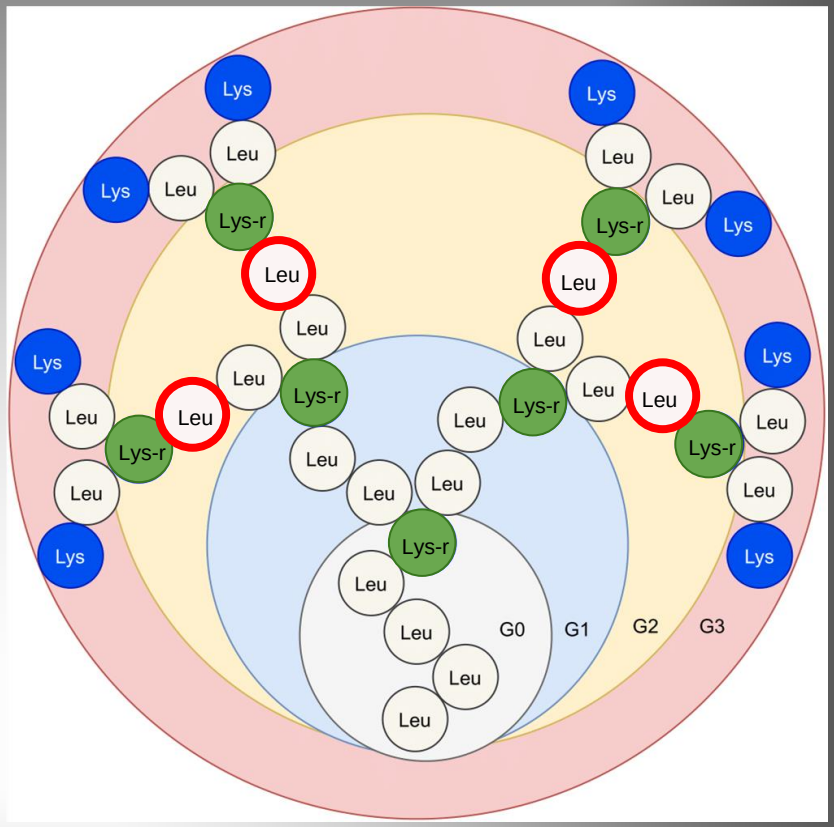
Prof. Tamis Darbre (Univ. Bern, CH)

Adapted from 10.1016/j.mattod.2015.06.003

⌑ Peptide dendrimers for siRNA transfection



MH18



MH47

⌑ Peptide dendrimers for siRNA transfection

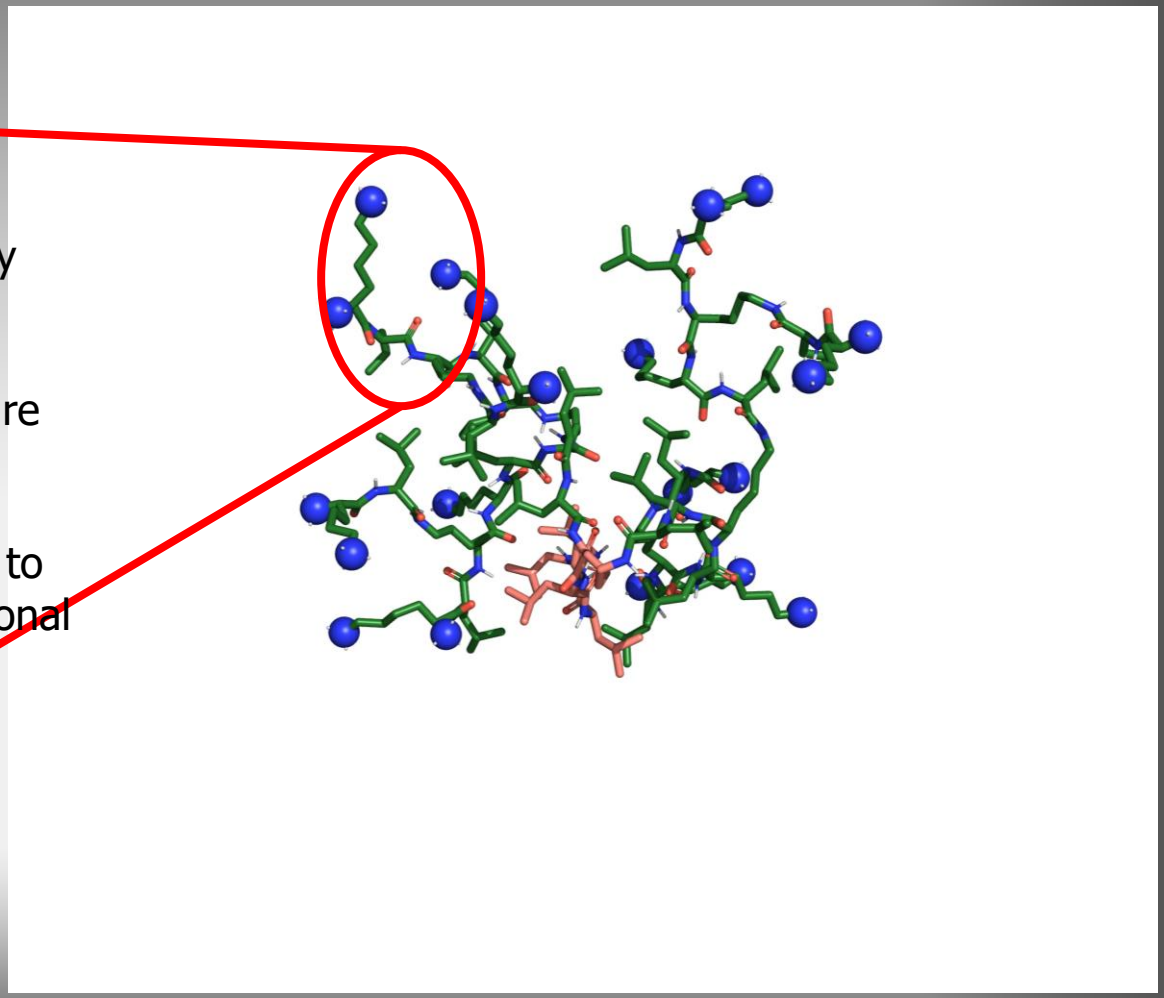
Lys side chain:
pK_a ≈ 10.4

High conformational variability

No obvious secondary structure

The radius of gyration seems to capture best their conformational space

NTerminus:
pK_a ≈ 8.0



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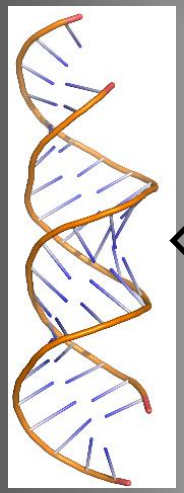
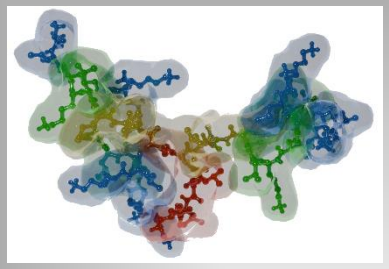
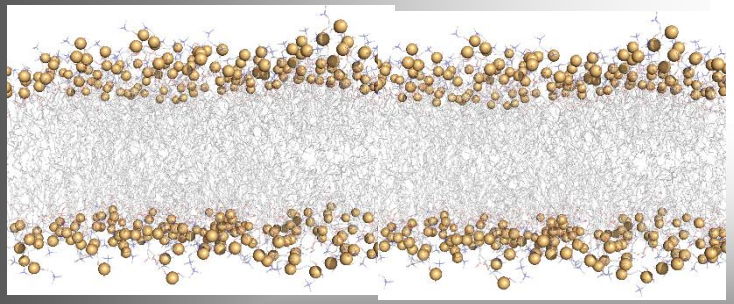


siRNA binding

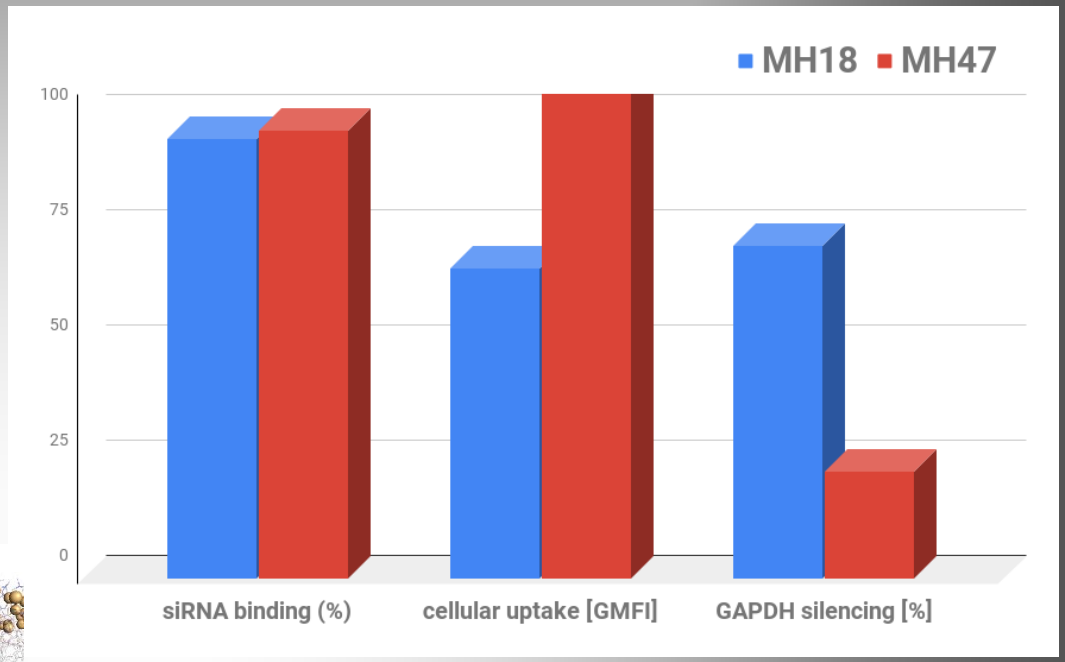


cell uptake
(pH ≈ 7.4)

late endosome
destabilization
(pH ≈ 5.0)

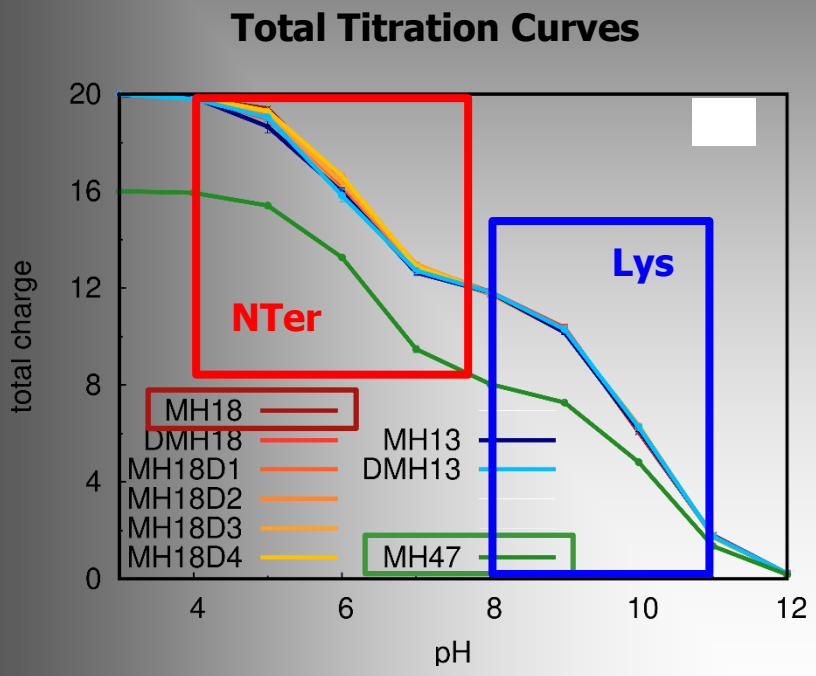


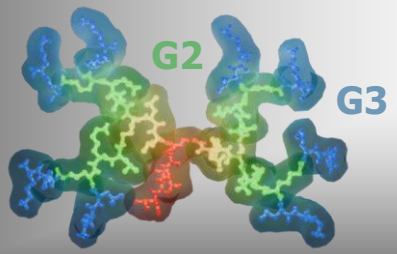
Steps to Transfection: Experimental Data

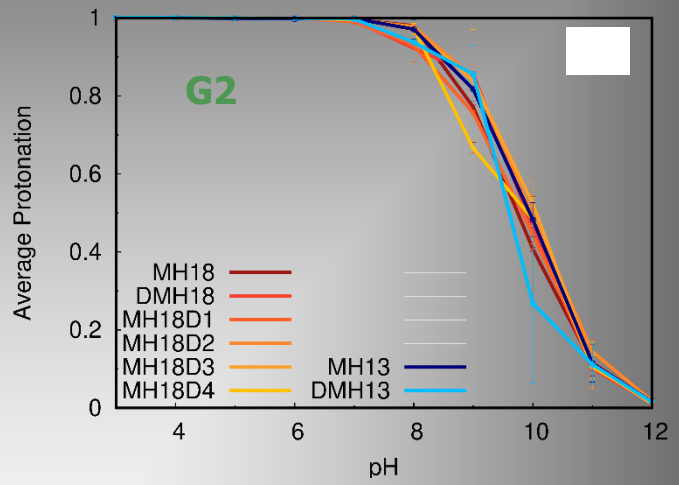


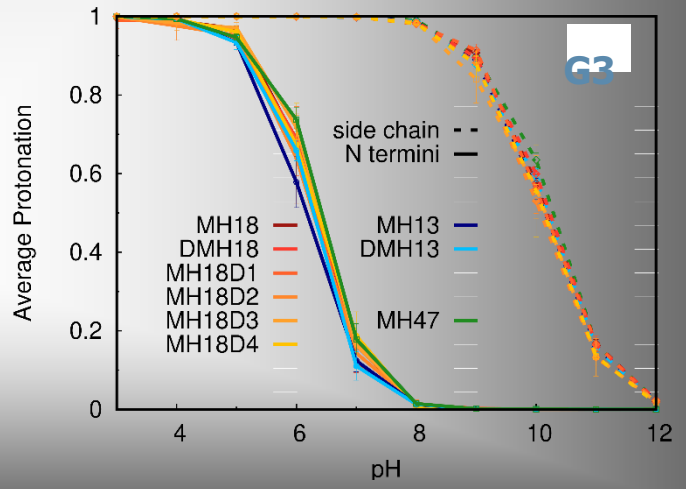
Heitz, M.; Javor, S.; Darbre, T.; Reymond, J.-L. Biochem. J. 2019, 30, 2165-2182

⌑ Peptide dendrimers in water

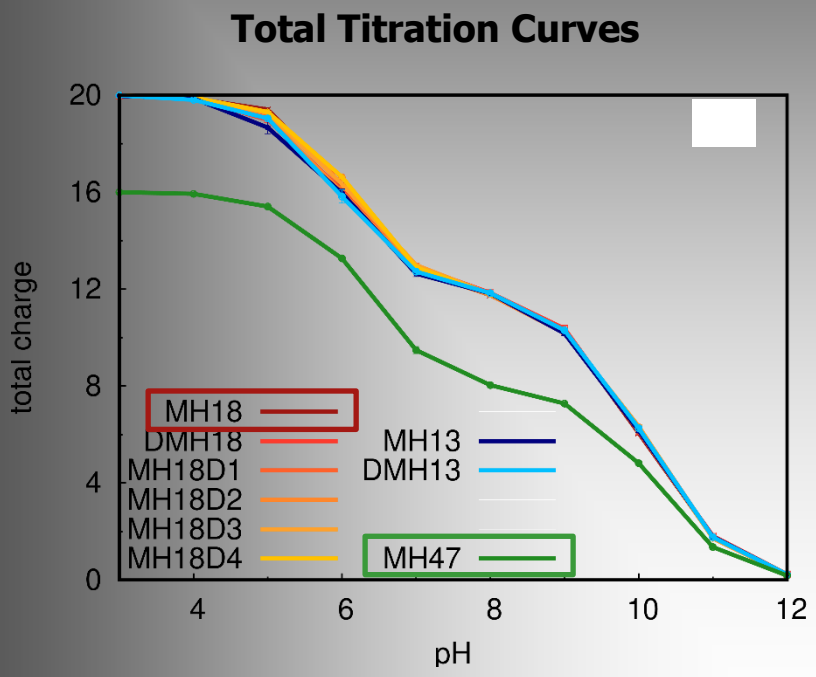


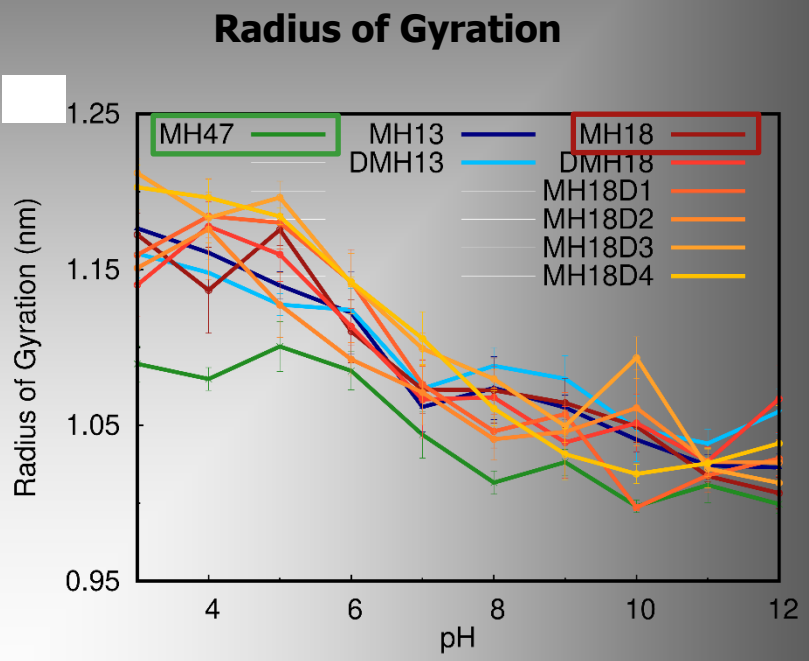






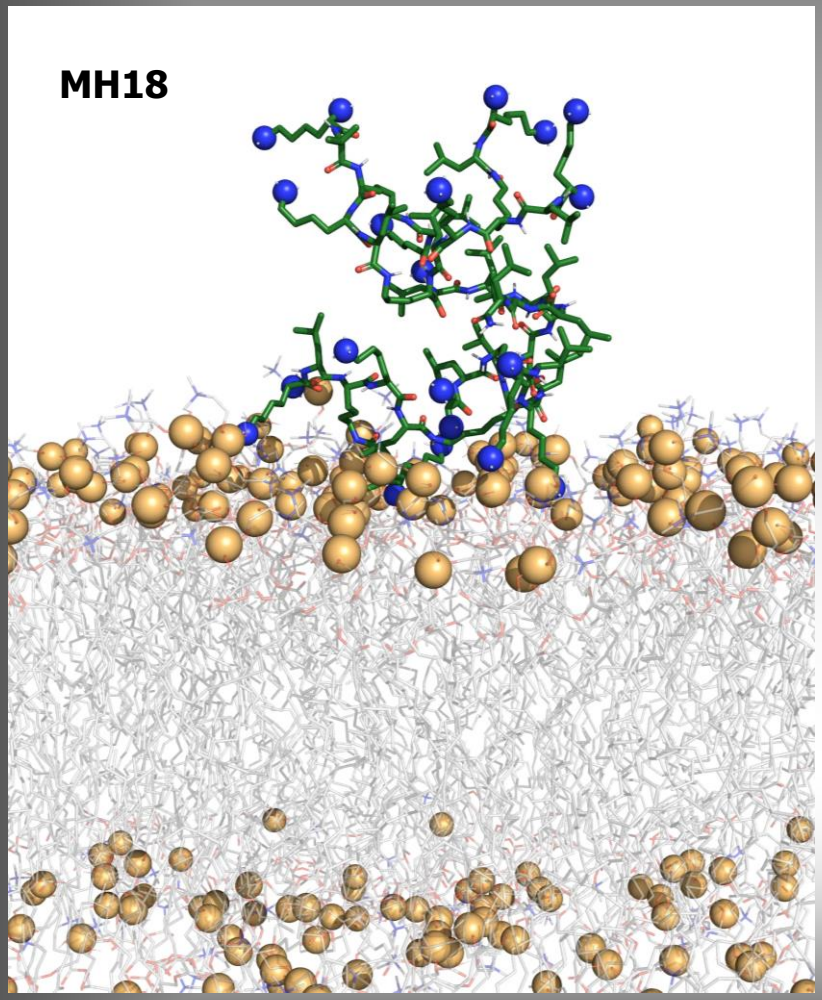
⌑ Peptide dendrimers in water

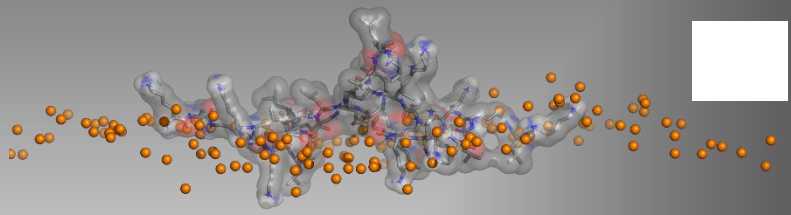




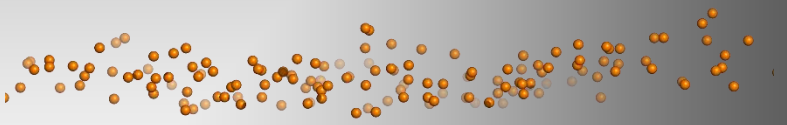
But, what happens when these peptide dendrimers see a POPC membrane?

⌑ Peptide dendrimers in a membrane

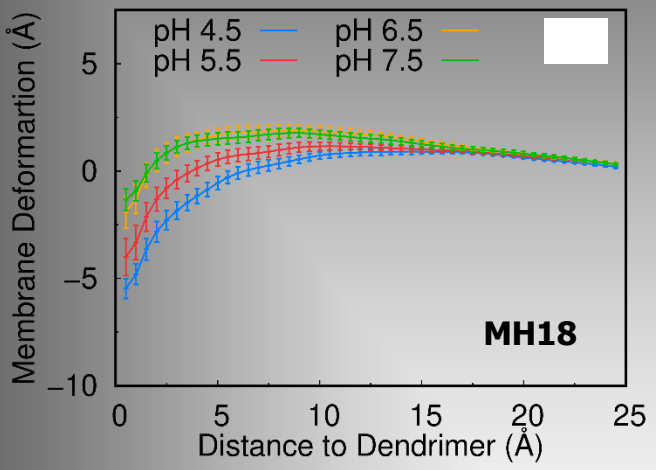


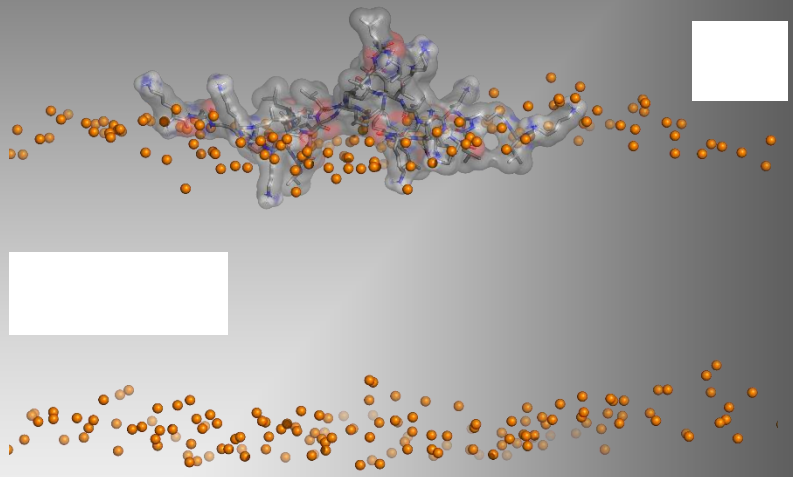


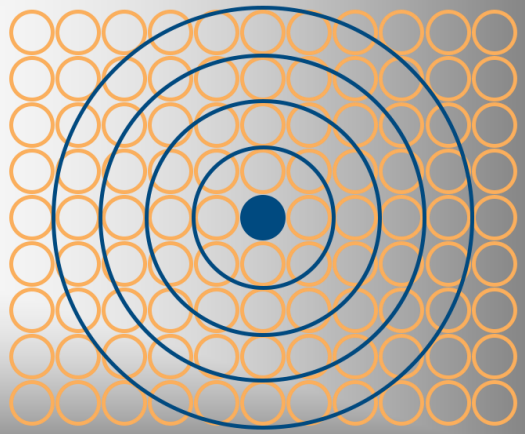
pH = 7.5



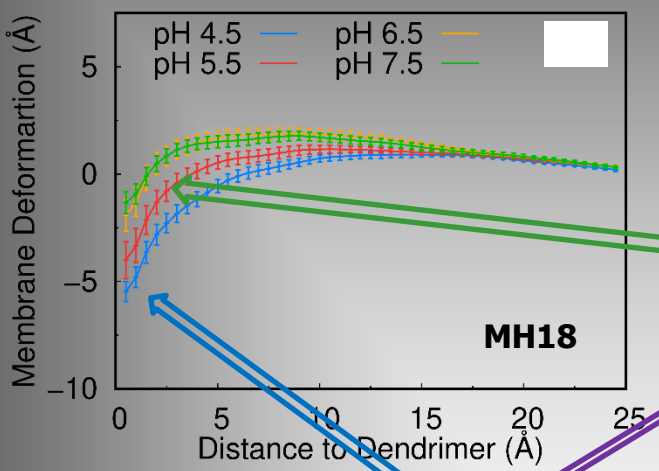
⌑ Peptide dendrimers in a membrane

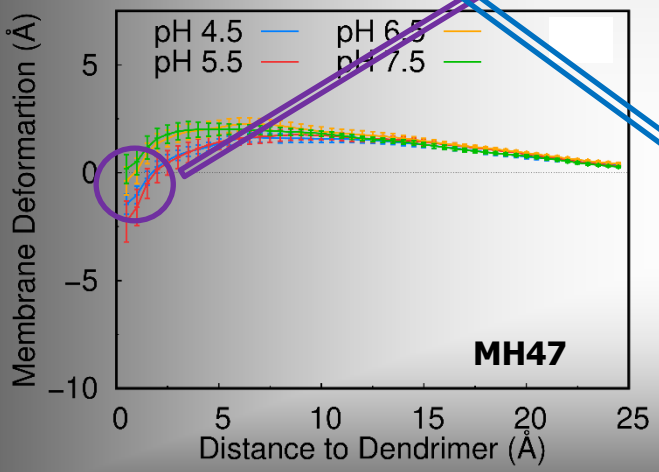


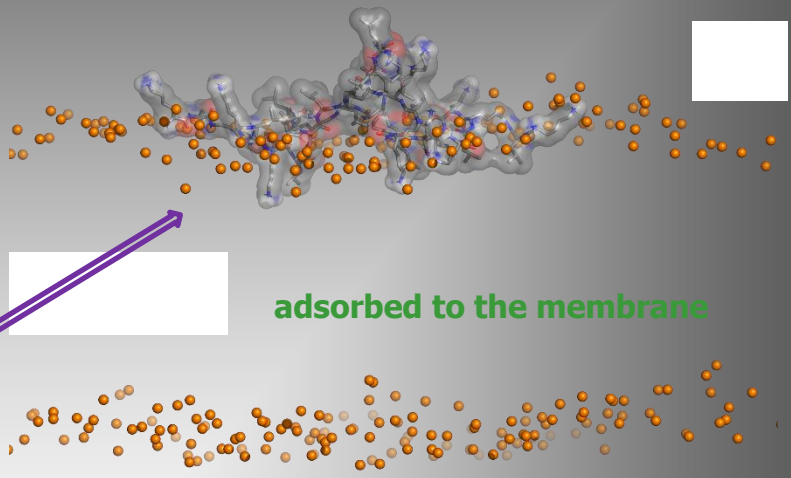




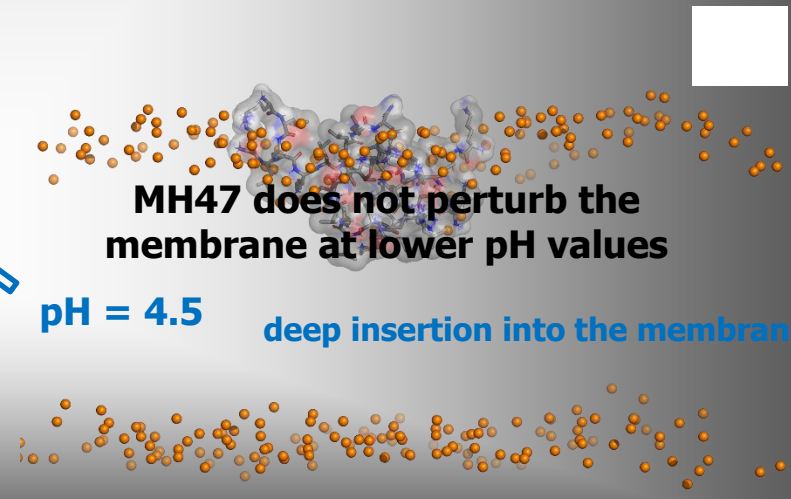
⌑ Peptide dendrimers in a membrane







adsorbed to the membrane



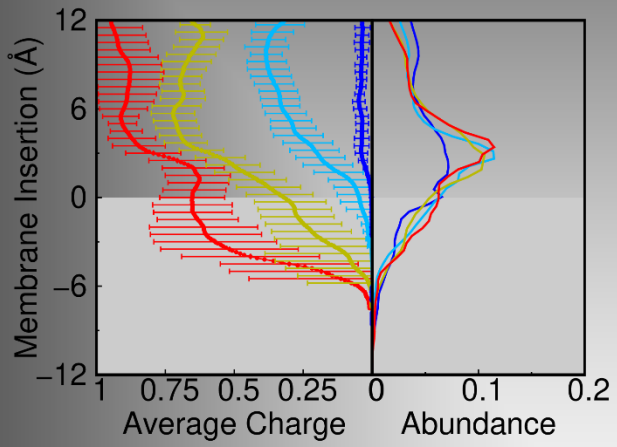
MH47 does not perturb the membrane at lower pH values

pH = 4.5

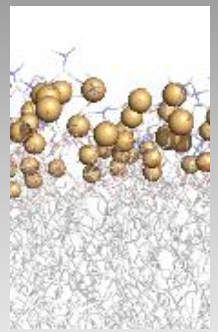
deep insertion into the membrane

⌑ Peptide dendrimers in a membrane

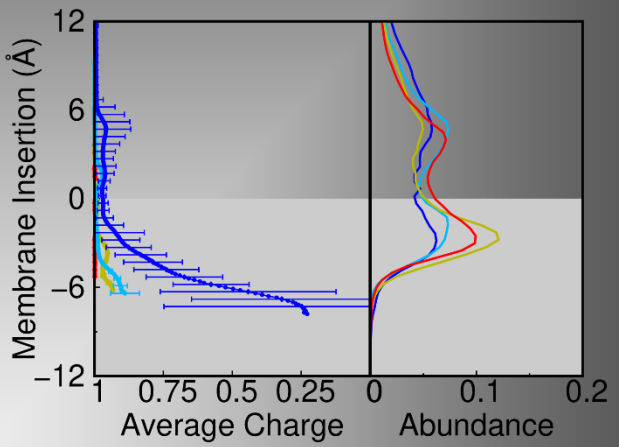
N-Termini

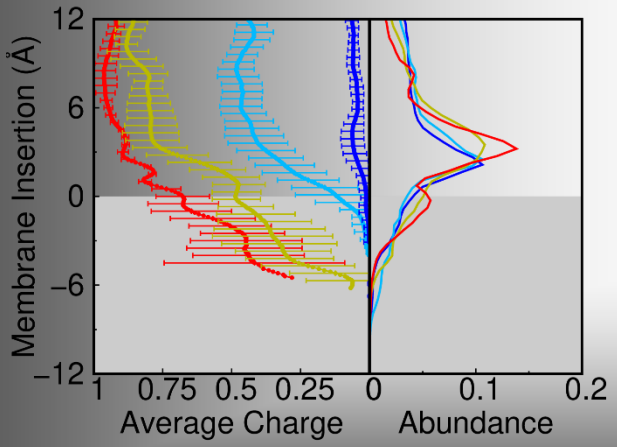


MH18



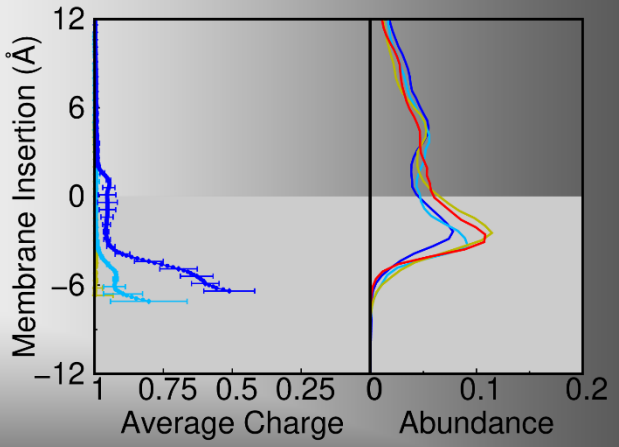
Lys side chain





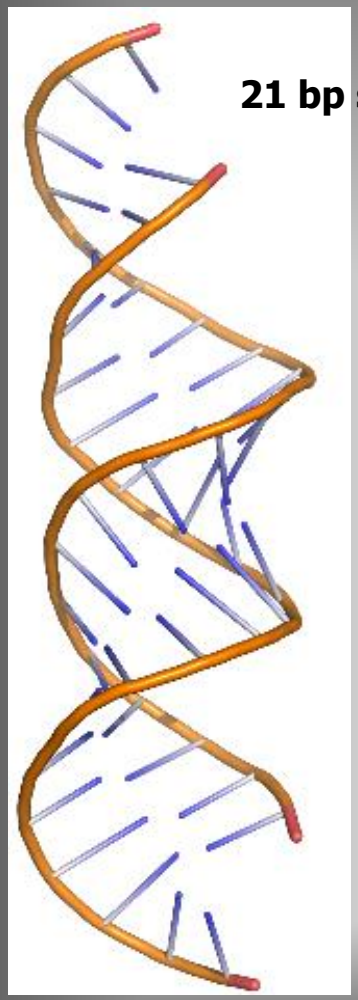
The higher charge density is key

MH47

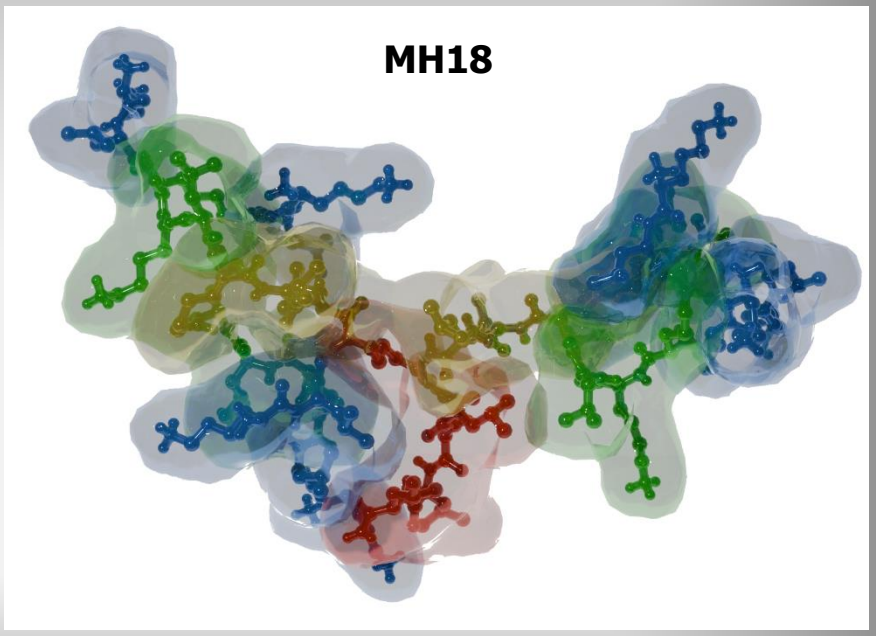


⌑ Peptide dendrimers / siRNA binding

Future Work

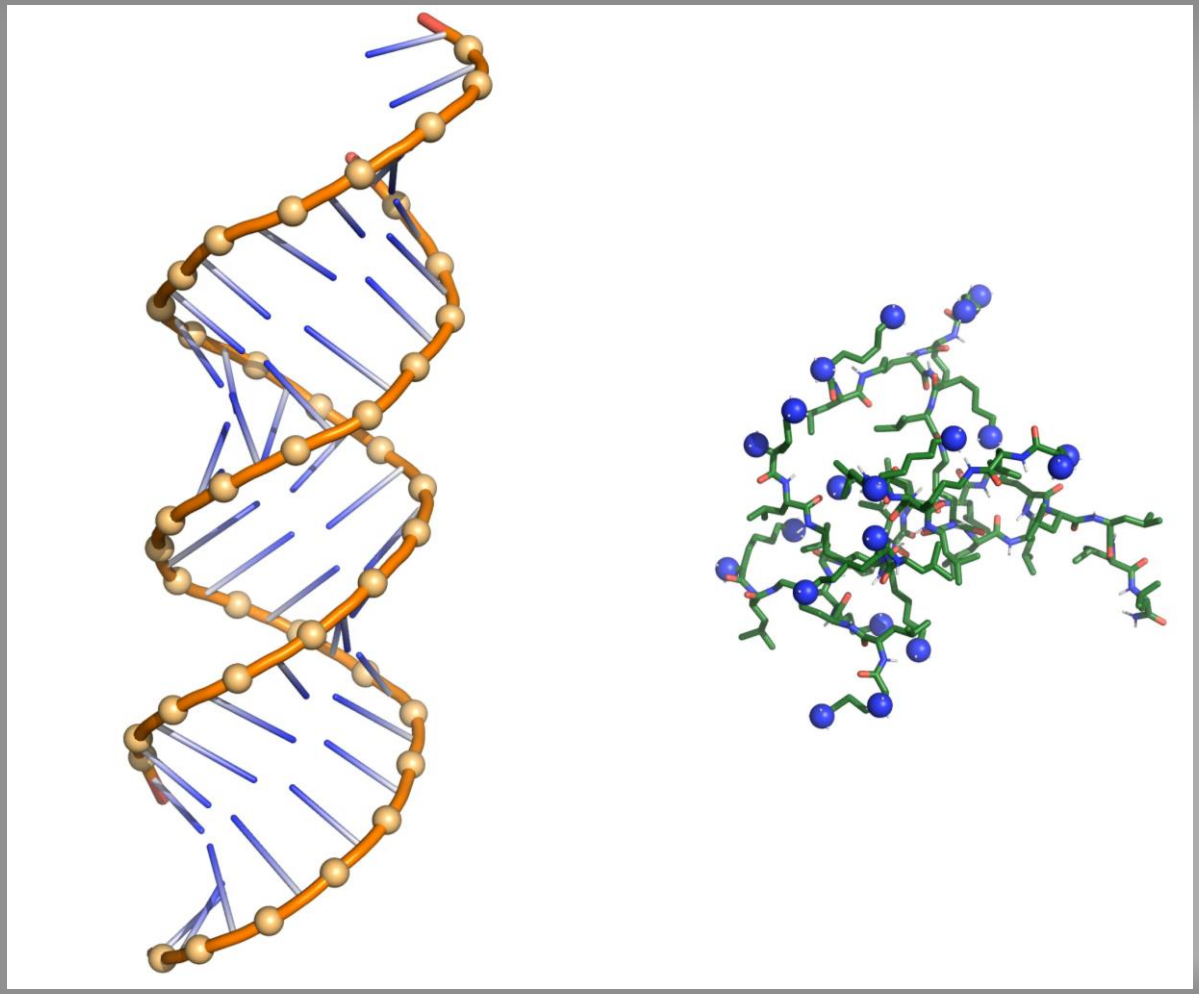


21 bp siRNA



⌑ Peptide dendrimers / siRNA binding

Future Work



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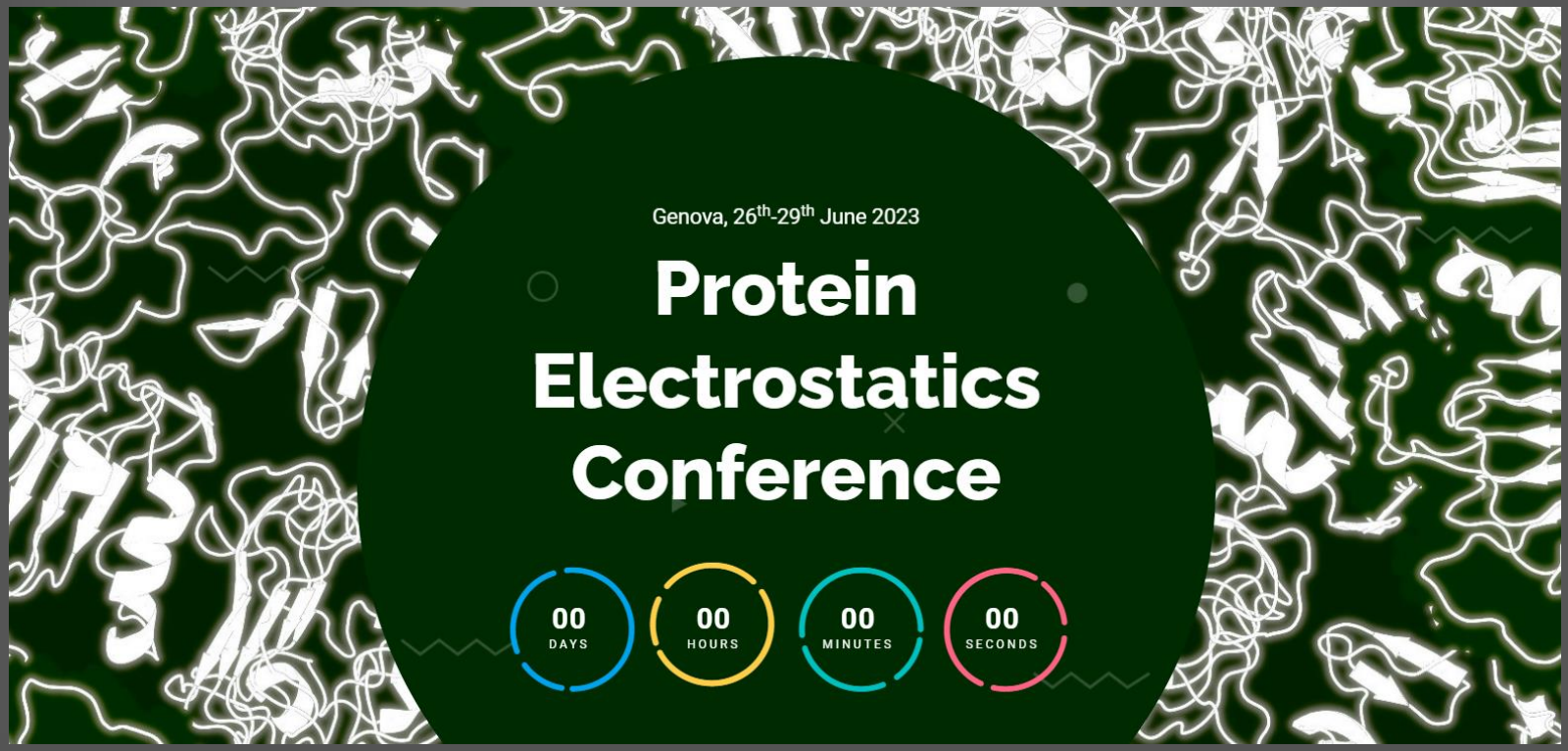

https://proteinelectrostatics.org