IECBM 2022
The 2nd International Electronic Conference on Biomolecules:
BIOMACROMOLECULES AND THE MODERN WORLD CHALLENGES
08–22 JULY 2022 | ONLINE

Self-Association Evidence for Antimicrobial Peptides via Long-Scale MD Simulations: a Case Study

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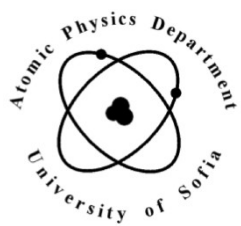
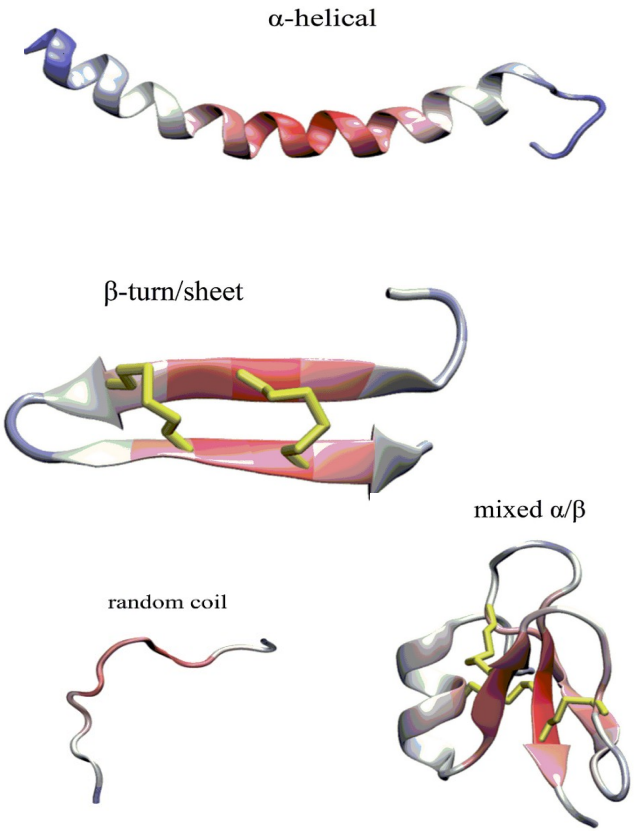


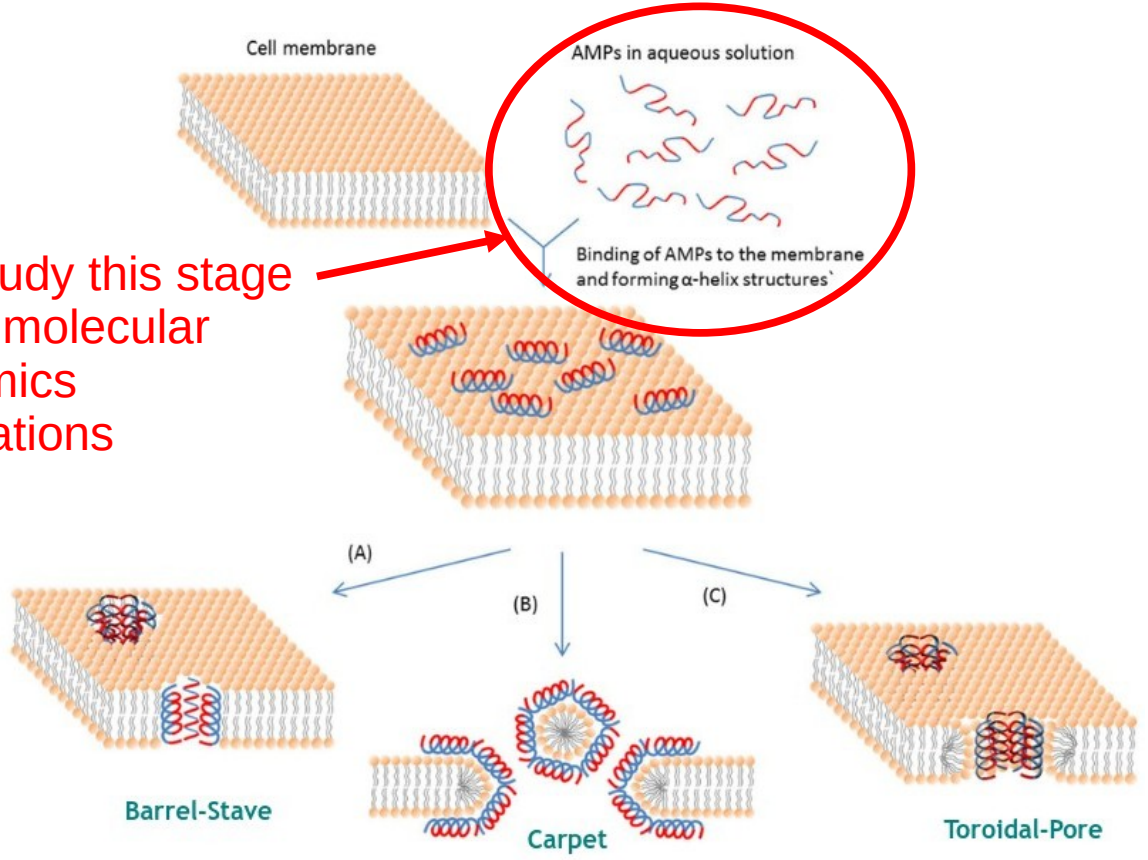
Antimicrobial peptides

Structure



Mechanism of action

We study this stage using molecular dynamics simulations



Our model

- AMPs do not exist in monomeric form after secretion in the bodily fluids as part of multicomponent mixtures
- They aggregate and form nanosized clusters in solution, prior to their attack on the target membrane
- The clusters are how AMPs get delivered to the membrane in high enough local concentration

Case study

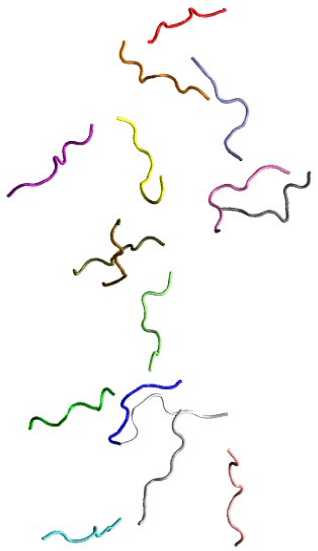
Recently discovered glycine-rich peptides, isolated from the mucus of the garden snail *Helix Aspersa*

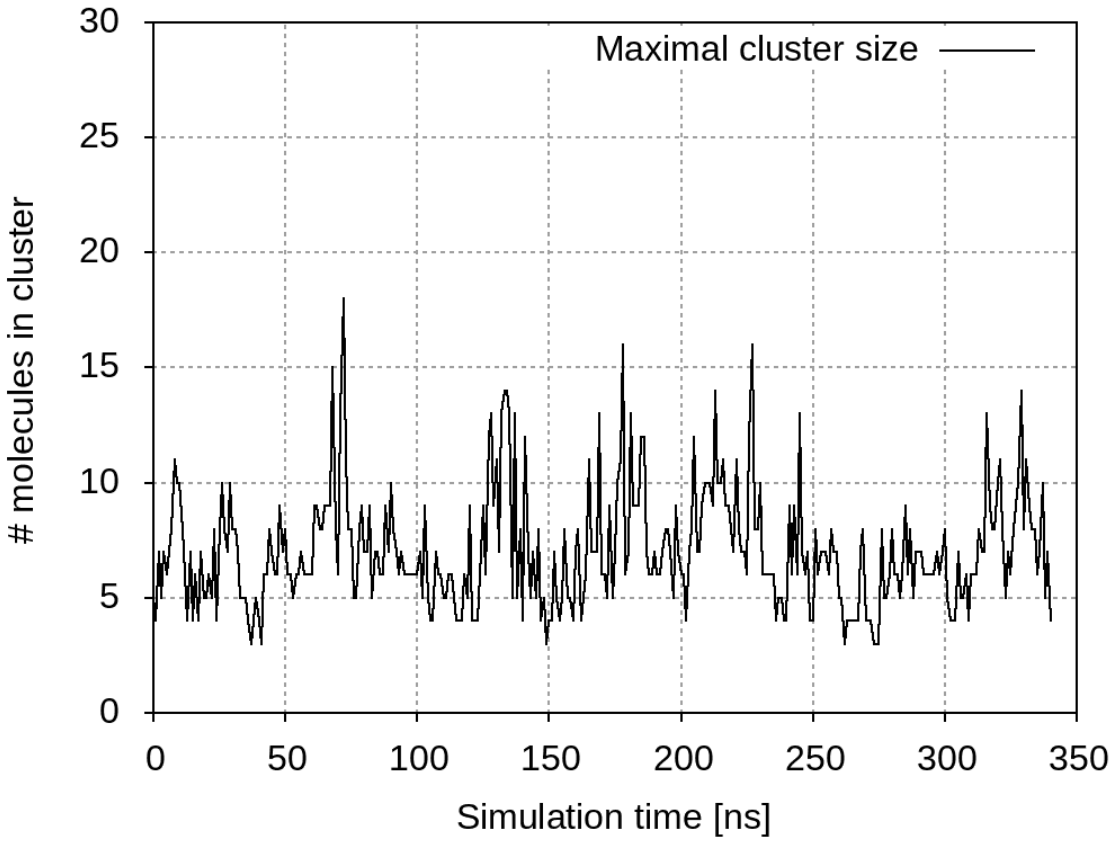
Peptide	Sequence	Charge	M_r [kDa]
p1	KVKDNQWRP	+2	1.17
p2	VNVVGGGGGIVGGGIGGGGM	0	1.57

Ilieva, N. et al. (2020). In Silico Study on the Structure of Novel Natural Bioactive Peptides. In: LSSC 2019. LNCS, 11958, 332–339, Springer, https://doi.org/10.1007/978-3-030-41032-2_38

Monocomponent p1 solution

27 p1 peptides in a cubic box, C_m = 48 mM

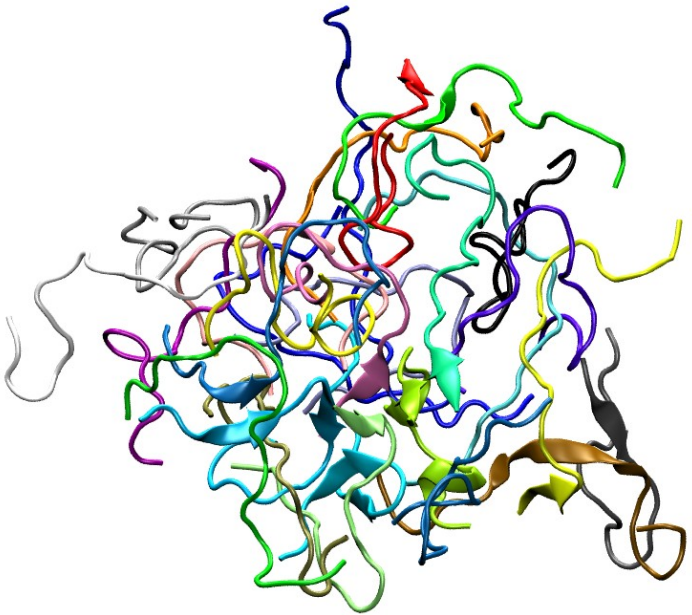




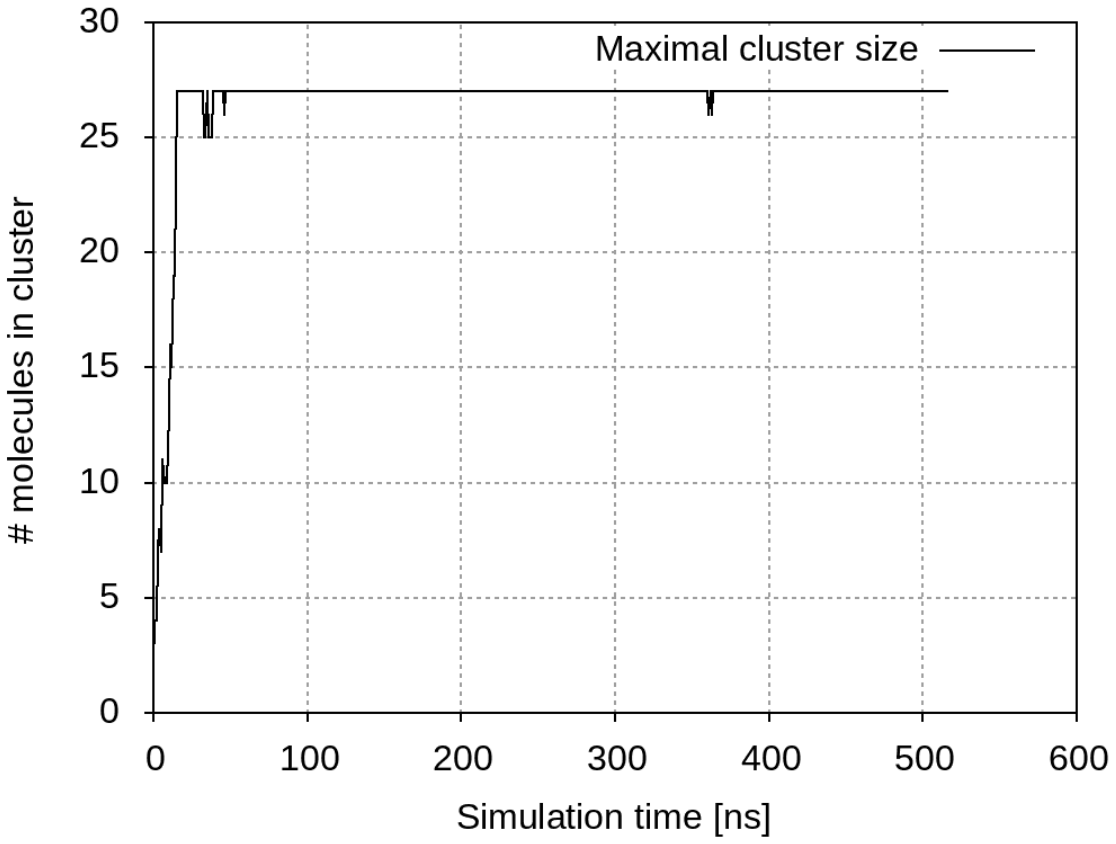
Largest p1 aggregate – fairly loose, monomers are well separated.

Monocomponent p2 solution

27 p2 peptides in a cubic box, C_m = 29 mM

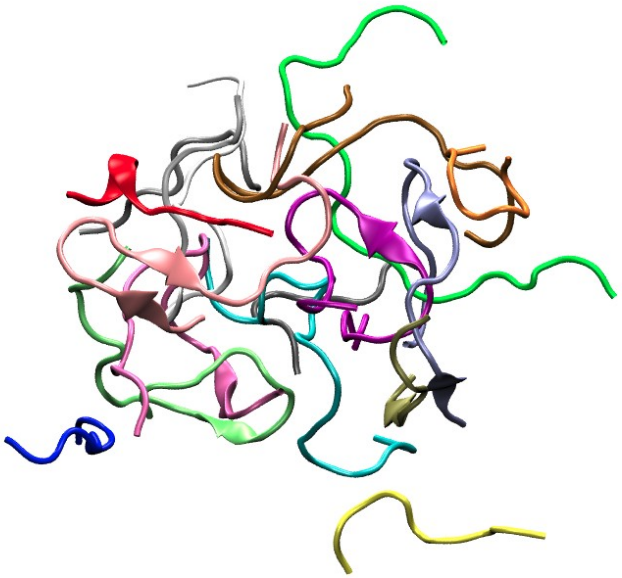


Largest p2 aggregate – tightly packed globule

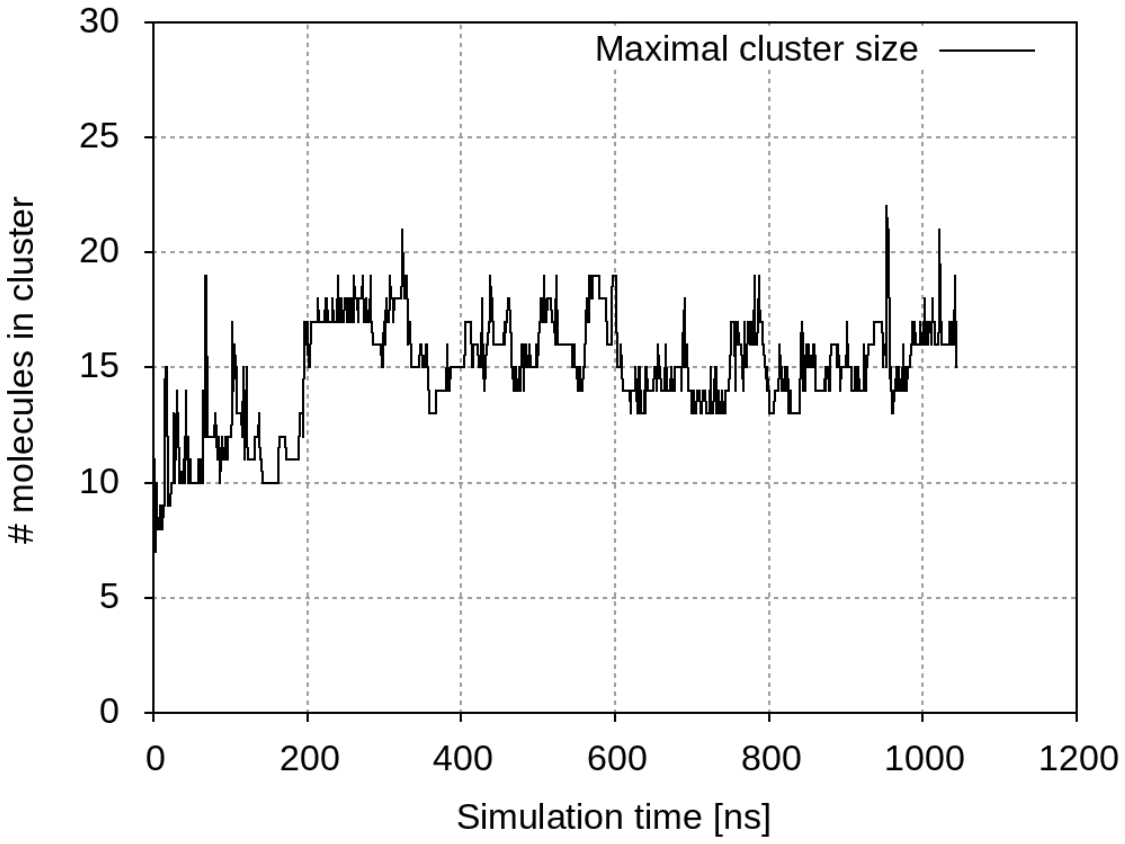


Multicomponent p1+p2 solution

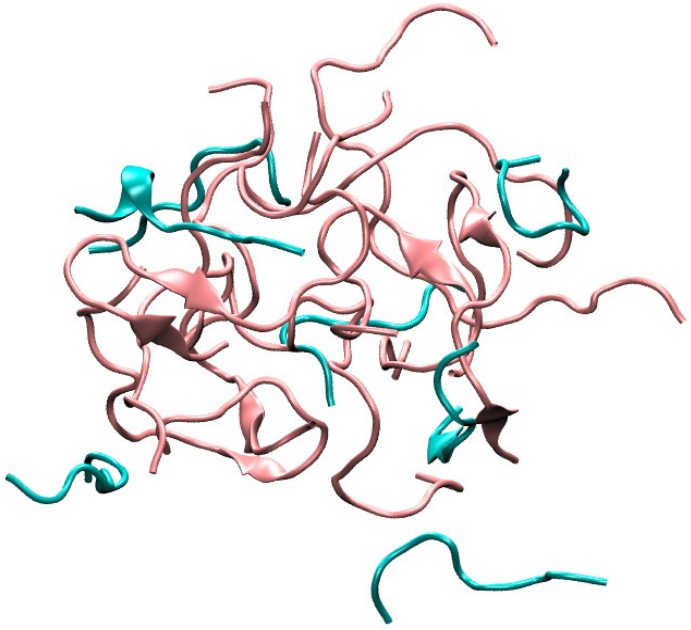
25 p1 and 10 p2 peptides in a cubic box



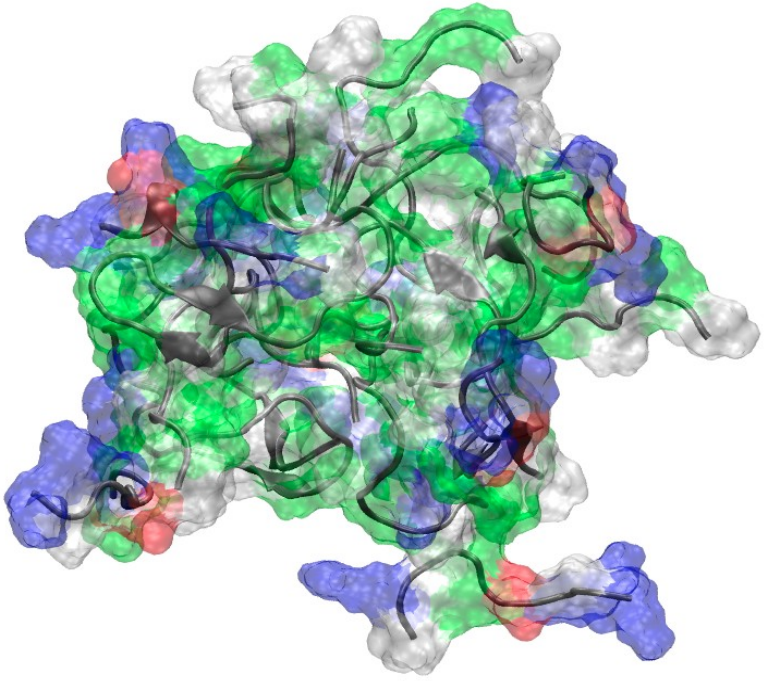
Mixed aggregate – 7 p1 and 9 p2 peptides



Multicomponent p1+p2 solution



Mixed 16-mer aggregate – coloured by peptide type (p1 – cyan, p2 – pink)



Residue type surface representation (basic aa – blue, acidic – red, polar – green, non-polar – white

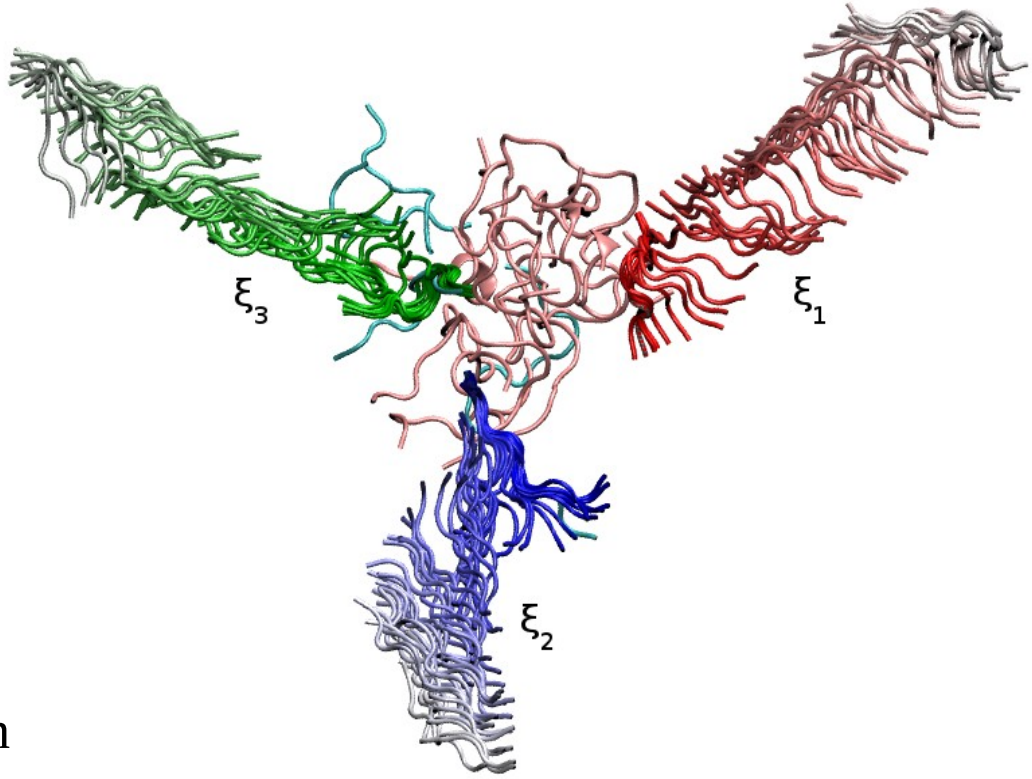
US dissociation free energies

Quantitative cluster stability assessment:

- p2 highly hydrophobic → no dissociation expected from the cluster.

- p1 peptides might dissociate due to the electrostatic repulsion from other p1 monomers potentially overcoming the attraction due to the hydrophobic effect.

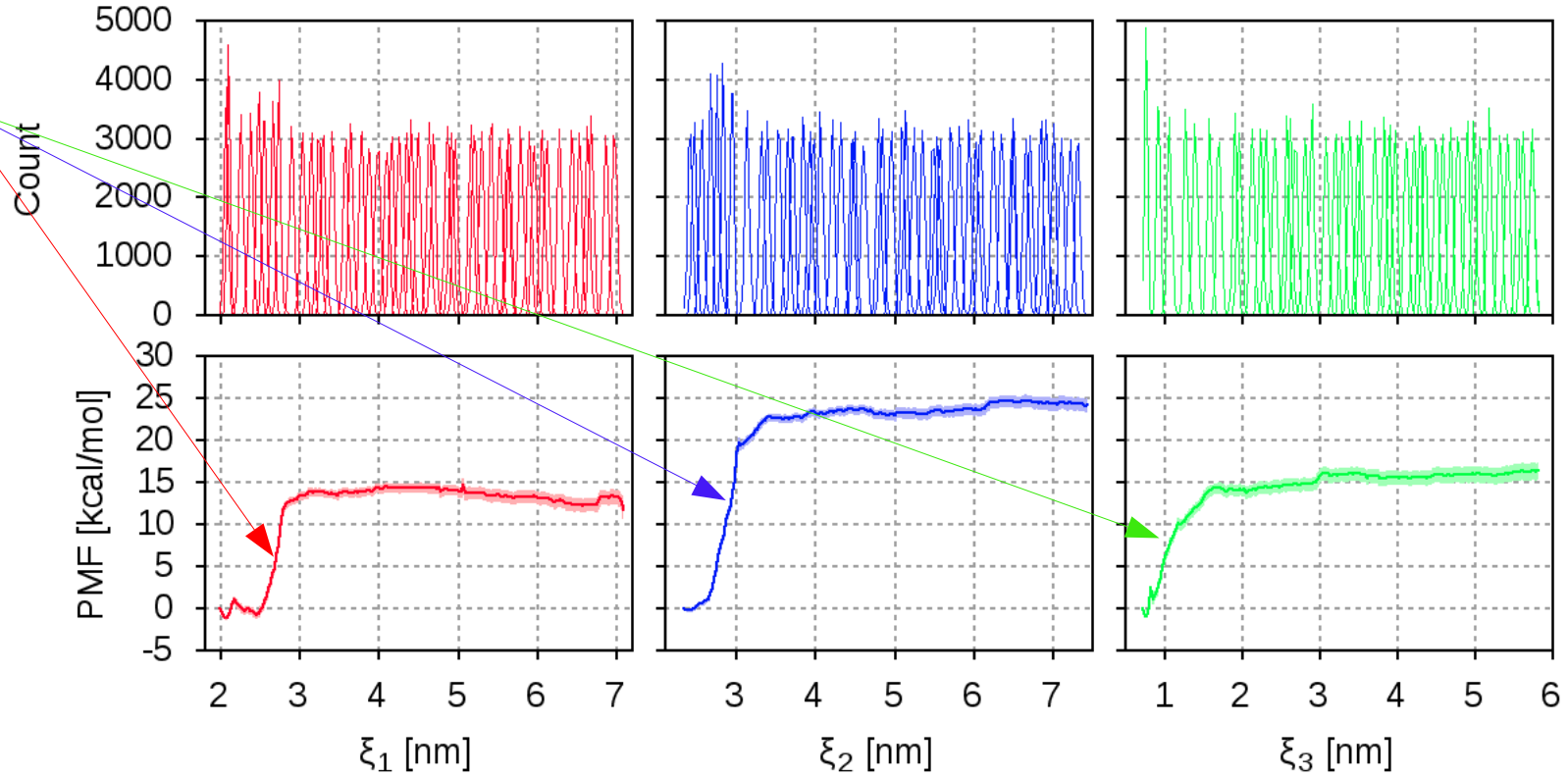
=> Calculate the dissociation free energy of the most solvent exposed p1 monomers, using umbrella sampling to estimate the potential of mean force along the collective variables ξ1, ξ2, ξ3, (COM distance between the respective p1 monomer and the aggregate)



US dissociation free energies

ΔG_d>13 kcal/mol

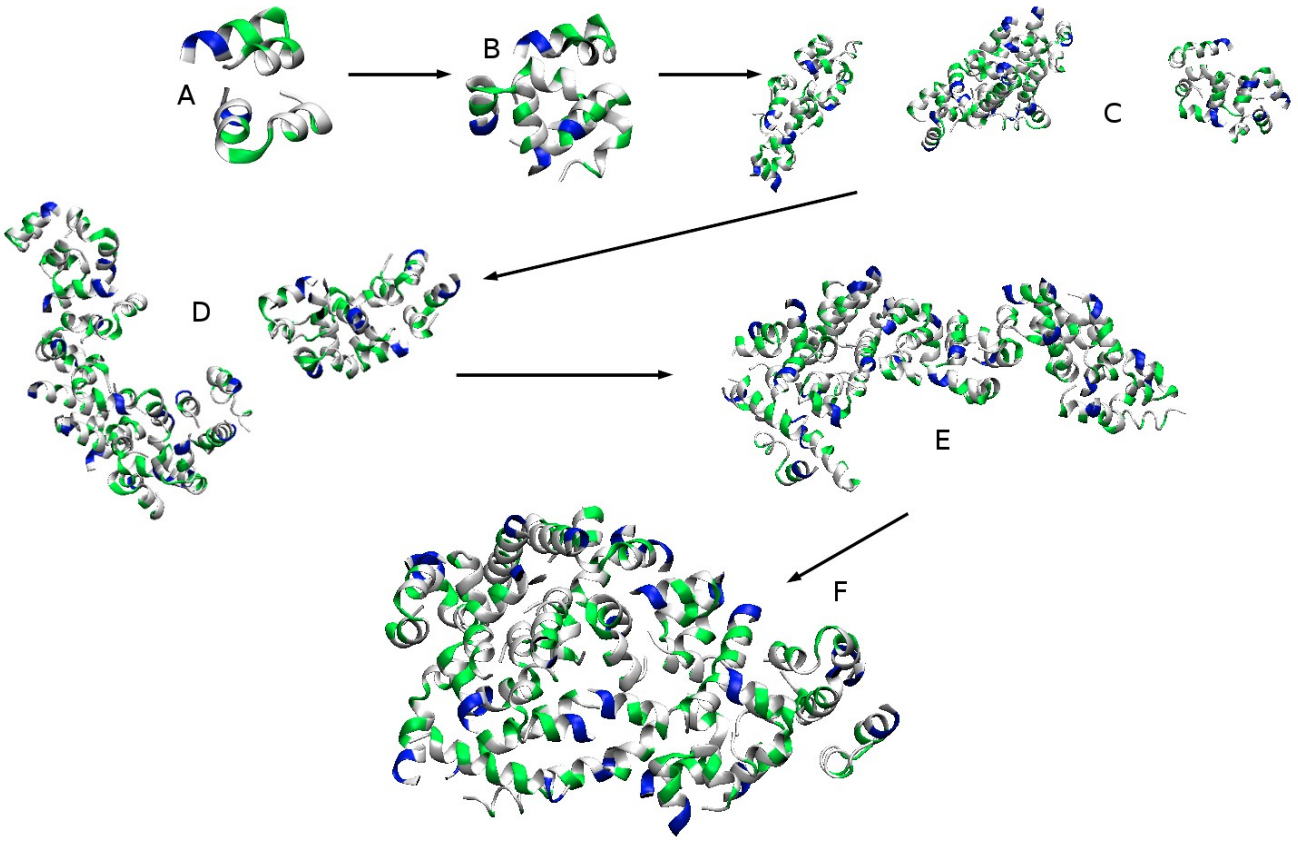
=> All three most solvent-exposed cationic peptides are stably bound to the aggregate and no spontaneous dissociation is to be expected.



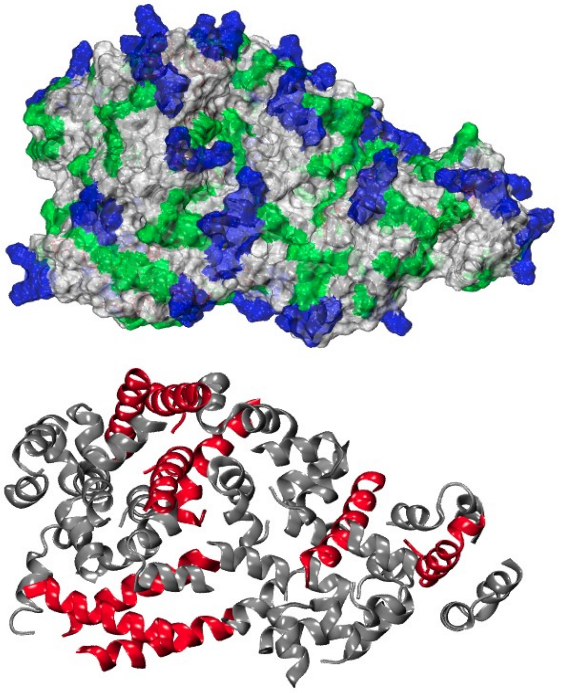
Histograms and PMFs for the three umbrella sampling simulations.

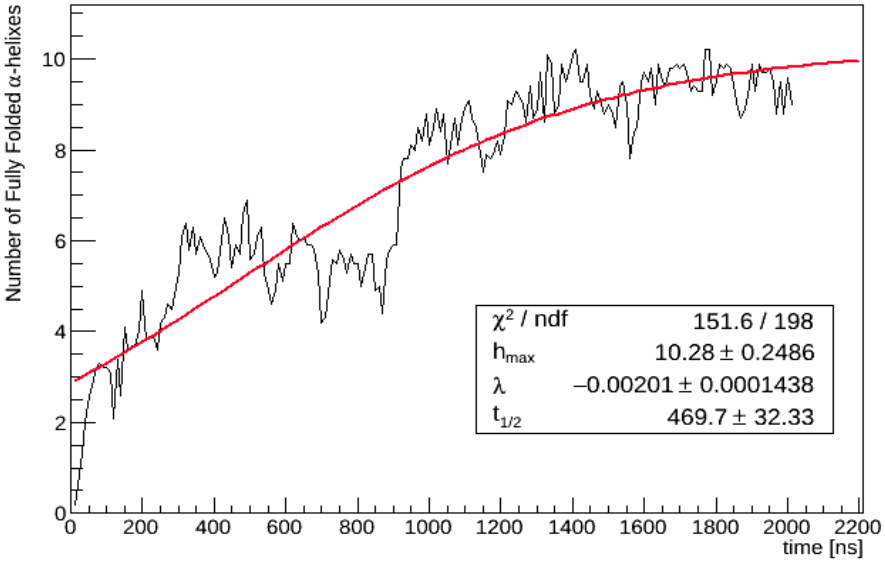
Conclusions

AMPs aggregate and form stable clusters with structure, similar to that of globular proteins – a non-polar hydrophobic core and exposed to the solvent charged and polar residues.



Conclusions





Aggregation leads to inter-peptide hydrogen bonds number increase, which causes formation of new and stabilization of alr1eady formed secondary structure elements, delivering AMPs in biologically active conformation to the target membrane in high local concentration.

Conclusions

Two possible scenarios for the role of the different peptides in the antimicrobial mixture:

Scenario	**I**	**II**
Cationic peptides	**Antimicrobial properties**	Coat the clusters and act as a navigation system, that is electrostatically attracted by the negatively charged phospholipids in the bacterial membranes
Non-cationic peptides	Act as “glue” for the cationic AMPs and deliver high enough local concentrations to the target membrane	**Antimicrobial properties**

Conclusions

- Regardless of the scenario, we propose that the so-formed structures provide the perfect transport system – locking the hydrophobic uncharged residues in the core of the cluster prevents the interaction with the eukaryotic membranes with lower surface charge density, and positioning the charged residues on the cluster surface enables for electrostatic interaction with the bacterial surface.
- In addition, the peptide folding, promoted by the amphiphilic structure in the aggregates, allows for high enough local concentration of AMPs to be delivered to the target membrane in a functionally active conformation.

Acknowledgments

- This work was supported in part by the Bulgarian National Science Fund under Grant KP-06 OPR-03-10/2018.
- Computational resources were provided by BioSim HPC cluster at the Faculty of Physics, Sofia University “St. Kliment Ohridski” and by CI TASK (Centre of Informatics – Tricity Academic Supercomputer & networK), Gdansk (Poland).