TOWARDS PEPTIDE-BASED THERAPEUTICS AGAINST CARDIAC DISEASE

Prediction and simulation of the S100A1ct peptide with a membrane environment

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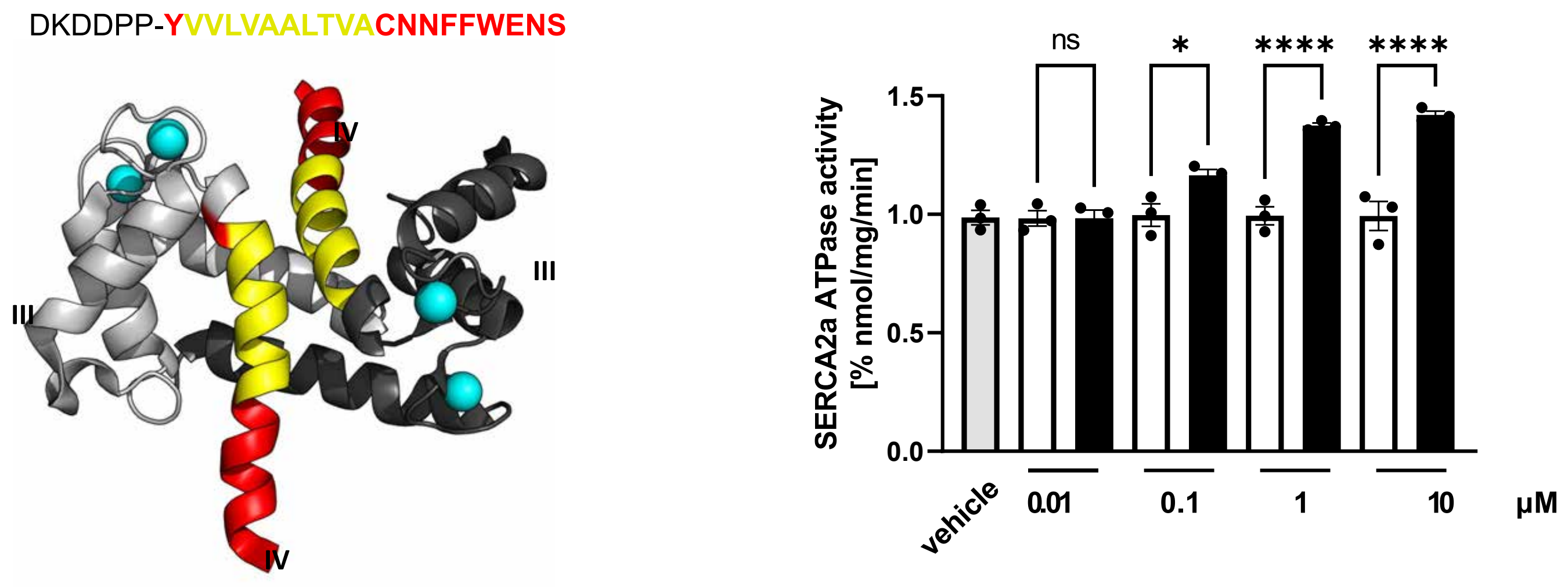
https://doi.org/10.17952/37EPS.2024.P1283

Introduction

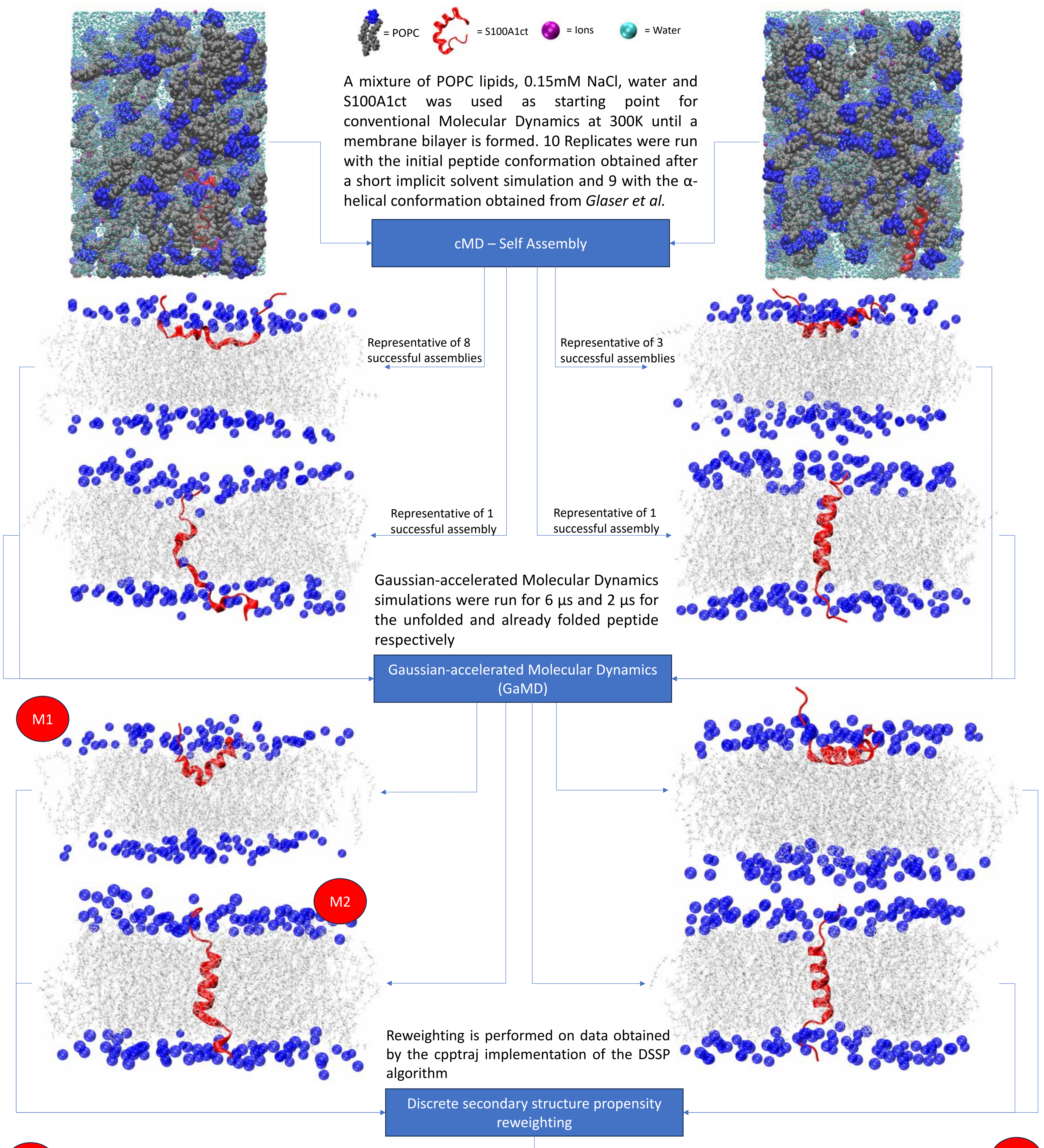
Cardiovascular diseases are the leading cause of death worldwide according to WHO estimates[1]. Heart failure accounted for over 60 million deaths in 2017[2] and can be classified into Heart Failure with reduced (HFrEF), preserved (HFpEF) and mid-range (HFmrEF) Ejection Fraction, with HFrEF accounting for many of these hospitalizations [3]. S100A1ct is a peptide derived from S100A1, a member of the Ca^{2+} binding EF-hand protein family, that has been observed to exert an inotropic effect on cardiomyocytes, both *in vivo and in vitro*, influencing with RyR2 and SERCA2a similarly to the parent protein[4,5].



On the left: S100A1ct sequence. In bold the S100A1-derived sequence, with red and yellow colours representing the polar and apolar portions. Below, the peptide is highlighted with the same colour scheme on the parent protein[5]. On the right: dose-dependent augmentation of SERCA2a activity by different concentrations of S100A1ct in cardiac SR vesicles, compared to control[5].

Peptide drugs can achieve a high degree of selectivity and specificity, however they come with drawbacks such as difficulties in oral and nasal administration in the absence of modifications or carriers [6]. S100A1ct is not immune to such weaknesses, therefore further modifications are needed to improve its potency and pharmacokinetics. In order to do so, a deeper characterization of S100A1ct interactions is needed.

S100A1ct conformations in a membrane bilayer model



A mixture of POPC lipids, 0.15mM NaCl, water and S100A1ct was used as starting point for conventional Molecular Dynamics at 300K until a membrane bilayer is formed. 10 Replicates were run with the initial peptide conformation obtained after a short implicit solvent simulation and 9 with the α-helical conformation obtained from *Glaser et al.*

cMD – Self Assembly

Representative of 8 successful assemblies

Representative of 3 successful assemblies

Representative of 1 successful assembly

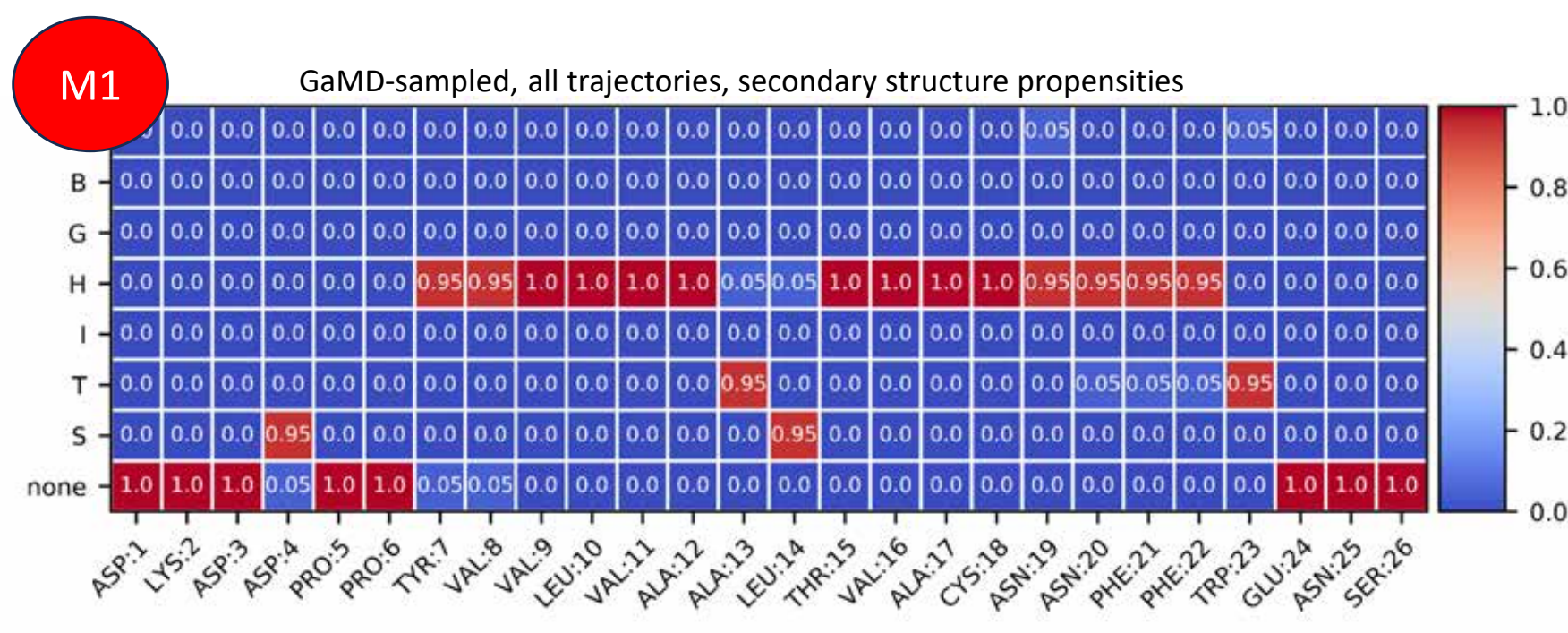
Representative of 1 successful assembly

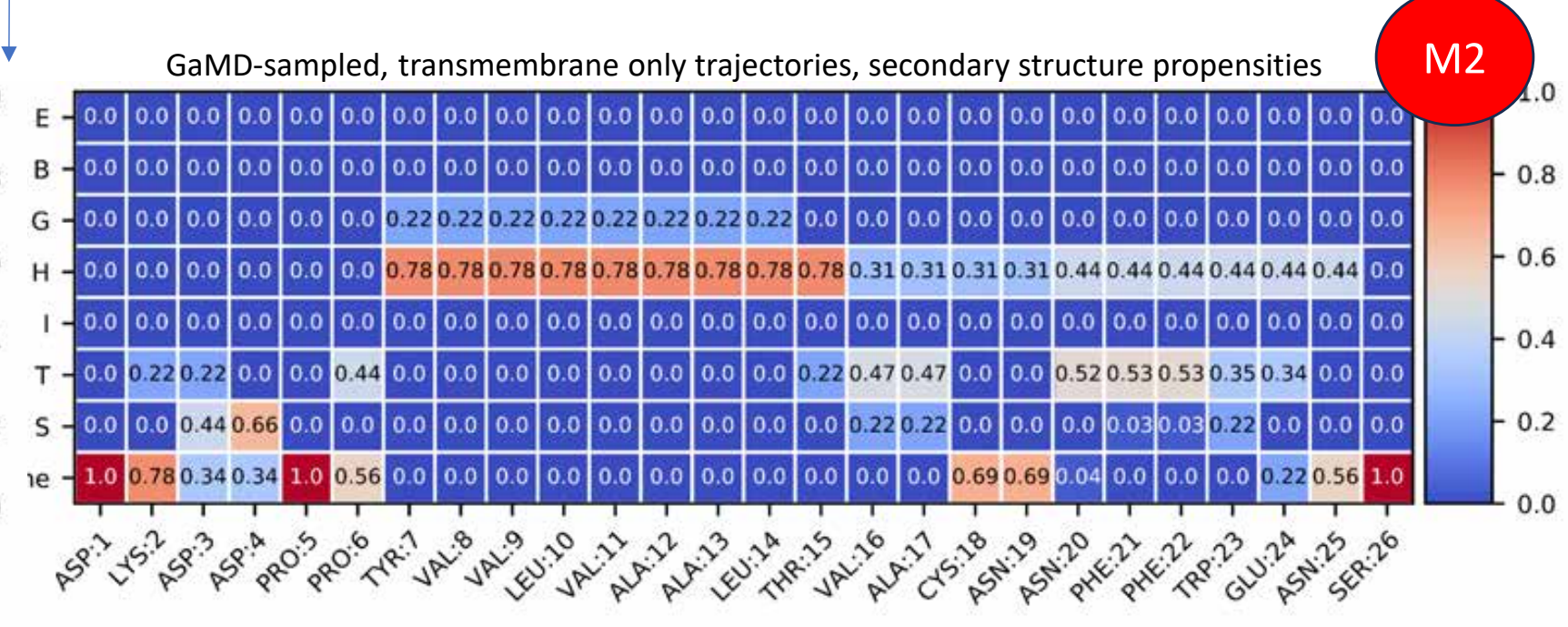
Gaussian-accelerated Molecular Dynamics simulations were run for 6 µs and 2 µs for the unfolded and already folded peptide respectively

Gaussian-accelerated Molecular Dynamics (GaMD)

Reweighting is performed on data obtained by the cpptraj implementation of the DSSP algorithm

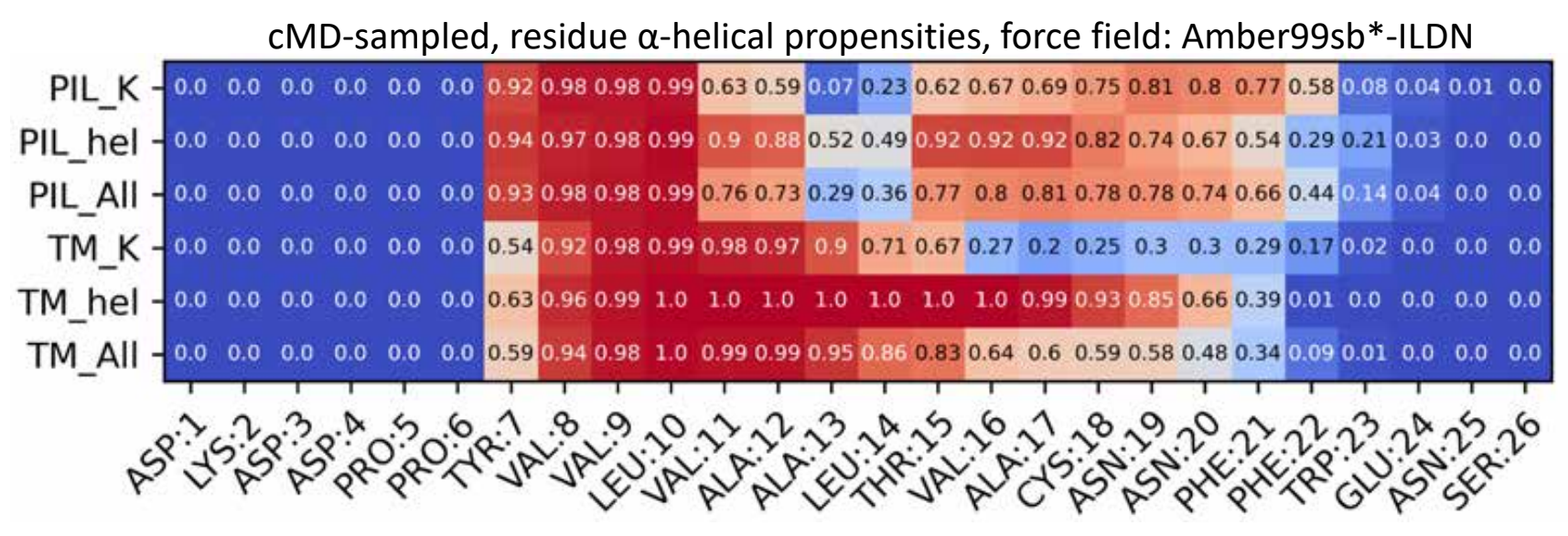
Discrete secondary structure propensity reweighting

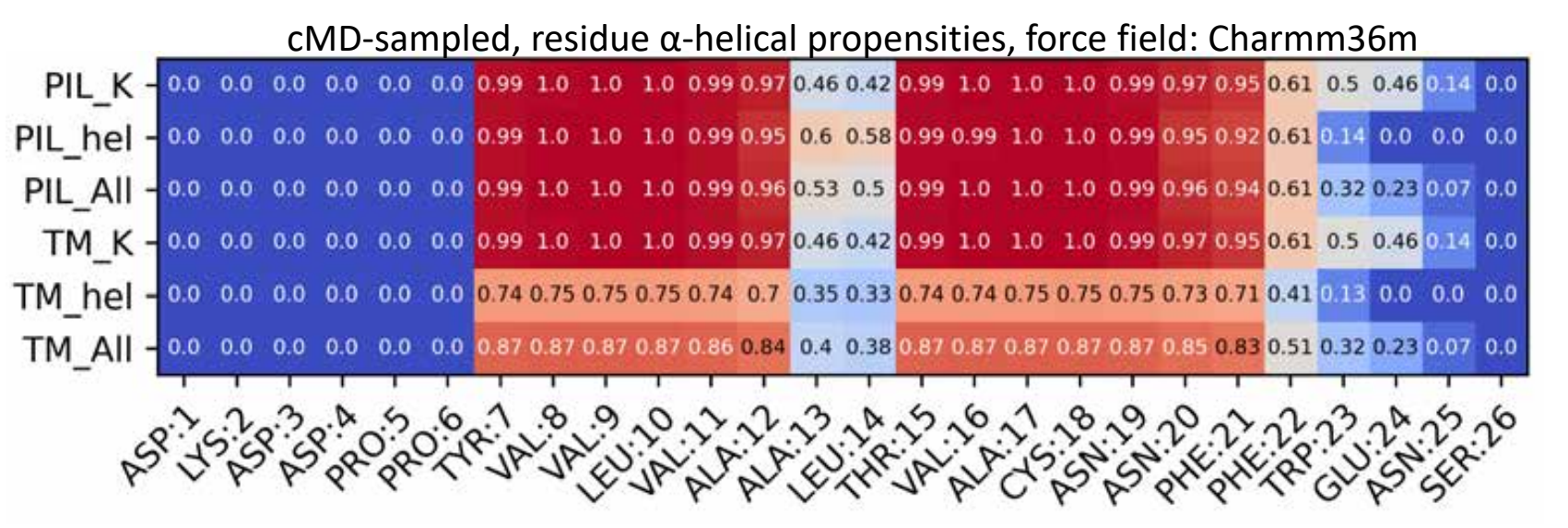




Confirmation of helical propensity in different force fields

In each force field, conventional Molecular Dynamics simulations were run for 600 ns from 8 starting points, each with two replicates. Four starting points with "M1"-like and "M2"-like conformations inserted in the membrane leaflet and transmembrane and other four with an helical conformation inserted in the same positions.





Outlook/Ongoing

- Evaluation of the impact of amino acid mutations on the peptide's preferred conformations in membrane with Monte Carlo simulations
- Simulation of both insertion and passage through the phospholipid bilayer in a more realistic cardiomyocyte model
- Clarify the role of the different kinked states in the peptide pharmacological properties
- Design and evaluation of peptidomimetics derived from such peptide.
- Investigating the possible role of cooperative effects in the switch between stable conformations

Acknowledgement

This study was supported by the Klaus Tschira Foundation Informatics for Life consortium (subproject 2 to RCW and PM)

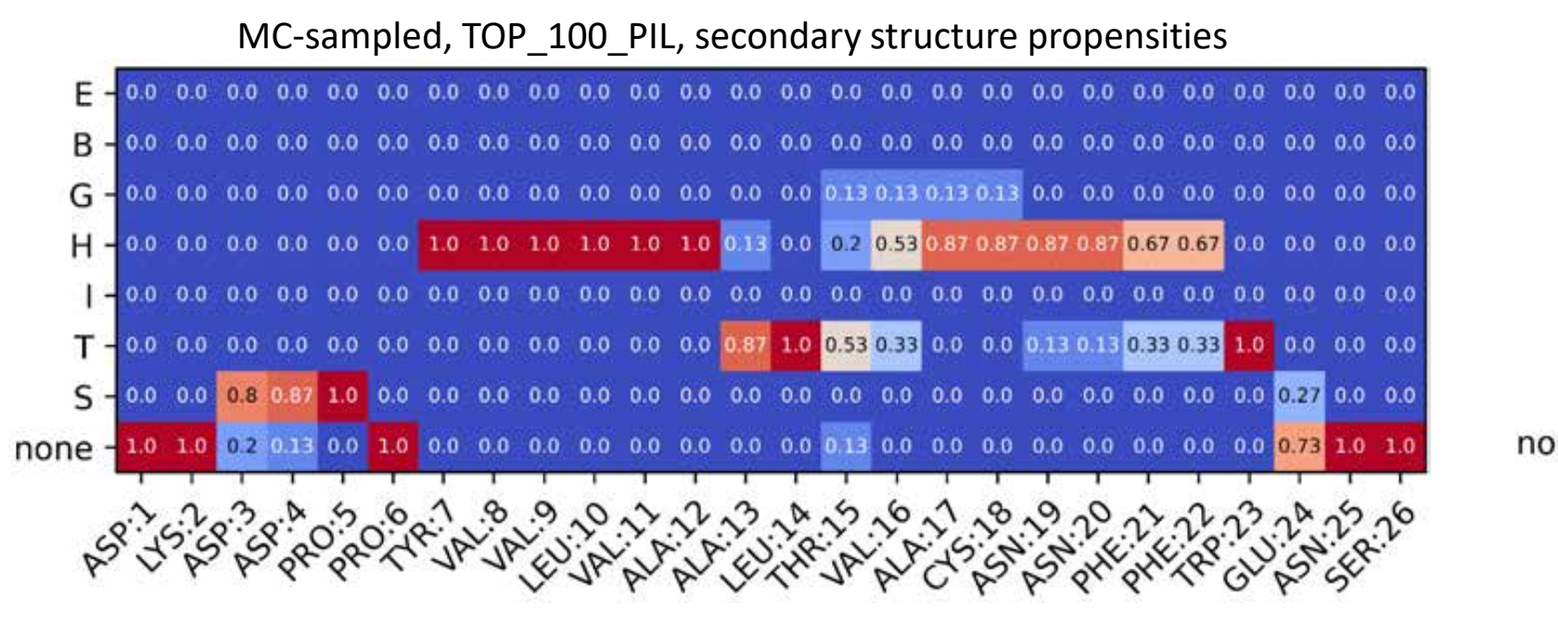
Monte Carlo simulations in implicit membrane

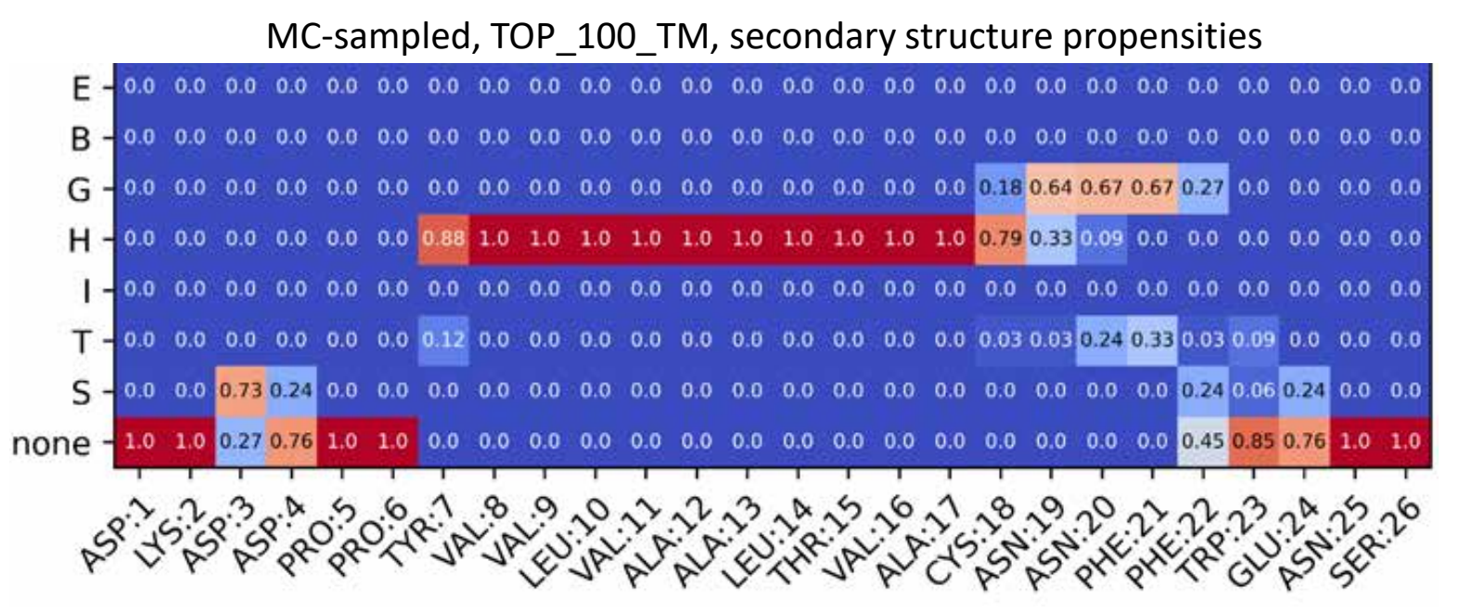
80 different runs were obtained from Monte Carlo (MC) simulations, 20 for each starting point (S100A1ct in the leaflet, transmembrane, in water and in the middle of the implicit membrane). The simulations were run at 310K using SLIM implicit membrane model[6] and Amber99sb*-ILDN force field using SIMONA software[8]. The obtained conformations were clustered by energy after high energy outliers (over 3 times the standard deviation) were removed.

Conformation Ensemble	Mean energy Kcal/mol *	Standard Deviation Kcal/mol*
TOP_100_PIL	679.21	1.55
TOP_100_TM	645.26	1.18
TOP_20_WATER	602.00	9.0

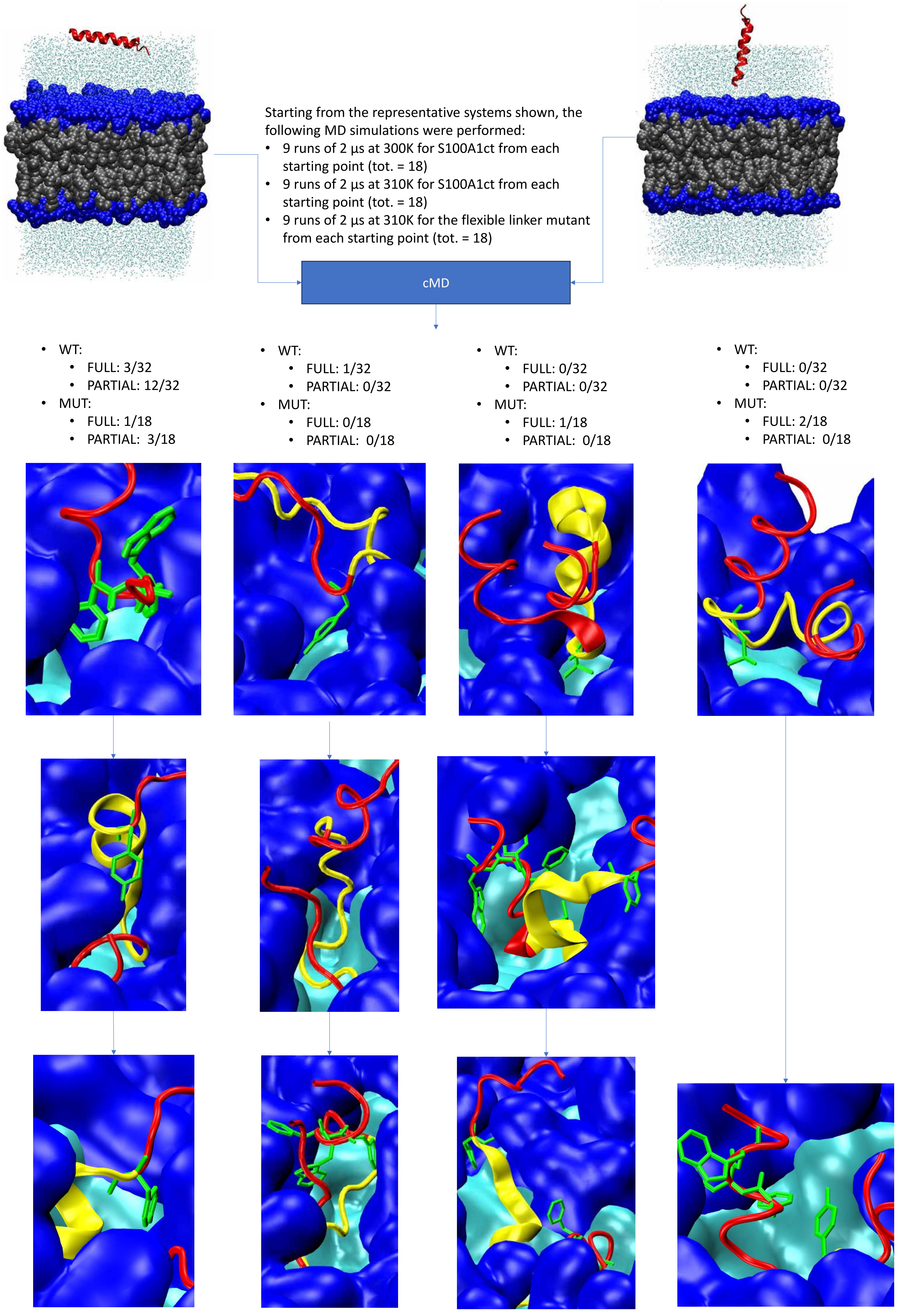
PIL = Peptide in the leaflet
TM = Transmembrane

* The energy here reported comes from the force field and can be used to compare different mutants, it does not represent the real one





S100A1ct insertion in a membrane bilayer model



Starting from the representative systems shown, the following MD simulations were performed:

- 9 runs of 2 µs at 300K for S100A1ct from each starting point (tot. = 18)
- 9 runs of 2 µs at 310K for S100A1ct from each starting point (tot. = 18)
- 9 runs of 2 µs at 310K for the flexible linker mutant from each starting point (tot. = 18)

cMD

- WT:
  - FULL: 3/32
  - PARTIAL: 12/32
- MUT:
  - FULL: 1/18
  - PARTIAL: 3/18

- WT:
  - FULL: 1/32
  - PARTIAL: 0/32
- MUT:
  - FULL: 0/18
  - PARTIAL: 0/18

- WT:
  - FULL: 0/32
  - PARTIAL: 0/32
- MUT:
  - FULL: 1/18
  - PARTIAL: 0/18

- WT:
  - FULL: 0/32
  - PARTIAL: 0/32
- MUT:
  - FULL: 2/18
  - PARTIAL: 0/18

Conclusions

S100A1ct peptide has been observed to interact with SERCA2a and predicted to do so with a transmembrane (TM) helical conformation. Here we show that:

- A TM peptide arrangement can be found using an unbiased "assembly" approach and it is stable across the GaMD trajectories
- S100A1ct appears, however, to be found more frequently at the interface between a single membrane leaflet and the aqueous environment
- These findings were confirmed with short MD simulations in two different force fields
- Monte Carlo simulations employing an implicit membrane model also show agreement with these results, providing an efficient means to quickly explore how mutations and different chemical modifications can impact the identified states

The process of insertion in the membrane follows a pathway that relies on the appearance of gaps between the polar heads, as recently observed for another peptide[9]. Importantly:

- Aromatic residues play a major role in the process, especially when a rigid linker is present at the N-terminus
- When such linker is more flexible, pathways relying on hydrophobic stretches appear, highlighting the importance of carefully designing the 'non-active' parts of the peptide

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