Chapter 2 - Supplementary information

1 Simulations of CaM C lobe and helix hA of K_v7.2 channel

Molecular dynamics simulations of CaM C lobe bounded to helix hA were performed to understand the energetic interactions that would arise from the binding of the Native (W344R-N) and Tilted (W344R-N) configurations, each situation was simulated following the same workflow and can be respectively found in the folders: **MUT-n MUT-t WT**.

Simulations were performed using CHARMM36 force field [1] in the NAMD 2.13 software [2]. To build wild type system, the experimental structural reference is the one with PDB ID 6FEG[3], in which the CaM and the channel were split and only the C lobe and helix hA were kept, to build the mutant ones, same workflow was followed but extracting one frame of the rosetta output that exhibited W344R-T conformation. Afterwards, the systems were solvated in TIP3P water [4] in a cubic box assuring that the protein was at least 1.2 nm away from the sides, also, to mimic neuron physiology, concentrations of 120 mM of KCl and 5 mM of NaCl were introduced. Throughout all simulation steps, SHAKE bond length constraints were applied to all bonds and nonbonded interactions were calculated by the particle-mesh Ewald method with a cutoff of 1.2 nm for separating the long range and short range interactions.

The three folders contain the same information for each simulation:

***./*:** parent folder of the simulation with coordinate (.pdb) and topology files (.psf) with the most probable configuration of the run.

step1_eminth_nvt/: Minimization of the input structure using 5000 steepest descent steps followed by a 0.5 ns of NVT thermalization at 298K, keeping the temperature using Langevin dynamics with a Langevin damping of $0.5\,ps^{-1}$ [5] and a time step of 1 fs. Separated into two steps.

- → 'eqMT01.conf': first half of the first step of thermalization.
- → 'eqMT02.conf': second half of the first step of thermalization.
- → 'eqMT02.restart.coor': final coordinates of the first step of thermalization.

step2_th_npt/: NPT thermalization at 298K, controlling the temperature using Langevin dynamics with a Langevin damping of $0.5\,ps^{-1}$ [5] and the pressure with a langevin piston with a target pressure of 1 atm [6], and a time step of 1 fs, for 0.5 ns separated into two runs.

- → 'eqMT03.conf': first half of the second step of thermalization.
- → 'eqMT04.conf': second half of the second step of thermalization.
- → 'eqMT04.restart.coor': final coordinates of the second step of thermalization.

step3_prod/: Production was performed with the same parameters as the previous NPT step but for 100 ns.

→ 'md.conf': input run for the production run.

→ 'md.restart.coor': binary file with the end of the run.

→ 'angles.png': time series of the angle of the run, angle definition can be found on the main text, chapter 3, Fig. 3.3.

→ 'rmsf.png': root mean square fluctuation of each amino acid on the sequence.

→ 'rms.png': total root mean square deviation of the sequence.

References

[1] Jing Huang and Alexander D MacKerell Jr. "CHARMM36 all-atom additive protein force field: validation based on comparison to NMR data". eng. In: *Journal of computational chemistry* 34.25 (Sept. 2013). PMC3800559[pmcid], pp. 2135–2145. ISSN: 1096-987X. DOI: 10.1002/jcc.23354. URL: https://doi.org/10.1002/jcc.23354.

[2] James C. Phillips et al. "Scalable molecular dynamics on CPU and GPU architectures with NAMD". In: *The Journal of Chemical Physics* 153.4 (2020), p. 044130. DOI: 10.1063/5.0014475.

[3] Ganeko Bernardo-Seisdedos et al. "Structural basis and energy landscape for the Ca^{2+} gating and calmodulation of the Kv7.2 K^+ channel". In: *Proceedings of the National Academy of Sciences* 115.10 (2018), pp. 2395–2400. DOI: 10.1073/pnas.1800235115.

[4] Pekka Mark and Lennart Nilsson. "Structure and Dynamics of the TIP3P, SPC, and SPC/E Water Models at 298 K". In: *The Journal of Physical Chemistry A* 105.43 (Nov. 2001), pp. 9954–9960. ISSN: 1089-5639. DOI: 10.1021/jp003020w. URL: https://doi.org/10.1021/jp003020w.

[5] Axel Brünger. “X-PLOR Version 3.1. A System for X-ray Crystallography and NMR”. In: Yale University Press, 1993. ISBN: 978-0300054026.

[6] Scott E. Feller et al. “Constant pressure molecular dynamics simulation: The Langevin piston method”. In: *The Journal of Chemical Physics* 103.11 (1995), pp. 4613–4621. DOI: 10.1063/1.470648.