Chapter 2 - Supplementary information

1 Simulations of double mutant CRD of K_V7.2 channel

Molecular dynamics simulations of helices hA, TW, and hB were performed for several double mutant sequences, each situation was simulated following the same workflow and can be respectively found in the folders:
Q341A-W344R_double_mutant Q341L-W344R_double_mutant Q341P-W344R_double_mutant Q341C-W344R_double_mutant Q341M-W344R_double_mutant Q341V-W344R_double_mutant Q341I-W344R_double_mutant Q341N-W344R_double_mutant W344R_single_mutant

All molecular dynamics simulations were performed with the software NAMD 2.13 [1] and the CHARMM36 all-atom force field [2]. The input structures were built from PDB 6FEG [3] by eliminating the N-terminal residual amino acids up to residue number 328, W344R and the other mutation on residue Q341 were introduced by means of the visualization software VMD [4],simulations were carried out in periodic cubic boxes of TIP3P water [5] so that the minimum distance of any protein atom and the edge of its box was at least 6.1 Å. To mimic neuron physiology, concentrations of 120 mM of KCl and 5 mM of NaCl were introduced. SHAKE bond length constraints were applied to all bonds, nonbonded interactions were calculated by the particle-mesh Ewald method with a cutoff of 12 Å for separating the

long range and short range interactions.

The folders contain the same information for each simulation, which is the following:

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

step1_min_nvt: Minimization of the input structure using 1000 steepest descent steps followed by a NVT thermalization at 298K, keeping the temperature using Langevin dynamics with a Langevin damping of $0.5\,ps^{-1}$ [6] and a time step of 1 fs, for 0.5 ns.

→ 'emint1_341_mut_NVT_base.conf': input file for the minimization and first step of thermalization.

step2_npt: NPT thermalization at 298K, controlling the temperature using Langevin dynamics with a Langevin damping of $0.5\,ps^{-1}$ [6] and the pressure with a langevin piston with a target pressure of 1 atm [7], for 1.5 ns.

→ 'emint1_341_mut_NPT_base.conf': input files for the second step of thermalization.

step3_prod: Production with the same parameters as the previous NPT step, this last step was repeated three times to obtain three different replicas that make up to $\sim 450ns$ of production time.

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

→ **replica_1**: results of the first production replica.

⋆ '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.

→ **replica_2**: results of the second production replica, same as replica 1.

→ **replica_3**: results of the third production replica, same as replica 1.

References

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[2] 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.

[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.

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[5] 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.

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[7] 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.