Chapter 4 - Supplementary information

1 Full channel simulations

Molecular dynamics simulation of the whole channel structures of KCNQ2 and KCNN4 were performed, each run contains three 500 ns long replicas, whose supplementary information is stored in:

KCNQ2_Apo KCNQ2_Retigabine KCNQ2_Ztz240 SK4_APO SK4_Riluzole

The building of the systems have been described in the main text, the molecular dynamics simulations were performed in GROMACS 2016.4 [1], for the simulations, temperature was regulated by means of the Nose-Hoover thermostat [2, 3]; and pressure, by the Parrinello-Rahman barostat [4], for temperature three reference groups were defined, namely the solvent, protein and membrane, the pressure was coupled semi isotropically, exploiting the symmetry around Z axis. The leap-frog integrator was used, with a time step of 2 fs. Long-range electrostatics was treated with the PME method [5], and short-range Coulomb and Van der Waals cutoffs were set at 1.2 nm. Periodic boundary conditions and the minimum image convention were employed. All gromacs inputs for equilibration and production runs were generated with CHARMM-GUI [6].

Input and output files of the whole protocol can be found in the following folders:

./: Parent folder.

→ 'topol.top': GROMACS topology file.

→ 'docking_parameters.pdf': parameters of the docking calculations to place riluzole in its binding pocket, in the case of SK4_Riluzole folder.

***toppar/*:** topology and parameter files that are required by the topology file.

***Therm/*:** 6 steps of NPT thermalization, with constraints applied to the membrane and protein, these constraints are gradually decreased in each step.

- → 'step6.0_minimization.mdp': input parameters for the minimization.
- → 'step6.1_equilibration.mdp'... 'step6.6_equilibration.mdp': input parameter files for the equilibration steps.
- → 'step6.6_equilibration.gro': final structure after thermalization, will be the input for production.
- → 'rms_therm.png': root mean square deviation of the protein atoms during the last step of thermalization.

***prod/*:** 500 ns long production runs at NPT ensemble.

- → 'step7_production.mdp': input parameters for the production runs.
- → '**replica1/**': output of the first production replica
  - ⋆ '${NAME}-1.gro.xtc': final coordinates of the run, in binary gromacs .xtc file
  - ⋆ 'rmsd-${NAME}-1.png': root mean square of the protein atoms, separated into calmoduline, transmembrane domain and calmodulin binding domains.
  - ⋆ 'rmsf${NAME}-1.png': root mean square fluctuations of the amino acids of the sequence of the channel.
- → '**replica2/**': output of the second production replica, same content as replica1.

→ '**replica3/**': output of the third production replica, same content as replica1.

References

[1] Mark James Abraham et al. "GROMACS: High performance molecular simulations through multi-level parallelism from laptops to supercomputers". In: *SoftwareX* 1-2 (2015), pp. 19–25. ISSN: 2352-7110. DOI: https://doi.org/10.1016/j.softx.2015.06.001.

[2] William G. Hoover. "Canonical dynamics: Equilibrium phase-space distributions". In: *Phys. Rev. A* 31 (3 Mar. 1985), pp. 1695–1697. DOI: 10.1103/PhysRevA.31.1695.

[3] Shūichi Nosé. "A molecular dynamics method for simulations in the canonical ensemble". In: *Molecular Physics* 52.2 (1984), pp. 255–268. DOI: 10.1080/00268978400101201.

[4] M. Parrinello and A. Rahman. "Polymorphic Transitions in Single Crystals: A New Molecular Dynamics Method". In: *J. Appl. Phys. (Melville, NY, U. S.)* 52 (1981), pp. 7182–7190. DOI: 10.1063/1.328693.

[5] Tom Darden, Darrin York, and Lee Pedersen. "Particle Mesh Ewald: An N log(N) Method for Ewald Sums in Large Systems". In: *J. Chem. Phys.* 98 (1993), pp. 10089–10092. DOI: 10.1063/1.464397.

[6] Jumin Lee et al. "CHARMM-GUI Input Generator for NAMD, GROMACS, AMBER, OpenMM, and CHARMM/OpenMM Simulations Using the CHARMM36 Additive Force Field". In: *Journal of Chemical Theory and Computation* 12.1 (Jan. 2016), pp. 405–413. ISSN: 1549-9618. DOI: 10.1021/acs.jctc.5b00935.