Chapter 4 - Supplementary information

1 REST2 simulations

For building the systems, a small fragment of KCNQ2 wild type NPAAGLIQSA**W**RFYATN and the mutated one NPAAGLIQSA**R**RFYATN were simulated, following the same workflow which can be respectively found in the folders: **W344R** and **WT** . For building the systems, the sequences were introduced into a dodecahedral box with TIP3P water molecules [1], with a minimun distance between protein and walls of 1.5 nm, also K^+ and Cl^- ions were added to achieve a physiological concentration of KCl of 0.15 M.

To observe the folding of the small fragment of the KCNQ2 channel, we needed to use a simulation method that would enhance the conformational sampling, and the one chosen was the replica exchange with solute scaling (REST2) [2], in which each replica is run at a different Hamiltonian that mimics a different temperature. All REST2 simulations followed the same workflow, 15 replicas were simulated with the first one at an effective temperature of $T_0 = 298\ K$ and a maximum one of $T_{14} = 373\ K$, the temperatures were spanned with the following relation:

$$T_m = T_0 \cdot \exp\left(m \cdot \frac{\ln(T_{n-1}/T_0)}{n-1}\right). \tag{1}$$

The scaling factor T_m is used in practice to scale potential energy

terms of the Hamiltonians:

$$E_m^{REST2} = \frac{T_0}{T_m} E_{hh} + \sqrt{\frac{T_0}{T_m}} E_{hc} + E_{cc}, \tag{2}$$

Where the scaling only affects one part of the system, known as the "hot" subsystem E_{hh}, and also its interface with the "cold" one E_{hc}, finally the interactions of the "cold" one E_{cc} are left unchanged.

All simulations were done using the CHARMM-36 force field [3] in GROMACS 2021.3 [4, 5, 6] patched with PLUMED 2.7.2 [7]. For the simulations, temperature was regulated by means of the velocity-rescaling thermostat [8]; and pressure, by the Parrinello-Rahman barostat [9]. The leap-frog integrator was used, with a time step of 2 fs. Snapshots were collected every 50 ps, and exchanges between neighboring replicas were attempted every 100 ps. Long-range electrostatics was treated with the PME method [10], and short-range Coulomb and Van der Waals cutoffs were set at 1.0 nm.

The workflow followed in REST2 simulations for each replica is the following, to begin with, steepest descent energy minimization was done until the maximum force was less than 100.0 kJ/(mol·nm), afterwards, 100 ps of NVT followed by another 100 ps of NPT were carried out with restrictions in the heavy atom, temperatures were kept at 298 K using a velocity-rescaling thermostat [8] and pressure to 1 bar using the Parrinello-Rahman barostat [9] in the NPT thermalization, finally 200 ns at NPT ensemble were collected as production for each replica, with exchanges attempted each 100 ps

Inside of each folder some input and output files of the whole protocol can be found:

***Min/*:** Each replica was energy-minimized by means of the steepest descent method until the maximum force was less than 100.0 kJ/(mol·nm).

→ 'min.mdp': input parameters for the minimization.

→ 'min.gro': output structure of the minimization.

***NVT/*:** Then each replica was equilibrated at NVT ensemble for 100 ps at a physical temperature of 298 K, restraining the positions of the heavy atoms, restrictions are defined in the topology and they have a value of 1000 kcal/mol along the three axes.

- → 'nvt.mdp': input parameters for the NVT equilibration.
- → 'nvt.gro': output structure of the NVT equilibration
- → 'processed.top': processed topology for equilibration, atom names of atoms comprising hot subsystem have _ attached for scaling with plumed script.

***NPT/*:** Then each replica was equilibrated at NPT for 100 ps at 298 K and a pressure of 1 bar, restraining the positions of the heavy atoms, restrictions are defined in the topology and they have a value of 1000 kcal/mol along the three axes.

- → 'npt.mdp': input parameters for the NPT equilibration.
- → 'npt.gro': output structure of the NPT equilibration
- → 'processed.top': processed topology for equilibration, atom names of atoms comprising hot subsystem have _ attached for scaling with plumed script.

***Prod/*:** Production runs were carried out for 200 ns per replica in the NPT ensemble, at the same physical temperature and pressure than the equilibrations, exchanges between neighboring replicas were attempted every 100 ps.

- → 'md.mdp': input parameters for the production.
- → 'md.gro': final snapshot of the production run.
- → 'processed.top': processed topology for production, atom names of atoms comprising hot subsystem have _ attached for scaling with plumed script.

→ 'exch_0.png': exchanges of the first replica, following its path along the replicas through time.

→ 'exch_all.png': exchanges of all replicas through time.

→ 'exchange_count.xvg': number of exchanges between the replicas and its probability.

./: in the parent folder the script for making the different topologies is given, named "gentopHREX.sh", it needs a running gromacs compilation patched with plumed, effective temperature range can be selected in the script, it is run with the command bash gentopHREX.sh and generates the scaled topologies in different folders R0...R${N} where N is the number of replicas minus one.

References

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