Chapter 3 - Supplementary information

1 REST2 simulations

To observe the folding of the small fragment of the SK2 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) [1], 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. Enhancing the conformational sampling.

All simulations were done using the CHARMM-36 force field [2] in GROMACS 2021.3 [3, 4, 5] patched with PLUMED 2.7.2 [6], once the

systems were built, solvation was performed by introducing TIP3P water molecules [7] in dodecahedral boxes 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. 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.

In this set of calculations, several sequences were simulated, in the following table information of each sequence can be found, like the entry name and ID of UniProt (https://www.uniprot.org/), folder name where the results are in and finally its sequence.

Entry name	ID	Folder name	Sequence
KCNN1_HUMAN	Q92952	sk1	NAAANVLRETWLIYKHT
KCNN4_HUMAN	O15554	sk4	ESAARVLQEAWMFYKHT
KCNQ1_HUMAN	P51787	kcnq1	PAAASLIQTAWRCYAAE
KCNQ2_HUMAN	O43526	kcnq2	NPAAGLIQSAWRFYATN
MYH7_HUMAN	P12883	myosin	SRIITRIQAQSRGVLAR
CAC1C_HUMAN	O13936	cav12	FYATFLIQEYFRKFKKR
INVS_HUMAN	Q9Y283	inversin	DIAAFKIQAVYKGYKVR
IQGA2_HUMAN	Q13576	iqgap2-2	EENVVKIQAFWKGYKOR

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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