

# 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  $T_0 = 298\text{ K}$  and a maximum one of  $T_{14} = 373\text{ K}$ , the temperatures were spanned with the following relation:

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

The scaling factor  $T_m$  is used in practice to scale potential energy terms of the Hamiltonians:

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

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 minimum 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.

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

***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

- [1] Lingle Wang, Richard A. Friesner, and B. J. Berne. “Replica Exchange with Solute Scaling: A More Efficient Version of Replica Exchange with Solute Tempering (REST2)”. In: *The Journal of Physical Chemistry B* 115.30 (Aug. 2011), pp. 9431–9438. ISSN: 1520-6106. DOI: [10.1021/jp204407d](https://doi.org/10.1021/jp204407d).
- [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](https://doi.org/10.1002/jcc.23354).
- [3] M. J. Abraham et al. “GROMACS: High Performance Molecular Simulations through Multi-Level Parallelism from Laptops to Supercomputers”. In: *SoftwareX* 1 (2015), pp. 19–25. DOI: [10.1016/j.softx.2015.06.001](https://doi.org/10.1016/j.softx.2015.06.001).
- [4] Herman J. C. Berendsen, David van der Spoel, and R. van Drunen. “GROMACS: A Message-Passing Parallel Molecular Dynamics Implementation”. In: *Comput. Phys. Commun.* 91 (1995), pp. 43–56. DOI: [10.1016/0010-4655\(95\)00042-E](https://doi.org/10.1016/0010-4655(95)00042-E).
- [5] David van der Spoel et al. “GROMACS: Fast, Flexible and Free”. In: *J. Comput. Chem.* 26 (2005), pp. 1701–1718. DOI: [10.1002/jcc.20291](https://doi.org/10.1002/jcc.20291).
- [6] Gareth A. Tribello et al. “PLUMED2: New Feathers for an Old Bird”. In: *Comput. Phys. Commun.* 185 (2014), pp. 604–613. DOI: [10.1016/j.cpc.2013.09.018](https://doi.org/10.1016/j.cpc.2013.09.018).
- [7] 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](https://doi.org/10.1021/jp003020w).

- [8] Giovanni Bussi, Davide Donadio, and Michele Parrinello. “Canonical Sampling through Velocity Rescaling”. In: *J. Chem. Phys.* 126 (2007), p. 014101. DOI: [10.1063/1.2408420](https://doi.org/10.1063/1.2408420).
- [9] 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](https://doi.org/10.1063/1.328693).
- [10] 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](https://doi.org/10.1063/1.464397).