Chapter 4 - Supplementary information

1 Cotranslational REST2 simulations

To observe the cotranslational 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) [1], in which each replica is run at a different Hamiltonian that mimics a different temperature. The building of the system is detailed in the main text of the thesis. Wild type and mutation W344R were simulated following the same workflow and can be respectively found in the folders: **W344R** and **WT** . 15 replicas were simulated with the first one at an effective temperature of $T_0 = 300\ K$ and a maximum one of $T_{14} = 450\ K$, 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 [2] in GROMACS 2021.3 [3, 4, 5] patched with PLUMED 2.7.2 [6]. For the simulations, temperature was regulated by means of the velocity-rescaling thermostat [7]; and pressure, by the Parrinello-Rahman barostat [8]. 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 [9], 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, 5000 steepest descent energy minimization was done, without restrictions on the atoms. Afterwards, 100 ps of NVT followed by another 200 ps of NPT were carried out with restrictions in the heavy atom, temperatures were kept at 298 K using a velocity-rescaling thermostat [7] and pressure to 1 bar using the Parrinello-Rahman barostat [8] in the NPT thermalization, finally, three independent REST2 simulations of 300 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/*:** → 'min.mdp': input parameters for the minimization.

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

→ 'topol_min.top': topology used for minimization (without position restraints.

***therm/*:** → 'nvt.mdp': input parameters for the NVT equilibration.

→ 'nvt.gro': output structure of the NVT equilibration

→ 'npt.mdp': input parameters for the NPT equilibration.

→ 'npt.gro': output structure of the NPT equilibration

→ 'topol_therm.top': processed topology for equilibration.

→ 'rms.png': RMSD of nascent chain and ribosome during equilibration steps.

***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.

→ 'prod.mdp': input parameters for the production.

→ 'processed_prod.top': processed topology for production, atom names of atoms comprising hot subsystem have _ attached for scaling with plumed script.

→ **replica1**: results of the first production replica.

⋆ '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.

⋆ 'exch_count.xvg': statistics of the exchanges.

⋆ 'rms.png': total root mean square deviation of the sequence.

⋆ 'rmsf.png': total root mean square deviation of the sequence.

→ **replica2**: results of the second production replica.

→ **replica3**: results of the third production replica.

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.

[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] 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.

[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.

[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.

[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.

[7] 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.

[8] 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.

[9] 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.