Chapter 3 - Supplementary information

1 Metadynamics simulations

Two describe the free energy profile of the angle formed by the triptophan with CaM, well-tempered metadynamics simulations [1] were carried out, there was no need for thermalize the systems, since the previously equilibrated 'one turn' and 'two turns' systems were used. Simulations were ran with CHARMM36 force field [2] in GROMACS 2021.3 patched with PLUMED 2.7.2 [3].

Gaussian functions to the Hamiltonian were added each picosecond, being these centered around the value of the angle at that simulated time and with a height of $2.0\,kJ/mol$ and a width of 0.5 rad. We observed that sometimes the effect of the addition of these Gaussians provoked the dissociation of the CaM C lobe and the channel fragment, therefore we had to add a funnel like potential preventing it.

For the simulations, 1000 ns of NPT ensemble molecular dynamics were carried out, temperature was regulated by means of the velocity-rescaling thermostat [4]; and pressure, by the Parrinello-Rahman barostat [5]. The leap-frog integrator was used, with a time step of 2 fs. Long-range electrostatics was treated with the PME method [6], and short-range Coulomb and Van der Waals cutoffs were set at 1.0 nm. Periodic boundary conditions and the minimum image convention were employed.

Results for the "One turn" and "Two turns" can be found in the folders **OneTurn TwoTurns**, respectively, in both cases the same information is given:

***OneTurn/*:** production of the "One turn" simulation.

- → 'md.mdp': input parameters for the molecular dynamics.
- → 'md.gro': input coordinate file for the simulation.
- → 'topol.top': compressed topology of the system.
- → 'metadynamics.png': time series of the angles of the whole simulation and free energy surfaces obtained for the last 200ns.
- → 'plumed.dat': input parameters for the metadynamics, in which the collective variable, parameters for the added Gaussian functions and the funnel potential are defined.

***TwoTurns/*:** production of the "Two turn" simulation.

- → 'md.mdp': input parameters for the molecular dynamics.
- → 'md.gro': input coordinate file for the simulation.
- → 'topol.top': compressed topology of the system.
- → 'metadynamics.png': time series of the angles of the whole simulation and free energy surfaces obtained for the last 200ns.
- → 'plumed.dat': input parameters for the metadynamics, in which the collective variable, parameters for the added Gaussian functions and the funnel potential are defined.

References

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