MD Simulations

Date: 2022-06-14
Tags: *3_MD Simulation 1_Drylab*
Created by: Stephan Schott

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The protein was protonated with {pdb2pqr|protonation method} at a pH of {7.4|pH}, with protonation states as determined by {PROPKA|pKa method}.

The protonation state of the ligand was determined using {Epik|ligand protonation program}.

The system was prepared with {PACKMOL-Memgen|membrane packing tool},

orienting the protein in the membrane slab by using {memembed|membrane protein orienting tool}.

placing the protein at {25 Å|surface distance} from the membrane surface.

The membrane composition was {4:4:1|lipid ratio} {DOPC:DOPE:DOPG|lipid composition}. Lipids were represented using the {Lipid21|lipid force field}. The bilayer structure was assessed by evaluating the electron density distribution along the membrane normal and area per lipid during the simulations.

A concentration of {0.15M|salt concentration} {NaCl|salt} was added to the simulation box.

The charge of the system was neutralized by adding the corresponding amount of {NaCl|salt} counterions.

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To obtain charges for the ligand, a gas-phase geometry optimization was carried out at the {HF|QM method}/{6-31G(d)|QM basis set} level using {GAUSSIAN 09, Revision B.0110|QM software}

on {three|:ligand conformers:}

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The RESP procedure with two fitting stages (hyperbolic constraint values: 0.0005/0.001) was used for charge derivation.

The system was solvated using a {rectangular|box type} water box using {OPC|water type} water with a minimum distance of {12 Å|shell radius} from the edges of the periodic box to the outermost atom of the solute.

{All-atom molecular dynamics (MD)|simulation type} simulations were performed using the {AMBER22|:MD suite:}. The {ff14SB|force field} was used to describe the protein

and the {gaff2|ligand force field} to describe the ligand

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Unless specified otherwise, a time step of {4 fs|dt_fs} was used for integration.

The {Langevin|:thermostat:} was used for temperature control with a collision frequency of {1 ps-1|gamma_ln} and a target temperature of {300 K|temp0}.

The {Berendsen|:thermostat:} with a coupling constant of {1 ps|tautp} was used. Translational and rotational motions were removed every {1000|nscm} simulation steps to avoid the flying-ice-cube effect.

The pressure of the system was maintained by the {Berendsen|:barostat:} with a coupling constant of {1 ps|taup}.

Covalent bonds involving hydrogen atoms were constrained using the SHAKE algorithm.

The Particle Mesh Ewald method was used to describe long-range electrostatic interactions, and a cutoff of {10 Å|cut} was used for short-range electrostatics and van der Waals forces.

The system was minimized by a combination of steepest descent and conjugate gradient algorithms.

Positional harmonic restraints with a force constant of {25 kcal mol-1 Å-2|restraint_wt} were applied to the {protein atoms/membrane atoms/ligand atoms|restrained elements}.

Thereafter, {50 ps|simulation time} of {NVT|ensemble} simulations were conducted. The system was then heated up to {100 K|temp0}. The previous step is followed by {300 ps|simulation time} to adjust the density of the simulation box to a pressure of {1 atm|pres0} and to heat the system to {300 K|temp0}.

During these steps, a harmonic potential with a force constant of {10 kcal mol^{-1} $Å^{-2}$|restraint_wt} was applied to the solute atoms.

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As the final step in thermalization, {5 ns|simulation time} {NPT|ensemble} simulations were performed.

During this process, the restraint forces on solute atoms were gradually removed within the first {500 ps|simulation time}.

Afterwards, {5|:replicates:} of {1 s|simulation time} independent production {NVT|ensemble} simulations were carried out.

To evaluate the convergence of the simulations, the RMSD, RMSF and RMS average correlation metrics of the solute were evaluated using CPPTRAJ. Unless stated otherwise, averages for observables from the MD simulations are expressed as grand mean ± standard error (SEM), calculated from the time averages over the {5|:replicates:}.

The main motions of the studied system were obtained by calculating the principal components by a principal component analysis from the covariance matrix and eigenvectors as obtained from CPPTRAJ.

Umbrella sampling simulations were performed by using a harmonic potential with force constant of {4 kcal mol−1 Å−2|US force constant}; the {distance between the COM of C atoms of residues 25–38 of each monomer|US reaction coordinate} was used as a reaction coordinate over {25|US windows}, being restrained in every simulation. Values for the reaction coordinate were recorded every {2 ps|US record time} and post-processed with the {Weighted Histogram Analysis Method|PMF method} implementation. For each window, the first {100 ns|US equilibration time} were not considered and regarded as equilibration of the system. The kernel densities of the reaction coordinate distributions showed a median overlap of {8.2%|US distribution overlap} between contiguous windows. The convergence of the free energy profile was evaluated by calculating the cumulative PMF every {10 ns|PMF_convergence_step}. The error was estimated by separating the production simulation in {50 ns|US independent chunk} independent parts each and then calculating the standard error of the mean of

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the independently determined energy profiles.

For each simulation system, the change in effective energy due to ligand binding was calculated by the {single|traj_approach} trajectory approach using the {MMPBSA.py|MMPBSA module} module with the {GB|solvation_model} {5|igb} model as implemented in {AmberTools17|AmberTools_version}.

To determine the contribution of individual residues to the total binding energy, a per-residue decomposition was performed.

The effective binding energy was then expressed as an average over {1000|:snapshots:} extracted at equally spaced intervals from the trajectories. The polar contribution to the solvation free energy was calculated by the linear Poisson-Boltzmann (PB) equation using an ionic strength of {150 mM|saltcon}, internal dielectric constant of {1|e_int}, and an external dielectric constant of {80|e_ext}, using otherwise the software defaults. The nonpolar contribution to the solvation free energy was considered proportional to the solvent accessible surface area (SASA) with appropriate and parameters as specified for the used model. The SASA was calculated with a solvent probe radius of 1.4 Å, Tan & Luo radii for the protein, and mbondi radii for the ligands. The dispersion term was calculated by a surface-integration approach.

Changes in configurational entropy due to ligand binding were calculated by normal mode analysis (NMA) as implemented in MMPBSA.py.We chose {GB^HCT|nmode_igb} as a water model, and each snapshot was minimized until the convergence criteria of a difference in minimized energy of <{0.001|drms} kcal mol^{-1} is satisfied.

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[A]SMD simulations were performed with a constant pulling velocity of v={1|Å ns^{-1}|velocity} along {reaction_coordinate |reaction_coordinate} and work applied along the {x,y,z axis/the reaction coordinate|fxyz} axis is determined. A uniform force constant of k = {100|kcal mol^{-1} $Å^{-2}$ |force_constant} (_the parameter in the infile used for steeredMD within AmberMD is rk2, which actually refers ot force_constant_) was employed. The reaction coordinate was divided into {10|No of_Stages} stages. For each stage, {25|No_Replicas_per_Stage} replicas are simulated for {1|ns|Simulation_time_per_Stage} each. Within these stages, the trajectory closest to the Jarzynski average is determined, and the final state of this trajectory is used as starting point for the consecutive stage. The overall PMF is approximated by calculating the mean of the obtained work per stage and summed up along the reaction coordinate.