Membrane simulation: yes/no

Cycles of minimization: XX

<Section**|**Preparation and Environment **>**

The variants were protonated with {PROPKA| protonation method} according to {7.4| pH}, neutralized by adding counterions.

<if|membrane simulation|e|true>, The variants are embedded in a membrane consisting {POPC|Lipid type} lipids and solvated in a {rectangular|box type} water box using {TIP3P|water type} with a minimal shell of . {12 Å|shell radius} around the solute. <ELIF|membrane simulation|e|false>, The variants were solvated in an {octahedral|box type} water box using {TIP3P|water type} with a minimal shell of . {12 Å|shell radius} around the solute

All atom {molecular dynamics (MD)|simulation} simulations were performed using {AMBER14|suite} suite..

<if|water type|e|TIP3P>, The {ff14SB|force field} force field was used in combination with the {LIPID14|force field} force field. <ELIF|water type|e|OPC>, The {ff19SB|force field} force field was used.

<if|membrane simulation|e|true> During the {thermalization|period}, the time step for all MD simulations was set to {2 fs|dt} with a direct-space, nonbonded cutoff of {9 Å|cut}. During the {production|period}, the time step for all MD simulations was set to {4 fs|dt} as hydrogen mass repartitioning was used with a direct-space, non-bonded cutoff of {8 Å|cut}.<ELIF|membrane simulation|e|false>, The time step for all MD simulations was set to {4 fs|dt} as hydrogen mass repartitioning was used with a direct-space, non-bonded cutoff of {8 Å|cut}.

To cope with long-range interactions, the Particle Mesh Ewald method was used; the SHAKE algorithm was applied to bonds involving hydrogen atoms.

<Section|Minimization>

At the beginning, {17,500|maxcyc} steps of steepest descent and conjugate gradient minimization were performed.

<for each|cycles of minimization print>, the value of {2500|maxcyc} step is set.

Steps positional harmonic restraints with a force constant of [<for each|cycles of minimization> print {25 kcal mol-1 Å-2|restraint\_wt},] were applied to the {MANUAL\_INPUT|(solute atoms) restrainmask}.

<Section|Thermalization>

Thereafter, {50 (ps)|simulation time} of {NVT|MD} simulations were conducted.

The system is then heated up to {100 K|temp0}.

The previous step is followed by {300|simulation time}simulations 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 {MANUAL\_INPUT|(solute atoms) restrainmask}.

As the final step in thermalization, {300 (ps)|simulation time} {NVT|MD} simulations were performed.

During this process, the restraint forces of {MANUAL\_INPUT|(solute atoms) restrainmask} were gradually reduced to {0 kcal mol-1 Å-2|restraint\_wt} within the first {100 (ps)|simulation time}.

<Section|Production>

Afterward, {5|overall repetitions} independent production {NVT|MD} simulations were performed.

For each production run, simulations of {2 ns|simulation time} were performed.