Membrane simulation: yes/no

Cycles of minimization: XX

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

The variants were protonated with {PROPKA| protonation method} according to pH {7.4| pH}, neutralized by adding counterions, [<if|membrane simulation|e|true> embedded in a membrane consisting of {POPC | Lipid type}] and solvated in a [<if|membrane simulation|e|true> {rectangular|box type} <ELIF|membrane simulation|e|false> {octahedral|box type}] of {TIP3P| water type} water with a minimal shell of {12 Å|shell radius} around the solute].

All atom {molecular dynamics (MD)|simulation} simulations were performed using the {AMBER14|suite} and the [<if|water type|e|TIP3P> {ff14SB|force field} <ELIF|water type|e| OPC> {ff19SB|force field}] force field [<if|membrane simulation|e|true>, in combination with the {LIPID14|lipid force field}] force field.

[<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 runs, the <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 minimizazion were performed. During [<for each|cycles of minimization print> {2500|maxcyc},] 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} [value nstlim\* value dt/1000] from {12,500|nstlim} and {4 fs|dt} of [<if|ntp|e|0> {NVT|MD} with {0|ntp} <elif|ntp|gt|0> {NPT|MD}] were conducted to heat up the system to {100 K|temp0}.

The previous step is followed by {300|simulation time} [value nstlim\* value dt/1000] ps from {75,000|nstlim} and {4 fs| dt} 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} [value nstlim\* value dt/1000] from {75,000|nstlim} and {4 fs|dt} of [<if|ntp|e|0> {NVT|MD} with {0|ntp} <elif|ntp|gt|0> {NPT|MD}] simulations were performed while gradually reducing the restraint forces of {MANUAL\_INPUT|(solute atoms) restrainmask} to {0 kcal mol-1 Å-2|restraint\_wt} within the first {100 (ps)|simulation time} [value nstlim\* value dt/1000] from {25,000|nstlim} and {4 fs|dt} of this step.

<Section|Production>

Afterward, {5|overall repetitions} independent production runs of [<if|ntp|e|0> {NVT|MD} with {0|ntp} <elif|ntp|gt|0> {NPT|MD}] simulations with {2|simulation time} [value nstlim\* value dt/1.000.000] ns from {500,000|nstlim} and {4 fs|dt} length each were performed.