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.

---

*Proposed reformulation:*

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

<if|membrane simulation|e|true>, the variants (or criterions?) are embedded in a membrane consisting of {POPC|Lipid type} and solvated in a {rectangular|box type}. <ELIF|membrane simulation|e|false>, the variants (or criterions?) are solvated in an {octahedral|box type}.

The variants (or criterions?) uses {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 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.

---

*Proposed reformulation:*

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

<if|water type|e|TIP3P>, {ff14SB|force field} was used, otherwise <ELIF|water type|e|OPC>, {ff19SB|force field} was used.

<if|membrane simulation|e|true>, the structure in combination with the {LIPID14|lipid force field} was prepared.

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[<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.

---

*Proposed reformulation:*

During the {thermalization|period}, <if|membrane simulation|e|true> 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}, <if|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. 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}.

---

*Proposed reformulation:*

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} [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}.

---

*Proposed reformulation:*

Thereafter, {50 (ps)|simulation time} were conducted, this number is derived from the formula [value nstlim\* value dt/1000] with parameters {12,500|nstlim} and {4 fs|dt}. There are certain conditions:

<if|ntp|e|0>, then {NVT|MD} with {0|ntp} was performed, or else <elif|ntp|gt|0>, then {NPT|MD} was performed.

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

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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}.

---

*Proposed reformulation:*

The previous step is followed by {300|simulation time}, calculated by [value nstlim\* value dt/1000] ps using the parameters of {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}.

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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.

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*Proposed reformulation:*

As the final step in thermalization, {300 (ps)|simulation time} is performed and this duration is calculated by [value nstlim\* value dt/1000] using the parameters of {75,000|nstlim} and {4 fs|dt}.

<if|ntp|e|0>, then {NVT|MD} with {0|ntp} is performed. <elif|ntp|gt|0>, {NPT|MD} simulation is performed.

The MD simulation is 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}, which is calculated using the formula [value nstlim\* value dt/1000] with parameters of {25,000|nstlim} and {4 fs|dt} for this step.

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<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.

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*Proposed reformulation*

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

<if|ntp|e|0>, then {NVT|MD} simulation with {0|ntp} was performed. Otherwise, <elif|ntp|gt|0>, then {NPT|MD} was performed.

For the production, simulations with {2 ns|simulation time} was performed which was derived from the formula of [value nstlim\* value dt/1.000.000] with parameters of {500,000|nstlim} and {4 fs|dt} length.