## Molecular dynamics simulations of tauro-CDC/GluN2DLBD and tauro-CDC/GluN3BLBD complexes

### <Section|General MD Environment>

1. Perform all {molecular dynamics (MD)|simulation} using the mixed single {precision/fixed precision GPU (CUDA) PMEMD|software}[[1]](#footnote-1) in the {Amber14|suite} of molecular simulation programs[[2]](#footnote-2).
2. Enable a time step of 4 fs, which can be represented using the parameter {0.004(ps)|dt} in PMEMD.
3. Use the Langevin thermostat[[3]](#footnote-3), which can be configured using ntt flag parameter {3|ntt}, with a collision frequency of 0.01 ps−1 {0.01(ps)|gamma\_ln} and a target temperature of *T* = 300 K {300|temp0} for temperature control.
4. constrained covalent bonds involving hydrogen atoms using the SHAKE algorithm[[4]](#footnote-4) {2| ntc}.
5. Estimated long-range electrostatic interactions using the Particle Mesh Ewald method[[5]](#footnote-5), and use a cutoff of {8 (Å)|cut} for short-range electrostatics and van der Waals forces.
6. Perform all the following steps {10|overall repetitions} for each of the {2|complexes quantity} specifically {tauro-CDC/GluN2DLBD, CDC/GluN3BLBD |complex name} so as to obtain 10 independent simulations per complex.

### <Section|Minimization>

1. Restrain the coordinates of all {MANUAL\_INPUT|(solute molecules) restraintmask} by a harmonic potential with a force constant of  {2.0 (kcal mol−1 Å−2)|restraint\_wt}.
2. Carry out {2000|(steps) ncyc} of steepest descent minimization, configured in PMEMD using {1|ntmin} flag.
3. Perform 3000 steps of conjugate gradient minimization, which has already been configured via the ntmin flag above. The total cycle is then {5000|maxcyc} resulted from accumulation of steepest descent minimization and conjugate gradient minimization.
4. Repeat the previous procedure with another set of restraints with the restraints switched from the solute to the solvent molecules, using {MANUAL\_INPUT|(solvent molecules) restraint} by a harmonic potential with a force constant of  {2.0 (kcal mol−1 Å−2)|restraint\_wt}.
5. Carry out {2,000|(steps) ncyc} of steepest descent minimization, configured in PMEMD using {1|ntmin} flag.
6. Perform 3,000 steps of conjugate gradient minimization, which has already been configured via the ntmin flag above. The total cycle is then {5000|maxcyc} resulted from accumulation of steepest descent minimization and conjugate gradient minimization.
7. Remove the restraints.
8. Perform {3,000|(steps)ncyc} steps of steepest descent minimization, configured in PMEMD using {1|ntmin} flag.
9. Perform 7,000 steps of conjugate gradient minimization, which has also been configured via the ntmin flag above. The total cycle is then {10000|maxcyc}.

### <Section|Equilibration>

1. Perform 20 ps of NVT-MD {0|ntp}. That is, using {5000|nstlim} when the time step (dt) is set to 5 fs. (the solute was restrained with a force constant of  {2.0 (kcal mol−1 Å−2)|restraint\_wt}) while heating the system from {0(K)|tempi} to {300(K)|temp0}.
2. Perform additional 5 ps using {1250|nstlim} of NVT-MD {0|ntp} at {300(K)|temp0} which also have the same {300(K)|tempi} value.
3. Achieve the density adaptation by 75 ps using {18750|nstlim} of NPT-MD {3|ntp} (solute restrained, force constant  {2.0 (kcal mol−1 Å−2)|restraint\_wt}).
4. Perform an additional 1.7 ns using {425000|nstlim} of restrained NPT-MD {3|ntp} before switching to the NVT ensemble {0|ntp}.
5. Perform 3.2 ns using {800000|nstlim} of restrained MD prior to the start of the production phase, with harmonic restraints (force constant:  {2.0 (kcal mol−1 Å−2)|restraint\_wt}) applied to only those Cα atoms that are closest to the center of mass of a secondary structure element (α-helix or β-sheet).

### <Section|Production>

1. Perform The subsequent production phase with the length of 500 ns using {800000|nstlim} of NVT-MD {0|ntp} (restraints as in the final NVT step of the equilibration phase. Normally, during the production run we do not use restraints.), resulting in an aggregate simulation time of {5 μs|simulation time per complex} or {10 μs|simulation time in total}.
2. Save coordinates for analysis and post-processing every 20 ps using {5000|ntwx}.

### <Section|Post Processing >

1. Perform post-processing and analysis of the MD trajectories was in CPPTRAJ[[6]](#footnote-6) as implemented in AmberTools15.
2. Inspect trajectories were visually with VMD[[7]](#footnote-7).

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