Protein Model with Polarizability and Transferability (*proMPT*)
Gromacs Tutorial for Trp-cage in 25 wt% [Choline][Cl]

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* For the original paper, please see: Pei-Yin Lee, Onkar Singh, Harry Bermudez, and Silvina Matysiak. *Manuscript in preparation. 2023.*
* Python3 and Gromacs 2019.4 are used.

[Create topology for Trp-cage]
Use "*create_indent.py*" to generate a CG conformation as an extended strand for Trp-cage. File needed to provide: "*seq.txt*". A file named "*protein.gro*" will be generated and this is our CG protein topology for Trp-cage.

[Create force field parameter files]
1. File "*fixedff_tc_Cl0.8_cho1.3_noTQ0CP.itp*" is the force field file that is derived from *ProMPT*. Additional parametrization were done to adjust the non-bonded interaction parameters related to [Choline][Cl].
2. Use "*genitp_md.py*" to generate the itp file for Trp-cage. Files needed to provide: "*seq.txt*" and "*1l2y.pdb*" (PDB file for Trp-cage). A file named "*output.itp*" will be generated as the itp file for Trp-cage. Here we need to manually modify the table number for the backbone dihedral potential according to the desired secondary structure. In the [dihedral] section, the second to last value is the table number for the backbone dihedral. 1 is for alpha helix, 2 for 3-10 helix, 4 for beta-sheet, and 6 for double-well (same preference for alpha helix and beta sheet). Currently only alpha helix, 3-10 helix, and beta sheet automation based on the pdb file is implemented. We recommend to check the [dihedral] section before running simulations to make sure the assigned secondary structure is desired. Here the force constant used for the dihedral backbone potential is 5.
3. "*martini_v2.0_ions.itp*" and "*water.em.itp*"/"*water.md.itp*" are from MARTINI for [Cl] anion and water, respectively.
4. File "choline_PNa.itp" is the force field for [Choline].

[Construct the simulation system for Trp-cage in 25 wt% [Choline][Cl]]
1. Create a protein in a box where the protein is at the center and the cubic box length is 1 nm larger than both sides of protein:
gmx_mpi editconf -f protein.gro -o box1.gro -c -d 1.0 -bt cubic
2. Add [Choline] cations:
gmx_mpi insert-molecules -f box1.gro -ci choline.gro -o box2.gro -nmol 252
3. Solvate water:
gmx_mpi solvate -cp box2.gro -cs water_001.npt.gro -o box3.gro -p newprotein.top -maxsol 1653

Here the "*water_001.npt.gro*" is taken from MARTINI. Extra water molecules are added to be replaced by [Cl] anions later.

4. Add [Cl] ions:

gmx_mpi grompp -f ions.mdp -c box3.gro -p newprotein.top -o ions.tpr

gmx_mpi genion -s ions.tpr -o ready.gro -p newprotein.top -nname CL -nn 253 (select group PW to be replaced)

Note that the number of cations, anions, and water depend on the size of the box. Just make sure that the concentration of [Choline][Cl] is 25 wt%. In addition, the water molecules used here is coarse-grained, so there is a 1 to 4 mapping to the atomistic water molecules. When calculating the needed CG water molecules, this should be taken into consideration.

[Energy minimization]

1. Generate tpr file for energy minimization: gmx_mpi grompp -f em.mdp -c ready.gro -p newprotein.top -o em.tpr

2. Run simulation:

gmx_mpi mdrun -s em.tpr -c em.gro -tableb ./table_a/* ./table_d* -v

Here the angular potential and the dihedral potential files need to be provided

[NPT equilibration]

1. First need to change "*water.em.itp*" to "*water.md.itp*" in "*newprotein.top*".
2. Generate tpr file for NPT equilibration: gmx_mpi grompp -f npt_posres_befion.mdp -p newprotein.top -c em.gro -o npt.tpr -maxwarn 1 -r em.gro -r em.gro.

3. Run simulation:

mpirun gmx_mpi mdrun -s npt.tpr -cpi state.cpt -tableb . /table_a/table_a*.xvg ./table_d*.xvg -deffnm npt_eq

[MD production run]

1. An NVT ensemble is used here, but an NPT ensemble can also be used. The simulation temperature is set at 350K, but it does not correspond to the real world 350K.
2. Generate tpr file for MD production run: gmx_mpi grompp -f md.mdp -p newprotein.top -c npteq.gro -o md.tpr
3. Run simulation: mpirun gmx_mpi mdrun -s md.tpr -cpi npt_eq.cpt -tableb ./table_a/table_a*.xvg ./table_d*.xvg -deffnm md