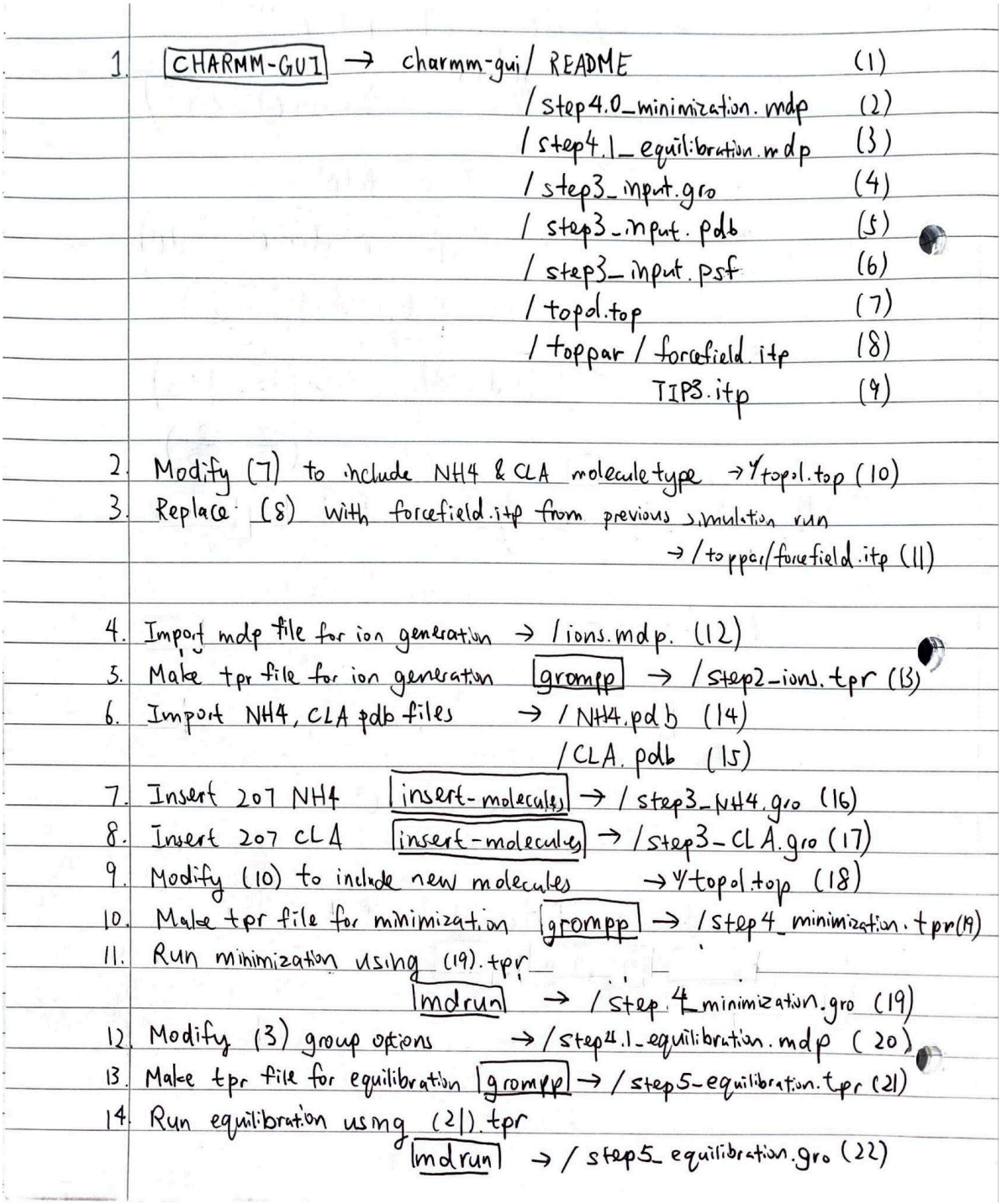
Creating NH4Cl and NaCl solution boxes

Part 1. NH4Cl

0. Overview



1. Use CHARMM-GUI solution builder to create a water box

Generated GROMACS input → ~/charmm-gui

Water Box Only System

Select Water Box Type: Rectangular

X: 70 Å Y: 70 Å Z: 70 Å

Include Ions

(NH4Cl cannot be added directly from 'Include Ions')

System Size:

Box Type	Rectangle		
Crystal Type	CUBIC		
System Size	A	70	Dimension along the A (X) axis
	B	70	Dimension along the B (Y) axis
	C	70	Dimension along the C (Z) axis
Crystal Angle	Alpha	90.0	Angle between the axis B and C
	Beta	90.0	Angle between the axis A and C
	Gamma	90.0	Angle between the axis A and B

Periodic Boundary Condition Options:

Generate grid information for PME FFT automatically

Explicit grid information for PME FFT

X Y Z

Force Field Options:

CHARMM36m

WYF parameter for cation-pi interactions

Hydrogen mass repartitioning

Y. Gao, J. Lee, I.P.S. Smith, H. Lee, S. Kim, Y. Qi, J.B. Klauda, G. Widmalm, S. Khalid, and W. Im *Journal of Chemical Information and Modeling* 2021 61 (2), 831-839. DOI.

Multi-site Ca^{2+}

Currently only available for CHARMM, NAMD, GROMACS, and OpenMM Zhang, A., Yu, H., Liu, C. *et al.* The Ca^{2+} permeation mechanism of the ryanodine receptor revealed by a multi-site ion model. *Nat. Commun.* 11, 922 (2020). DOI.

Input Generation Options:

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- NAMD
- GROMACS
- AMBER
- OpenMM
- CHARMM/OpenMM
- GENESIS
- Desmond
- LAMMPS
- Tinker

Equilibration Input Generation Options:

NVT Ensemble

Dynamics Input Generation Options:

NPT Ensemble

NVT Ensemble

Temperature: 303.15 K

2. Modify the topology file ~/charmm-gui/topol.top to include NH4 and CLA [moleculetype] {topol.top}

```
;;
;; Generated by CHARMM-GUI FF-Converter
;;
;; Correspondance:
;; jul316@lehigh.edu or wonpil@lehigh.edu
;;
;; The main GROMACS topology file
;;

; Include forcefield parameters
#include "toppar/forcefield.itp"

[ moleculetype ]
; name  nrexcl
NH4          2

[ atoms ]
; nr       type      resnr    residu   atom     cgnr      charge  mass
     1       HGP2      1      NH4     HZ1      1     0.330000     1.0080   ; qtot  0.330
     2      NG3P3      1      NH4      NZ      2    -0.320000    14.0070   ; qtot  0.010
     3       HGP2      1      NH4     HZ2      3     0.330000     1.0080   ; qtot  0.340
     4       HGP2      1      NH4     HZ3      4     0.330000     1.0080   ; qtot  0.670
     5       HGP2      1      NH4     HZ4      5     0.330000     1.0080   ; qtot  1.000

[ bonds ]
; ai        aj          funct     b0          Kb
    2     1     1
    2     3     1
    2     4     1
    2     5     1

[ angles ]
; ai        aj          ak          funct     th0         cth         S0          Kub
    1     2     3     5
    1     2     4     5
    1     2     5     5
    3     2     4     5
    3     2     5     5
    4     2     5     5

[ moleculetype ]
; name  nrexcl
CLA          1

[ atoms ]
; nr       type      resnr    residu   atom     cgnr      charge  mass
     1        CLA    230      CLA     CLA      1    -1.000000    35.4500   ; qtot -1.000

#include "toppar/TIP3.itp"

[ system ]
; Name
Title

[ molecules ]
; Compound      #mols
TIP3            10850
```

3. Replace the original forcefield.itp file with forcefield.itp file from previous membrane-protein-NH4Cl run, to add new atomtypes

{original}

```
[ atomtypes ]
; name  at.num  mass    charge  ptype    sigma    epsilon ;          sigma_14          epsilon_14
     HT     1     1.0080     0.417     A    4.00013524445e-02    1.924640e-01
     OT     8    15.9994    -0.834     A    3.15057422683e-01    6.363864e-01
```

{modified}

```
[ atomtypes ]
    CLA    17    35.4500    -1.000     A    4.04468018036e-01    6.276000e-01
   HGP2     1     1.0080     0.330     A    4.00013524445e-02    1.924640e-01
  NG3P3     7    14.0070    -0.320     A    3.29632525712e-01    8.368000e-01
```

4. Copy mdp file from the tutorial for ions generation to ~/ions.mdp

{ion.mdp}

```
; ions.mdp - used as input into grompp to generate ions.tpr
; Parameters describing what to do, when to stop and what to save
integrator  = steep         ; Algorithm (steep = steepest descent minimization)
emtol       = 1000.0        ; Stop minimization when the maximum force < 1000.0 kJ/mol/nm
emstep      = 0.01          ; Minimization step size
nsteps      = 50000         ; Maximum number of (minimization) steps to perform

; Parameters describing how to find the neighbors of each atom and how to calculate the
interactions
nstlist         = 1         ; Frequency to update the neighbor list and long range forces
cutoff-scheme   = Verlet    ; Buffered neighbor searching
ns_type         = grid      ; Method to determine neighbor list (simple, grid)
coulombtype     = cutoff    ; Treatment of long range electrostatic interactions
rcoulomb        = 1.0       ; Short-range electrostatic cut-off
rvdw            = 1.0       ; Short-range Van der Waals cut-off
pbc             = xyz       ; Periodic Boundary Conditions in all 3 dimensions
```

5. Make tpr file for ion generation

```
!gmx grompp -f ions.mdp -c charmm-gui/step3_input.gro -p
charmm-gui/topol.top -o step2_ions.tpr
```

6. Copy NH4+ and CLA- pdb files from library to ~/NH4.pdb and ~/CLA.pdb

{NH4.pdb}

```
ATOM      1  HZ1 NH4 N   1      -0.571   0.792   0.358  1.00  0.00      NH4
ATOM      2  NZ  NH4 N   1      -0.000  -0.000  -0.000  1.00  0.00      NH4
ATOM      3  HZ2 NH4 N   1       0.993   0.135   0.279  1.00  0.00      NH4
ATOM      4  HZ3 NH4 N   1      -0.355  -0.892   0.401  1.00  0.00      NH4
ATOM      5  HZ4 NH4 N   1      -0.067  -0.035  -1.037  1.00  0.00      NH4
TER       6      NH4     1
```

{CLA.pdb}

```
ATOM      1  CLA CLA C   1       0.000   0.000   0.000  1.00  0.00      CLA
TER       2      CLA     1
```

7. Insert 1M of NH4+ to ~/step3_NH4.gro (207 inserted)

Polyatomic ions cannot be inserted by gmx genion

31 inserted for 0.15M NH4+

```
!gmx insert-molecules -f step2_ions.tpr -ci NH4.pdb -conc 1 -o
step3_NH4.gro -try 30
```

8. Insert 1M of CLA- to ~/step3_NH4Cl.gro (207 inserted)

31 inserted for 0.15M CLA-

```
!gmx insert-molecules -f step3_NH4.gro -ci CLA.pdb -conc 1 -o
step3_NH4Cl.gro -try 30
```

9. Modify the topology file ~/topol.top to account for new ions

```
[ molecules ]
; Compound      #mols
TIP3          10850
NH4           207
CLA           207
```

10. Make tpr file for minimisation

```
!gmx grompp -f charmm-gui/step4.0_minimization.mdp -c
step3_NH4Cl.gro -p charmm-gui/topol.top -o step4_minimization.tpr
-maxwarn 1
```

{charmm-gui/step4.0_minimization.mdp}

```
define                  = -DPOSRES -DPOSRES_FC_BB=400.0 -DPOSRES_FC_SC=40.0
integrator              = steep
emtol                   = 1000.0
nsteps                  = 5000
nstlist                 = 10
cutoff-scheme           = Verlet
rlist                   = 1.2
vdwtype                 = Cut-off
vdw-modifier            = Force-switch
rvdw_switch             = 1.0
rvdw                    = 1.2
coulombtype             = PME
rcoulomb                = 1.2
;
constraints             = h-bonds
constraint_algorithm    = LINCS
```

11. Run minimisation

```
!gmx mdrun -v -deffnm step4_minimization -s step4_minimization.tpr
```

12. Modify equilibration mdp group options to account for NH4 CLA molecule groups {charmm-gui/step4.1_equilibration}

```
define                  = -DPOSRES -DPOSRES_FC_BB=400.0 -DPOSRES_FC_SC=40.0
integrator              = md
dt                      = 0.001
nsteps                  = 125000
nstxout-compressed      = 5000
nstxout                 = 0
nstvout                 = 0
nstfout                 = 0
nstcalcenergy           = 100
nstenergy               = 1000
nstlog                  = 1000
;
cutoff-scheme           = Verlet
nstlist                 = 20
rlist                   = 1.2
vdwtype                 = Cut-off
vdw-modifier            = Force-switch
rvdw_switch             = 1.0
rvdw                    = 1.2
coulombtype             = PME
rcoulomb                = 1.2
;
tcoupl                  = v-rescale
tc_grps                 = NH4 CLA TIP3
tau_t                   = 1.0 1.0 1.0
ref_t                   = 303.15 303.15 303.15
;
constraints             = h-bonds
constraint_algorithm    = LINCS
;
nstcomm                 = 100
comm_mode               = linear
comm_grps               = TIP3 NH4 CLA
;
gen-vel                 = yes
gen-temp                = 303.15
gen-seed                = -1
```

13. Make tpr file for equilibration

```
!gmx grompp -f charmm-gui/step4.1_equilibration.mdp -o
step5_equilibration.tpr -c step4_minimization.gro -r step3_NH4Cl.gro
-p charmm-gui/topol.top -maxwarn 1
```

14. Run equilibration

```
!gmx mdrun -v -deffnm step5_equilibration -s step5_equilibration.tpr
```

Part 2. NaCl

1. Generate box using CHARMM-GUI

Water Box Only System

Select Water Box Type: Rectangular

X: 70 Å Y: 70 Å Z: 70 Å

Include Ions

Ion Concentration: 1 M NaCl

Ion Placing Method: Distance

Periodic Boundary Condition Options:

Generate grid information for PME FFT automatically

Explicit grid information for PME FFT

X Y Z

Force Field Options:

CHARMM36m

WYF parameter for cation-pi interactions

Hydrogen mass repartitioning

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NVT Ensemble

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NPT Ensemble

NVT Ensemble

Temperature: 303.15 K