**Non\_bonded prodrug**

**1. define the bonded parameters of PEG-MA-CIT or PEG-MA-ORN**

**Model**

*/opt/jchem/bin/****msketch*** *PEG-MA-CIT.cdx → PEG-MA-CIT.mol2*

***chimera*** *PEG-MA-CIT.mol2 → add charge* ***(DO NOT add H)***

***using bio2byte.be website*** *to creat itp-file and gro-file, top-file*

*(user)*

*For* ***PEG-MA-CIT, net charge is 0;***

***For PEG-MA-ORN, net charge is +1;***

b. Setup

*# Place the solute in a simulation box*

*gmx editconf -f PEGMACIT\_GMX.gro -bt cubic -d 1.4 -o PEGMACIT\_inbox.gro*

*# Prepare minimization in vacuum*

*gmx grompp -f em\_vacuum.mdp -c PEGMACIT\_inbox.gro -p topol.top -o em\_vacuum.tpr*

*# Make minimization in vacuum*

*gmx mdrun -s em\_vacuum.tpr -deffnm em\_vacuum -v*

*# Fill the box with water*

*gmx insert-molecules -f em\_vacuum.gro -ci spc216.gro -o PEGMACIT\_inslv.gro -nmol 5000*

gmx grompp -f ions.mdp -c *PEGMACIT\_inslv.gro* -p topol.top -o ions.tpr

# Add ions into the box

*gmx genion -s ions.tpr -p topol.top -neutral -conc 0.15 -pname NA -nname CL -o PEGMACIT\_WI.gro*

*# Prepare the minimization in the solvent*

*gmx grompp -f em\_solvent.mdp -c PEGMACIT\_WI.gro -p topol.top -o em\_solvent.tpr*

*# Make minimzation in the solvent*

*gmx mdrun -s em\_solvent.tpr -deffnm em\_solvent -v*

c. Equil

*# Prepare Berendsen equilibration*

*gmx grompp -f berendsen.mdp -c em\_solvent.gro -p topol.top -o berendsen.tpr*

*# Make Berendsen equilibration* ***(1 ns)***

*gmx mdrun -s berendsen.tpr -deffnm berendsen -v*

*# Prepare Nose-Hoover equilibration* ***(1 ns)***

*gmx grompp -f nosehoover.mdp -c berendsen.gro -p topol.top -o nosehoover.tpr*

*# Make Nose-Hoover equilibration*

*gmx mdrun -s nosehoover.tpr -deffnm nosehoover -v*

d. Prod **(byrd04: 1 days)**

*# Prepare production run* ***(200 ns)***

*gmx grompp -f md\_prod.mdp -c nosehoover.gro -p topol.top -o md\_prod.tpr*

*# Make production run*

*gmx mdrun -s md\_prod.tpr -deffnm md\_prod -v*

e. Alys

1. Choose an atom-to-bead mapping, create mapping.ndx file from em\_vaccum.gro

2. Put the PEG-MA-CIT into the center of box and generated .gro and .xtc files

**#### ./extr.sh ###**

gmx convert-tpr -s md\_prod.tpr -o md\_solute.tpr

*gmx trjconv -f nosehoover.gro -s md\_prod.tpr -o md\_solute.gro -center -pbc cluster*

*gmx trjconv -f md\_prod.xtc -s md\_prod.tpr -o md\_solute.xtc -center -pbc cluster*

3. Coarse-grain target atomistic data.

Create a 1kda\_mapped.itp file with a directive for "[bonds]" containing (multiple) pairs of CG beads, "[angles]" containing triples and "[dihedrals]" quartets.

**### ./mapped.sh ###**

*seq 0 8 | gmx traj -f md\_solute.gro -s md\_solute.tpr -oxt md\_mapped.gro -n mapping.ndx -com -ng 9*

*seq 0 8 | gmx traj -f md\_solute.xtc -s md\_solute.tpr -oxt md\_mapped.xtc -n mapping.ndx -com -ng 9*

*gmx grompp -f PEGMACIT\_minim.mdp -c md\_mapped.gro -p PEGMACIT\_mapped.top -o PEGMACIT**\_mapped.tpr*

1. **Analysis bonds and angles distributions**

creat bonds.ndx and angles.ndx

### ./bondanalysis.sh ###

### ./angleanalysis.sh ###

rm -rf ANGLEDISTRIBUTIONS

mkdir ANGLEDISTRIBUTIONS

NANGLES=6

IANGLE=0

while [ $IANGLE -lt $NANGLES ]

do

echo $IANGLE | gmx angle -f md\_mapped.xtc -n angles.ndx -type angle -ov ANGLEDISTRIBUTIONS/angle\_$IANGLE.xvg

gmx analyze -f ANGLEDISTRIBUTIONS/angle\_$IANGLE.xvg -dist ANGLEDISTRIBUTIONS/distr\_$IANGLE.xvg -bw 1.0

rm -rf \#\*

let IANGLE=$IANGLE+1

done

exit

**CG model**

cp ../Alys/md\_mapped.gro

### ./mono.sh ###

*#!/bin/bash*

*cat md\_mapped.gro water.gro > cg\_PEGMACIT.gro*

**10. Create CG simulation**

Extract one frame from your mapped trajectory and solvate it in the same solvent as the atomistic simulation. **Create a top file** (include the general martini itp files and the newly created molecule itp and add the right number of molecules (solute and solvent))

**We can use the itp file of all-atom simulation in octanol**

**### ./cgmd.sh ###**

### minimization ###

*gmx grompp -f minim.mdp -c cg\_TEMPocta.gro -p cg\_TEMPocta.top -o minim.tpr*

*gmx mdrun -v -deffnm minim*

(if the atom is not cooperated, run the commands below)

*gmx grompp -f minim.mdp -c cg\_ptxocta.gro -p cg\_ptxocta.top -o minim.tpr -maxwarn 10)*

### relax the trajectory in the same solvent ###

*gmx grompp -f relax.mdp -c minim.gro -p cg\_TEMPocta.top -o relax.tpr*

*gmx mdrun -v -deffnm relax*

### run MD simulation ###

*gmx grompp -f cg\_md.mdp -c relax.gro -p cg\_TEMPocta.top -o cg\_md.tpr*

*gmx mdrun -v -deffnm cg\_md*

**11. Calculate the average and standard deviation for all the distributions**

### ./bondanalysis.sh ###

**1. creat the itp-file of 114 PEG\_OH**

a. generate 114 PEO itp-file from PEO martini.itp

polyply gen\_itp -f PEO.martini.3b.itp -seq PEO:114 -o PEO\_114.itp -name PEO

b. creat linker between ~~(~~CH2-O-CH2~~)-~~ and ~~(~~CH2-O-CH2~~)-~~ in OH\_link.ff

[ link ]

[ molmeta ]

by\_atom\_id true

[ bonds ]

114 115 1 0.28000 7000

[ angles ]

113 114 115 2 140 25

c. creat linker between ~~(~~CH2-O-CH2~~)-~~ and OH end in OH\_end.itp

[ moleculetype ]

OHend 1

[ atoms ]

1 TP1 1 OHend OH 1 0.0000 36

d. Combine all the parts of PEG (114 PEO, linker, OH end)

polyply gen\_itp -f PEO\_114.itp OH\_end.itp OH\_link.ff -seq PEO:1 OHend:1 -o PEG\_114\_OH.itp -name PEGOH

**2. creat the itp-file of PEG modified Orn/Cit copolymers in random order**

a. creat itp-file of PEG-amino acid linker in MA.itp and MA-ORN.ff or methylamine\_CIT.ff

#MET.itp

[ moleculetype ]

MA 1

[ atoms ]

1 TN0d 1 MA MA 1 0.0000 ; 36.0

#MA-ORN.ff

[ link ]

[ molmeta ]

by\_atom\_id true

[ bonds ]

1 233 1 0.25000 7000 {"group": "link"} ;MA-ORN

233 234 1 0.28000 7000 {"group": "link"} ;MA-PEO

[ angles ]

235 234 233 2 123.0 50.0 {"group": "link"} ; EO-EO-MA

234 233 1 2 132.0 50.0 {"group": "link"} ; EO-MA-BB

233 1 2 2 100.0 50.0 {"group": "link"} ; MA-BB-SC1

233 1 3 2 160.0 15.0 {"group": "link"} ; MA-BB-BB

#MA-CIT.ff

[ link ]

[ molmeta ]

by\_atom\_id true

[ bonds ]

1 233 1 0.25000 7000 {"group": "link"} ; MA-CIT

233 234 1 0.28000 7000 {"group": "link"} ; MA-PEO

[ angles ]

235 234 233 2 123.0 50.0 {"group": "link"} ;PEO-PEO-MA

234 233 1 2 132.0 50.0 {"group": "link"} ; PEG-MA-BB

233 1 2 2 107.0 50.0 {"group": "link"} ; MA-BB-SC1

233 1 4 2 128.0 20.0 {"group": "link"} ; MA-BB-BB

b. combine all the itp-files of PEG\_OH, MET, Orn/Cit in script\_all.py

polyply gen\_itp -f PolyOC\_seq\_ratio\_90\_\*.itp MA.itp PEG\_114\_OH.itp MA\_CIT.ff -seq PolyOC\_seq\_ratio\_90\_\*:1 MA:1 PEG\_114\_OH:1 -o PEG114\_PolyOC\_seq\_ratio\_90\_\*.itp -name PEG\_Crn\_Cit

**Build new system:**

24 chains PEG\_Orn/Cit copolymer in random order

**150 mM NaCl**

**2.5 mM PEG\_Orn/Cit**

**Box size: 25 25 25 nm^3**

**1. #Creat simulation box with box size as 28.1 nm in script\_all.py**

polyply gen\_coords -p system.top -o PEG\_Orn\_Cit.gro -name PEG\_Orn\_Cit -box 25.2 25.2 25.2 -r PEG\_Orn\_Cit.gro

**2. Minimization of new PEG\_Orn/Cit copolymers** **in PEG\_Orn\_Cit\_min.sh**

#energy minimized amino acid backbone and PEG

gmx grompp -f minimization-vac.mdp -c PEG\_Orn\_Cit.gro -p system.top -o minim\_vac.tpr -r PEG\_Orn\_Cit.gro

gmx mdrun -v -deffnm minim\_vac

#add water solvent int othe box

gmx solvate -cs water.gro -cp minim\_vac.gro -o PEG\_Orn\_Cit\_W.gro -radius 0.21 2>&1

**3. Add IONs in to the box** **in #PEG\_Orn\_Cit\_ION.sh**

#add 150 mM NaCl into the box

gmx grompp -f minimization.mdp -p system.top -c PEG\_Orn\_Cit\_W.gro -o PEG\_Orn\_Cit\_W.tpr -maxwarn 1

echo WN | gmx genion -s PEG\_Orn\_Cit\_W.tpr -neutral -conc 0.15 *-pname TNA -nname TCL* -p system.top -o start.gro

edit start.gro, let it as below

eg. 20931TCL CL29087 …..

**4. Do Minimization of the new system**

# Minim.sh

gmx grompp -f minimization.mdp -p system.top -c start.gro -o minimization.tpr

gmx mdrun -v -deffnm minimization

**5. Do equimibration for the new system (NVT equilibration) 10 ns**

#Equilibration1 allows the water to solvate the copolymer and amino acid

gmx grompp -f equilibration.mdp -p system.top -c minimization.gro -o equilibration.tpr -n system.ndx

gmx mdrun -v -deffnm equilibration

check the temperature distribution of system:

gmx energy -f eq1.edr -o temp.xvg (around 280 K)

**~~5. Do equimibration2 for the new system (~~****~~NPT equilibration) 10 ns~~**

~~#equilibration2 with positional restraints released~~

~~gmx grompp -f eq2.mdp -p system.top -c eq1.gro -o eq2.tpr -n system.ndx~~

~~gmx mdrun -v -deffnm eq2~~

check the temperature and pressure distributions of system:

gmx energy -f eq1.edr -o temp.xvg (around 280 K)

gmx energy -f eq1.edr -o pressure.xvg (around 1 bar)

**6. start production of system**

gmx grompp -f dynamic.mdp -p system.top -c equilibration.gro -o dynamic.tpr -maxwarn 1

gmx mdrun -v -deffnm dynamic

Analysis

*gmx clustsize -f dynamic.xtc -s dynamic.tpr -mc maxclust.xvg -ac avcluste.xvg -nc nclust.xvg -cut 0.6 -n traj1.ndx -pbc*

