Double drug loaded system

1. Define the bonded parameters of **TEMPO\_digly**

a. Model

*/opt/jchem/bin/****msketch*** *TEMPO-digly.cdx → TEMPOdigly.mol2*

***chimera*** *gemdigly1k.mol2 → add H and charge*

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

b. Setup

*# Place the solute in a simulation box*

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

*# Prepare minimization in vacuum*

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

*# Make minimization in vacuum*

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

*NB: error report, Atom type ho not found*

*Solution: copy the oh and ho parameters from octanol\_GEM.itp to TEMPOdigly\_GMX.itp*

*# Fill the box with octanol*

*gmx insert-molecules -f em\_vacuum.gro -ci octanol\_GMX.gro -o TEMPOdigly\_inslv.gro -nmol 5000*

*# Prepare the minimization in the solvent*

*gmx grompp -f em\_solvent.mdp -c TEMPOdigly\_inslv.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: 2 days)**

*# Prepare production run* ***(500 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*

d. Alys

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

2. Put the TEMPO\_digly 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 TEMPOdigly\_minim.mdp -c md\_mapped.gro -p TEMPOdigly\_mapped.top -o* *TEMPOdigly\_mapped.tpr*

1. **Analysis bonds and angles distributions**

creat bonds.ndx and angles.ndx

### ./bondanalysis.sh ###

### ./angleanalysis.sh ###

rm -rf ANGLEDISTRIBUTIONS

mkdir ANGLEDISTRIBUTIONS

NANGLES=31

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 simulation perpétration in octanol

1. Create the CG itp file

We can copy the 1kda\_mapped.itp file of CG simulation in water

1. Mapping Octanol beads and GemPi1kda beads

For each octanol, one octanol mapped into 2 beads

**### indexer.py ###**

#!/usr/bin/env python

nmols = 1138 # Number of molecules

atspermol = 9 # AA atoms per molecule

beadspermol = 2 # CG beads per molecule

firstbead = 4 # AA atoms in the first bead

atsperbead = 4 # AA atoms per CG bead

lastbead = 5 # AA atoms in the last bead

outname = "mapping.ndx" # Output filename

with open(outname,'w') as NDX:

for mol in range(nmols):

for bead in range(beadspermol):

# The number of atoms (nats) might depend on whether it's a first,

# last, or middle bead. NDXs are 1-based

if bead==0:

nats = firstbead

startat = 1

elif bead==beadspermol-1:

nats = lastbead

startat = atspermol-lastbead+1

else:

nats = atsperbead

startat = (bead-1)\*atsperbead + firstbead + 1

startat += mol\*atspermol

ats = range(startat, startat + nats)

ats = " ".join(map(str, ats))

NDX.write("[ mol%dbead%d ]\n%s\n" % (mol+1,bead+1, ats))

**8. Creat octanol box**

**### ./mapp.sh ###**

*seq 0 2275 | gmx traj -f octanolnoh.gro -s octanolnoh.gro -oxt cg\_octanol.gro -n mapping.ndx -com -ng 2276*

where 2275 is the number of CG beads in your molecule and the 'seq 0 2274' avoids having to type all index groups. Inspect the file mapping.ndx to see how the atoms of toluene are grouped into the CG beads

**9. Combine the trajectories of GemPi1kda and octanol**

cp ../Alys/md\_mapped.gro

mv md\_mapped.gro cg\_TEMPOdigly.gro

put cg\_TEMPOdigly.gro and cg\_octanol.gro into the same file cg\_TEMPocta.gro, then edit cg\_gemocta.gro, detect the three lines

### ./mono.sh ###

*#!/bin/bash*

*cat cg\_TEMPOdigly.gro cg\_octanol.gro > cg\_TEMPocta.gro*

*edit cg\_TEMPocta.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\_PEGMACIT.gro -p PEGMACIT\_mapped.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 PEGMACIT\_mapped.top -o relax.tpr*

*gmx mdrun -v -deffnm relax*

### run MD simulation ###

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

*gmx mdrun -v -deffnm cg\_md*

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

### ./bondanalysis.sh ###

### ./angleanalysis.sh ###

***11. Analyze the bond distribution and angle distribution, change the value of itp files, repeat step 9 until get a good result.***

***Biphase\_TEMPO(water to octanol)***

***copy water\_newbox.gro, TEMPOdigly\_mapped.itp, cg\_md.gro***

**# put TEMPO in the center of box**

**gmx make\_ndx -f cg\_md.gro -o TEMPO.ndx**

***gmx trjconv -f cg\_md\_all.xtc -s cg\_md.tpr -o TEMPO.gro -center -pbc mol -n TEMPO.ndx***

*Select group for centering: 4 ;(TEMPO)*

*Select group for output: 4; (TEMPO)*

gmx editconf -f TEMPO.gro -o TEMPO\_new.gro -box 11.84 11.84 23.68 -center 5.92 5.92 5.92

*gmx solvate -cp TEMPO\_new.gro -cs water\_newbox.gro -o TEMPO\_water.gro*

*Note: cope* ***octanol\_new.gro*** *from*

~/ProDrug/Ptx\_digly\_PI\_SG1/PtxPi2kda/Solution/Biphase\_Ptx2kda\_W\_OCO/octanol\_new.gro

***#assemble two box***

gmx solvate -cp TEMPO\_water.gro -cs octanol\_new.gro -o TEMPO\_Solv.gro

*>* ***gmx make\_ndx -f TEMPO\_Solv.gro -o system.ndx***

*name 2(2kda) ProDrug*

***3(W) | 4(WF) | 5(ION) | 6 (OCO) ------- Solvent***

***3(W) | 4(WF) | 5(ION) ------------ WATER***

***# Biphase minimization***

*gmx grompp -f minimization.mdp -c TEMPO\_Solv.gro -p system.top -o minimization.tpr -n system.ndx -maxwarn 1*

*gmx mdrun -deffnm minimization -v*

***# position restraint***

*gmx genrestr -f minimization.gro -n system.ndx -o TEMPO\_res.itp*

***edit system.top***

*; Ligand position restraints*

*#ifdef POSRES\_TEMPO*

*#include "TEMPO****\_res****.itp"*

*#endif*

***edit equilibration.mdp***

*define = -DSTRONG\_POSRES*

***# Biphase equilibration***

*gmx grompp -f* ***equilibration****.mdp -c minimization.gro -p system.top -o* ***equilibration****.tpr -n system.ndx -r minimization.gro -maxwarn 1*

*gmx mdrun -deffnm* ***equilibration*** *-v*

***Umbrella sampling***

***#define the em.mdp, em2.mdp, eq.mdp file.***

*Add restraint conformation and pull code*

***; Bond parameters***

*continuation = no ; continuing from NPT*

*constraints =* ***none***

*constraint-algorithm = lincs*

***; pressure***

*Pcoupl = parrinello-rahman*

*Pcoupltype =* ***semiisotropic*** *; semiisotropic*

*tau-p = 12.0*

*compressibility =* ***4e-5 4e-5*** *;3e-4*

*ref-p =* ***1.0 1.0*** *;1.0*

***; Pull code***

*pull = yes*

*pull\_ncoords = 1 ; only one reaction coordinate*

*pull\_ngroups = 2 ; two groups defining one reaction coordinate*

*pull\_group1\_name = WATER*

*pull\_group2\_name = ProDrug*

*pull\_coord1\_type = umbrella ; harmonic potential*

*pull\_coord1\_geometry = distance ; simple distance increase*

*pull\_coord1\_dim = N N Y*

*pull\_coord1\_groups = 1 2*

***pull\_coord1\_start = no ; define initial COM distance > 0***

*pull-coord1-init = 0.0*

***pull\_coord1\_rate = 0,0 ; 0.01 nm per ps = 10 nm per ns***

*pull\_coord1\_k = 1000 ; kJ mol^-1 nm^-2*

***pull-pbc-ref-prev-step-com = yes***

***pull-group1-pbcatom = 6495***

***#pull.mdp#***

*> gmx grompp -f pull.mdp -c ../equilibration.gro -p ../../Parameters/system.top -o pull.tpr -n ../system.ndx*

*> gmx mdrun -nt 1 -deffnm pull -v &> pullrun.log*

**#em.mdp#**

*> gmx grompp -f em.mdp -c pull.gro -p ../../Parameters/system.top -o em.tpr -n ../system.ndx*

*> gmx mdrun -nt 1 -deffnm em -v &> emrun.log*

**#em2.mdp#**

*> gmx grompp -f em2.mdp -c em.gro -p ../../Parameters/system.top -o em2.tpr -n ../system.ndx*

*> gmx mdrun -nt 1 -deffnm em2 -v &> em2run.log*

***#eq.mdp#***

*> gmx grompp -f eq.mdp -c em2.gro -p ../../Parameters/system.top -o eq.tpr -n ../system.ndx*

*> gmx mdrun -nt 1 -deffnm eq -v -px eq\_x -pf eq\_f &> eqrun.log*

*gmx grompp -f pull.mdp -c ../equilibration.gro -p ../../Parameters/system.top -o pull.tpr -n ../system.ndx*

*gmx mdrun -nt 1 -deffnm pull -v &> pullrun.log*

*gmx grompp -f em.mdp -c pull.gro -p ../../Parameters/system.top -o em.tpr -n ../system.ndx*

*gmx mdrun -nt 1 -deffnm em -v &> emrun.log*

*gmx grompp -f em2.mdp -c em.gro -p ../../Parameters/system.top -o em2.tpr -n ../system.ndx*

*gmx mdrun -nt 1 -deffnm em2 -v &> em2run.log*

*gmx grompp -f eq.mdp -c em2.gro -p ../../Parameters/system.top -o eq.tpr -n ../system.ndx*

*gmx mdrun -nt 1 -deffnm eq -v -px eq\_x -pf eq\_f &> eqrun.log*

***Production***

*gmx grompp -f md.mdp -c ../../Initial/$window/eq.gro -p ../../Parameters/system.top -o md.tpr -n ../../Initial/system.ndx*

*gmx mdrun -nt 2 -deffnm md -v -px md\_x -pf md\_f -cpi md.cpt &> mdrun.log*

***Analysis***

***ls -d ../Production/\*/md.tpr > tpr-files.dat***

***ls -d ../Production/\*/md\_x.xvg > pullx-files.dat***

***gmx wham -ix pullx-files.dat -it tpr-files.dat -bsres -bins 200 -temp 300 -unit kJ -b 100 -nBootstrap 100 -zprof0 0.0 -min 0 -max 12***

***Biphase\_TEMPO(octanol to water)***

***# Biphase equilibration***

*gmx grompp -f* ***equilibration****.mdp -c eq.gro -p system.top -o* ***equilibration****.tpr -n system.ndx -r eq.gro -maxwarn 1*

*gmx mdrun -deffnm* ***equilibration*** *-v*

***Gem\_propa\_PI\_TEMPO\_digly\_Ptx***

1. Model

*/opt/jchem/bin/****msketch*** *GemPI-TEMPO-Ptx.cdxml → Gem\_propa\_PI\_TEMPO\_digly\_Ptx.mol2*

***chimera*** *Gem\_propa\_PI\_TEMPO\_digly\_Ptx.mol2 → add H and charge*

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

***user/charge 0***

2. Setup

*# Place the solute in a simulation box*

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

*# Prepare minimization in vacuum*

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

*# Make minimization in vacuum*

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

**result : em\_vacuum.gro**

3.Coarse-grain target atomistic data: Coarse\_mapping preparation

copy the bonded parameters from 1kda\_mapped.itp

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

About mapping.ndx file, see the schemetic represention ( printed picture) for numbering details.

**Here the mapp.sh script was edited as follow:**

**### ./mapp.sh ###**

*seq 0 54 | gmx traj -f em\_vacuum.gro -s em\_vacuum.tpr -oxt em\_mapped.gro -n mapping.ndx -com -ng 55*

**3.  *GemPI2kda\_TEMPO\_digly\_Ptx*  CG MD**

*A) Minimization the new* ***GemPI2kda\_TEMPO\_digly\_Ptx*** *coarse-grained model*

#####cgmd.sh######

*gmx grompp -f 2kda\_minim.mdp -c em\_mapped.gro -p 2kda\_mapped.top -o 2kda\_mapped.tpr*

*gmx mdrun -s 2kda\_mapped.tpr -deffnm 2kda\_mapped -v*

***4. Preparation of*** ***GemPI2kda\_TEMPO\_digly\_Ptx with 72 polymers***

*Conditions:*

*150 mM NaCl + water (10% WF)*

*5 mM ProDrug (72 chains of* ***GemPI2kda\_TEMPO\_digly\_Ptx****)*

*15 microsecond expected*

Here, we are going to replicate the polymer chain until 72 polymers into the box. The box size has been fix at ~ 28.8 nm for x,y,z dimensions for a concentration of [ProDrug] at 5 mM.

# Place the solute in a simulation box

*gmx editconf -f 2kda\_mapped.gro -bt cubic -d 1 -o prodrug\_inbox.gro*

# put additional 23 prodrug in the box

*gmx insert-molecules -f prodrug\_inbox.gro -ci 2kda\_mapped.gro -o system0.gro -nmol 71 -box 28 28 28*

# define the size of box

*gmx editconf -f system0.gro -o system\_temp.gro -d 1.0 -bt cubic -box 28.8 28.8 28.8*

1. **Minimization steps**

# Minimization in vaccum following by minimization in solution

*gmx grompp -f minimization-vac.mdp -c system\_temp.gro -p system.top -o minimization-vac.tpr*

*gmx mdrun -deffnm minimization-vac -v*

# Solvatation

*gmx solvate -cp minimization-vac.gro -cs water.gro -radius 0.21 -o system\_W.gro*

gmx grompp -f ions.mdp -c system\_W.gro -p system.top -o ions.tpr

# Add ions into the box

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

Then select the water group, here it’s the #3.

# Adding anti-freeze water:

To take account of anti-freeze water into the simulation, it is necessary to edit the topology file.

Ex: if the system contained 100000 water molecules, the final system\_WI.gro should contain 10% of anti-freeze water molecules (WF) and 90% of martini water.

1. # modofy of topology file:

2kda\_mapped.top

NB: the gro file atomname changes is not necessary if the use a index file.

2. modify the system\_WI.gro

But you can edit the name of water molecule W to WF using bash command as vi.

:286207,317919s/W/WF/g

# minimization

*gmx grompp -f minimization.mdp -c system\_WI.gro -p system.top -o minimization.tpr -maxwarn 1*

*gmx mdrun -deffnm minimization -v*

1. **Equilibration and production step**

Set 50000 step for the equilibration following by 1µs of production

# Equil

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

gmx mdrun -deffnm equilibration -v

# Prod

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

*gmx mdrun -deffnm dynamic -v*