***Biphase\_SG1(water to octanol)***

***~/ProDrug/Gem\_PI\_SG1/GemPi1kDa/Solution/Gem1/Biphase\_SG1***

***ln -s ../1chain\_water/dynamic.xtc***

***ln -s ../1chain\_water/dynamic.tpr***

***ln -s ../1chain\_water/dynamic.gro***

**# put SG1 in the center of box**

**gmx make\_ndx -f dynamic.gro -o SG1.ndx**

***gmx trjconv -f dynamic.xtc -s dynamic.tpr -o SG1.gro -center -pbc mol -n SG1.ndx***

*Select group for centering: 6 ;(SG1)*

*Select group for output: 7; (SG1\_W\_WF\_IONS)*

**#extend the box size (*11.84 11.84 23.68*)**

**and define the position of SG1 (5.92 5.92 5,92)**

*gmx editconf -f SG1.gro -o SG1\_newbox.gro -box 11.84 11.84 23.68 -center 5.92 5.92 5.92*

*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 SG1\_newbox.gro -cs octanol\_new.gro -o SG1\_Solv.gro

*>* ***gmx make\_ndx -f SG1\_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 SG1\_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 SG1\_res.itp*

***edit system.top***

*; Ligand position restraints*

*#ifdef POSRES\_SG1*

*#include "****SG1\_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*

*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\_digly\_glycol(water to octanol)***

***/home/ping/ProDrug/Gem\_PI\_SG1/GemPi1kDa/Solution/Gem1/Biphase\_Digly\_glycol***

***ln -s ~/ProDrug/Ptx\_digly\_PI\_SG1/PtxPi2kda\_new/Solution/dynamic.xtc***

***ln -s ~/ProDrug/Ptx\_digly\_PI\_SG1/PtxPi2kda\_new/Solution/dynamic.tpr***

***ln -s ~/ProDrug/Ptx\_digly\_PI\_SG1/PtxPi2kda\_new/Solution/dynamic.gro***

**# put Digly\_glycol in the center of box**

**gmx make\_ndx -f dynamic.gro -o Digly\_glycol.ndx**

***gmx trjconv -f dynamic.xtc -s dynamic.tpr -o Digly\_glycol.gro -center -pbc mol -n Digly\_glycol.ndx***

*Select group for centering: 6 ;(****Digly\_glycol****)*

*Select group for output: 7; (****Digly\_glycol****\_W\_WF\_IONS)*

**#extend the box size (*11.84 11.84 23.68*)**

**and define the position of SG1 (5.92 5.92 5,92)**

*gmx editconf -f* ***Digly\_glycol.gro*** *-o* ***Digly\_glycol****\_newbox.gro -box 11.84 11.84 23.68 -center 5.92 5.92 5.92*

*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 ***Digly\_glycol****\_newbox.gro* -cs octanol\_new.gro -o ***Digly\_glycol***\_Solv.gro

*>* ***gmx make\_ndx -f Digly\_glycol****\_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* ***Digly\_glycol****\_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* ***Diglyglycol****\_res.itp -fc 100000 100000 100000*

***edit system.top***

*; Ligand position restraints*

*#ifdef POSRES\_* ***Diglyglycol***

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

*#endif*

***edit equilibration.mdp***

*define = -DSTRONG\_POSRES*

***edit : 2kda\_mapped.itp***

[ position\_restraints ]

; i funct fcx fcy fcz

1 1 100000 100000 100000

2 1 100000 100000 100000

3 1 100000 100000 100000

***# 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***