#MD simulation and MM/PB(GB)SA binding free energy calculation
1. #Assuming AMBER ((https://ambermd.org/Installation.php)) has been properly installed.
2. cd your/work/directory

#Assign atomic charges to the ligand
3. antechamber -i ligand.mol2 -fi mol2 -o gaussian.mol2 -fo mol2 -nc 0 -c bcc -rn MOL
4. parmchk2 -i gaussian.mol2 -f mol2 -o frcmod

#Build the initial system
5. tleap -f tleap.in

#Run MM minimization (min1-min4) and MD simulation (md1-md4)
6. #The following calculations can be submitted through qsub, 'source run.qsub'
pmemd.cuda -O -i min1.in -p water.top -c water.crd -r min1.rst -o min1.out -ref water.crd
pmemd.cuda -O -i min2.in -p water.top -c min1.rst -r min2.rst -o min2.out -ref min1.rst
pmemd.cuda -O -i min3.in -p water.top -c min2.rst -r min3.rst -o min3.out -ref min2.rst
pmemd.cuda -O -i min4.in -p water.top -c min3.rst -r min4.rst -o min4.out
pmemd.cuda -O -i md1.in -p water.top -c min4.rst -o md1.out -x md1.trj -r md1.rst -ref min4.rst
pmemd.cuda -O -i md2.in -p water.top -c md1.rst -o md2.out -x md2.trj -r md2.rst -ref md1.rst
mpirun -np 14 pmemd.MPI -O -i md3.in -p water.top -c md2.rst -o md3.out -x md3.trj -r md3.rst
pmemd.cuda -O -i md4.in -p water.top -c md3.rst -o md4.out -x md4.trj -r md4.rst
(#md4.trj is the production MD trajectory for the further MM/PB(GB)SA binding free energy analysis)

7. MM/PB(GB)SA calculation
mpirun -np 10 MMPBSA.py.MPI -O -i gbsa.in -o gbsa.txt -sp water.top -cp complex.top -rp receptor.top -lp ligand.top -y md4.trj
mpirun -np 10 MMPBSA.py.MPI -O -i pbsa.in -o pbsa.txt -sp water.top -cp complex.top -rp receptor.top -lp ligand.top -y md4.trj
(#results of MM/GBSA and MM/PBSA (ε=1) can be found in gbsa.txt and pbsa.txt, respectively.)

8. entropy calculation
mpirun -np 10 MMPBSA.py.MPI -O -i nmode.in -o entropy.txt -sp water.top -cp complex.top -rp receptor.top -lp ligand.top -y md4.trj