

University of North Carolina at Chapel Hill

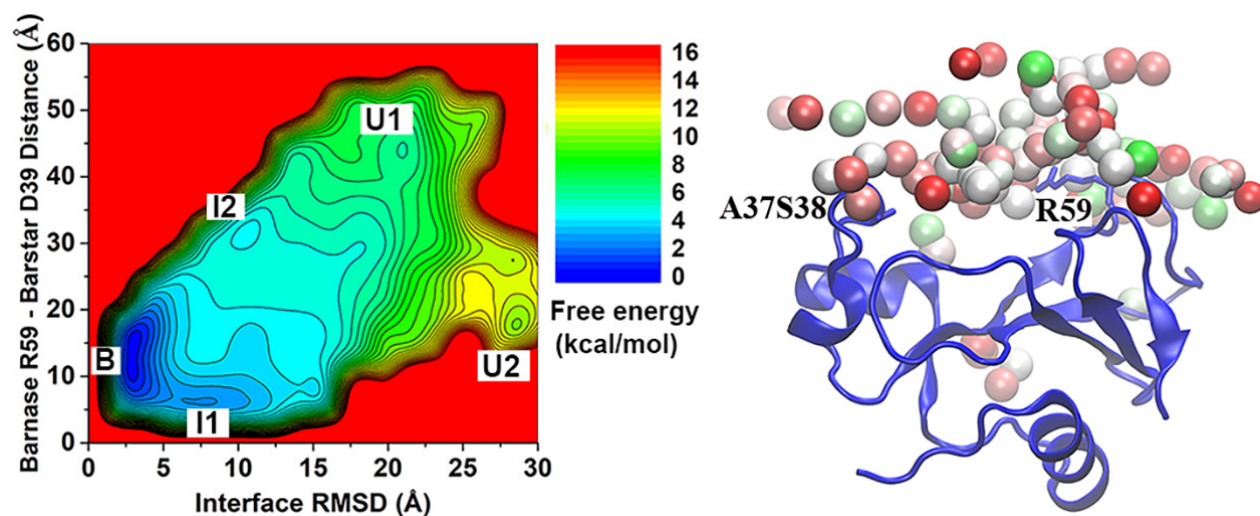
Department of Pharmacology and

Computational Medicine Program

GaMD Workshop 2024

Protein-Protein Interaction Gaussian accelerated Molecular Dynamics (PPI-GaMD) Tutorial

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Abstract

Protein-Protein Interaction Gaussian accelerated Molecular Dynamics (PPI-GaMD) is a biomolecular enhanced sampling method that works by selectively boosting the interaction potential energy between protein partners to facilitate their slow dissociation, while another boost potential is applied to the remaining potential energy of the entire system to facilitate protein rebinding. This tutorial explains how you can perform PPI-GaMD simulations using the Amber24 simulation package. Barnase-barstar is used as a model system for demonstration. You will learn how to run PPI-GaMD simulations to sample multiple conformational states of barnase-barstar in explicit solvent and reweight PPI-GaMD simulations to calculate the system free energy profiles. Knowledge about standard Molecular Dynamics (MD) simulations is required. Experiences with Amber and understanding of PPI-GaMD theory are preferred for learning the tutorial.

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1. Introduction

Gaussian accelerated Molecular Dynamics (GaMD)¹ is a biomolecular enhanced sampling method that works by adding a harmonic boost potential to smooth the system potential energy surface. GaMD greatly reduces energy barriers and accelerates biomolecular simulations by orders of magnitude. GaMD does not require predefined reaction coordinates or collective variables and is thus advantageous for unconstrained enhanced sampling of large biomolecular complexes. Since the GaMD boost potential exhibits a Gaussian distribution, biomolecular free energy profiles can be properly recovered through cumulant expansion to the second order (“Gaussian approximation”)². Please refer to Ref. 1 for details of the GaMD method.

Based on GaMD, a new Protein-Protein Interaction GaMD (PPI-GaMD) method has been developed for improved sampling simulations of PPIs³. PPI-GaMD works by selectively boosting the interaction potential energy between protein partners to facilitate their slow dissociation, while another boost potential is applied to the remaining potential energy of the entire system to facilitate protein rebinding. PPI-GaMD has been demonstrated on a model system of the ribonuclease barnase interactions with its inhibitor barstar (**Figure 1**).

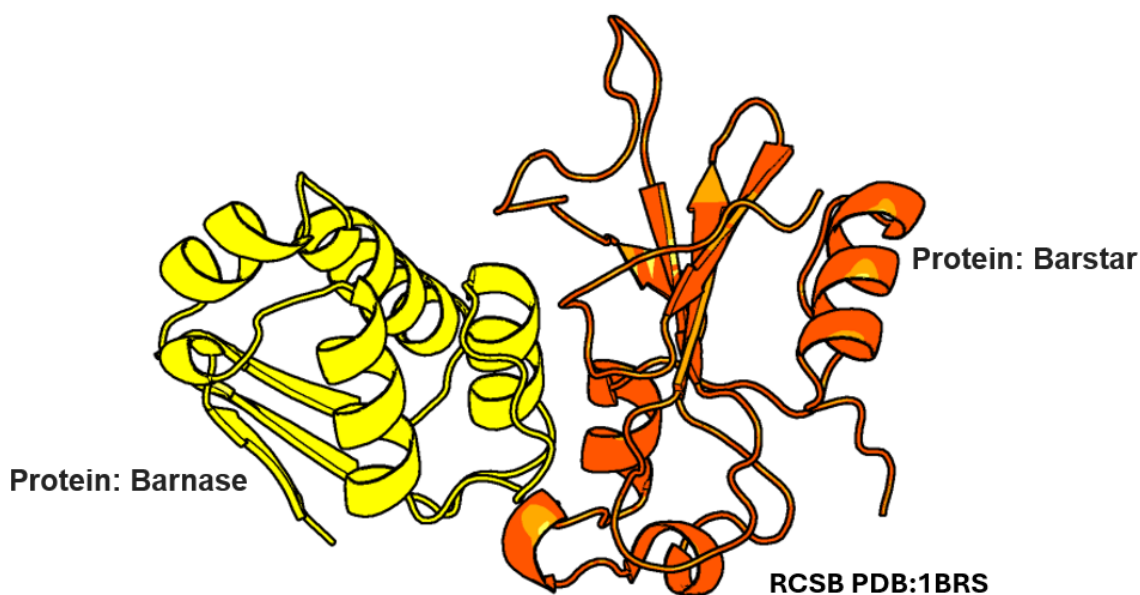


Figure 1: An X-ray structure of the barnase–barstar complex (RCSB PDB: 1BRS). The barnase and barstar are shown in yellow and orange cartoons, respectively.

In this tutorial, we will use barnase-barstar complex as a model system to learn the following-

- How to run PPI-GaMD simulations?
- How to analyze simulation trajectories?
- How to reweight PPI-GaMD simulations for Potential of Mean Force (PMF) free energy calculations?

Simulation length in this tutorial is intentionally reduced for demonstration; however, it is important to note that they are typically insufficient for practical applications. Additionally, sufficient equilibration of the system with cMD simulations before running PPI-GaMD simulations is strongly recommended.

Required files-

- Files required for the PPI-GaMD tutorial can be downloaded at ppigamd.tar.bz2 (14MB) or copied from the following folder on the UNC cloud server: `/shared/data/ppigamd`. Further instructions on how to obtain these files will be discussed in subsequent sections (**Figure 2**).
- AMBER24+ installed (<https://ambermd.org>)
- Python3 with NumPy and SciPy installed (<https://www.python.org/>)

As shown in **Figure. 2**, the `ppigamd` folder contains two sub-folders- simulation and analysis

- The **simulation** folder includes eight files: *complex.prmtop* and *cmd.rst*, representing the system's topology and coordinate files after 14ns cMD; *md.in* and *gamd-restart.in*, representing the input files for PPI-GaMD equilibration and production; *run-equil.pbs* and *run-prod.pbs*, the scripts used to submit jobs in the UNC cloud via SLURM; *run-equil.sh* and *run-prod.sh*, the bash scripts used to run PPI-GaMD equilibration and production simulations using your own workstations.
- The **analysis** folder includes 15 files: *native-amber.pdb* (initial PDB file for visualization), *analysis.in* (input file for *cpptraj*), *gamd-all.log* (PPI-GaMD log file), *K27-D39.dat* (reaction coordinate file calculated with *cpptraj*), *R59-D39.dat* (reaction coordinate file calculated with *cpptraj*), *run-[1-2]d-pmf.pbs* (SLURM submitting scripts for UNC cloud), *run-[1-2]d-pmf.sh* (bash scripts for 1-2D PMF calculations using your own workstations), *reweight-[1-2]d.sh* and *do-reweighted-[1-2]d.sh* (bash scripts for reweighting calculations), *PyReweighting-[1-2]D.py* (Python scripts for reweighting calculations).

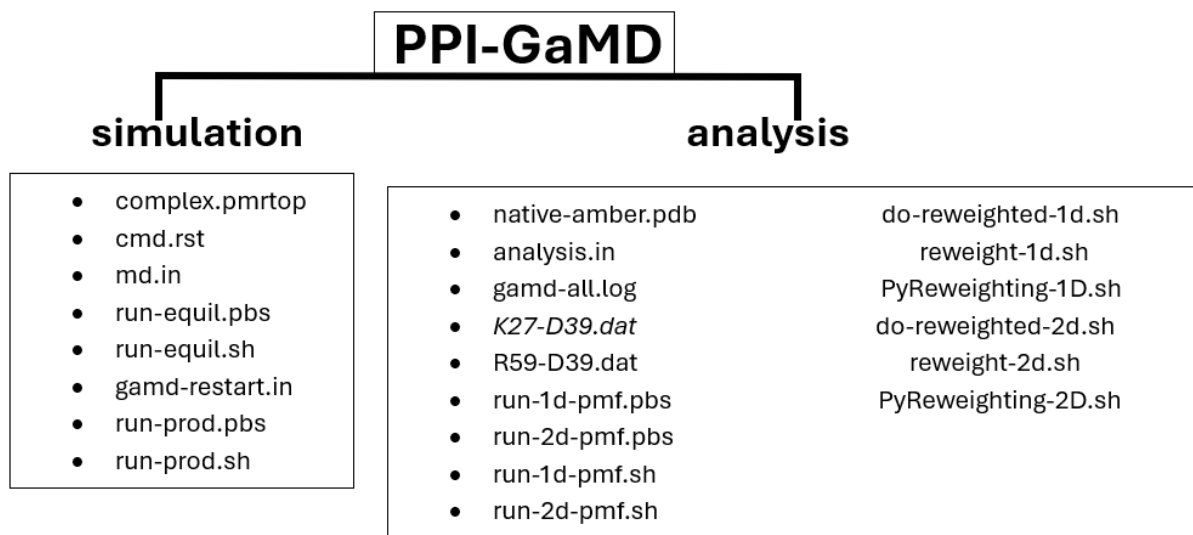


Figure 2. Directory structure of PPI-GaMD tutorial files.

- PyReweighting scripts can be downloaded at [PyReweighting24.tar.bz2](#) (12KB) or copied from the following folder on the UNC cloud server: /shared/data/PyReweighting24. As shown in (Figure 3), there are three folders: 1d-reweighted, 2d-reweighted, and 3d-reweighted, each corresponding to the calculation of 1D, and 2D. Each folder includes two bash scripts (*do-reweighted-[1-2]d.sh* and *reweight-[1-2]d.sh*), and a Python script (*PyReweighting-[1-2]D.py*) for performing the respective calculations.

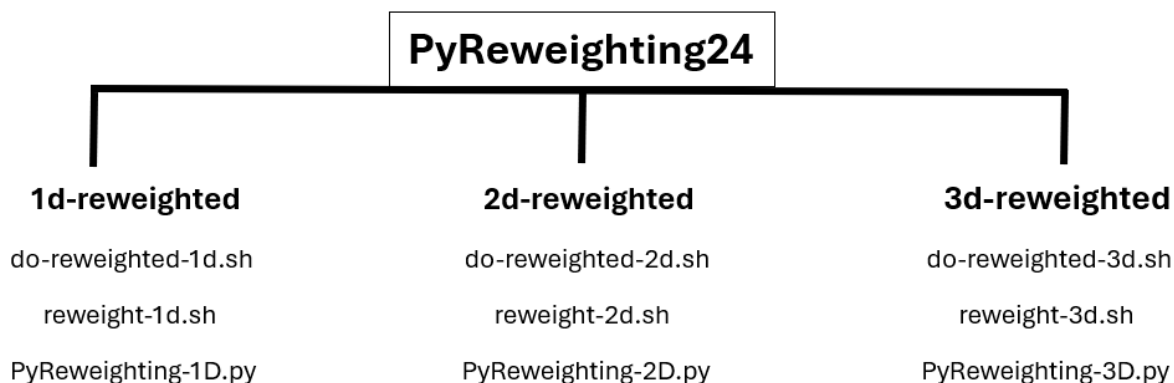


Figure 3. Directory structure of PyReweighting24 files.

2. Run PPI-GaMD Simulations on Barnase-Barstar

2.1 System Setup

Before running PPI-GaMD simulation, a short conventional MD (cMD) simulation is needed to obtain a well equilibrated structure. Two recommended approaches for setting up the system are through *tleap* in AMBER or the CHARMM-GUI website. The following tutorials are highly recommended-

- *tleap*: <https://ambermd.org/tutorials/pengfei/index.php>
- CHARMM-GUI: <https://ambermd.org/tutorials/CHARMM-GUI.php>

For simplicity, we have prepared the system files after energy minimization, heating, equilibration with restraints, and 14 ns cMD simulation. These files can be found in the “simulation” folder, including the system topology (*complex.prmtop*) and coordinates (*cmd.rst*). Since cMD is not the focus of this tutorial, we use these files directly.

The following are instructions for using the UNC cloud server-

Make sure that the AMBER environment is properly configured by running the appropriate *source* command. Copy the “ppigamd” folder to your home directory.

```
source /shared/apps/software/amber24/amber.sh
cd ~/
cp -r /shared/data/ppigamd .
cd ~/ppigamd/simulation
```

The following are instructions for users using your own workstation-

Make sure that you have downloaded **ppigamd.tar.bz2** to your home directory and extract the tutorial files.

```
source /shared/apps/software/amber24/amber.sh  ## Users must modify according to their local system
cd ~/
tar -xvjf ppigamd.tar.bz2
cd ~/ppigamd/simulation
```

In the simulation folder, you should see the following files- *cmd.rst*, *complex.prmtop*, *run-equil.pbs*, *run-equil.sh*, *run-prod.pbs*, *run-prod.sh*, *md.in*, and *gamd-restart.in*.

2.2. PPI-GaMD Equilibration

The *pmemd.cuda* program in AMBER 24+ will be used to perform PPI-GaMD equilibration. The users will require three files for running the simulation-

- *complex.prmtop* (System topology file)
- *cmd.rst* (Coordinate file, after 14 ns of cMD simulations)
- *md.in* (PPI-GaMD input file)

The following are instructions for using the UNC cloud server-

Users must be in the *~/ppigamd/simulation* directory and must have the required files- *run-equil.pbs* and *md.in*. Users can use the following script *run-equil.pbs* to run the job. Below is the command-

```
cd ~/ppigamd/simulation
sbatch run-equil.pbs
squeue
```

A copy of the *run-equil.pbs* script is shown below-

```
#!/bin/sh
#SBATCH --job-name=equilbration
#SBATCH --ntasks=1
#SBATCH --cpus-per-task=1
#SBATCH --time=00:20:00
#SBATCH --output=log.%x.%j
#SBATCH --gres=gpu:1
#SBATCH --qos=gpu_access
source /shared/apps/software/amber24/amber.sh
which pmemd.cuda
# gamd equilibration
pmemd.cuda -O -i md.in -p complex.prmtop -c cmd.rst -o md-1.out -x md-1.nc -r gamd-1.rst -gamd gamd-1.log
```


The following are instructions for users using your own workstation-

Users must be in the ~/ppigamd/simulation directory and must have the required files- *run-equil.sh* and *md.in*. Users can use the following script *run-equil.sh* to run the job. Below is the command-

```
cd ~/ppigamd/simulation
chmod +x run-equil.sh
nohup ./run-equil.sh &
```

A copy of the *run-equil.sh* script is shown below-

```
#!/bin/sh
#Make sure to modify the path of amber24 to the correct location
#source /shared/apps/software/amber24/amber.sh
which pmemd.cuda
# gamd equilibration
pmemd.cuda -O -i md.in -p complex.prmtop -c cmd.rst -o md-1.out -x md-1.nc -r gamd-1.rst -gamd gamd-1.log
```

You should obtain the following files: *md-1.out*, *gamd-1.rst*, *md-1.nc*, *gamd-1.log* and *gamd-restart.dat*.

- *md-1.out*: Simulation output file containing the system energies.
- *md-1.nc*: Trajectory file storing the MD simulation frames.
- *gamd-1.rst*: Restart file including both coordinate and velocity information.
- *gamd-1.log*: Logfile of the boost potential values used for reweighting.
- *gamd-restart.dat*: Stores the potential statistics used to apply boosts for the PPI-GaMD simulations.
- *mdinfo*: Updates the current state of the simulation, useful for monitoring progress and checking the simulation status.

The *gamd-restart.dat* file is generated in the working directory, which will be used for calculating the parameters to apply boost, *thus it is very important*.

Here is an example for *md.in* input file-

.....
GaMD equilibration simulation

&cntrl

```
imin=0,      ! No minimization
irest=0,     ! This IS a new MD simulation
ntx=1,       ! read coordinates only
```

```

! Temperature control
ntt=3,      ! Langevin dynamics
gamma_ln=1.0, ! Friction coefficient (ps^-1)
tempi=310.0, ! Initial temperature
temp0=310.0, ! Target temperature
ig=-1,      ! random seed

! Potential energy control
cut=9.0,    ! nonbonded cutoff, in Angstroms

! MD settings
nstlim=24800000, ! simulation length
dt=0.002,      ! time step (ps)

! SHAKE
ntc=2,      ! Constrain bonds containing hydrogen
ntf=1,      ! Do not calculate forces of bonds containing hydrogen

! Control how often information is printed
ntr=100,    ! Print energies every 1000 steps
ntwx=100,   ! Print coordinates every 1000 steps to the trajectory
ntwr=5000,  ! Print a restart file every 10K steps (can be less frequent)
! ntvr=-1,   ! Uncomment to also print velocities to trajectory
! ntfr=-1,   ! Uncomment to also print forces to trajectory
ntxo=2,     ! Write NetCDF format
ioutfm=1,   ! Write NetCDF format (always do this!)

! Wrap coordinates when printing them to the same unit cell
iwrap=1,
ntwprt = 3161,
! Constant pressure control. Note that ntp=3 requires barostat=1
barostat=1, ! Berendsen... change to 2 for MC barostat
ntp=1,      ! 1=isotropic, 2=anisotropic, 3=semi-isotropic w/ surften
pres0=1.0,  ! Target external pressure, in bar
taup=0.5,   ! Berendsen coupling constant (ps)

! Constant surface tension (needed for semi-isotropic scaling). Uncomment
! for this feature. csurften must be nonzero if ntp=3 above
! csurften=3, ! Interfaces in 1=yz plane, 2=xz plane, 3=xy plane
! gamma_ten=0.0, ! Surface tension (dyne/cm). 0 gives pure semi-iso scaling
! ninterface=2, ! Number of interfaces (2 for bilayer)

! Set water atom/residue names for SETTLE recognition
!watnam='TIP3', ! Water residues are named TIP3 !owtnm='OH2', ! Water oxygens are named OH2

! GaMD parameters
icfe = 1, ifsc = 1,gti_cpu_output=0,gti_add_sc=1,
timask1 = ':111-199',scmask1 = ':111-199',
bgpro2atm=1,edpro2atm=1727,
timask2 = "",scmask2 = "",

```

**igamd = 17, iE = 1, irst_gamd = 0, iEP = 2, iED = 2,
ntcmd = 800000, nteb = 24000000, ntave = 160000,
ntcmdprep = 320000, ntebprep = 320000,
sigma0P = 2.7, sigma0D = 7.0,**

PPI-GaMD input parameters-

igamd	Flag to apply boost potential = 17 Dual boost on both the protein-protein interactions and the remaining potential energy of the entire system.
iEP and iED	Flag to set the threshold energy E = 1 (default) set threshold energy to lower bound $E=V_{\max}$ = 2 set threshold energy to upper bound $E=V_{\min}+(V_{\max}-V_{\min})/k_0$
irst_gamd	Flag to restart PPI-GaMD simulation = 0 (default) new simulation = 1 restart simulation
ntcmd	Number of cMD steps to calculate V_{\max} , V_{\min} , V_{avg} , σV
nteb	Number of biasing equilibration steps
sigma0P	Upper limit of the standard deviation of the first potential boost (Default- 6.0 kcal/mol).
sigma0D	Upper limit of the standard deviation of the second potential boost (Default- 6.0 kcal/mol).
timask1	Specifies protein 1 residues. Default is an empty string.
scmask1	Specifies protein 1 residues using soft core in ambmask format. Default is an empty string.
bgpro2atm	Starting atomic number for protein 2.
edpro2atm	End atomic number for protein 2.
icfe	Basic flag for free energy calculations.
ifsc	Flag for soft core potentials = 0 SC potentials is not used (default) = 1 SC potentials is used.
gti_cpu_output	Softcore λ -derivative terms will be combined and output the same result for each TI region (to match the CPU-version output) = 1 (default) = 0 (the softcore λ -derivative terms will be output for each TI region)

Guidelines for setting the input parameters for the md.in input file-

- ***ntave***: 4 x atom
- ***ntcmd***: 5 x ntave
- ***ntcmdprep***: 2 x ntave
- ***ntebprep***: 2 x ntave
- ***nteb***: 150 x ntave
- ***nstlim***: ntcmd + nteb

NB: For PPI-GaMD simulations, user should set ***ntf***= 1 and ***barostat***= 1 to control pressure, as these settings have been widely tested.

NB: The gamd-restart.dat should contain the boost potential statistics like this-

-187.33074947353452	-308.41071121115237	-254.45473150954271	6.6378783467078906
-103001.87040691008	-105006.80930861726	-103948.88830739332	79.383568681198128

2.3 PPI-GaMD Production Simulations

For running PPI-GaMD production simulations, users will need two different input files (*gamd-restart.in* and *gamd-1.rst*)-

- complex.prmtop (System topology file)
- gamd-1.rst (Coordinate file, after ~1 ns of PPI-GaMD equilibration simulation)
- gamd-restart.in (PPI-GaMD input file)

The following are instructions for using the UNC cloud server-

Users must be in the ~/ppigamd/simulation directory and must have the necessary files- *run-prod.pbs* and *gamd-restart.in*. Users on UNC cloud will use *run-prod.pbs* script to submit the production job through SLURM. Below is the command to submit the job-

```
cd ~/ppigamd/simulation
sbatch run-prod.pbs
squeue
```

A copy of the *run-prod.pbs* script is shown below-

```
#!/bin/sh
#SBATCH --job-name=prod
#SBATCH --ntasks=1
#SBATCH --cpus-per-task=1
#SBATCH --time=00:20:00
#SBATCH --output=log.%x.%j
#SBATCH --gres=gpu:1
#SBATCH --qos=gpu_access
source /shared/apps/software/amber24/amber.sh
which pmemd.cuda
#gamd production
pmemd.cuda -O -i gamd-restart.in -p complex.prmtop -c gamd-1.rst -o md-2.out -x md-2.nc -r gamd-2.rst -
gamd gamd-2.log
```

The following are instructions for users using your own workstation-

Users must be in the *~/ppigamd/simulation* directory and must have the required files- *run-prod.sh* and *gamd-restart.in*. Users can use the following script *run-prod.sh* to submit the job. Below is the command used-

```
cd ~/ppigamd/simulation
chmod +x run-prod.sh
nohup ./run-prod.sh &
```

A copy of the *run-prod.sh* script is shown below-

```
#!/bin/sh
#source /shared/apps/software/amber24/amber.sh
which pmemd.cuda
#gamd production
pmemd.cuda -O -i gamd-restart.in -p complex.prmtop -c gamd-1.rst -o md-2.out -x md-2.nc -r gamd-2.rst -
gamd gamd-2.log
```

Here is an example for gamd-restart.in input file-

.....
GaMD production simulation

&cntrl

```
imin=0,      ! No minimization
irest=0,     ! This IS a new MD simulation
ntx=1,       ! read coordinates only
```

```
! Temperature control
ntt=3,       ! Langevin dynamics
gamma_ln=1.0, ! Friction coefficient (ps^-1)
tempi=310.0, ! Initial temperature
temp0=310.0, ! Target temperature
```

```

ig=-1,      ! random seed

! Potential energy control
cut=9.0,    ! nonbonded cutoff, in Angstroms

! MD settings
nstlim=50000000, ! simulation length
dt=0.002,    ! time step (ps)

! SHAKE
ntc=2,      ! Constrain bonds containing hydrogen
ntf=1,      ! Do not calculate forces of bonds containing hydrogen

! Control how often information is printed
ntr=100,    ! Print energies every 1000 steps
ntwx=100,   ! Print coordinates every 1000 steps to the trajectory
ntwr=5000,  ! Print a restart file every 10K steps (can be less frequent)
! ntwv=-1,   ! Uncomment to also print velocities to trajectory
! ntwf=-1,   ! Uncomment to also print forces to trajectory
ntxo=2,     ! Write NetCDF format
ioutfm=1,   ! Write NetCDF format (always do this!)

! Wrap coordinates when printing them to the same unit cell
iwrap=1,
ntwpri = 3161,

! Constant pressure control. Note that ntp=3 requires barostat=1
barostat=1, ! Berendsen... change to 2 for MC barostat
ntp=1,      ! 1=isotropic, 2=anisotropic, 3=semi-isotropic w/ surften
pres0=1.0,  ! Target external pressure, in bar
taup=0.5,   ! Berendsen coupling constant (ps)

! Constant surface tension (needed for semi-isotropic scaling). Uncomment
! for this feature. csurften must be nonzero if ntp=3 above
! csurften=3, ! Interfaces in 1=yz plane, 2=xz plane, 3=xy plane
! gamma_ten=0.0, ! Surface tension (dyne/cm). 0 gives pure semi-iso scaling
! ninterface=2, ! Number of interfaces (2 for bilayer)

! Set water atom/residue names for SETTLE recognition
!watnam='TIP3', ! Water residues are named TIP3
!owtnm='OH2', ! Water oxygens are named OH2

! GaMD parameters
icfe = 1, ifsc = 1, gti_cpu_output = 0, gti_add_sc = 1,
timask1 = ':111-199', scmask1 = ':111-199',
bgpro2atm = 1, edpro2atm = 1727,
timask2 = "", scmask2 = "",

igamd = 17, iE = 1, irest_gamd = 1, iEP = 2, iED = 2,
ntcmd = 0, nteb = 0, ntave = 160000,
ntcmdprep = 0, ntebprep = 0,

```

sigma0P = 2.7, sigma0D = 7.0,

3. Simulation Analysis

We have already used *cpptraj* to calculate the reaction coordinate files and you should see those files named ***R59-D39.dat*** and ***K27-D39.dat***. Additionally, we have the combined GaMD log file named ***gamd-all.log***. Double check the analysis folder to ensure that you have those files.

Here, we provide a sample *cpptraj* script to calculate the reaction coordinate files, but you don't need to run this script as we have generated the relevant results for you in the *~/ppigamd/analysis* folder.

```
parm /shared/data/ppigamd/analysis/native-amber.pdb
reference /shared/data/ppigamd/analysis/native-amber.pdb
trajin /shared/data/trajectory/ppigamd.nc
rms      reference      out      rmsd-I-rmsd-CA.dat      ':27-28,35-40,51,56-62,82-85,101-105,137-157,179,183,190&@CA'
rms      reference      out      rmsd-I-rmsd-backbone.dat  ':27-28,35-40,51,56-62,82-85,101-105,137-157,179,183,190&@CA,N,O'
distance R59-D39 :59@CZ :149@CG out R59-D39.dat
distance K27-D39 :27@NZ :149@CG out K27-D39.dat
```

3.1 Reweight PPI-GaMD Simulations to Calculate 1D Free Energy Profile

To calculate 1D PMF free energy profile, the following files are needed-

- *R39-D39.dat*
- *gamd-all.log*
- *Pyreweighting-1D.py*
- *do-reweighted-1d.sh*
- *reweight-1d.sh*

The following are instructions for using the UNC cloud server-

Users must be in the *~/ppigamd/analysis* directory and must have the following files- *run-1d-pmf.pbs*, *do-reweighted-1d.sh*, *PyRweighting-1D.py*, *R59-D39.dat* and *gamd-all.log*. Users will use SLURM to submit the 1D reweighting job on UNC cloud by using the following script- *run-1d-pmf.pbs*-

```
cd ~/ppigamd/analysis
sbatch run-1d-pmf.pbs
```

A copy of the *run-1d-pmf.pbs* script is shown below-

```
#!/bin/sh
#SBATCH --job-name=pmf-1d
#SBATCH --ntasks=1
#SBATCH --cpus-per-task=1
#SBATCH --time=00:20:00
#SBATCH --output=log.%x.%j

source /shared/apps/software/amber24/amber.sh
cd ~/ppigamd/analysis
chmod +x do-reweighted-1d.sh
./do-reweighted-1d.sh R59-D39.dat gamd-all.log
```

The following are instructions for users using your own workstation-

Users must be present in the ~/ppigamd/analysis directory and must have the following files- *do-reweighted-1d.sh*, *PyReweighting-1D.py*, *R59-D39.dat* and *gamd-all.log*. Users can use the script- *run-1d-pmf.sh* to do 1D reweighting calculation as shown below-

```
cd ~/ppigamd/analysis
chmod +x run-1d-pmf.sh
./run-1d-pmf.sh &
```

A copy of the *run-1d-pmf.sh* script is shown below-

```
#!/bin/sh
cd ~/ppigamd/analysis
chmod +x do-reweighted-1d.sh
./do-reweighted-1d.sh R59-D39.dat gamd-all.log
```

The reweighted PMF output files can be found in folder *pmf-R59-D39-bin1.0-cutoff50* as shown below-

- *pmf-c2-Phi.dat-reweight-disc1.0.dat.xvg* corresponds to the reweighting PMF.
- *pmf-Phi.dat-noweight-disc1.0.dat.xvg* represents the non-reweighted PMF.

Users can plot the files using OriginPro (**Figure 4**).

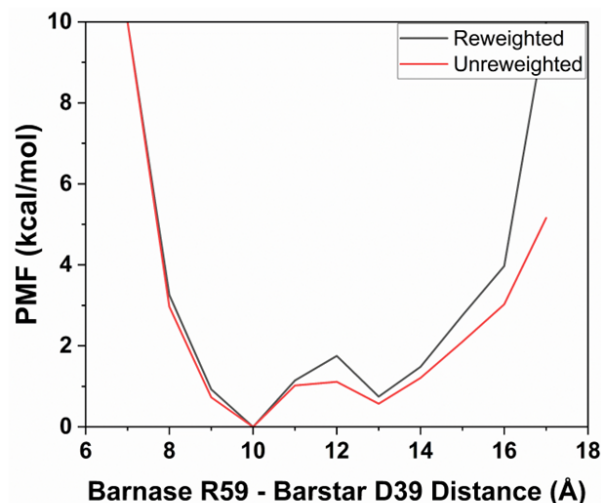


Figure 4. 1D Potential of mean force (PMF) profile regarding the barnase R59 and barstar D39 residue distance from 50 ns PPI-GaMD simulation.

3.2 Reweight PPI-GaMD Simulations to Calculate 2D Free Energy Profile

To calculate 2D PMF free energy profile, the following files are needed-

- *R39-D39.dat*
- *K27-D39.dat*
- *gamd-all.log*
- *Pyreweighting-2D.py*
- *do-reweighted-2d.sh*
- *reweight-2d.sh*

The following are instructions for using the UNC cloud server-

Users must be present in the `~/ppigamd/analysis` directory and must have the required files- *run-2d-pmf.pbs*, *do-reweighted-2d.sh*, *PyRweighting-2D.py*, *K27-D39.dat*, *R59-D39.dat* and *gamd-all.log*. Users will use SLURM to submit the job with the following script- *run-2d-pmf.pbs*-

```
cd ~/ppigamd/analysis
sbatch run-2d-pmf.pbs
squeue
```

A copy of the *run-2d-pmf.pbs* script is shown below-

```
#!/bin/sh
#SBATCH --job-name=pmf-2d
#SBATCH --ntasks=1
#SBATCH --cpus-per-task=1
#SBATCH --time=00:20:00
#SBATCH --output=log.%x.%j

source /shared/apps/software/amber24/amber.sh
cd ~/ppigamd/analysis
chmod +x do-reweighted-2d.sh
./do-reweighted-2d.sh K27-D39.dat R59-D39.dat gamd-all.log
```

The following are instructions for users using your own workstation-

Users must be present in the ~/ppigamd/analysis directory and must have the required files- *do-reweighted-2d.sh*, *PyReweighting-2D.py*, *K27-D39.dat*, *R59-D39.dat* and *gamd-all.log*. Users can use the script *do-reweighted-2d.sh* with the following command line-

```
cd ~/ppigamd/analysis
chmod +x run-2d-pmf.sh
./run-2d-pmf.sh &
```

A copy of the *run-2d-pmf.sh* script is shown below-

```
#!/bin/sh
cd ~/ppigamd/analysis
chmod +x do-reweighted-2d.sh
./do-reweighted-2d.sh K27-D39.dat R59-D39.dat gamd-all.log
```

The reweighted PMF output files can be found in folder *pmf-2d-binx1.0-biny1.0-cutoff50* as shown below-

- *pmf-2D-c2-Phi_Psi-reweight-discx1.0-discy1.0.xvg* corresponds to the reweighting PMF.
- *pmf-2D-Phi_Psi-noweight-discx1.0-discy1.0.xvg* represents the non-reweighted PMF.

Users can plot the files using OriginPro (**Figure 5**).

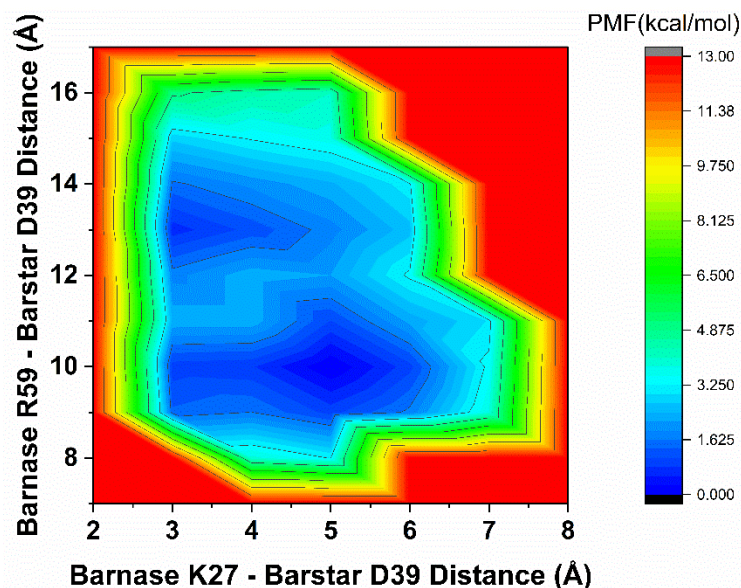


Figure 5. 2D PMF profile regarding the distance between the barnase K27 and barstar D39 residue and the distance between the barnase R59 and barstar D39 residue from 50 ns PPI-GaMD simulation.

4. QUESTIONS

If you encounter any issues or have further questions, please post them to GaMD mailing list: gamd-discuss@lists.sourceforge.net after subscription at <https://sourceforge.net/projects/gamd/lists/gamd-discuss>.

For updates and latest tutorials of GaMD, visit the GaMD website: <https://www.med.unc.edu/pharm/miaolab/resources/gamd/>.

5. REFERENCES

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- (3) Wang, J., & Miao, Y. (2022). Protein-protein interaction-Gaussian accelerated molecular dynamics (PPI-GaMD): Characterization of protein binding thermodynamics and kinetics. *Journal of chemical theory and computation*, 18(3), 1275-1285.