Tutorial 2
Replica Exchange Molecular Dynamics (REMD)

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This tutorial comes in three parts. In the first, the theory behind REMD simulations will be briefly described. Then we will look at how to perform t-REMD on alanine dipeptide in vacuum condition with 4 replica between 300 K and 1000 K using gromacs package patched with plumed. the AMBER14SB force field will be used and each replica will run for 2 ns. Finally, we will analyze the output files and construct free energy. This tutorial assumes that the reader is comfortable with basic usage of Gromacs, Plumed and Linux commands.

1 Theory

Conformational sampling to simulate protein folding, drug binding processes using molecular dynamics is hampered by the slow barrier crossing conformational transitions. To overcome this, several enhanced sampling methods have been devised. Among these, global tempering approaches enhances the sampling of all the degrees of freedom of the system. Parallel tempering replica exchange molecular dynamics is a widely used global tempering method, where several copies of the system are simulated at different temperatures simultaneously and independently, while exchange of coordinates between two adjacent replicas is attempted after certain time intervals based on metropolis criteria. In this manner, high temperature replicas deliver new conformations to the lower temperature replicas which are otherwise very rarely sampled at low-temperatures.

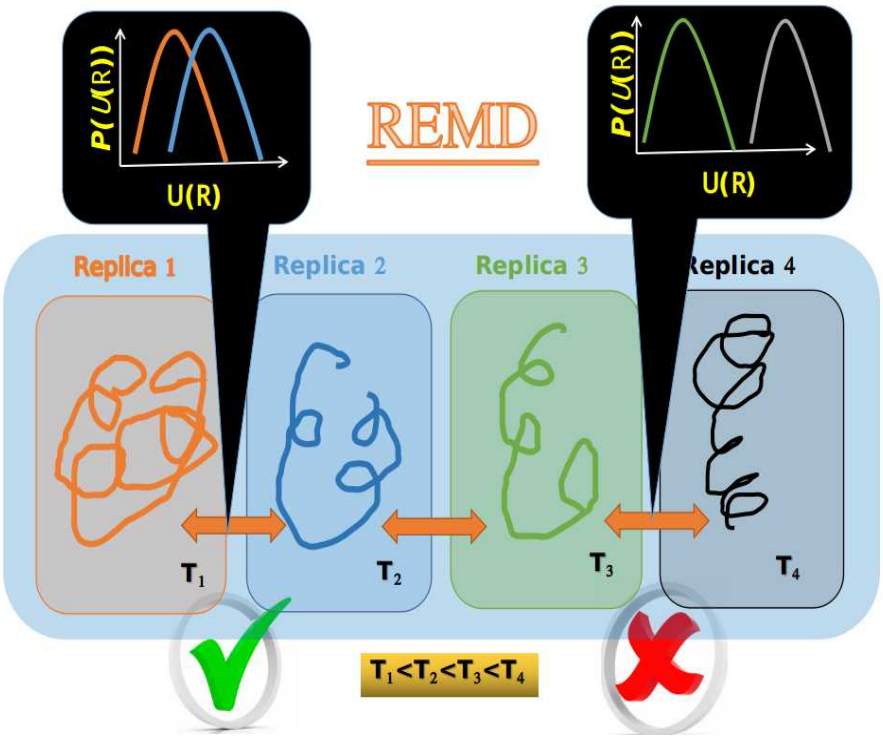
In REMD, the exchange of adjacent replica will be attempted based on metropolis criterion with following acceptance probability:

$$p(i \rightarrow j) = min(1, e^{-\Delta}) \tag{1}$$

Where,

$$\Delta = (\beta_i - \beta_j)(U(R_i) - U(R_j) \tag{2}$$

where $U(R_i)$ and $U(R_j)$ are the potential energies of replica i and j at temperatures β_i and β_j respectively, where $\beta = \frac{1}{k_B T}$.



Schematic of Replica Exchange Molecular Dynamics(REMD) Simulation.

Things to be noted before starting REMD Simulation:

There are number of inter-connected issues to be considered while setting up the simulation.

1. What range of temperatures do we need to span?
2. How many replicas do we need?
3. What exchange probability is needed?

Temperatures should be distributed across all the replicas in a geometric progression which means keep the exchange rate constant across the temperature range. Depending on the number of processors available and the range of

temperature to sample, choose a exponential distribution:

$$T_i = T_0 * e^{ci} \tag{3}$$

Where, c is the desire acceptance ratio and T_0 is the starting temperature, these two parameters can be tuned to obtain reasonable temperature intervals. The exponential allows the increase in temperature intervals. As distribution of total energy increases with temperature and thus exchange rate increases. In the case of larger systems you may have to use large range of temperatures in such scenario it is wise to use below link to get temperature range: Temperature Generator for REMD:(http://folding.bmc.uu.se/remd/).

The literature suggest that an exchange acceptance probability is around 0.2 (i.e 20%) is a good idea. You will have to experiment with the number of replicas you want to use to span the desired temperature range with 0.2 exchange probability.

In this tutorial we will use only 4 replicas with temperatures 300, 366, 547, 996. These temperatures gives reasonable exchange probabilities as we are dealing with alanine di-peptide in vacuum conditions. Whereas you might need to use more number of replicas when you are looking for larger systems in explicit solvent.

2 Setting up REMD Simulation:

Note: To run the REMD simulations smoothly we will have to install **gromacs** with mpi version and should be patched with **plumed**(optional).

In this part, we will build the initial structures using gmx2pdb tool and perform REMD Simulation after short minimization and equilibration steps.

2.1 Preparation of Starting Structure

The necessary steps for preparing the starting structure is dependent of whether you want to use explit solvent or vacuum or implicit solvent model. As in this tutorial we will be preparing alanine di-peptide in vacuum conditions, the preparation of initial structure are limited to generation of gromacs topology (**topol.top**) and coordinates (**conf.gro**) files.

2.1.1 Topology Generation

The below command will generate the topology and coordinate files, by default they are **topol.top**, **conf.gro** and **porse.itp** file (which is used

for positions restraints). During this step gromacs command will prompt for choosing the force field and solvent model. Choose the option for **AMBER14SB** force fields and option **None** for the solvent model, as we don't required the solvent model for the vacuum simulations.

```
gmx pdb2gmx -f ala-dipeptide.pdb -ter -ignh
```

2.2 Energy Minimization

```
; Minimization input for
; vacuum condition (min.mdp)
integrator          = steep
emto                = 1000.0
emstep              = 0.01
nsteps              = 50000
nslist              = 10
cutoff-scheme   = group
ns_type             = grid
coulomtype          = cutoff
rcoulomb            = 2.0
rvdw                = 2.0
pbc                 = no
rlist               = 2.0
```

min.mdp:Input file for gromacs minimization in vacuum condition.

The above input(**min.mdp**) is to run 50000 steps of energy minimization using the steepest descent algorithm for the vacuum condition. (refer input parameter section of gromacs manual for the more details.) Now, run these gromacs commands to perform the minimization run:

```
gmx grompp -f min.mdp -p topol.top -c conf.gro -o min.tpr
gmx mdrun -v -deffnm min
```

2.3 Equilibration of all the Replica

In principle, we will have to equilibrate the initial structure at every temperature before starting the REMD simulation. As we are running vacuum simulation this step can be skipped.

You can use this **nvt.mdp** file as input for the equilibration runs.

```
; Equilibration input for
; vacuum condition (nvt.mdp)
title                    = Ala-d
cpp                      =/lib/cpp
integrator    = md
dt                       = 0.002
nsteps                   = 1000000
nstxout                  = 100
nstvout                  = 100
nstfout                  = 100
nstlog                   = 100
nstenergy                = 100
nstxtcout                = 100
xtc_groups    = system
energygrps    = Protein
nslist                   = 10
ns_type                  = grid
coulomtype    = cutoff
rcoulomb                 = 2.0
rvdw                     = 2.0
pbc                      = no
rlist                    = 2.0
cutoff-scheme            = group
comm-mode  = Angular
tcoupl                   = v-rescale
tc-grps                  = Protein
tau_t                    = 0.1
ref_t                    = 300
gen_vel                  = yes
gen_temp                 = 300
gen_seed                 = 173529
constraints              = all-bonds
```

nvt.mdp:Input file for gromacs equilibration in vacuum condition.

You need to prepare 4 nvt.mdp files (nvt0.mdp, nvt1.mdp, nvt2.mdp and

nvt3.mdp) for the respective temperature replica. In the above **nvt.mdp** file modify **ref_t** tag for different temperatures. And copy the **mini.gro** from the minimization run and use this command to run Equilibration:

```
gmx grompp -f nvt.mdp -p topol.top -c mini.gro -o nvt.tpr
gmx mdrun -v -deffnm nvt
```

2.4 REMD Simulation

We will be running REMD simulation using 4 replica with temperature range 300, 366, 547, 996. We have to prepare the input files as said above.

Prepare 4 sets of inputs

[ala0.gro, nvt0.mdp] corresponds to ref_t=300

[ala1.gro, nvt1.mdp] corresponds to ref_t=366

[ala2.gro, nvt2.mdp] corresponds to ref_t=547

[ala3.gro, nvt3.mdp] corresponds to ref_t=996

For temperature-REMD we need to run a number of simulations that can communicate. This is done via **mdrun -multi** option in gromacs, and **-replex** tag also needs to be used to provide desired exchange frequency.

Construct 4 *.tpr files using above gromacs command.

we can also write a small shell script to do that using loops: **submit.sh**

```
for i in 0 1 2 3;do
gmx grompp -f nvt$i.mdp -p topol.top -c mini.gro -o remd$i.tpr
-maxwarn 10;done

mpirun -np 4 gmx_mpi mdrun -v -deffnm remd -multi 4 -replex
100
```

2.4.1 REMD with Plumed

In the case of using plumed you will have to give additional **-plumed** tag in the command and make sure gromacs is patched with plumed and you place plumed input file (**plumed.dat**) in the same working directory. Whereas the plumed input for alanine di-peptide phi psi as collective variables (**CVs**):

```
# set up two variables for Phi and Psi dihedral angles
phi: TORSION ATOMS=5,7,9,15
psi: TORSION ATOMS=7,9,15,17

# monitor the two variables
PRINT STRIDE=10 ARG=phi,psi FILE=COLVAR
```

And the submit script will look like: (**submit.sh**)

```
for i in 0 1 2 3;do
gmx grompp -f nvt$i.mdp -p topol.top -c mini.gro -o remd$i.tpr
-maxwarn 10;done

mpirun -np 4 gmx_mpi mdrun -v -deffnm remd -plumed
plumed.dat -multi 4 -replex 100
```

Where,
-np = No of processors used
-multi= Instruct the program to preform multi (4) runs
-replex = Instruct the system to attend an exchange at every 100 steps.

3 Post Processing & Analysis:

3.1 Observing Replica Exchange Statistics

You can find the replica exchange statistics such as exchange probabilities and the exchanges of replica involved in every 100 steps in the remd$i.log files

```
grep -A9 "average probabilities" *.log
```

The output of this command should print as bellow.

```
remd_0.log:Repl  average probabilities:
remd_0.log-Repl      0    1    2    3
remd_0.log-Repl       .52  .20  .06
remd_0.log-Repl  number of exchanges:
remd_0.log-Repl      0    1    2    3
remd_0.log-Repl      2559  994  276
remd_0.log-Repl  average number of exchanges:
remd_0.log-Repl      0    1    2    3
remd_0.log-Repl       .51  .20  .06
remd_0.log-
--
remd_1.log:Repl  average probabilities:
remd_1.log-Repl      0    1    2    3
remd_1.log-Repl       .52  .20  .06
remd_1.log-Repl  number of exchanges:
remd_1.log-Repl      0    1    2    3
remd_1.log-Repl      2559  994  276
remd_1.log-Repl  average number of exchanges:
remd_1.log-Repl      0    1    2    3
remd_1.log-Repl       .51  .20  .06
remd_1.log-
--
remd_2.log:Repl  average probabilities:
remd_2.log-Repl      0    1    2    3
remd_2.log-Repl       .52  .20  .06
remd_2.log-Repl  number of exchanges:
remd_2.log-Repl      0    1    2    3
remd_2.log-Repl      2559  994  276
remd_2.log-Repl  average number of exchanges:
remd_2.log-Repl      0    1    2    3
remd_2.log-Repl       .51  .20  .06
remd_2.log-
--
remd_3.log:Repl  average probabilities:
remd_3.log-Repl      0    1    2    3
remd_3.log-Repl       .52  .20  .06
remd_3.log-Repl  number of exchanges:
remd_3.log-Repl      0    1    2    3
remd_3.log-Repl      2559  994  276
remd_3.log-Repl  average number of exchanges:
remd_3.log-Repl      0    1    2    3
remd_3.log-Repl       .51  .20  .06
remd_3.log-
```

Log:::Replica exchange statistics extracted from log files.

Attention:
In the above replica exchange statistics, the exchanges were accepted with an exchange probability 0.5 (i.e 50%). In case you could find those probabilities as 0.0, which means that, your chosen temperature range may not be sufficient enough for the potential energy overlap to accept the exchanges with reasonable exchange probability. In such scenario, you might need to go back and repeat the simulations with reasonable temperature range.

3.2 Concatenate the trajectories

By concatenating all the log files into single log file (**REMD.log**) and by using gromacs built-in tool **demux.pl** will generate the **replica_index.xvg** and **replica_temp.xvg** files. We need a trajectory with continuous coordinates despite the jumps in the ensemble space due to attempted exchanges. This trajectory can be generated using **gmx trjcat** tool with **-demux** tag and above index files.

You can use the below commands to do this:

```
# Concatenate log files
cat *.log > REMD.log
demux.pl REMD.log

# De-multiplexing a REMD trajectory
gmx trjcat -f *.xtc -demux replica_index.xvg
```

3.3 Checking Potential Energy Overlap

As we have discussed above (referring Schematic of REMD) the metropolis criteria is dependent of the potential energy deference of the replicas ($\Delta U(\mathbf{R})$). Which means that to make sure the adjacent replicas to be sampled the continuous coordinates their potential energy distributions should overlap else the metropolis criteria will reject the exchange.

And also make a note that checking distributions of potential energy overlap is the wise idea to check whether the taken temperature range is required enough or not. See the PE overlap of 4 replica:

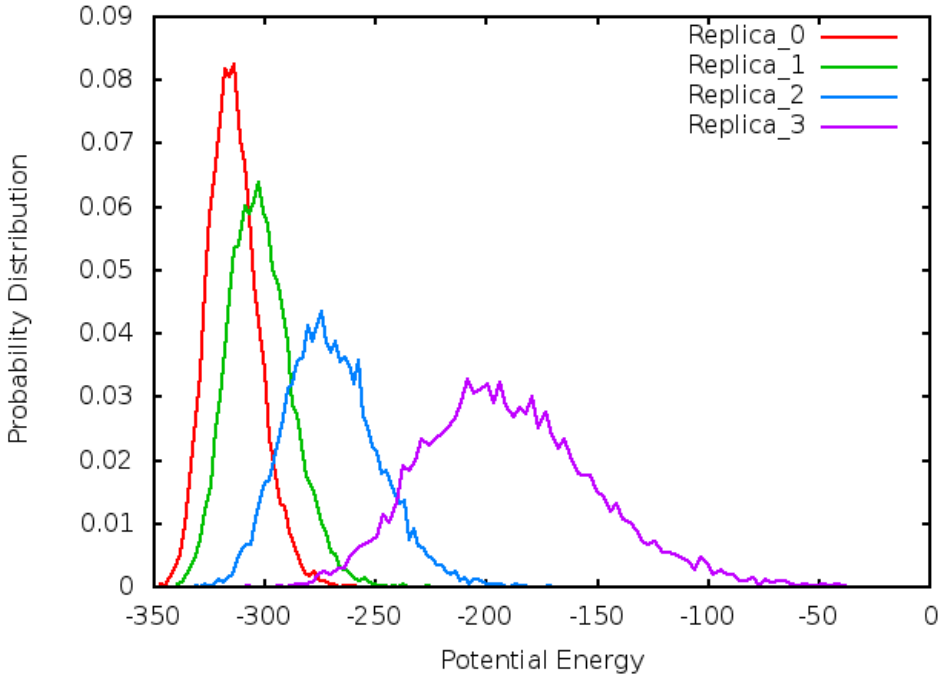


Fig:4:Overlap of distributions of potential energy of each replica.

You can get the potential energy of each replica with time using **gmx energy** tool and then you can use **xmgrace** or self written program to get the distribution of it.

Gromacs Commands:

```
# Choose potential energy on screen to print data into file PE.xvg

gmx energy -f remd.edr -s remd.tpr -o PE.xvg
```

3.4 Construction of FES

In the case of using **PLUMED**, you can see the COLVAR.$i files for thr respective temperature replica. you can plot the CV values with time to check their evaluation. **Gnuplot** commands to plot CV v/s Time of 300 K replica are as follows:

```
# Open Gnuplot on terminal
Terminal$ gnuplot


plot "COLVAR.0" u 1:2 w l lw 2 title"PHI"
plot "COLVAR.0" u 1:3 w l lw 2 title"PSI"
```

Whereas, the 1D free energy surface along Phi and Psi of the replica at 300 K, can be constructed using plumed in-built tool **sum_hills** with following command:

```
# Construct FES using sum_hills


plumed sum_hills –histo COLVAR.0 –idw phi –sigma 0.2 –kt
2.5 –outhisto fes_phi.dat

plumed sum_hills –histo COLVAR.0 –idw psi –sigma 0.2 –kt 2.5
–outhisto fes_psi.dat
```

sum_hills is the plumed biult-in tool which can be used to post process the existing HILLS or COLVAR files produced by plumed. Refer the plumed website[2,3] for the more details about sum_hills.

Where,
–**histo** = Calculates the histogram (i.e Probability $P(s)$) of the given CV with given file name COLVAR.
–idw = Specifies the variables to be used for calculating the histogram.
-sigma = Specify the bin width while binning histogram.
–kt = The Temperature of system in the energy units ($kJmol^{-1}$). In the above case kt = 2.5 ($kJmol^{-1}$) is corresponds to 300 K

–outhisto = Specify the file name to write the histogram data.

These command will produce the following figures:

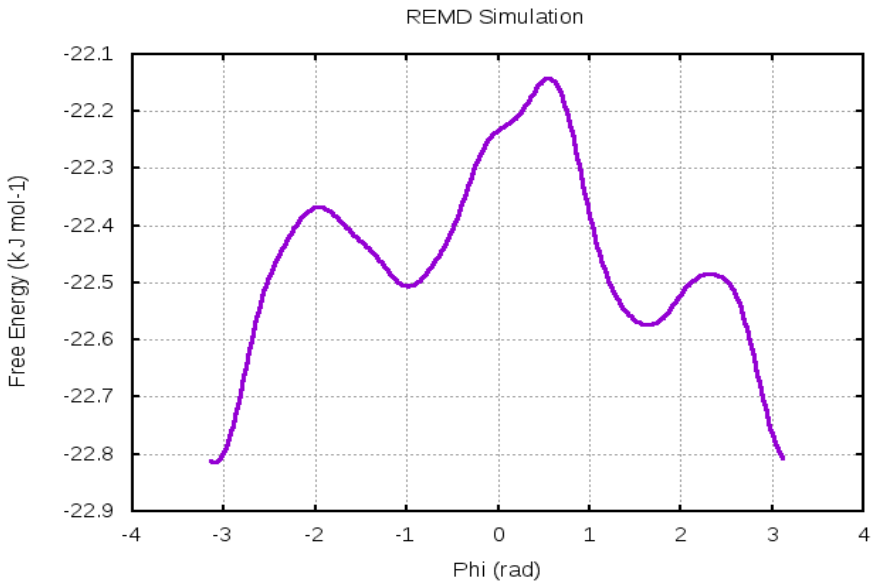


Fig:5:Free energy along Phi.

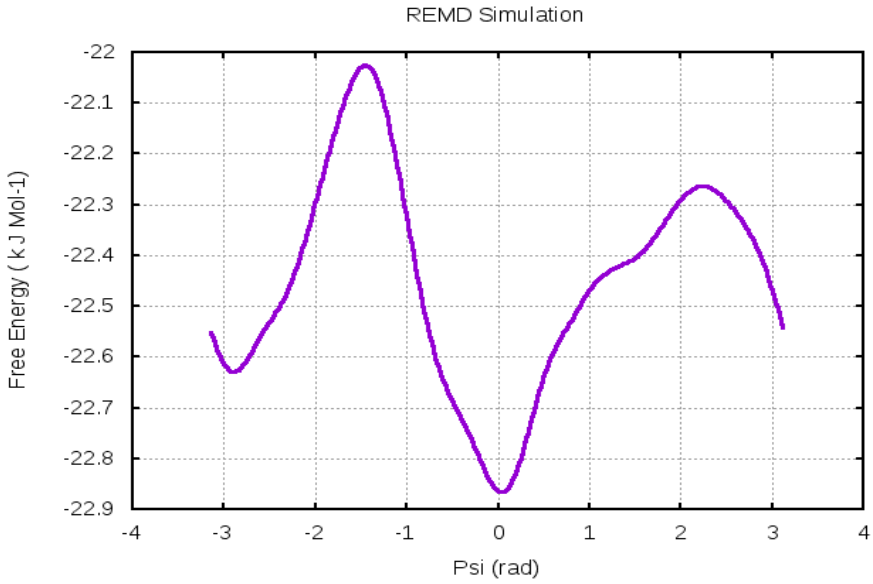


Fig:6:Free energy along Psi.

2-D Free Energy:
The 2D free energy surface(Fig.7) can also be constructed using same plumed sum_hills tool.

 # Construct 2D FES using sum_hills

mv COLVAR.0 COLVAR
plumed sum_hills –histo COLVAR –sigma 0.2,0.2 –kt 2.5
–outhisto fes_2D.dat

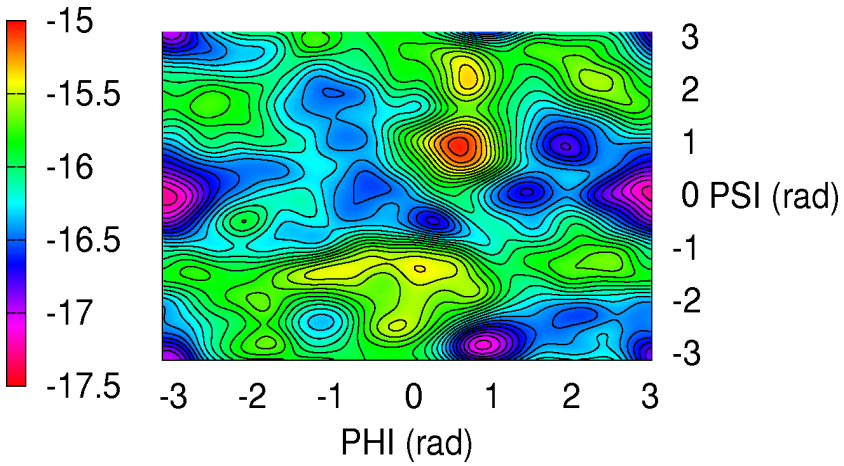


Fig:7. **2D FES (k J mol-1) of Alanine di-peptide corresponding to the replica at 300 K produced from 2ns REMD simulation**

4 Summary:

To be conclude we have discussed the underlying theory of REMD and setting up the simulations along with using the post processing commands and tools. A shell script to run this complete tutorial by using ala_dipep.pdb as input, can be found at Nisanth Nair Research Group Github Page(Ref 4).

5 References:

1. Sugita, Y.; Okamoto, Y. Chem. Phys. Lett. 1999, 314 (1–2), 141–151.
2. Plumed: [http://www.plumed.org/]
3. More about sum_hills: Click here
4. Nisanth Nair Research Group (GitHub Page): Click here.

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