

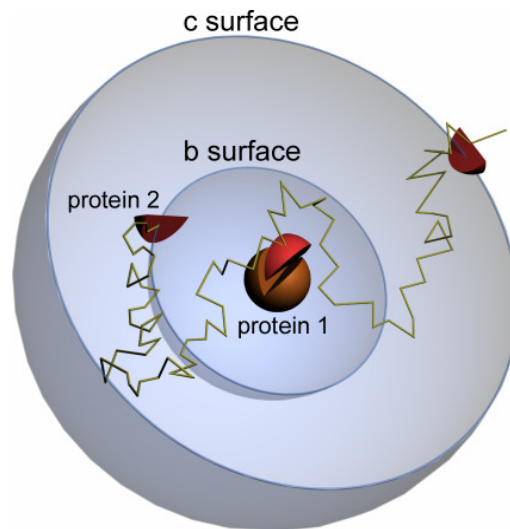
EMBO Practical Course -2010

„Biomolecular Simulation“

June 26th-July 3th

Brownian Dynamics Simulation
of Barnase-Barstar association

Second part of Practical on Brownian Dynamics
Simulation



Rebecca Wade

Mykhaylo Berynskyy

Contributors to previous versions:

Domantas Motiejunas

Razif Gabdoulline

Anna Feldman-Salit

Divita Garg

HITS gGmbH
Schloss-Wolfsbrunnenweg 35
69118 Heidelberg
Germany

Email: firstname.lastname@h-its.org

Introduction

The aim of this practical is to run Brownian dynamics simulations of protein-protein association and to visualize the trajectories of the proteins during these simulations. Simulations will be performed for the proteins, barnase and barstar.

Why simulate protein-protein association?

-Protein-protein association is the most ubiquitous event in protein function. Its rate is relevant for the function of proteins.

- When proteins must find one another by diffusion in order to associate, the speed of association is limited by the rate of bimolecular diffusional association.

- Brownian dynamics simulations allow computation of the association rate constants and their dependence on environmental conditions and the effects of mutations (1,5). They can also be used for protein-protein docking (9).

How are proteins modeled in Brownian dynamics simulations of their association?

-In Brownian dynamics simulations (3), the diffusional motion of proteins is generally modeled by assuming the proteins to be rigid bodies. The interaction of the proteins with solvent molecules is modeled implicitly by random forces experienced by the proteins due to collisions with water molecules.

- During BD simulations, proteins interact with one another by exclusion and electrostatic forces. Attractive electrostatic forces may result in fast association rates that are sensitive to mutation of the protein or to changes in ionic strength. Electrostatic forces are computed from a Poisson-Boltzmann continuum model under the effective charge approximation (6). Other contributions to intermolecular forces eg. nonpolar (hydrophobic) interactions (10) may be included in BD runs.

How can I simulate protein-protein association?

For these simulations, you will use the SDA (Simulation of Diffusional Association) Program (2) written at EMBL and further developed at HITS gGmbH (see: <http://mcm.h-its.org/software/SDA>). Protein electrostatic potentials will be computed with the UHBD (University of Houston Brownian Dynamics) program (8) and used as input for the Brownian dynamics simulations with SDA. Trajectories will be visualized using VMD (11).

Why barnase and barstar?

-Barnase is an extracellular ribonuclease. Barstar is an intracellular inhibitor of barnase. To stop barnase working inside the cell, barstar binds to barnase very tightly (high affinity) and very quickly (high association rate).

-The binding of barnase and barstar has been very well characterized experimentally in terms of structure, energetics and kinetics.

-Barnase and barstar thus provide a good system for validating theoretical methods to compute association rates and for learning which factors are important for proteins to have high association rates (1).

-The attractive complementary electrostatic interactions between barnase and barstar are important for the speed of their association, and for the sensitivity of association rates to ionic strength and to mutation of charged amino acid residues.

Literature:

1. Gabdoulline, R.R. and Wade, R.C. *Biophys. J.* (1997) 72, 1917-1929. Simulation of the Diffusional Association of Barnase and Barstar
2. Gabdoulline, R.R., Wade, R.C. (1998) *Methods*, 14, 329-341. Brownian dynamics simulation of protein-protein diffusional encounter.
3. J.D. Madura, J.M. Briggs, R.C. Wade, R. Gabdoulline. (1998) in *The Encyclopedia of Computational Chemistry*, Schleyer, P.v. R.; Allinger, N. L.; Clark, T.; Gasteiger, J.; Kollman, P. A.; Schaefer III, H. F.; Schreiner, P. R., Eds.; John Wiley & Sons, Chichester, 1998, Brownian dynamics.
4. <http://mcm.h-its.org/software/SDA>
5. Gabdoulline, R.R. and Wade, R.C. (2001) *J. Mol. Biol.* 306, 1139-1155. Protein-protein Association: Investigation of Factors Influencing Association Rates by Brownian Dynamics Simulations.
6. Gabdoulline, R.R. and Wade, R.C. (1996) *J. Phys. Chem.* 100, 3868-3878. Effective charges for Macromolecules in Solvent
7. Gabdoulline, R.R. and Wade, R.C. (2002) *Curr. Opin. Struct. Biol.*, (2002), 12, 204-213. Biomolecular diffusional association
8. Madura, J.D.; Briggs, J.M.; Wade, R.C.; Davis, M.E.; Luty, B.A.; Ilin, A.; Antosiewicz, J.; Gilson, M.K.; Bagheri, B.; Scott, L.R. and McCammon, J.A. *Comp. Phys. Comm.* (1995) 91, 57-95. Electrostatics and Diffusion of Molecules in Solution: Simulations with the University of Houston Brownian Dynamics Program
9. Motiejunas D, Gabdoulline RR, Wang T, Feldman-Salit A, Johann T, Winn PJ, Wade RC. *Proteins* (2008) 71, 1955-1969. Protein-protein docking by simulating the process of association subject to biochemical constraints.
10. Gabdoulline RR and Wade RC. *J. Am. Chem. Soc.* (2009) **131**, 9320-9238. On the contributions of diffusion and thermal activation to electron transfer between *Phormidium laminosum* plastocyanin and cytochrome f : Brownian dynamics simulations with explicit modeling of nonpolar desolvation interactions and electron transfer events.
11. Humphrey, W., Dalke, A. and Schulten, K., *J. Molec. Graphics*, 1996, vol. 14, pp. 33-38. "VMD - Visual Molecular Dynamics"

Brownian dynamics simulations of barnase-barstar association

[The SDA \(Simulation of Diffusional Association\) program](#) permits simulation of the diffusional association of two molecules given the atomic structure of the bound complex of the two molecules. The molecules may be proteins, DNA or low molecular weight compounds. The program can be used to compute association rate constants. A range of „reaction criteria” can be specified for rate constant computation. The program permits analysis of diffusional trajectories and formation of encounter complexes. The program computes electrostatic forces from electrostatic potentials computed with UHBD or APBS and charges generated with ECM (Effective Charges for Macromolecules). The ECM program is part of the SDA distribution. Nonpolar desolvation forces can also be computed. SDA can be used for computing electron transfer rates and for molecular docking.

The aim of this part of the practical is to simulate the relative diffusional motion of two proteins, barnase and barstar, in order to compute their bimolecular association rate constant and learn how they form an encounter complex.

0. Set up the required files:

0.1. Create a working directory for BD in your \$HOME directory:

```
mkdir bd
cd bd
```

0.2. Copy the following file from /opt/tutorial-BD/ to bd/ directory by typing:

```
cp /opt/tutorial-BD/practical-bd.tar .
```

0.3. Untar tutorial files in your home directory and go to sda/ directory:

```
tar xvf practical-bd.tar
cd practical-bd/
```

Check that the following directories are in this directory: bin, data
bin: uhbd, ecm and sda executables and scripts
data : input and parameter files for uhbd, ecm, sda and vmd

1. Run the Brownian dynamics simulations:

```
./bin/run_bd_com_apbs
```

In ~ 2 mins, this script will:

- compute electrostatic potentials of the proteins using APBS
- prepare the necessary force files: charges for both proteins and reaction atom pairs
- run BD simulations with SDA
- write rate information to file „rates”. In this file, columns are:
reaction distance in Å, rate constant in $M^{-1}s^{-1}$; blocks are for 1 contact, 2 contact, 3 contact and 4 contact reaction criteria (many more trajectories should be run to obtain results with good accuracy)
- write one trajectory to file `trajectories`
- rewrite the trajectory to file `trajectories.200`, so that the trajectory snapshots are separated in time by (approximately) 200 ps
- convert this file to a standard format dynamics trajectory file called `cmxs.DCD`
- run two additional BD simulations (one longer simulation, the other with different initial conditions)

2. Visualize a Brownian Dynamics simulation trajectory with VMD:

2.1. Start VMD by typing:

```
vmd
```

2.2. Load the protein coordinates by typing in the VMD console window:

```
play data/vmd187.in
```

3 pdb files will be loaded and coloured so that the target protein (barnase) is green, the moving protein (barstar) is red, and the docked position for barstar is blue. For visualization purposes, barnase will be kept stationary

throughout the trajectory. [In simulations to compute rate constants, the rotational and translational motion of both proteins is considered].

2.3. Display the trajectory

- The time step between trajectory snapshots is 200 ps. During this time, barstar moves ca. 6Å (1Å=0.1nm) by random diffusion.

Click on the forward arrow button in the VMD Main window. The movie will play continuously.

Stop the movie by relicking on the forward arrow button.

Explore different parts of the trajectory using the sliding bar.

Alter the speed by sliding the button.

- During the animation, you can alter the viewing perspective as follows:
to rotate, type 'r' on VMD_OpenGL window and then press left-mouse button;
to scale, type 's' on VMD_OpenGL window and then press left-mouse button, while moving the mouse horizontally;
to translate, type 't' VMD_OpenGL window and then press left-mouse button.

2.4. Explore the trajectory in detail

- At the beginning of the simulation, barstar is on the same side of barnase as its binding site, but its orientation is not suitable for precise docking. Barstar spends a lot of time interacting with barnase, until it achieves the right orientation.

To see this, type 500 (and press <Enter>) in the frame number field and play the movie at slower speed. Barstar moves away and then comes back in ~15 ns to adopt an arrangement allowing subsequent docking. Type 603 (and press <Enter>) in the frame number field to see this docked encounter complex arrangement.

- In Brownian dynamics simulations, association to formation of a diffusional encounter complex is simulated. The subsequent step of docking to form a fully bound complex is not simulated and would require a more detailed force field accounting for attractive shortrange interactions and side-chain rearrangements. This means that after satisfaction of contact criteria for formation of a diffusional encounter

complex, barstar eventually diffuses away from barnase in the Brownian dynamics simulations. In this simulation only the electrostatic forces are included but also desolvation and hydrophobic forces can be computed with SDA.

In this simulation, barstar remains in close proximity to its docked position for ca. 12 ns (steps 570-630), which is significantly longer than the time required for side-chain rearrangement upon docking of these proteins.

- Analyse a single frame to identify the closest residues of barnase and barstar
 1. Type 570 in the frame number field
 2. Go to „ Graphics“ -> „ Representation...“ . Select the molecule 1: 2.pdb and undisplay it by double-clicking left button of mouse (alternatively, type in „ none“ for Selected_Atoms list). Select the molecule 2: 2b.pdb and display it in all-atom representation by selecting „ Drawing Method“ „ Lines“ . Change coloring to atom-type based scheme selecting „ Coloring Method“ „ Type“ . Hide the hydrogens by typing “noh” for Selected_Atoms list. Do the same with the molecule 0:1.pdb. When done, close this window („ Graphical Representations“).
 3. Experiment with toggling „ r“ , „ t“ and „ s“ that change the mode of 3D view manipulation with the mouse (left button pressed). Press „ c“ and pick some atom of barnase to change the centering of the molecules. Press „ r“ afterwards and check if by this centering you can analyse 3D structure better.
 4. Select „ Mouse“ -> „ Label“ -> „ Atoms“ in the VMD_Main control window. Pick an atom of barstar in close contact with barnase. Pick an atom of barnase in close contact with barstar. Note their names.
 5. Now find out the way to remove unwanted labels (inspect selecting „ Graphics“ -> „ Labels“ in VMD_Main window menu).
- Play the movie stepwise and watch how the mutual arrangement of residues changes.

Repeat the previous analysis step and select „ Mouse“ -> „ Label“ -> „ Bonds“ and then pick 2 closest atoms. Play the movie stepwise and notice the changes in the distance.

- Monitor 2 contacts formation during the Brownian dynamics simulations.

Remove unwanted labels and type in VMD command window:

```
label add Bonds 0/1535 2/537
label add Bonds 0/548 2/690
```

The distance between 2 pairs from required reaction atom pair list will be labeled.

- Play the movie stepwise and find the frame at which both pair distances are less than 7Å.
- Define the distance between these atoms in a docked complex of barnase and barstar.

3. Making movies of protein motions with VMD

- Make a movie of the trajectory part from frame 550 to 620. For this:

Select the molecule 2 (2b.pdb), select „ Delete_Frames“ by clicking right mouse button and remove frames 0-549 and then 71-2031 (last); make sure that you have the trajectory with 71 frames.

Change visualization options, for example, showing or hiding barstar in bound conformation (molecule 1:2.pdb), choosing different representations, adding/removing labels, rescaling VMD OpenGL window. Orient the molecules properly and watch the movie on the screen once again.

In VMD_Main window select Extensions -> Visualization -> Movie_Maker. Set Working Directory to your „ \$HOME/bd/practical-bd“ directory, name the file, set Movie_Settings to Trajectory and then make the movie.

Now find the generated movie in practical-bd/ directory and play it using **mplayer**.

```
mplayer movie_name.mpg
```

If needed, remake movie to show the dynamics of proteins better.

4. Analyse results:

- Look at the script bin/run_bd_com to follow the procedure.

Type „ grep ^# bin/run_bd_com_apbs“ to see comments;

Type „ grep -v echo bin/run_bd_com_apbs | grep -v clear“ to see commands other than those writing to console.

- Look at the parameter and input files in the data/ directory.

Find out why there are 2 pqr files and when are they used.

The files are similar to the files used in the previous tutorial.

Look at all *.in files (in data/ directory) and locate in the script where they are used.

- Look at files „ rates-2“ , „ rates“ and „ rates-50“ and try to figure out what are the rates of forming 2 contacts at 6A. Relate this to experimental rate of $3e8 \text{ M}^{-1}\text{s}^{-1}$.

To understand exactly which parameters are used in the simulations, refer to the on-line sda documentation at:

<http://projects.villa-bosch.de/mcmsoft/sda/6.00/index.html>

Compare data/sda6.in and data/sda6-2.in and data/sda6-50.in and relate this to differences in rates*.

- Analyse the file „ data/rates-8000“ - the result of Brownian dynamics simulations with 8000 runs (this needs long simulations and therefore were done beforehand). It has the following information: reaction distance in A, rate constant in $\text{M}^{-1}\text{s}^{-1}$, standard error calculated from several runs, the rates obtained from each individual run; blocks are for 1 contact, 2 contacts, etc reaction criteria.
- Find out what are the rates of forming 2 contacts at 6A and standard error of this rate.

Start **gnuplot** and plot the rates. For that, type in „ gnuplot“ and give the plot command:

```
se log y; p „data/rates-8000“ w l 1, „data/rates-8000“ u  
1:2:3 w e 3, 3e8 w l 0
```

(l is el, 1 is one, so it is better to cut'n'paste this line).

- Find out what commands you actually typed in using help command of gnuplot.

- Find out what is the maximal rate of forming contacts and estimate how much larger is the rate of forming 2 contacts compared to the rate of forming 3 contacts.
- Locate the contact distance at which 1, 2, 3, 4 contacts are formed at experimental rate.

Questions to Simulation and Analysis:

1. Why does it take only 4 times longer to simulate 50 trajectories compared to 6 (compare „ Execution time“ in files „ sda6.ou“ and „ sda6-50.ou“)? How long will it take to run 100 trajectories?
2. Why do the rates from different simulations differ (compare rates in files „ rates“ and „ rates-2“)? Which one is correct?
3. Is it necessary to write the trajectory (as we did in this practical) to calculate the association rate?
4. What does the rate $0.3405e+10 \text{ M}^{-1}\text{s}^{-1}$ mean? Is this fast or slow?