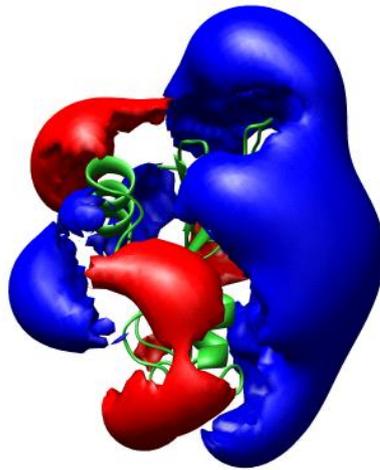


# Second Part of the Practical on Brownian Dynamics Simulation

## Brownian Dynamics Simulations: Rate of Association of Barnase and Barstar



Neil Bruce, Gaurav Ganotra, Rebecca Wade

Email: [firstname.lastname@h-its.org](mailto:firstname.lastname@h-its.org)

HITS gGmbH

Schloss-Wolfsbrunnenweg 35

69118 Heidelberg

Germany

## **Introduction**

The aim of this practical is to run Brownian dynamics (BD) simulations to investigate the bimolecular association of two proteins, and to visualize the trajectories of these proteins during the simulations. Simulations will be performed for the proteins, barnase and barstar, using the software SDA (1,2).

### **Why do we perform Brownian dynamics simulations of protein-protein association?**

- Protein-protein association one of the most ubiquitous events in protein function. The rate at which these associations occur is important for understanding cellular function at the molecular level.
- For many protein-protein interactions, the speed of association is limited by the rate of bimolecular diffusional association.
- BD simulations provide a way of modelling the diffusion of solutes computationally. It has been applied to the estimation of the bimolecular association rate constants of proteins, and for making predictions on their dependence on changing environmental conditions or mutations in protein sequences (1,3). They can also be used to perform protein-protein docking, to predict the structures of bound complexes (4).

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

- In Brownian dynamics simulations (1,5), the diffusional motion of proteins is often modeled by assuming the proteins to be rigid bodies.
- The interactions of proteins with water molecules is modeled implicitly, using random forces that mimic the forces that act on proteins due to collisions with water molecules.
- These random forces act together with a molecular force field, which describes the intermolecular forces acting between proteins, to drive the dynamics of the system.
- When proteins are assumed to be rigid bodies, the forces acting between pairs of proteins can be precomputed, and modelled as the interactions of a set of interaction sites on one protein with precomputed grids on another (5).

- The predominant forces acting between proteins are due to long-range electrostatic interactions. These can be modelled as the interaction between an electrostatic potential field derived by solving the Poisson-Boltzmann equation for one protein, and a set of “effective charges” on another protein (6).
- The repulsive interactions that prevent proteins from overlapping with each other can be modelled by a simple volume exclusion term (1).
- Other contributions to intermolecular forces can also be included in BD simulations. For a description of the forces used in SDA, see the Appendix to Ref (2).

### **How can I simulate protein-protein association?**

- For these simulations, you will use the SDA (Simulation of Diffusional Association) Program (1,2,7), written at EMBL and further developed at HITS (see: <http://mcm.hits.org/sda7/>).
- Protein electrostatic potentials will be computed with the APBS (Adaptive Poisson-Boltzmann Solver) program (8) and used as input for the Brownian dynamics simulations with SDA.
- Trajectories will be visualized using VMD (9).

### **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 (3,10).
- 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:***

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## **Brownian dynamics simulations of barnase-barstar association**

[The SDA \(Simulation of Diffusional Association\) program](#) allows the simulation of the diffusional association of two molecules given their atomic structures. 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 a Poisson-Boltzmann equation solver, such as APBS or UHBD, and charges generated with ECM (Effective Charges for Macromolecules). The ECM program is part of the SDA distribution. Non-polar desolvation forces can also be computed. SDA can also 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. We will also look at the effect that an amino acid mutation on barnase has on the computed association rate constants

### **0. Set up the required files:**

#### **0.1. Move into the directory for this practical from your \$HOME directory:**

```
cd practical-bd/bd
```

#### **0.2. Check the contents of the following directories:**

**bin:** Contains the scripts that will be used to run the simulations in this practical. To run these scripts you need to have VMD installed on your computer, and available in your executable path.

**data:** Contains input and parameter files for SDA and VMD.

**results:** Currently an empty folder. This is where the results of your simulations will be

created.

**prepared\_results:** An example set of BD simulation results.

**sda\_dir:** Contains the Brownian dynamics software SDA, which will be used in the practical.

**0.4. Copy all the electrostatic potential files that you created in the last practical into the data directory (replace *<directory>* with the location of your files from the last practical):**

```
cp <directory>/electrostatics/apbs/* data/
```

Now, you should have all files of the previous APBS preparation tutorial additionally in the data/ folder. (If you had problems with the APBS tutorial, download the prepared results from the last practical, and copy these)

## **1. Move into the results directory, and run the Brownian dynamics simulations:**

```
cd results/  
../bin/run_first.sh
```

In about 5 minutes, this script will:

- Prepare the necessary force field files for both proteins.
- Find the native contacts in the bound complex to generate a set of reaction criteria.
- Run an initial BD simulation with SDA 7.
- Write computed rate information to the file “assoc\_rates\_1.out”. In this file, the columns are:

reaction distance in Å

rate constant (in  $M^{-1}s^{-1}$ )

standard error in the computed rates

The blocks in the files show the rate constants for forming 1, 2, 3 and 4 contacts.

- This first script runs a single simulation of 500 trajectories. We want to run some others for comparison, which we run with a second script

```
../bin/run_second.sh
```

- The following BD simulations will be run:
  - A second simulation of 500 trajectories.
  - A longer simulation of 2000 trajectories.
  - A simulation of 2000 trajectories using a mutant barnase structure (R87A).

(NOTE: ideally many more trajectories should be run to obtain results with good accuracy)

- While the second script is running, open a new terminal window and move back to the results directory to start the next section.

```
cd practical-bd/bd/results
```

## **2. Visualize a Brownian Dynamics simulation trajectory with VMD:**

### **2.1. Start VMD and load the protein structures and trajectories**

```
vmd -e ../data/vmd.in
```

Three pdb files will be loaded and colored so that the target protein (barnase) is green and the docked position for barstar is blue. The simulated trajectory of barstar is shown in red. 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].

## 2.2. Display the trajectory

- Move the scroll bar that controls the trajectory to the beginning (note: the first frame is the bound complex structure and not part of the trajectory)
- Press the play button to run the trajectory movie.
- Stop the movie by re-clicking on the forward arrow button.
- Explore different parts of the trajectory using the scroll bar.
- Alter the speed by sliding the switch.
- During the animation, you can alter the viewing perspective as follows:
  - to rotate, type 'r' in the VMD\_OpenGL window and then press left-mouse button
  - to scale, type 's' in the VMD\_OpenGL window and then press left-mouse button, while moving the mouse horizontally
  - to translate, type 't' in the VMD\_OpenGL window and then press left-mouse button.
  - If you want to change the center of rotation of the view, type 'C' in the VMD\_OpenGL window and click on a position on one of the molecules.

## 2.3. Explore the trajectory in detail

The simulations, especially the specific trajectories, can be different when you run the same simulation on different computers with different processors. Also, small changes in the preparation of the electrostatic potential grids used in the calculation can result in different trajectories being generated. If your trajectory does not match the one described below, you can use the previously run simulation in the `prepared_results/` directory.

At the beginning of the calculated trajectory, barstar is far away from barnase, but after a while barstar comes closer to the binding site of barnase. Once it reached the binding site, barstar spends some time interacting with barnase, then diffuses away.

In Brownian dynamics simulations, protein association to form 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 that accounts for short range

interactions and simulations that allow 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 and a polar desolvation term are included, but hydrophobic forces can also be computed with SDA.

- Analyze the frames close to the encounter of barnase and barstar:
  - Type 950 in the frame number field of the VMD Main window.
  - Move the trajectory forwards a frame at a time, and find the frame in which barstar is most closely aligned to the bound structure.
  - Go to "Graphics" -> "Representation...". Select Molecule 1: barstar.pdb and hide it by double-clicking the left button of mouse (alternatively, type in "none" for Selected\_Atoms list). Select Molecule 2: barstar\_traj.pdb and display it in all-atom representation by selecting "Drawing Method" "Lines". Change coloring to atom-type based scheme selecting "Coloring Method" "Type". Do the same with the molecule 0:barnase.pdb. When done, close this window ("Graphical Representations").
  - 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 the 3D structure better.
  - Select "Mouse" -> "Label" -> "Atoms" in the VMD\_Main control window (or press "1"). Pick an atom of barstar in close contact with barnase. Pick an atom of barnase in close contact with barstar.
  - 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"(or press "2") and then pick 2 closest atoms. Play the movie stepwise and notice the changes in the distance.
  - You can plot how this distance changes over the trajectory by selecting

“Graphics” -> “Labels” -> “Bonds”, and choosing the “graph” tab.

- Monitor 2 contacts formation during the Brownian dynamics simulations. Remove unwanted labels and type in VMD command window (“Extensions” → “Tk Console”):

```
label add Bonds 0/284 2/358
```

```
label add Bonds 0/788 2/316
```

- The distances between 2 pairs from the required reaction atom pair list will be labeled. (These 2 pairs are barnase: OG Ser 38 and barstar: O Val 45 and barnase: NE2 His 102 – barstar: OD2 Asp 39).
- Play the movie stepwise and find a frame at which both pair distances are less than 6.5Å.
- Find the distances between these atoms in a docked complex of barnase and barstar (Hint: you can repeat the label commands above, changing the final atom selections to 1/358 and 1/316)

### **3. Analyze results:**

Now we will analyse the results of the simulations run with both of the run scripts. To understand exactly which parameters are used in the simulations, refer to the SDA 7 documentation at:

[http://mcm.h-its.org/sda7/doc/doc\\_sda7/doc.html](http://mcm.h-its.org/sda7/doc/doc_sda7/doc.html)

- Look at the scripts bin/run\_first.sh and bin/run\_second.sh to follow the procedure used.
- Look at the parameter and input files in the data/ directory.
- Look at all \*.in files, especially the sda\*.in files (in the data/ directory), and locate where in the run scripts they are used.
- Look at the files “assoc\_rates\_1.out“, “assoc\_rates\_2.out“ and “assoc\_rates\_2000.out“ and find out what are the rates of forming 2 contacts at 6.5 Å. Relate this to experimental rate of  $3 \times 10^8 \text{ M}^{-1}\text{s}^{-1}$ .

- The file “assoc\_rates\_mut\_2000.out” shows the data obtained from a simulation in which the Arg 87 residue of barnase has been mutated to alanine. How does this compare to the wild-type simulation? In VMD, look at the location of this amino acid within the structure of barnase (type “resid 87” as the selected atoms in the Graphical Representation menu).
- Analyze the file “prepared\_results/assoc\_rates\_20000.out” the result of Brownian dynamics simulations with 20000 trajectories (this takes longer to run, so the simulation was done beforehand). It has the following information: reaction distance in Å, rate constant in  $M^{-1}s^{-1}$ , 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 6.5 Å and standard error of this rate.
- Go to the prepared\_results/ folder, start **gnuplot** to plot the rates:

```
gnuplot
```

- At the gnuplot> prompt, pressing enter after each command:

```
set logscale y
set xlabel 'Contact distance (A) '
set ylabel 'Rates '
set xrange [4.0:12.0]
set yrange [1e6:1e10]
plot      'assoc_rates_20000.out'      with      line,
'assoc_rates_20000.out' using 1:2:3 with yerrorbars, 3e8 with
line
```

**Note:** If copy-pasted, the quotes need to be retyped in the gnuplot console. Otherwise, it will not plot properly.

To find out what commands you type do, you can use:

```
help "command"
```

- Locate the contact distance at which 1, 2, 3, 4 contacts are formed at experimental rate ( $3 \times 10^8 M^{-1}s^{-1}$ ).

#### **4. Questions on Simulations and Analyses:**

- Why is it possible that the run times for 500 runs can be different (compare times under “Mytimer Total” in files “sda.out” and “sda\_2.out”, and remember the differences in the sda\*.in files)?
- Considering the electrostatic potentials you looked at in the previous practical, can you explain the difference between the rates constants that were calculated in simulations containing wild-type and mutant barnase?
- Is it necessary to write the trajectory (as we did in this practical) to calculate the association rate?
- What does a rate constant of  $3 \times 10^8 \text{ M}^{-1}\text{s}^{-1}$  mean? Is this fast or slow?

#### **5. Optional exercise**

There is a webserver (webSDA) that can be used to run SDA simulations (<http://mcm.hits.org/webSDA>). You can use the webserver to run an additional Brownian dynamics simulation to look at the effect of changing the ionic strength has on rates.

- On the front page, scroll down to the solute upload section, and enter “barnase” as the solute name. In the PDB ID field, enter “1BRS” and make sure only chain C is selected. Keep the pH at 7. Press Upload.
- Repeat the previous step, but enter “barstar” as the name and select chain F.
- Click on the SDA Association option at the bottom of the page.
- On the next page, enter a name for your simulation, select barnase as the target solute and barstar as the mobile solute.
- Click the advanced options button, and set the ionic strength to 0 mM. Click next page.
- On the following page, you do not need to make any changes, and can press next page again. The webserver will now calculate the grid files needed for the calculation.

- On the next page click advanced options and set the number of runs to “500”. Leave the other options as their default values, and click Run SDA.
- When the simulation is finished, press Run the Bootstrap to generate the association rate data.

How do the rates that are calculated at zero ionic strength compare to those calculated at an ionic strength of 100 mM?

You could also use webSDA to perform docking simulations to see if you can predict the docked structure of the barnase-barstar complex (What effect does the choice of interaction terms selected in the simulation have on the predicted complexes?) or to run a simulation of many barnase and barstar molecules in a periodic simulation box.