Chemistry 430 — Simulation in Chemistry & Biochemistry

Laboratory #11 — Diffusional Association of the Barnase-Barstar Complex

In this lab you will use Brownian dynamics (BD) simulations to compute the association rate constant (*i.e.,* k_{ON}) and residence time (related to k_{OFF}) for the dimerization interaction of the barnase and barstar proteins. The protocol involves computation of the electrostatic potential of each protein using the APBS Poisson-Boltzmann solver, and then generation of BD trajectories via the SDA7 (***S***imulation of ***D***iffusional ***A***ssociation) program package developed by Prof. Rebecca Wade's group at the Heidelberg Institute of Theoretical Studies (H-ITS). This lab involves a number of fairly sophisticated calculations, and our major goal (in addition to estimating the above rates) is to understand the individual components.

Protocol

(1) Online documentation for the SDA7 package is available from a link under the SDA7 entry in the Software Resources section of the course web site. Two papers that explain the SDA calculation protocol and its application to barnase-barstar are also provided on the web site for this lab (**`biophysj-72-1917-97.pdf`** and **`methods-14-329-98.pdf`**). You will want to refer to all of these as you start to analyze your data.

(2) Download the **`sda7.tar.gz`** file from the web site for this lab. Move the file to your home directory. Use gunzip to decompress the file (if necessary), and unpack the resulting **`.tar`** file via the command **`tar xvf sda7.tar`**. This will put the SDA7 package into an **`/sda7`** directory. Inside this directory will be the **`/bin`** subdirectory with SDA7-related executables, **`/bin-apbs`** with the same APBS executable we used in an earlier lab, and an **`/examples`** directory containing several test cases and examples.

(3) In the **`/examples`** directory created in step 2, we will do some of the barnase-barstar calculations. The files for this system are located at **`/examples/bnbs`**. Under the **`/bnbs`** subdirectory, we first prepare the system via a script in the **`/prepare_grids_and_ecm`** area, then compute the association constant in the **`/bnbs_assoc`** directory, and finally determine the residence time of the dimer complex in the **`/bnbs_koff`** directory.

(4) The **`/prepare_grids_and_ecm`** directory contains the two proteins, barnase and barstar, as PDB files without any hydrogen atoms: **`p1_noh.pdf`** and **`p2_noh.pdb`**. Use VMD or another file viewer to look at these proteins. Which one is barnase and which is barstar? Just from looking at the individual proteins, can you predict which portions of the structures interact upon forming the dimer? There are multiple crystal structures of the dimer complex in the PDB, for example PDB codes 1BRS and 2ZA4. Download one of the dimer structures from the PDB web site, and take a look in VMD. Can you now rationalize the binding mode?

(5) Still in the **`/prepare_grids_and_ecm`** directory, run the **`run_ed_hd_ecm.sh`** script at the command line in a terminal window. The script will run APBS on each protein, compute the electrostatic potential on a grid, and then find the ECM (Effective Charge

Model) for each protein. For information on ECM, click on the ecm.html link on the web page for this lab. Take a look at the **`run_ed_hd_ecm.sh`** script and see if you can follow the sequence of steps it performs.

(6) Now move to the **`/bnbs_assoc`** directory, run the **`script_assoc.sh`** script at the command line in a terminal window. The script will run 2500 Brownian dynamics trajectories for the pair of proteins, starting at a moderate separation distance, and checking to see if each trajectory results in complex formation (or not). As before, take a look at the **`script_assoc.sh`** script and see if you can understand what it is doing. The script first does 2500 trajectories, and then uses a bootstrapping statistical method to get the average association rate as a function of distance and number of contacts, which is written to the file **`rates_average`**. It then performs five separate blocks of 500 trajectories and computes the average and standard deviation of the association rate as a function of distance and number of contacts, as written to the file **`rates_combined`**. In both cases the distance is varied from 3.0 to 20.0 Ång, and the number of contacts is varied from 1 (the first section of results in the output files) to 4 (the last section).

(7) Finally, go to the **`/bnbs_koff`** directory and run the **`script_koff.sh`** script. Inspect the script, and figure out which calculations it is performing. In this case, the final output file is **`residence_time`**, which contains four sections, representing 1 to 4 contacts. For each section, the off rate is given as a function of distance, which again ranges from 3.0 to 20.0 Ång.

Questions:

(1) The results from your association BD simulations are for 1 to 4 residue-residue contacts between the two proteins at distances between 3 and 20 Ång. Plot the reaction rates for 1-, 2-, 3-, and 4-contacts as a function of distance. Do the curves ever cross? Why?

(2) The experimentally observed association rate is about 2.86×10^8 M^{-1} s^{-1}. At what reaction distance does your simulated 3-contact rate equal this measured value? Why is the association rate higher for distances greater than this point (or less for shorter distances)?

(3) Explain briefly how the SDA calculations for the association rate (k_{on}) and residence time (related to k_{off}) differ.