

EMBO Practical Course -2008

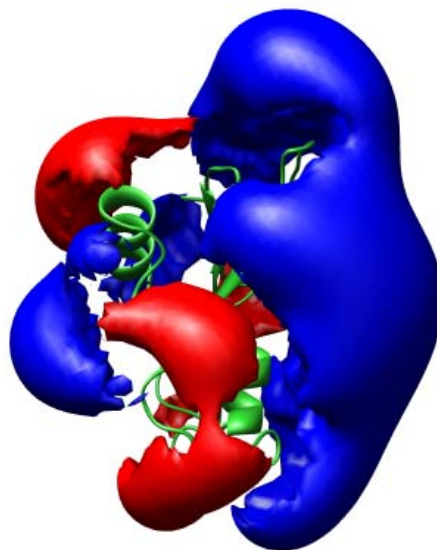
„Biomolecular Simulation“

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Protein Electrostatic Potentials:

Calculation with UHBD and Analysis

**First Part of the Practical on Brownian Dynamics
Simulation**



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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).

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

-In Brownian dynamics simulations (3), the diffusional motion of proteins is generally modelled 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 may 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).

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 EML Research (see: <http://projects.villa-bosch.de/mcm/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.

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://projects.villa-bosch.de/mcm/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

Calculation of electrostatic potentials with UHBD

[The UHBD \(University of Houston Brownian Dynamics\) program](http://adrik.bchs.uh.edu.uhbd.html) is capable of solving the linear and non-linear Poisson-Boltzmann equation using a finite-difference method. In addition, the program can be used to perform Brownian dynamics simulations of the association of two molecules and of the internal dynamics of a protein. Steady and non-steady state rate constants of encounter of a molecule (e.g. substrate/inhibitor) with a target (e.g. enzyme) can be computed from the Brownian dynamics simulations. The UHBD code can also be used to perform stochastic dynamics calculations or molecular mechanics energy minimizations using Poisson-Boltzmann and/or other molecular mechanics forces. The program also has the ability to compute electrostatic free energies of binding for two molecules as well as non-electrostatic surface area dependent terms.
(<http://adrik.bchs.uh.edu.uhbd.html>)

The aim of this part of the practical is to compute and examine the electrostatic potentials of two proteins, barnase and barstar, which associate quickly to form a high-affinity complex.

0. Set up the required files.

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

```
mkdir uhbd
cd uhbd
```

0.2. Copy the following files from /opt/tutorial-UHBD/ to uhbd directory by typing:

```
cp /opt/tutorial-UHBD/1h.pdb .
cp /opt/tutorial-UHBD/2h.pdb .
cp /opt/tutorial-UHBD/uhbd.inp .
cp /opt/tutorial-UHBD/uhbd.qtable.dat .
```

1h.pdb	coordinates of barnase prepared for uhbd calculations by adding polar hydrogen atoms
2h.pdb	coordinates of barstar prepared for uhbd calculations by adding polar hydrogen atoms
uhbd.inp	input script for uhbd
uhbd.qtable.dat	file defining atomic charges and radii (OPLS parameter set)

1. Analyze the input files:

uhbd.qtable.dat, uhbd.inp, 1h.pdb and 2h.pdb.

- 1.1. Why does the first residue in 1h.pdb have two names?
- 1.2. Which other residues in 1h.pdb have non-standard names? (there are two more of these)
- 1.3. What charge is assigned to the NH1 atom of residue Arg ? And to the NH2 atom of residue Arg ? (refer to uhbd.qtable.dat)
- 1.4. What is the size of the grid (in Angstroms) over which the electrostatic potential will be computed?

2. Run the UHBD calculation:

2.1. Type:

```
/opt/tutorial-UHBD/uhbd.exe < uhbd.inp > uhbd.out
```

This calculation will take about 30 seconds.

3. Examine the 3 output files that appear in your directory:

- 3.1. Referring to the file uhbd.inp, try to figure out why the files uhbd1.grd and uhbd2.grd are so large and how one could make them 8 times smaller. (note that no protein atom should extend beyond the grid)
- 3.2. Browse the uhbd output file (uhbd.out) to find out what the net charges of barnase and barstar are.
- 3.3. Browse the uhbd output file to check if the NH1 atoms of Arg residues are indeed assigned charge of -0.8e.
- 3.4. Browse the uhbd output file to check if convergence of the electrostatic potentials was achieved and, if so, how many iterations were required.

4. Visualize the electrostatic potentials:

- 4.1. The UHBD grid files are in a binary format and must be converted to ASCII file format in order to be readable into visualization software, e.g. VMD or PYMOL.

4.2. Convert the grid output files:

4.2.1. Copy the script „rua.tcl” from /opt/tutorial-UHBD/ into your working directory

4.2.2. /opt/tutorial-UHBD/bin2ascii uhbd1.grd uhbd1A.grd

4.2.3. /opt/tutorial-UHBD/bin2ascii uhbd2.grd uhbd2A.grd

4.3. Display electrostatic potential isocontours in VMD (version 1.8.4):

4.3.1. Start VMD by typing:

```
vmd
```

4.3.2. Load 1h.pdb and 2h.pdb (coordinates of barnase and barstar, respectively)

4.3.3. Type in the console window „source rua.tcl”

4.3.4. Load electrostatic potentials by typing „readUHBD uhbd1A.grd” and „readUHBD uhbd2A.grd” in the console window.

4.3.5. Open the „Graphical Representations” menu as follows:

In the „VMD Main” window, go to the „Graphics” pull-down menu and click on „Representations”.

4.3.6. Select the molecule for which the electrostatic potential will be displayed using the „Selected Molecule” menu.

4.3.7. Create a new representation by clicking the „Create Rep” button and then set

- „Drawing Method” to „Isosurface” (you should see the boundary of the grid used to compute the electrostatic potential)
- „Boundary” to „None”
- „Representation Method” to „Solid Surface”
- „Isovalue” to desired values, e.g. -0.6 (equivalent to KT in kcal/mol/e at 300K for a unit point charge). Press return to

have a new value of the isovalue accepted.

- „Coloring Method” to „ColorID” and then give color e.g. „1” (red) for negative potential isocontours and „0” (blue) for positive potential isocontours.

4.3.8. Create further electrostatic potential isocontours at different contour levels by repeating 4.3.6. and 4.3.7.

4.3.5. Toggle the different contours on and off by double-clicking on the line describing the chosen representation in the blue menu.

4.3.6. When two proteins and their grid files are loaded into VMD, rotate them independently and together by using the „Molecule Toggle Fixed” and „Molecule Toggle Active” options in the VMD main window.

Can you identify the electrostatic complementarity between barnase and barstar at the interface?

Are the isocontours consistent with the net charges of the proteins (see 3.2)?

Do the proteins' electrostatic potentials appear to be predominantly monopolar, dipolar, quadropolar or higher order multipolar?

5. Dependence of protein electrostatic potentials on solvent properties

Try investigating the effects of altering the solvent properties on the electrostatic potentials. You can do this by editing file `uhbd.inp` and then repeating sections 2, 3 and 4.

In `uhbd.inp` you can try changing the ionic strength (`ios`; e.g. set „`ios = 0`”, `ios` is given in mM) or the solvent relative dielectric constant (`sdie`; e.g. Set „`sdie=10`”). Note how the extent of the electrostatic potential isocontours changes according to these parameters.

6. Investigating the interface with MolSurfer

- Investigate barnase-barstar interface in more details using the MolSurfer Tutorial.

<http://projects.villa-bosch.de/mcm/software/molsurfer>.

You can also explore the demonstrations on the website which show how

MolSurfer can be used to study protein-protein and protein-DNA interfaces and the results of molecular dynamics simulations of ligand egress from a buried binding site in a protein.

NB – to run Molsurfer, you need to have java-enabled web browser. Currently, any version for Windows, and java versions 1.3.* (or 1.5.*, but not 1.4.*) or below for Linux and Irix can work.

7. PIPSA

If you want to compare the electrostatic potentials of structurally related proteins, you might want to try using PIPSA, either as a standalone software or from the webPIPSA webserver. See:

<http://pipsa.eml.org>