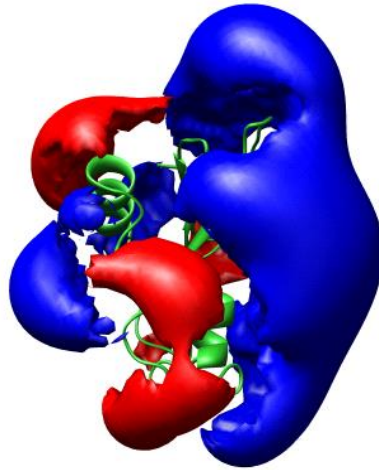


# First Part of the Practical on Brownian Dynamics Simulation

## Protein Electrostatic Potentials: Calculation with APBS and Analysis



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## **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 estimation of the bimolecular association rate constants of proteins, and for predictions to be made of 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 which 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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## **Calculation of electrostatic potentials with APBS**

[The APBS \(Adaptive Poisson-Boltzmann Solver\)](#) is a software package for modeling biomolecular solvation and calculating electrostatic potentials through solution of the Poisson-Boltzmann equation (PBE), one of the most popular continuum models for describing electrostatic interactions between molecular solutes in salty, aqueous media. Continuum electrostatics plays an important role in several areas of biomolecular simulation, including:

- Simulation of diffusional processes to determine ligand-protein and protein-protein binding kinetics (Brownian dynamics).
- Implicit solvent molecular dynamics of biomolecules.
- Solvation and binding energy calculations to determine ligand-protein and protein-protein equilibrium binding constants.
- Computational titration of biomolecules, for example to estimate the pKa of protein amino-acid side chains.

APBS was designed to efficiently evaluate electrostatic properties for such simulations for a wide range of length scales, enabling the investigation of molecules with tens to millions of atoms. APBS also includes implicit solvent models of nonpolar solvation which accurately account for both repulsive and attractive solute-solvent interactions.

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. Move from your home directory to the directory for this practical, create a working directory for APBS, and copy a required file into it:

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

```
mkdir apbs
cd apbs
cp ../tutorial-APBS/multiplyGrids.pl .
```

0.2. Download the atomic structure of barnase and barstar complex from the Protein Data Bank (1BRS) and extract chain C for barnase and chain F for barstar :

- Download the PDB structure:

```
wget http://www.rcsb.org/pdb/files/1BRS.pdb
```

- Extract chain C:

```
awk '/^ATOM/ && $5=="C"' 1BRS.pdb > barnase.pdb
```

- Extract chain F:

```
awk '/^ATOM/ && $5=="F"' 1BRS.pdb > barstar.pdb
```

0.3. Convert the two PDB files into the PQR format, which includes atomic charges and radii:

- Stand-alone version:
  - In apbs folder run the following commands to get PQR files.
  - For barnase:

```
../pdb2pqr-linux-bin64-2.0.0/pdb2pqr --with-ph=7.0 --ph-calc-  
method=propka --ff=amber --ffout=amber barnase.pdb  
barnase.pqr
```

- For barstar:

```
../pdb2pqr-linux-bin64-2.0.0/pdb2pqr --with-ph=7.0 --ph-calc-  
method=propka --ff=amber --ffout=amber barstar.pdb  
barstar.pqr
```

- Web-server version (if the stand-alone version is unavailable):
  - Start your web browser and go to: <http://nbcrc-222.ucsd.edu/pdb2pqr 2.0.0/>
  - Upload barnase.pdb and submit (choose the AMBER force field and output naming scheme). After ca. 30 sec. you should be able to download the barnase.pqr file. Save it in the apbs directory.
  - Upload barstar.pdb and submit. After ca. 30 sec. you should be able to download the barstar.pqr file. Save it in the apbs directory.

0.4. Copy the input file for apbs:

```
cp ../tutorial-APBS/apbs.inp .
```

## 1. **Analyze the input files:**

barnase.pqr	Coordinates of barnase prepared for APBS calculations by adding all hydrogen atoms, and defining atomic charges and radii.
barstar.pqr	Coordinates of barstar prepared for APBS calculations by adding all hydrogen atoms, and defining atomic charges and radii.
apbs.inp	Input script for APBS.

1.1. What is the net charge of the residue 3 (Val) in barnase.pqr?

1.2. In what protonation state are the histidine residues in the PQR files? Is this assignment reasonable? How can you check it?

1.3. What charge is assigned to the NH1 and NH2 atoms of Arg residues? (refer to the PQR

files)

1.4. What is the size of the grids (in Angstroms) over which the electrostatic potentials will be computed? (Check in apbs.inp)

## **2. Run the APBS calculation:**

APBS can be run with the web-server or standalone. Here, we will use a locally installed apbs executable.

2.1. Run the calculation.

```
apbs apbs.inp > apbs.out
```

This calculation will take about 30 seconds.

## **3. Examine the output files that appeared in your directory:**

3.1. Referring to the file apbs.inp, try to figure out why the files barnase.apbs.grd and barstar.apbs.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 APBS output file (apbs.out) to find out what the net charges of barnase and barstar are (can also be seen in input pqr file).

3.3. Browse the APBS output files to check if convergence of the electrostatic potentials calculations was achieved and, if so, how many iterations were required (see file io.mc).

## **4. Visualize the electrostatic potentials:**

The APBS grid files are in ASCII format and directly readable to visualization software, e.g. VMD or PyMOL.



4.1. Multiply each electrostatic grid with the corresponding exclusion grid to zero electrostatic values inside the protein:

```
perl multiplyGrids.pl barnase.excl.grd barnase.apbs.grd >  
barnase.ep_excl.grd
```

```
perl multiplyGrids.pl barstar.excl.grd barstar.apbs.grd >  
barstar.ep_excl.grd
```

4.2. Display electrostatic potential isocontours in VMD:

4.2.1. Start VMD by typing:

```
vmd
```

4.2.2. Load barnase.pqr and barstar.pqr as "New Molecules"

4.2.3. Load the electrostatic potentials into the respective pqr files

- Right click on barnase.pqr and select 'Load Data Into Molecule'.
- Choose the file barnase.ep\_excl.grd and select file type 'UHBD grid'.
- Repeat for barstar.

4.2.4. Open the "Graphical Representations" menu as follows:

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

4.2.5. Select the molecule for which the electrostatic potential will be displayed using

the "Selected Molecule" menu.

4.2.6. Create new representations (by clicking the "Create Rep" button) to visualize negative and positive isocontours

- Set "Drawing Method" to "Isosurface" (you should see the boundary of the grid used to compute the electrostatic potential)
- Set "Show" to "Isosurface"
- Set "Draw" to "Solid Surface"
- Set "Isovalue" to desired values, e.g. -1.0 (units are  $KT/e^-$  at 300 K). Press Enter to have a new value of the isovalue accepted.
- Set "Coloring Method" to "ColorID" and then give color e.g. "1" (red) for negative potential isocontours
- Repeat the same steps up above to get positive isocontour by applying 1.0 for "Isovalue" and "0" (blue) for "ColorID".

4.2.7. Create further electrostatic potential isocontours at different contour levels (change Isovalue) by repeating 4.2.6.

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

4.2.9. When two proteins and their grid files are loaded into VMD, rotate them independently and together by using the "Molecule Toggle Fixed" (F) and "Molecule Toggle Active" (A) options in the VMD main window. Further, you can press "c" to set center of rotation on a specific atom, "r" to rotate a molecule, or "t" to translate it.

- 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 apbs.inp and then repeating sections 2, 3 and 4. The

In apbs.inp you can try changing the ionic strength (e.g. replace conc 0.100 (100mM) with conc 0.000 in ionic concentration, it is given in M) 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.

5.1. In apbs/ folder create a new folder and copy the files needed for another APBS run:

```
mkdir solvent_properties
cd solvent_properties
cp ../barnase.pqr ../barstar.pqr ../apbs.inp .
```

5.2. Edit you apbs.inp file:

```
gedit apbs.inp
```

replace "conc 0.100" (100mM) with "conc 0.000" or edit the solvent relative dielectric constant to "sdie 10"

5.3. Repeat sections 2, 3 and 4

## **6. Optional exercise: Investigating the interface with MolSurfer**

Investigate the barnase-barstar interface in more details using the MolSurfer map request page, and uploading the pqr files you have created.

<http://projects.h-its.org/dbase/molsurfer/submit-elec.html>

Note: if Java is not working on your computer, you can view the 2D maps of the interaction surface.

- How complementary do you electrostatic potentials of the two proteins appear at the interface.

## **7. Optional exercise: Calculation of the electrostatic potential of ubiquitin**

- Create a new folder:

```
mkdir ubiquitin
```

```
cd ubiquitin
```

- Download the PDB structure:

```
wget http://www.rcsb.org/pdb/files/1UBQ.pdb
```

- Convert your PDB file to PQR (note the extra --drop-water option):

```
../pdb2pqr-linux-bin64-2.0.0/pdb2pqr -with-ph=7.0 --ph-calc-method=propka --ff=amber --ffout=amber --drop-water 1UBQ.pdb 1UBQ.pqr
```

- Copy and modify apbs.inp file

```
cp ../apbs.inp .
```

- Run APBS calculation:

```
apbs apbs.inp > apbs.out
```

- Check your outputs and grids. What is the net charge of ubiquitin? Is ubiquitin's electrostatic potential monopolar, dipolar or multipolar?

## **8. PIPSA**

If you want to compare the electrostatic potentials of structurally related proteins, you might want to try using PIPSA, either as a stand-alone software tool, or from the webPIPSA webserver. See: <http://pipsa.h-its.org/pipsa/>