Chemistry 430 — Simulation in Chemistry & Biochemistry

Laboratory #6 — APBS Poisson-Boltzmann Calculations on Lysozyme

In this lab you will run Poisson-Boltzmann continuum electrostatics calculations on the enzyme lysozyme using the APBS solver. The electrostatic potential will be computed and displayed in the VMD software. APBS calculations are then used to estimate the pK_a of the ASP-66 residue in the lysozyme active site, as well as other nearby residues.

Protocol

(1) Download the lysozyme PDB file from the Protein Data Bank. The PDB website is **`https://www.rcsb.org`**, and the PDB code for lysozyme is **`2LZT`**. Edit the file to remove the water molecules and ions that are present (*i.e.*, using a text editor, delete the **`HETATM`** lines below the protein itself, near the bottom of the file).

(2) Copy the APBS (**`apbs`**) executable and the PDB2PQR package (**`pdb2pqr.tar.gz`**) from the lab area on the course web site. Make a directory where you will perform the lab, and move the PDB file, the APBS executable and the PDB2PQR package to this directory. Make sure the **`apbs`** file has the "executable" attribute by issuing the terminal command: **`chmod +x apbs`**. In the same directory uncompress (using **`gunzip pdb2pqr.tar.gz`**) and then expand (using **`tar xvf pdb2pqr.tar`**) the PDB2PQR package.

(3) Process your lysozyme PDB file (**`2lzt.pdb`**) by using PDB2PQR to produce a PQR file (**`2lzt.pqr`**). A sample command to run PDB2PQR is contained in the file **`RUN-PDB2PQR`** on the lab web page.

(4) Open the PQR file in the VMD software, which is already be installed on lab computers. You can then follow the instructions from the tutorial on "APBS Electrostatics in VMD", which can be found on the lab web site as the file **`tutorial-vmd.pdf`**. Substitute your lysozyme protein for the one in the tutorial. Enter the full path name to the downloaded APBS executable as the "APBS Location", and set the "Working Directory" to **`/scratch`**. Note that after entering an input value into a box in the VMD interface, you must press the "Enter/Return" key for the value to be used.

Continue with the "Isocontour Visualization" section of the tutorial until you are able to display the electrostatic potential, with a red surface for "negative" potential and blue for "positive" potential. Save a screen shot of the potential surface to include in your lab report (on macOS, use **`Shift-Command-4`**, then drag to highlight the area to be saved).

Work through the "Visualizing Surface Potentials" section until you generate a molecular surface colored according to the electrostatic potential, analogous to the one shown in the tutorial. Save a screen shot of the colored molecular surface for your report.

(5) Repeat the APBS calculation from VMD in step 4 using different ionic strength values. The ionic strength can be set in the APBS setup "Mobile Ions" window prior to launching APBS. Try running the calculation at 0 mM, 150 mM and 1 M ionic strength. Again, save screen shots for your report on this lab.

(6) Next, we will follow the instructions from the APBS Tutorial titled "Lysozyme pK_a Example" to estimate the pK_a of the ASP-66 amino acid residue. A copy of the tutorial can be found on the lab web site as the file **`tutorial-pka.pdf`**.

(7) There will be six separate APBS calculations needed for pK_a estimation for the ASP-66 residue. Note ASP is the deprotonated, negatively charged residue with a carboxylate anion, while ASH is the protonated form with a carboxylic acid. The six calculations are:

- **(A)** the protein with ASP-66
- **(B)** the protein with ASH-66 in place of ASP-66
- **(C)** an isolated ASP residue
- **(D)** an isolated ASH residue
- **(E)** the protein with the charge of ASP-66 atoms set to zero
- **(F)** the protein with the charge of ASH-66 atoms set to zero

(8) First, prepare the input files for the six APBS calculations. It is suggested that you give the input file for each calculation a different name. The input for calculation **(A)** is given in the tutorial, and on the lab site as **`RUN-APBS`**. For calculations **(B)**, **(D)** and **(F)** use a text editor to change ASP to ASH at residue 66 in the PDB. For calculations **(C)** and **(D)**, you should remove from the input PDB file all residues except for residue 66. Then rerun PDB2PQR for all of these modified PDF files to make the corresponding PQR files. Finally, for calculations **(E)** and **(F)**, the atomic charges for residue 66, found in the next to the rightmost column of the PQR file, should be set to zero. Then run APBS six times, for each of the **(A)** to **(F)** systems using the command:

```
apbs foo.in | tee foo.out
```

where "foo" is replaced in turn by each of your six input file names.

(9) Once you have the APBS electrostatic energy from the output of each of the above calculations, construct a thermodynamic cycle to estimate the pK_a of the ASP-66 residue in the protein. You will also need the "intrinsic" pK_a value an isolated ASP amino acid, which can be found in a biochemistry textbook or on the internet.

(10) If you have time, repeat the tutorial protocol to compute the pK_a of residues HIS-15, GLU-35 and ASP-52.

Questions

(1) Explain why a set of six APBS calculations is needed to compute the pK_a of a single protein residue. How are the individual calculation results combined to estimate the pK_a? There is a review article in the "Readings" section on the course web site from the group of the APBS developer, Nathan Baker, which has a short description of Poisson-Boltzmann pK_a calculations (*Methods in Cell Biology*, ***84***, 843-870, 2008).

(2) Why is it usually recommended to remove any water molecules from a PDB file prior to running APBS calculations?

(3) How does the electrostatic potential around lysozyme change as a function of ionic strength? Find the active site of lysozyme, and describe the potential in the active site.

(4) Experimental pK_a values for residues ASP-66, HIS-15, GLU-35 and ASP-52 are given in the "Lysozyme pK_a Example" tutorial. How do the values you computed compare with the measured experimental values? See if you can find published literature references for these specific pK_a values. What kinds of methods are used to experimentally measure the pK_a values of protein residues?