Chemistry 478 — Molecular Modeling

Laboratory #8 — Poisson-Boltzmann Calculation on Lysozyme Using APBS

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 residues in the lysozyme active site.

Protocol

(1) Download the lysozyme PDB file (**`2LZT`**) from the Protein Data Bank. Edit the file to remove water molecules and ions that are present (*i.e.*, the HETATM lines below the protein itself, near the bottom of the file).

(2) Copy the 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 exercise, move the PDB file, the APBS executable and the PDB2PQR package to this directory. Uncompress (**`gunzip`**) and expand (**`tar xvf`**) the **`pdb2pqr.tar.gz`** file into the same directory.

(3) Process your lysozyme PDB file (**`2lzt.pdb`**) to 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 visualization software. You can then follow the instructions from the APBS tutorial for using APBS with VMD (the VMD program is already installed on all of the lab machines), substituting your lysozyme protein for the one in the tutorial. Continue until you are able to display the electrostatic potential, colored red for "negative" and blue for "positive". Save a screen shot (use Shift-Command-4, then drag to highlight the area to be saved) of the colored potential surface to include in your lab report.

(5) Follow the instructions from the APBS tutorial on computing pK_a values to estimate the pK_a of ASP 66. Repeat the tutorial protocol to compute the pK_a of HIS 15 an GLU 35. There will be six separate APBS calculations needed for each pK_a estimation.

Questions

(1) *Briefly* outline the set of APBS calculations needed to compute a single protein pK_a value. How are the individual calculation results combined to estimate the pK_a? There is a review article on the course web site from the lab 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) Repeat the APBS calculation from VMD in step 4 using different ionic strength values. How does the electrostatic potential surrounding the lysozyme change as a function of ionic strength? Find the active site of the lysozyme molecule. Describe the nature of the potential around the active site.

(4) Describe using the "thermodynamic cycle" the procedure you use to obtain pK_a values for the APBS calculations. What methods are used to experimentally measure pK_a values of protein residues? How do your estimates for ASP 66, HIS 15 and GLU 35 compare with the measured experimental values?