

# Software

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## Lysozyme pKa example

### Background

Hen egg white lysozyme (HEWL) is a very popular system for pKa calculations as it has a number of interesting values for its titratable residues. Early pKa work on this enzyme is presented in Tanford C and Roxby R ([Interpretation of protein titration curves. Application to lysozyme. Biochemistry. 11 \(11\), 2192-8, 1972](#)) which also contains the pKa values used in this example. More recent pKa calculations and a review of some of the methodology can be found in Nielsen JE and Vriend G ([Optimizing the hydrogen-bond network in Poisson-Boltzmann equation-based pKa calculations. Proteins. 43 \(4\), 403-12, 2001](#)). Finally, the biological relevance of lysozyme is briefly reviewed at [Wikipedia](#).

HEWL has two active site residues GLU 35 and ASP 52 whose titration states determine the catalytic competency of the enzyme:

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created by Nathan Baker

#### [Version 1.8](#)

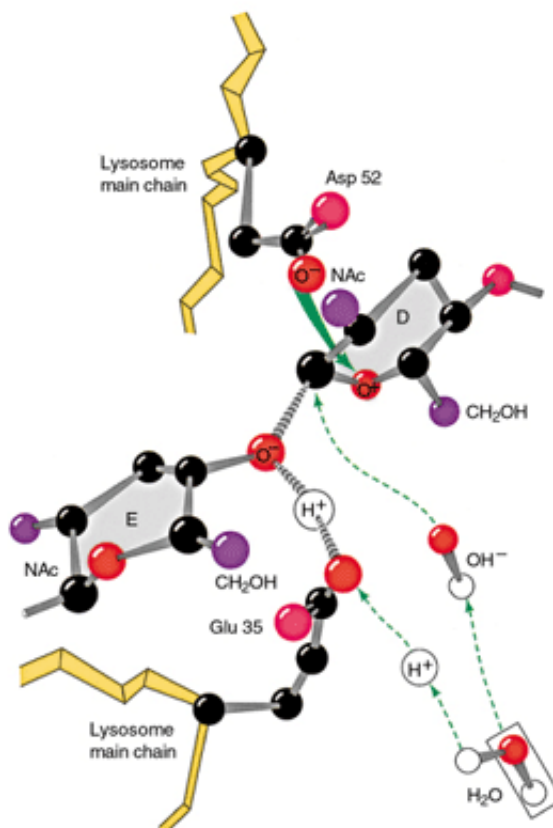
edited by Nathan Baker

#### [What does the message "WARNING! Unusually large potential values detected on the focusing boundary!" mean?](#)

edited by Nathan Baker

#### [New APBS 1.3 Windows Installer](#)

created by Nathan Baker



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In particular, the enzyme is only active when ASP 52 is ionized ( $pK_a \approx 1.2$ ) and GLU 35 is neutral ( $pK_a = 6.3$ ). Additionally, lysozyme has ASP 66 with a  $pK_a$  of 2.0 and HIS 15 with a  $pK_a$  of 5.7.

## Overview and disclaimer

In what follows, we'll calculate the *intrinsic*  $pK_a$  of ASP 66. This is the  $pK_a$  calculated without regard for the titration state changes in other lysozyme residues. Calculating actual  $pK_a$  changes requires analysis of the coupled titration state energetics of the entire system. This is an extremely labor-intensive process which is best left to computer programs such as WHATIF, PDB2PQR, MCCE, and related software.

## Preparing the PDB file

Download the PDB entry [2LZT](#) from the PDB; save it as `2LZT-ASP66.pdb`. If you have time, you should also visit the [PDBSum](#)

[parm](#)

edited by Nathan Baker

[View All](#)[analysis page](#) as well for additional information about the structure.

**Warning:** Problems with explicit water! It is very important that you remove all explicit water from the PDB file before proceeding. (Why?)

We're going to need to generate a protonated form of ASP 66 to perform our pKa calculations. We will do this with the [PDB2PQR web server](#). Unless otherwise directed, PDB2PQR adds hydrogens to residues based on model pKa values. Therefore, we will need to specify the titration state of ASP 66 for our pKa calculation by changing the residue name from ASP to ASH using your favorite text editor. Save the result as `2LZT-ASH66.pdb`.

We're now ready to run PDB2PQR to generate protonated versions of our PDB files. Use the command line version of PDB2PQR or one of the web servers listed on the PDB2PQR homepage to generate protonated PQR files for `2LZT-GLU35.pdb` and `2LZT-GLH35.pdb`. Name your results `2LZT-GLU35.pqr` and `2LZT-GLH35.pqr`, respectively. Although it is always important to test sensitivity to various force fields, I'd recommend starting with PARSE.

**Note:** You can use PDB2PQR to assign titration states with PROPKA but don't do it for the above steps since we need to set the titration states explicitly for our calculations.

Recall that we're also going to need the isolated residue for our electrostatics calculations of intrinsic pKa. Use your favorite text editor to extract the entire ASP 66 and ASH 66 residue from `2LZT-ASP66.pqr` and `2LZT-ASH66.pqr`, respectively. Save the results as separate PQR files containing only the residue of interest: `ASP66.pqr` and `ASH66.pqr`, respectively.

Finally, we'll also need to perform electrostatics calculations on HEWL with and uncharged residue 66. Use your favorite text editor to zero out the charges in `2LZT-ASP66.pqr` and `2LZT-ASH66.pqr` to create `2LZT-noASP66.pqr` and `2LZT-noASH66.pqr`. This can be done by setting the second-to-last column in the PQR file to zero; e.g.

```
ATOM 1008 N ASP 66 -1.147
0.169 33.201 -0.4000 1.5000
```

```

ATOM 1009 CA ASP 66 -0.225 -
0.753 32.484 -0.0000 2.0000
ATOM 1010 C ASP 66 0.047 -
1.937 33.399 0.5500 1.7000
ATOM 1011 O ASP 66 0.881 -
2.784 33.058 -0.5500 1.4000
ATOM 1012 CB ASP 66 1.017 -
0.081 31.957 0.0000 2.0000
ATOM 1013 CG ASP 66 1.991
0.445 32.943 0.1000 1.7000
ATOM 1014 OD1 ASP 66 1.939
0.308 34.173 -0.5500 1.4000
ATOM 1015 OD2 ASP 66 2.951
1.077 32.481 -0.5500 1.4000
ATOM 1016 H ASP 66 -0.854
0.593 34.052 0.4000 1.0000
ATOM 1017 HA ASP 66 -0.721 -
1.091 31.694 0.0000 0.0000
ATOM 1018 HB2 ASP 66 1.494 -
0.751 31.351 0.0000 0.0000
ATOM 1019 HB3 ASP 66 0.718
0.685 31.351 0.0000 0.0000

```

in the lysozyme PQR file would become

```

ATOM 1008 N ASP 66 -1.147
0.169 33.201 0.0000 1.5000
ATOM 1009 CA ASP 66 -0.225 -
0.753 32.484 0.0000 2.0000
ATOM 1010 C ASP 66 0.047 -
1.937 33.399 0.0000 1.7000
ATOM 1011 O ASP 66 0.881 -
2.784 33.058 0.0000 1.4000
ATOM 1012 CB ASP 66 1.017 -
0.081 31.957 0.0000 2.0000
ATOM 1013 CG ASP 66 1.991
0.445 32.943 0.0000 1.7000
ATOM 1014 OD1 ASP 66 1.939
0.308 34.173 0.0000 1.4000
ATOM 1015 OD2 ASP 66 2.951
1.077 32.481 0.0000 1.4000
ATOM 1016 H ASP 66 -0.854
0.593 34.052 0.0000 1.0000
ATOM 1017 HA ASP 66 -0.721 -
1.091 31.694 0.0000 0.0000
ATOM 1018 HB2 ASP 66 1.494 -
0.751 31.351 0.0000 0.0000
ATOM 1019 HB3 ASP 66 0.718
0.685 31.351 0.0000 0.0000

```

## Setting up the total electrostatic energy calculations

We will be using focusing calculations to calculate the electrostatic potential and free energies for the systems of interest.

**Warning:** In what follows, we are evaluating total electrostatic free

energies -- e.g., energies which contain charge self-interaction terms. We will cancel these self-interaction terms in subsequent steps when we calculate solvation or transfer free energies. Therefore, it is very important that you use the same grid parameters (grid centers, dimensions, spacings, etc.) for every calculation.

Here is a template input that we will use for each of the solvation energy calculations:

```

read
  mol pqr compound.pqr # This is the
  compound for which we will calculate solvation
  energies
  mol pqr ref.pqr      # This is a
  compound used as a reference for grid centering
end
elec name inhom
  mg-auto             # Focusing
calculations
  dime 129 129 129   # This is a good
  grid spacing for this system
  cglen 52.0 66.0 79.0 # These are
  reasonable coarse grid settings for this system
  (PDB2PQR-recommended)
  fglen 51.0 59.0 67.0 # These are
  reasonable fine grid settings for this system
  (PDB2PQR-recommended)
  cgcent mol 2       # Center the grid
  on the reference molecule
  fgcent mol 2       # Center the grid
  on the reference molecule
  mol 1
  lpbe
  bcfl sdh
  pdie 20.00
  sdie 78.54
  srfm smol
  sdens 40.0
  chgm spl2
  srad 1.40
  swin 0.30
  temp 298.15
  calcenergy total
  calcforce no
end
# Print the final energy
print energy inhom end
quit

```

There are a number of aspects to this input file which are worth noting:

- In general, `compound.pqr` will change for each calculation but `ref.pqr` will not. Choose one molecule to be `ref.pqr` (`2LZT-ASP66.pqr` is a good choice) and use it in every

calculation.

- We are using a solute dielectric constant ( $\epsilon_p$ ) of 20 (see [pdie](#)). This is a common choice for pKa since <begin vigorous waving of hands> it is thought to implicitly represent internal relaxation and rearrangement of the solute </end vigorous waving of hands>.
- We are using a molecular surface ([srfm](#) smol) with a reasonably high density of surface discretization points ([sdens](#) 40.0]). pKa and other electrostatics results can be very sensitive to surface choice.

You now have enough information to calculate total electrostatic energies for all of the relevant molecules so far: [2LZT-ASP66.pqr](#), [2LZT-ASH66.pqr](#), [2LZT-noASP66.pqr](#), [2LZT-noASH66.pqr](#), [ASP66.pqr](#), and [ASH66.pqr](#). You should be able to construct APBS input files for each of these systems by modifying the template above. Once these input files are constructed, you can run the PB calculation by

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

where [foo.in](#) is the input file of interest and the output is saved in [foo.out](#).

## Setting up the transfer free energy calculations

Recall the transfer free energies can be evaluated by direct subtraction of total electrostatic energies for the different dielectric environments and components. This is usually the most stable route, assuming identical grids and solute conformations are used for all calculations. You should always check for a lack of convergence in the calculations and can be resolved by decreasing the grid spacing (e.g., increasing the number of grid points).

## Putting it all together

At this point, you should have everything you need to calculate the intrinsic pKa of interest. However, if you get stuck, I've attached some example files below that might be helpful:

- [2LZT-ASP66.pqr](#) and [2LZT-ASP66.in](#)  
PQR and input files for ASP 66 in the protein







- ASP66.pqr and ASP66.in  
PQR and input files for isolated ASP 66 in solution
- 2LZT-noASP66.pqr and 2LZT-noASP66.in  
PQR and input files for the protein with an uncharged ASP 66
- 2LZT-ASH66.pqr and 2LZT-ASH66.in  
PQR and input files for ASH 66 in the protein
- ASH66.pqr and ASH66.in  
PQR and input files for isolated ASH 66 in solution
- 2LZT-noASH66.pqr and 2LZT-noASH66.in  
PQR and input files for the protein with an uncharged ASH 66
- run-apbs.sh  
A Bash shell script for running the various APBS calculations
- process.sh  
A Bash shell script for transforming the output from run-apbs.sh into a pKa shift























## What's next?

Based on this brief introduction, you should be in a good position to go back and try to evaluate intrinsic pKas for HIS 15 and GLU 35. How well do your results for those residues agree with experiment? What's different with those residues?

So far, we've only examined intrinsic pKas in this example and have ignored coupling between titratable groups. Jens Nielsen has developed a very nice software package called [pKaTool](#) which allows you to explore coupling between titratable sites and its impact on titration events in a protein system. He has provided a [tutorial \(PDF\)](#) which you can use to explore coupled titration states and use to familiarize yourself with [pKaTool](#).



 2LZT-ASH66.in (Nathan Baker, M: v.2	
 2LZT-ASH66.... Nathan Baker, M: v.2	
 2LZT-ASP66.in (Nathan Baker, M: v.2	

 2LZT-ASP66.p... Nathan Baker, M:	v.2	
 2LZT-noASH6... Nathan Baker, M:	v.2	
 2LZT-noASH6... Nathan Baker, M:	v.2	
 2LZT-noASP6... Nathan Baker, M:	v.2	
 2LZT-noASP6... Nathan Baker, M:	v.2	
 ASH66.in (1k) Nathan Baker, M:	v.2	
 ASH66.pqr (1k) Nathan Baker, M:	v.2	
 ASP66.in (1k) Nathan Baker, M:	v.2	
 ASP66.pqr (1k) Nathan Baker, M:	v.2	
 Process.sh (1k) Nathan Baker, M:	v.2	
 Run-apbs.sh (0k) Nathan Baker, M:	v.2	

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