



## Tutorial: Computation of polypeptide titration curves

For this we will use the program **pepKalc** : A tool to calculate protonation states for intrinsically disordered peptides and proteins, which I have developed together with technician Pieter van der Meulen, PhD student Kamil Tamiola and my colleague Dr. Ruud Scheek at the University of Groningen (NL). The program can be found under *Tools* at <http://www.protein-nmr.org> as a web server implementation. Please read the explanation to the tool on the web site, and then click on the link at the bottom to be transferred to the actual computation engine. After the computation you will get the results of the calculation and graphic output. All titration curves etc. can be downloaded for further work up and reporting.

You can enter many things on the form, but often 'default' values are preprinted and might suffice. Hopefully the meaning of many of the parameters is familiar to you from class and reading the 2015 review By Hass & Mulder. Read also the explanations to the right of each entry field, as well as the explanation at the bottom of the page.

Here are a few exercises for you to perform:

### 1. Get familiar with the tool

- Enter a sequence of amino acids. Use uppercase one-letter abbreviations for the 20 natural amino acids. Use a small cap 'n' for N-terminal aminogroup and 'c' to include also a C-terminal carboxylate group. For example, FRANSLIKESNMR would be a peptide where the terminal charges are removed. To be correct you should enter: nFRANSLIKESNMRC.
- Use all the default values, and have a look at the output.

### 2. How far do electrostatic interactions reach in random coil polypeptides?

- Use all the default values, except for the following:
- Enter as sequence of amino acids: nGc. This would be the amino acid glycine.
- Scroll down and select Ionic Strength = 0.0
- Click on Submit button and inspect the results
- Can you rationalize the pKa shifts? How about the Hill parameters?
- How many neutral amino acids should the protein contain between the termini before the pKa shift is below 0.1 units? And below 0.01 units?

### 3. How effective is ionic strength at screening charge-charge interactions?

- Repeat the above exercise, but this time use  $I=0.1$  M.
- How much is the electrostatic interaction energy corresponding to the pKa shift of 0.1 unit? And of 0.01 unit?
- What is the difference in this energy for the amino acid glycine, in the presence or absence of 0.1 M salt?

4. Can negative cooperativity in titration curves of proteins be interpreted by a single coupling parameter?

- Enter a sequence of seven Asp residues: DDDDDDD. You now have seven acidic groups in a linear chain, all with the same intrinsic proton affinity.
- Set ionic strength to 0.0 M (to enhance the effect)
- Submit
- Look at the titration curves (blue). Can you rationalize the result?
- The derivatives to the blue curve (red) are called 'capacitance curves'. As you can see it is much easier to detect deviation from 'ideal' titration behavior from these curves. Can you rationalize the shape of the red curve?
- The green curve is a fit to the HH equation - including a Hill parameter, and seems to fit surprisingly well for all residues. Hill parameters are typically assigned to the interaction between two groups. What does this tell you about observing curves with small Hill parameters in proteins?

5. Where does asymmetry in protein titration curves come from?

- Enter the following sequence of four Asp and four Glu residues: DDDDEEEE. You now have seven acidic groups in a linear chain, but the Asp and Glu have intrinsic proton affinities that differ by 0.4 units.
- Set ionic strength to 0.0 M (to enhance the effect)
- Submit
- Can you rationalize the titration curves observed for D4 and E5?

6. The case of strong coupling in a low dielectric, and 'back-titration'

- Enter the following sequence of three acidic residues with identical intrinsic proton affinities: DDD. Since the acidic groups are in a linear chain, the pairwise interactions of the outer and central acid are different.
- Set ionic strength to 0.1 M
- Uncheck the box to calculate the dielectric constant, and enter epsilon of values from 20 down to 4 and observe the effect of increasing the interactions between the charged forms. Note both the individual titrations as well as the development of the total charge of the peptide.
- Can you rationalize the results?

7. Enter input to your taste to answers a meaningful question :)