These questions accompany Mathias A.S. Hass & Frans A.A. Mulder "Contemporary NMR studies of protein electrostatics" *Annu. Rev. Biophys*. **44** (2015) 53-75

1. "Where are the protons"? When studying the protonation state of a residue, NMR spectroscopy comes to mind. After all, ^{1}H NMR is a sensitive technique with chemical specificity, such that a signal for each proton would be expected and its intensity could be monitored as a function of pH. Explain why this is, unfortunately, not the case. In your answer you will need to combine elements of chemical stability, kinetics and NMR detection.
2. NMR can detect spin–1/2 nuclei, like ^{1}H, ^{13}C and ^{15}N. Discuss the advantages and disadvantages of using these nuclei. Make three columns for the different nuclei, and write down a number of elements in rows (for example: sensitivity). You should list at least four different elements, and fill in the boxes of the matrix.
3. What is indirect detection? What is the meaning of an NMR experiment with the acronym H(C)CO?
4. For which amino acid side chains are 'contemporary' NMR experiments based on indirect detection of heteronuclei now available? Can you briefly outline the experiment that has been developed for Tyr residues, and mention what rational was used to follow the CG chemical shift? It seems such an odd choice.
5. What are the two influences of salt on the titration curves (i.e. their positions and appearance) shown in Figure 2, and what are the reasons for those apparent changes (i.e. the electrostatic effects)?
6. Equation 10 expresses a deceptively simple equation for the chemical shift as a function of pH for two interacting sites. Unfortunately, such a curve will be underdetermined. Which physical parameters describing the protonation equilibria underlie the titration curve?
7. What is the Hill coefficient, and what is its relation to NMR titration curves?
8. Proton binding capacitance (PBC) curves provide an intuitive visual inspection means for analyzing titration curves. What does the PBC express?
9. Is it possible to obtain genuine microscopic parameters from NMR experimentally? Answer this question using argumentation from p.69-70.
10. Read the 5 Summary Points at the end. Have you understood each of them? Do you agree?
11. Read the 5 Future Issues. Do you agree? Do you have more pressing questions, or can you think of additional areas where NMR could play a key role?