For the following questions consisting of multiple parts (a, b, c, etc), answer each part separately (i.e. label each part of your answer.) Otherwise the answer provided will be graded as just for part a.

(1) Recent advances in NMR spectroscopy have made it possible to measure to an unprecedented level of detail the pH-dependent protonation states of individual acidic and basic groups in proteins. The development of a suite of dedicated pulse sequences provides access to microscopic protonation equilibria for the titratable amino acids. The exquisite data quality that can now be routinely obtained greatly facilitates and improves the modeling of the underlying energetics, which ultimately is needed to explain pH-dependent protein stability and function. Why is it important to determine these protonation states in protein to such detail?

(2) The imidazole ring of histidine has three commonly observed protonation states.
   (a) What are these 3 states?
   (b) Generally, how are the populations of three states distributed?
   (c) What are the factors that affect these populations?
   (d) How are these three states detected by NMR methods?
   (e) Why is it important to know the protonation states of histidine?

(3) The following chemical shift vs pH plots represent different protonation sites (N) in proteins.
   (a) Give the N value for each. From left to right: (i), (ii), (iii) and (iv). Label your answers as Ni, Nii, Niii and Niv.

   \[ N = \quad \quad \quad \quad \quad \]

   (b) Are the pKa values obtainable from each of these sets of data?

(4) For two interacting protonation sites in a protein, there are both the general microscopic model and the macroscopic model for the two sites.
   (a) How many states are there in the microscopic model? Explain your answer.
   (b) How many states are there in the macroscopic model? Explain your answer.

(5) In the review article by Hass and Mulder in 2015 (Annu Rev Biophy 2015. 44:53-75), they listed 5 future issues for contemporary NMR titration studies of proteins. Please discuss any two of these 5 issues.