

Answers to Problem Set #6

Three-Dimensional Solution Structure of the N-Terminal Receiver Domain of NTRC^{†,‡}

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Figure caption. Summary of restraints used for assignment and secondary structure determination of protein X. The relative intensity of sequential NOEs is indicated by the height of the solid box ($d_{\alpha N}^{(i,i+1)}$, $d_{\beta N}^{(i,i+1)}$, $d_{NN}^{(i,i+1)}$). Horizontal lines represent medium-range NOEs ($d_{\alpha N}^{(i,i+3)}$, $d_{\alpha\beta}^{(i,i+3)}$). Filled circles reflect amide protons which are observed in spectra collected at pH 6.4, 25°C at least 1 h after dissolving the sample in D₂O. Small (<6Hz) and large (≥ 9 Hz) values of $J_{\alpha H-NH}^3$ are represented by o and x, respectively.

1. Using the data in the above figure, identify the secondary structure elements in this protein and fill them in on the final line of the table (labeled 2°).

Hint: Start by identifying helices, then identify β -strands.

Notes: helices are easy to identify by the characteristic $i,i+3$ mid-range NOEs. Helices also have small values of $J_{\alpha H-NH}^3$ and strong sequential d_{NN} , $d_{\beta N}$. β -strands are more difficult to pick out. All secondary structure should be relatively protected (still observable after 1 h in D₂O, although strands at the edge of a sheet will be less well protected). β -strands are recognized by the strong sequential $d_{\alpha N}$ and large $J_{\alpha H-NH}^3$.

2. Structure determination requires assignments.

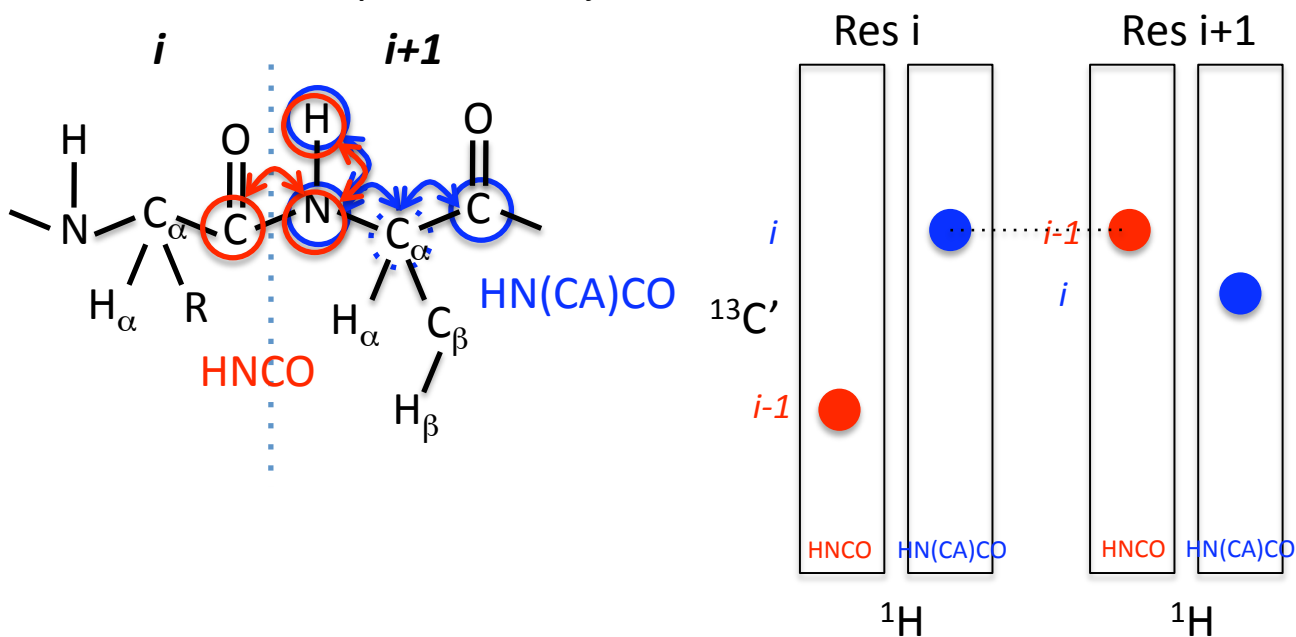
a. What interaction provides through-bond coupling useful for the assignment process?

J-coupling

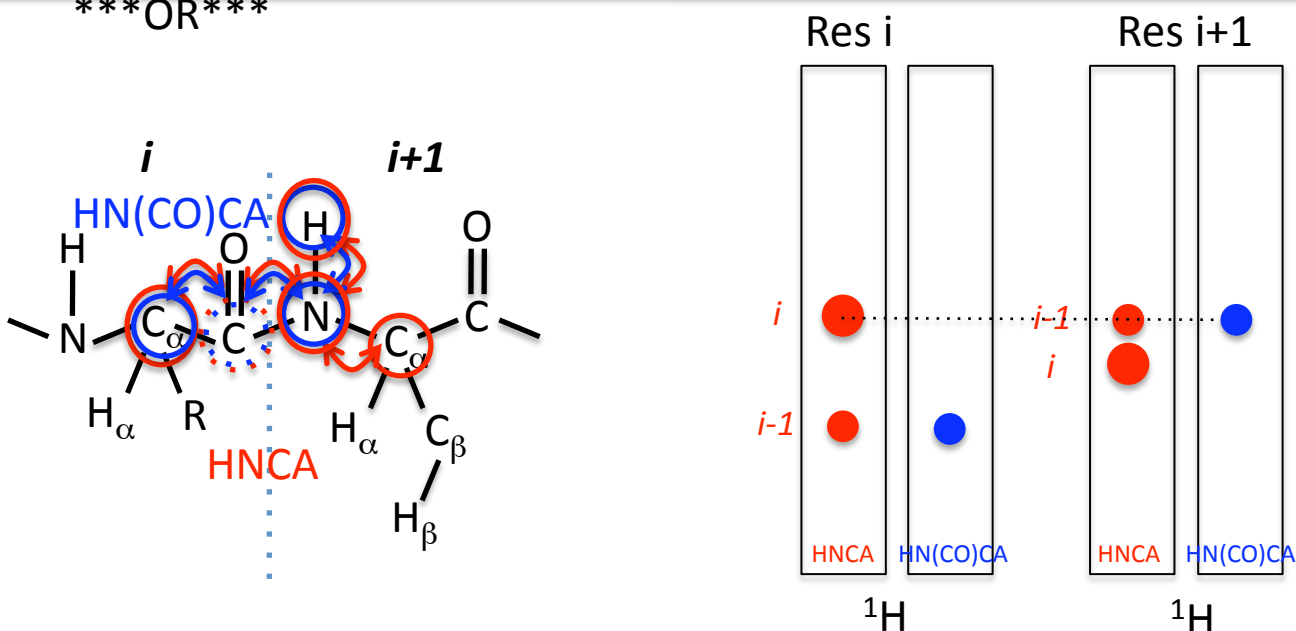
b. Name a pair of NMR experiments you would use to assign the backbone of a medium sized $^{13}\text{C}/^{15}\text{N}$ labeled protein.

HNCA, HN(CO)CA OR HNCO, HN(CA)CO

c. Draw a peptide segment and illustrate the through bond correlations you expect to observe with each experiment. Draw a sample strip of the spectrum with the expected resonances for each experiment for 2 adjacent residues, $i, i+1$.



OR



3. What does the splitting between the nuclear spin energy levels depend on? Is this splitting large or small? How does this contribute to the insensitivity of NMR as a technique?

$\Delta E = \gamma B_0$. Gyromagnetic ratio depends on the type of nucleus, B_0 is the spectrometer field strength. This splitting is small. Therefore, the population difference between the two states is small (Boltzmann distribution) and the net magnetic moment, which is the bulk magnetization we can manipulate, is quite small. This makes NMR insensitive.

4. Do you expect to see localized chemical shift changes or widespread chemical shift in the NMR spectrum of hemoglobin upon binding by oxygen? Why?

You would expect more widespread chemical shift changes. Binding oxygen will cause shifts in the local binding site. However, hemoglobin is allosterically regulated and small structural changes are propagated across the protein resulting in widespread chemical shift changes.

5. Protein X has 100 amino acids and no prolines. It is not very thermostable and is 30% unfolded at 30 °C according to CD measurements. You take an 15N-1H HSQC spectrum at 30 °C and see approximately 70 peaks that are well-dispersed plus 30 peaks that are much sharper and clustered more toward the middle of the spectrum.

a. Is your NMR data consistent with the estimate of the % unfolded you determined by CD?

Yes. The well-dispersed peaks correspond to folded amino acids, the sharp peaks clustered in the middle correspond to unfolded amino acids. So 70 folded and 30 unfolded amino acids.

b. Is unfolding a global or local process?

Local. 70 amino acids have folded properties, 30 amino acids have unfolded properties.

c. At 20 °C where protein X is fully folded, residue 23 has a proton chemical shift of 9.4ppm. At 30 °C this residues has a proton chemical shift of 8.4ppm. What is $\Delta\omega$ if you took your spectra on an 800 MHz spectrometer?

$\Delta\omega$ is 1ppm. On an 800 MHz spectrometer, 1ppm=800Hz. So the chemical shift difference corresponds to 800 Hz.

d. How fast must k_{ex} ($=k_{unfold} + k_{fold}$) be if folding/unfolding is in fast exchange?

Fast exchange means $k_{ex} \gg \Delta\omega$, so at least $8000 s^{-1}$. Under these conditions you would see a single peak at the population-weighted average position. This peak would shift from the folded to the unfolded position as the temperature is raised and the unfolded population increases.