BCH222 -- Structures by NMR

Reading

C.I. Branden and J. Tooze (1999) "Chapter 18: Determination of Protein Structures" in Introduction to Protein Structure, Second Edition, especially NMR section this week.

(optional) for more info on the glutaredoxin or eglin C NMR structures, see Xia et al., Protein Sci. **1**:310 (1992); or Hyberts et al., Protein Sci. **1**:736 (1992).

Graphics assignment - Note that all examples in this lesson are of proton NMR.

1. Open file Xia.kin and follow the text writeup in the kinemage file.

   A. **Skip KIN.1 -** don't worry about the "pseudoatoms"

   B. Kin. 2 - a pair of antiparallel beta strands in glutaredoxin, with protons color-coded by approximate chemical shift value (color coding is blue to left of a 1-D NMR spectrum, red toward the right).
   What do the triangles represent?____________
   Follow text instructions, to see the different groups of NOE's typical of β structure.

   C. Kin. 3 - an α-helix in glutaredoxin; protons color-coded as before, except that NH and CαH groups are not distinguished here. Again, follow the kinemage text to see the typical groupings of helical NOE's.

2. File Hyberts.kin (also see kinemage text)

   A. Kin. 1 - the central β-sheet, the helix below, and the somewhat isolated and extended binding loop of the protease inhibitor Eglin C. Note the relative tightness of the ensemble of NMR models in the various regions.

   B. Kin. 2 - shows that although the binding loop had a high rms deviation in an overall superposition, yet its internal structure is quite well defined, as in this local superposition based just on residues 42-46.

   C. Kin. 4 - compares one of the NMR models with 4 different x-ray structures (all done as complexes with a protease, and all 4 agreeing closely). Again, for residues 42-46, the X-ray and NMR structures agree locally (check phi, psi angles), but the loop orientation is different. Think about the issues of positive and negative evidence about the location and mobility of that loop. Is the NMR data compatible with, and/or does it prove, either that 1) the loop might always stay in the same location that the x-ray structures showed in complexes, or that 2) in solution the loop position is highly mobile as depicted in the overall NMR ensemble?

3. Look at file 5ptiPaPb.kin.

   This set of coordinates, including hydrogens, came from a joint crystal structure determination by X-ray and neutron scattering. Virtually the same structure was worked out also by NMR, and this is the molecule on which NMR protein-structure methods were first developed. We will look at one helical region and one antiparallel β region in order to understand the locations and lengths of some of the proton-proton distances typically used in NMR structure determinations. This file has side chains and hydrogens only for the part of the molecule studied in Terry Oas's folding intermediate, pαpβ. The NMR information from the synthetic pαpβ fragment is interpreted in terms of the coordinates from the crystal structure of the entire bpti molecule, basic pancreatic trypsin inhibitor.

   Choose View2 and locate the NH, αH and βH protons of Ala 48, on the helix. Measure the requested distances and insert them in the table below. $d_{\alpha N}$ for instance, is dist (αH_i-NH_{i+1}). Circle those entries you think are short enough to generate strong NOE's.

distance	Ala 48 (in helix)	Cys 30 (in sheet)
$d_{\alpha N}$		
$d_{\beta N}$		
d_{NN}		
$d(\alpha H_i - \beta H_{i+3})$		$d(\alpha H_{C30} - \alpha H_{Y23})$
$d(\alpha H_i - \beta H_{i+4})$		XXXXXXXXXX

Now choose View3 to center on Cys 30, which is in the β-ribbon. Measure the distances to fill in the table, and again circle the short ones. Compare the patterns for α-helix and antiparallel β and check that they fit what was described in the papers. For the β-sheet case, what other H-H distances look short enough to generate long-range NOE's between Cys 30 and Tyr 23? ____________________________

Now turn on "papb NOEs", which shows the long-range NOE's (but not the noe's within n ± 1 in sequence) measured in the isolated pαpβ fragment. Which NOE's were actually observed between Cys 30 and Tyr 23? ____________________________

4. File Tendami.kin

This kinemage contains 9 different distance-geometry models for the amylase inhibitor tendamistat, plus an average structure. These structures represent a second stage in the structure determination, with 800+ NOE's rather than 500+, and with stereospecific resonance assignments for the internal side chains (that is, distinguishing the two terminal methyl groups on a Leu or Val). The agreement among converged structures is good, but the distance-geometry calculations can get caught in bad local minima - these 9 models are the results of runs 46, 50, 66, 70, 73, 80, 94, 97, and 98 out of 100 runs (however, note that they apparently learned some things about how to do it as they went along). Simulated annealing is a current method that converges more reliably.

In the startup view, look at the overall structure, which is a small Greek key β barrel. Turn on the side chains. Is there anything strange about the side chains or the chain termini? ____________________________ (Hint: this is an average structure.)
Turn on some or all of the individual models, without side chains. What happens at the chain termini? ____________________________

Now turn on side chains and look at those that were normal vs. peculiar in the average structure (e.g., Tyr 37 vs. Tyr 20). This shows why people now use a minimized (re-idealized) average structure to represent the consensus of an NMR structure. Obviously, the distance constraints for tendamistat are very strong in some places (Leu 14 and Val 35 are especially well-located side chains, and the backbone in the β-sheets is extremely reproducible, including twist), fairly good in others, and almost non-existent at the N-terminus.

Center on Leu 14 and turn on the hydrogens. Find a few of the H pairs whose long-range NOE's must have helped fix it and its neighbors so accurately: ____________________________

One interesting place is near Asp 58 (View5); compare the 9 individual models. In 4 cases (models ________, ________, ________, and ________) the Asp 58 side chain is actually inside the barrel, while in the other 5 it is external. The local backbone conformations are different for these two subsets, and also the conformation of the nearby strand that includes Tyr 20 is different, in a correlated fashion. That both these possibilities are consistent with the list of constraints must mean that no side-chain NOE's were found for Asp 58, and also that the peptides surrounding it are not H-bonded. Note that both 57 and 59 are glycines, so they can do very little to resolve the ambiguity. Either 57-58 is a β-bulge or 58-59 is. Notice that it takes rather special circumstances to allow such ambiguity, and in fact in this case there is no doubt at all which version is correct.
Which? ____________________________
Why? ____

In a later NMR determination of this structure with much more data collected, all members of the ensemble agreed in this area and showed the more reasonable orientation.

Nowadays almost all NMR structures are done on samples isotopically labelled with N15 and C13, which are NMR-observable as well as the H1 protons and allow measurement of many more interatomic interactions, either through-bond couplings or through-space NOE's. These 3D and 4D experiments allow easier determination of larger structures, but the overall logic is similar to the 2D proton NMR illustrated above: first the NMR resonances must be "assigned" to individual specific atoms, and then the interactions measured which jointly determine an ensemble of possible structures.

It is also now possible to measure what is called residual dipolar coupling (or RDC) data in a partially-oriented protein sample, most often done for the backbone NH's, giving a measure of the angle between that individual bond and the magnetic field. That extra information helps greatly in determining the overall molecular shape accurately.