NMR facility

Arnout Kalverda and Steve Homans

Overview of facility

The NMR facility is equipped with one 600 MHz and two 500 MHz Varian Inova NMR spectrometers. Two of the spectrometers are housed in a purpose built extension to the Astbury building. All three instruments are setup to use ^{1}H, ^{13}C, ^{15}N and ^{2}H during normal operation. A mobile fifth channel (dedicated to deuterium) adds flexibility. One of the 500 MHz instruments is equipped with a triple resonance probe including a broadband channel to allow ^{31}P correlated experiments in nucleic acids. In the coming year the facility will be expanded with a 750 MHz NMR spectrometer.

NMR research with larger systems

By making use of the 600 MHz NMR instrument a number of projects have been instigated with larger systems. Ongoing projects are Verotoxin B subunit pentamer (35 kDa), arabinose binding protein (37 kDa), heat labile enterotoxin B subunit pentamer (58 kDa) and Class II FBP-aldolase (78 kDa). In all these cases, ^{2}H, ^{13}C, ^{15}N triply-labelled protein is needed. For the two proteins in the 30-40 kDa range it was found that a deuteration level of 50% was adequate for providing good sensitivity for triple resonance experiments. The presence of partially protonated side chains also allows both the experiments for backbone assignments as well as side chain assignments to be acquired with a single sample. By using TROSY based triple resonance experiments modified for use with partially deuterated proteins, backbone assignments for arabinose binding protein are nearing completion. In the case of systems with a size above 50 kDa it is crucial that perdeuteration is as complete as possible. This was key to the successful recording of TROSY HNCA, HN(CO)CA and NOE spectra on FBP-aldolase.

Relaxation measurements of protein dynamics

The key to affinity in protein-ligand interactions lies with the thermodynamic parameters of binding. NMR is uniquely poised to extend global thermodynamic parameters to a more detailed residue specific view. Changes in main chain and side chain dynamics make a contribution to the entropy changes upon ligand binding. ^{15}N relaxation measurements report on the dynamics of the backbone while ^{2}H and ^{13}C relaxation methods provide information on side chain dynamics. Changes in protein dynamics are being mapped out in two protein ligand systems, Verotoxin B subunit and mouse urinary protein. Both proteins can bind to an array of closely related ligands. This allows an attempt to correlate dynamic and thermodynamic differences between ligands.

NMR of protein folding and misfolding

Information about the structural features of partially folded states can be obtained indirectly through hydrogen exchange and/or denaturant titration experiments followed by ^{1}H-^{15}N HSQC spectra. Denaturant titrations were used to monitor the unfolding of a partially folded amyloid precursor of β_2-microglobulin. The experiment indicates that the 5 central β-strands of the native fold form the most stable region of the intermediate and that it unfolds in a highly non-cooperative manner. Native state hydrogen exchange has been used with the Colicin Immunity protein Im7 to show that the hydrogen exchange data provide information on the secondary structure of an intermediate state.

Publications

Gell, C., Brockwell, D.J., Beddard, G.S., Radford, S.E., Kalverda, A.P. & Smith, D.A. (2001) Accurate use of single molecule fluorescence correlation spectroscopy to determine molecular diffusion times. *Single Mol.* **2**, 177-181.

Giesen, A.W., Bae, L.C., Barrett, C.L., Chyba, J.A., Chaykovsky, M.M., Cheng, M.C., Murray, J.H., Oliver, E.J., Sullivan, S.M., Brown, J.M., Dahlquist, F.W. & Homans, S.W. (2001) Measurement of one-bond $^{1}H^{\alpha}$-$^{13}C^{\alpha}$ couplings in backbone labelled proteins. *J. Biomol. NMR* **19**, 255-260.

Kjellberg, A. & Homans, S.W. (2001) Residual ^{2}H quadrupolar couplings in weakly aligned carbohydrates. *J. Magn. Res.* **151**, 90-93.

Reader, J.S., VanNuland, N.A.J., Thompson, G.S., Ferguson, S.J., Dobson, C.M. & Radford, S.E. (2001) A partially folded intermediate species of the beta-sheet protein apo-pseudoazurin is trapped during proline limited folding. *Protein Science* **10**, 1216-1224.

Funding
We thank the Wellcome Trust and BBSRC for funding.