DEPARTMENT OF BIOCHEMISTRY
UNIVERSITY OF OXFORD

Biomolecular Solution-state NMR in the Department of Biochemistry

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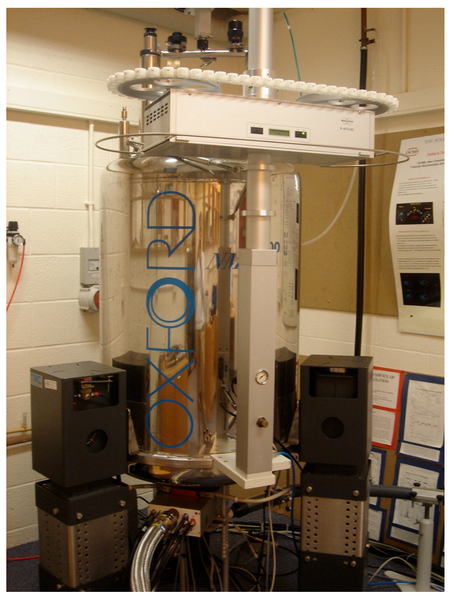
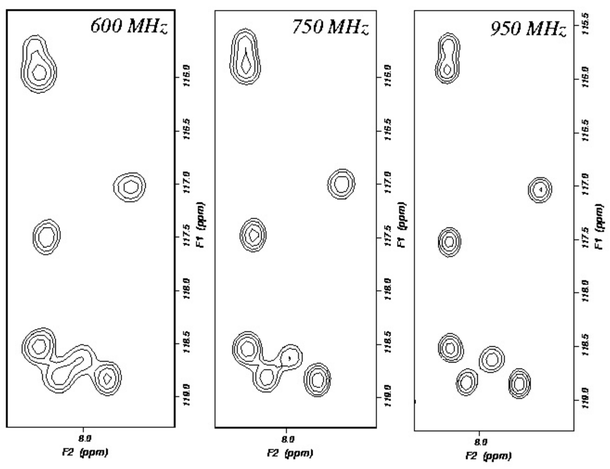
The solution-state NMR facility in the Department of Biochemistry has four NMR spectrometers operating at ^{1}H frequencies of 500, 600, 750 and 950 MHz. These spectrometers are used primarily for the study of proteins. The research interests and areas of expertise of the research groups using the facility include protein structure determination (both soluble globular and membrane proteins), protein backbone dynamics on both fast (ps-ns) and slower (us-ms) timescales, protein folding and partially folded proteins, and protein-ligand interactions (both small and macromolecule ligands). An overview of the facility will be presented along with examples of the research we carry out.



500 MHz system. Bruker AVANCE console and a TCI cryoprobe with an automatic sample changer. Also a 600 MHz system with a Bruker console & cryoprobe with ^{19}F capability.

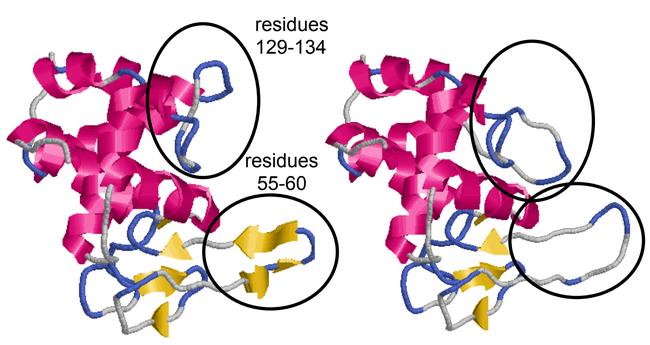
First commercial 750 MHz magnet. Equipped with a home-built console and an inverse ^{1}H/^{15}N/^{13}C triple resonance probehead with actively-shielded triple-axis gradients.

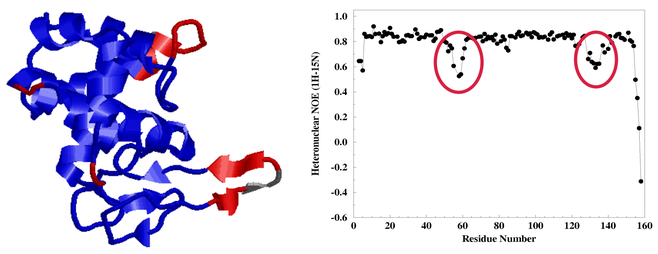
The first commercial 950 MHz (22.3 Tesla) magnet was delivered in 2005 (by Oxford Instruments). This spectrometer is equipped with a home-built console and an inverse ^{1}H/^{15}N/^{13}C triple resonance probehead with actively-shielded triple-axis gradients.



Higher field provides improved resolution as demonstrated by the expanded region from ^{1}H-^{15}N HSQC spectra of lysozyme from lambda phage collected on our 600, 750 and 950 MHz spectrometers.

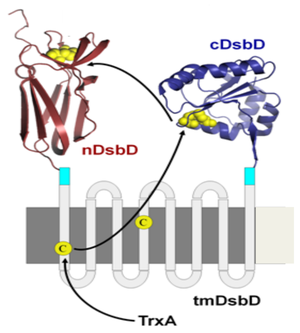
PROTEIN DYNAMICS



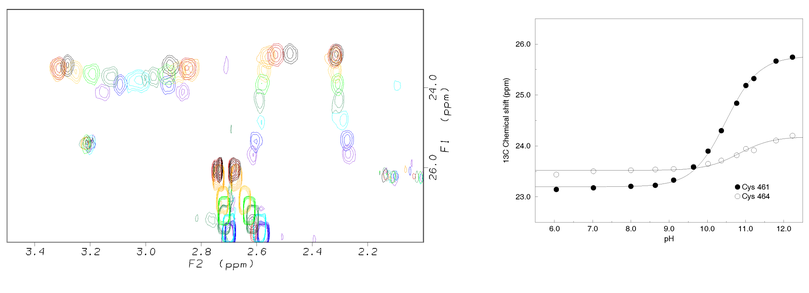


Molecules in open and closed conformations are observed in crystals of lysozyme from λ phage. ^{15}N relaxation measurements show two regions of increased flexibility in the backbone of λlys in solution.

PROBING ACTIVE-SITE REACTIVITY

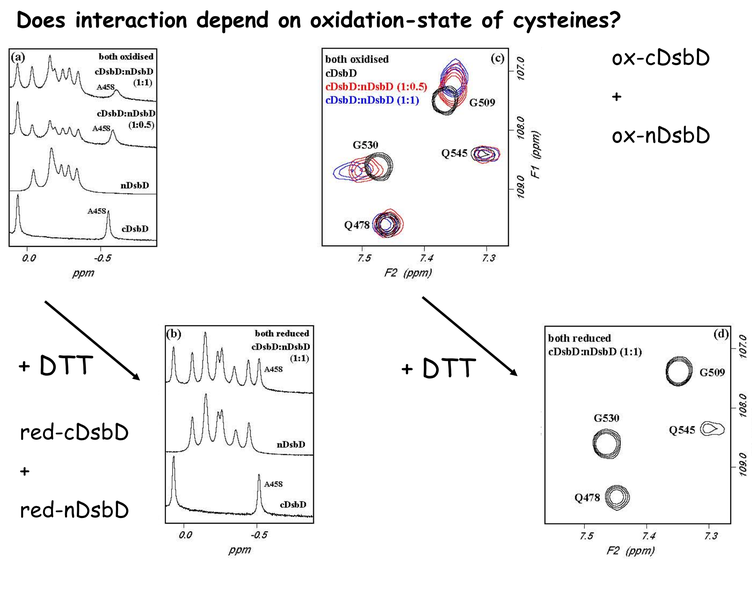


DsbD is a three-domain bacterial oxidoreductase that transfers reductant from cytoplasmic Trx to the Ccm and Dsb pathways in the periplasm.



Cysteine pK_a values in the active site CXXC motif of cDsbD can be measured using ^{1}H-^{13}C HSQC spectra. An unusually high pK_a value of 10.5 is measured for Cys 461 in the active site of DsbD. This high value makes C461 a poor nucleophile but protects it from futile re-oxidation by DsbA.

PROTEIN-PROTEIN INTERACTIONS



The affinities of the interaction between nDsbD and cDsbD depend on the oxidation states of the two domains. Line broadening and chemical shift changes are observed when both proteins are oxidised while no interaction is observed when both proteins are reduced.

References

1. L.J. Smith, A. Bowen, A. DiPaolo, A. Matagne and C. Redfield, *The Dynamics of Lysozyme from Bacteriophage Lambda in Solution probed by NMR and MD simulations*, ChemBioChem 14, 1780-1788 (2013).
2. D.A.I. Mavridou, J.M. Stevens, S.J. Ferguson and C. Redfield, *Active-site properties of the oxidized and reduced C-terminal domain of DsbD obtained by NMR spectroscopy*, J. Mol. Biol. 370, 643-658 (2007).
3. D.A.I. Mavridou, E. Saridakis, P. Kritsiligkou, A.D. Goddard, J.M. Stevens, S.J. Ferguson and C. Redfield, *Oxidation-state-dependent protein-protein interactions in disulfide cascades*, J. Biol. Chem. 286, 24943-24956 (2011).

For further information about the Department of Biochemistry NMR facility contact Christina Redfield via email (christina.redfield@bioch.ox.ac.uk) or by phone (x75330).