Lincoln College Biochemistry Tutorials

Dr. Mark C. Leake (m.leake1@physics.ox.ac.uk)

Michaelmas Term, Tutorial 4

Title: Protein structure – Basic 1D resonance techniques (Lincoln)

This week's work will cover simple resonance spectroscopy, mostly nuclear magnetic resonance (NMR) but also some electron spin resonance (ESR). It will extend a beyond just proteins to cover other areas as well. As usual, concentrate on applications rather than detailed theory. Will cover protein structure and dynamics by 2D and 3D NMR later.

You should cover (make notes on) the following areas --
1. Basic principles -- nuclear (I) and electron (S) spins and magnetic dipole moments, energy levels for magnetic dipoles in an applied magnetic field (2I+1 or 2S+1 levels), bulk behaviour of magnetic moments (magnetisation).
Types of spectroscopy :- NMR, radio-wave, absorption (CW) and emission (FT) spectroscopy, technical advantages of FT NMR. ESR, micro-wave, absorption and 1^{st} derivative (observed) spectra.
Types of species that can be observed :- NMR, nuclei, spin 1/2 (1H, 13C, 15N, 19F, 31P, etc.), spin > 1/2 (2H, etc.). ESR, unpaired electrons (nitroxide radical, metal ions, etc.)
2. Spectral parameters that can be observed -- what information can we get from each parameter?
NMR: chemical shift (frequency), intensity, fine-structure (J-coupling), linewidth and T1 and T2 relaxation rates. Effects of dynamic processes (motion, chemical exchange) on these. Effects of paramagnetic ions. Magnetisation transfer. For I > 1/2, quadrupolar couplings and line shape, effect of dynamics on line shape.
ESR: g-value (frequency), intensity, fine-structure (hyper-fine interactions), linewidth and relaxation rates, anisotropy.
3. Particular topics -- not an exclusive list, but some pointers.
NMR - covalent structure of natural products, etc.
ligand/drug binding, binding constants, rate constants, membrane studies, specific labelling with 13C or 2H, fluidity *in vivo*, intracellular pH, metabolites, ion transport imaging and microscopy, mostly biological and medical uses
ESR - dynamics, for both solution and membrane applications
angular orientation wrt membranes
distance determination between two spin-labels
spatial localisation using chemical reduction

References
Standard general texts + OLIS + WinSpirs + etc.
Books:

S. Chien and C. Ho (ed.), (1986) "NMR in biology and medicine", pub. Raven - more advanced specialist articles.

A. Derome, (1987) “Modern NMR techniques for chemical research”, pub. Pergamon.
P.J. Hore, (1996) “Nuclear Magnetic Resonance”, Oxford Chemistry Primers, pub. OUP - very simple introduction to the theory.
H.W.E. Rattle, (1995) “An NMR primer for the life sciences”, pub. Partnership – easy start to both theory and applications.

Much more advanced books :-
G.M. Clore and A.M. Gronenborn, (1993) “NMR of proteins”, pub. Macmillan.
J.S. Evans, (1995) “Biomolecular NMR spectroscopy”, pub. OUP.
Articles:
(1985) Biochem. Soc. Trans., **13**(3), p.542-633 - series of wide-ranging articles on ESR.
J.B. Aguayo et al., (1986) Nature, **322**, p.190-191 - “NMR imaging of a single cell”, only for interest to see what can be done.
P.S. Belton and R.G. Ratcliffe, (1985) Prog. in NMR Spec., **17**, p.241-279 - “NMR and compartmentation in biological tissues”, - more advanced but very good.
K.M. Brindle and I.D. Campbell, (1987) Quart. Rev. Biophys., **19**, p.159-182 - “NMR studies of enzyme kinetics in cells and tissues”.
J.M. Knight, I.C. Shaw and A.P. Jones, (1996) Chemistry in Britain, **32**(6), 37-40 - brief survey of NMR in living systems.
G.K. Radda, (1986) Science, **233**, p.640-645 - “The use of NMR spectroscopy for the understanding of disease”.
D.D. Thomas, T.M. Eads, V.A. Barnett, K.M. Lindhal, D.A. Momont and T.C. Squier, (1985) in "Spectroscopy and the dynamics of molecular biological systems", pub. Academic Press, p.239 - “Saturation transfer EPR and triplet anisotropy: complementary techniques for the study of microsecond rotational dynamics”, introduction is a nice account of general ESR.

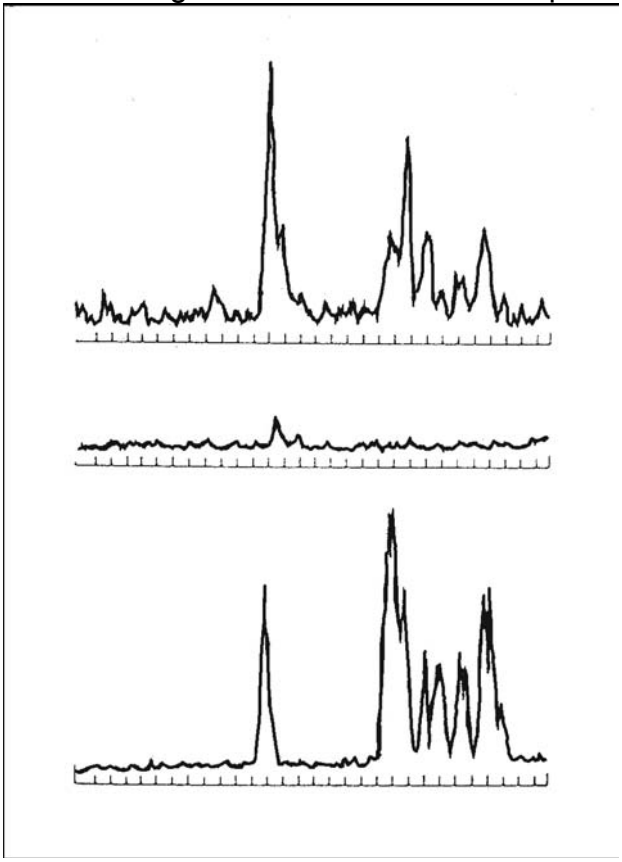
Problems
All submitted material to be attached as one bundle from each separate student, to be clearly marked with the title of the tutorial, the date, the name of the student, and to clearly display “FAO Dr. Mark Leake, Clarendon Lab” on the first page. To be handed in to either the receptionist, or placed in the “L” pigeon-hole, of the Clarendon Laboratory, Dept of Physics by *12 noon* the day before the tutorial.

1). Predict both the 1H and 13C NMR spectra of a) pure ethanol and b) pure ethanol with a trace of acid. Why do the two 1H spectra differ?

2). Compare and contrast the information that can be obtained on membrane proteins by NMR and ESR.

3). Explain why the 1H NMR spectra of small peptides/proteins tend to consist of sharp resonances while the 1H NMR spectra of large proteins tend to consist of very broad resonances. The Lac repressor is a 150,000 MW tetrameric protein

consisting of a core and a DNA-binding headpiece. Comment on the information that can be gained from the 1H NMR spectra below.



4). Explain the following observations when alpha-methyl-mannoside is added to a solution of mannose binding protein (MBP);
i. New resonances appear between 3 ppm and 5 ppm. These increase in intensity and change in chemical shift as more ligand is added. When the ligand is in vast excess, these resonances resemble those of a solution of the free ligand.
ii. Selected resonances between 7 ppm and 9 ppm, present in the spectrum of the free protein, change in chemical shift as ligand is added but do not change in intensity.

The following data is obtained for a protein resonance on titrating alpha-methyl-mannoside into MBP;

Ligand concentration (mM)	Chemical shift (ppm)
0	7.05557
0.1	7.05374
0.2	7.05411
0.5	7.04715
1.0	7.04092
2.0	7.03433
4.0	7.02407
8.0	7.01564
16.0	7.01198
32.0	7.01235

Calculate the dissociation constant of the protein:ligand complex.
The crystal structure of MBP shows the mannose residue flanked by two amino-acid side chains (His 189 and Ile 207). The anomeric position of the saccharide points towards the isoleucine. MBP will also bind a-methyl-fucoside and b-methyl-fucoside.
The following data was obtained for the ratio of b K_D/a K_D for methyl-fucoside binding to the wild type protein and two different mutants.

Mutant	K_D ratio b/a
Wild Type	4.3
His 189 → Ala	1.7
Ile 207 → Ala	3.7

What can be deduced from this about the structure of the MBP:fucose complex ?

5). The 39K NMR spectrum of human erythrocytes suspended in a medium containing 60 mM K+ shows a single very broad resonance. If 6 mM Dy3+ and 15 mM tripolyphosphate are added to the medium, two peaks are observed, separated by about 8 ppm, one peak being twice the intensity of the other. Identify these two peaks and explain their occurrence. How could this effect be used to study ion transport across the erythrocyte membrane?