# Oneshot45 and $T_1$-DOSY: the gun and the knife of mixture analysis

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DOSY (Diffusion-Ordered Spectroscopy)[1] is one of the most commonly-employed methods for identifying compounds in mixtures by NMR. Here, two techniques are presented to improve the capabilities of DOSY experiments.

DOSY experiments are very demanding of spectral quality, for example even very small phase errors in signals causing visible distortions in the diffusion domain. All the basic echo experiments used for diffusion measurement show multiplet phase distortions caused by J-modulation, requiring a trade-off between phase errors and gradient pulse width. Two methods are commonly used to suppress its effects, a purging spin-lock pulse[2,3] and a z-filter[4] (also known as a Longitudinal Echo Delay). Neither is entirely satisfactory: spin locking causes sample heating, and LED requires extensive phase cycling, and both lose sensitivity and mask warning signs of instrumental problems such as convection. We propose a simple and effective solution to remove the unwanted anti-phase terms; it avoids all the disadvantages noted, adding an orthogonal 45º purging pulse immediately before the onset of acquisition (Fig. 3). The new method is illustrated for a widely-used general purpose DOSY pulse sequence, Oneshot[5].

DOSY techniques struggle when there is severe spectral overlap. In order to improve resolving power, we have incorporated relaxation into diffusion experiments as a further dimension. This results, to a first approximation, in a locally trilinear data set which, in contrast with a bilinear data set (e.g. a standard DOSY data set) can be decomposed with model-free multivariate statistical methods such as PARAFAC (parallel factor analysis)[6]. This enables overlapping multiplets from different species, and by extension whole spectra, to be separated.[7]

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# From Clone to Clinic: Biophysical Methods in Fragment-Based Drug Design'

*Glyn Williams*, Astex Therapeutics

Compared to the biochemical assay methods used in drug-discovery, NMR spectroscopy requires high concentrations of the target and has low-throughput. However, in fragment-based drug discovery where molecular interactions are of low affinity and chemical libraries are small, these disadvantages are reduced and, in practice, are offset by the information content and variety of experimental formats provided by NMR. In conjunction with mass spectrometry, calorimetry and X-ray crystallography, NMR has been used at all stages of the Astex fragment process ('Pyramid™'), from characterisation of the target, to detection of fragment binding and the optimisation of the chosen leads into clinical candidates.

This talk will give an overview of the Pyramid process and discuss the way biophysical methods have been combined to generate clinical inhibitors of the chaperone, HSP90.

# Disorder and Dynamics in Inner-Earth Minerals Probed by DFT Calculations and Solid-State NMR

*John Griffin*, University of St. Andrews

For a number of years it has been hypothesised that the Earth's mantle may contain a vast amount of water.[1] Indeed, it is thought the total amount may be equivalent to or greater than that present on the Earth's surface in the oceans and the atmosphere. However, the exact mechanism by which water is stored is not understood. It is currently thought that defect sites within the nominally anhydrous silicates that make up the Earth's mantle may hold sufficient amounts of structurally-bound hydrogen to account for the inner-Earth water budget. We present a combined experimental and computational solid-state NMR study of a group of hydrous magnesium silicates that provide models for water incorporation within forsterite (a-$Mg_2SiO_4$) which exists in the Earth's mantle to a depth of 410 km. DFT calculations using CASTEP show that experimental $^{17}O$ high-resolution two-dimensional NMR spectra can be understood using a structural model that involves dynamic exchange of hydroxyl protons between two sites.[2] This contrasts previous diffraction studies on the same materials, which only identified disordered static occupancy of the two sites.[3] The effect of fluorine substitution into the crystal structure is also investigated. $^{2}H$ magic-angle spinning (MAS) NMR reveals that this restricts hydroxyl-group dynamics, resulting in only a single hydroxyl proton site. While this suggests a more ordered structure, $^{19}F$ MAS NMR reveals considerable disorder of the $F^-$ sites, which are assigned using DFT calculations. $^{19}F$ MAS spin echo experiments also indicate the existence of rarely observed 'through-space' J-couplings between $F^-$ sites; this permits the recording of homonuclear J-resolved spectra and refocused-INADEQUATE correlations, which give information about F-F proximities within the structure.

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# Smaller Samples of Small Molecules in Tiny Tubes and Other Things of Interest

*Gary Martin,* Merck, USA

Full presentation attached:

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# What Drives Ligand-Protein Interactions

*Steve Homans*, University of Leeds

The Human Genome Project is providing a wealth of information on nucleic acid and protein sequences. Identification of the roles of these molecules in the homeostasis of the cell is continuing to offer unprecedented opportunities for therapeutic intervention in disease. Correspondingly, there is much emphasis among the structural biology community concerning high-throughput structural analysis in tandem with gene sequencing. However, if this structural information is to be of value for rational drug design, it is necessary to obtain a deeper understanding of the molecular basis of ligand-protein interactions. Thus, while the crystal or NMR structure of a protein is unquestionably thought provoking in the process of ligand design, the key to understanding the affinity of a ligand for its receptor lies in the dynamics and thermodynamics of the association rather than a simple static picture. With the advent of technologies such as isothermal titration calorimetry (ITC), it is possible under ideal circumstances to obtain reliable experimental data on the global thermodynamic parameters governing a biomolecular association. However, from the point of view of ligand optimisation, it would be of immeasurable benefit to obtain these thermodynamic parameters experimentally on a per-residue, rather than global basis. This presentation will describe some of our recent results using NMR in combination with ITC, protein crystallography, computational chemistry and site-directed mutagenesis in order to delineate the thermodynamics of ligand-protein associations in model systems. Particular emphasis will be placed on the contribution of residual ligand entropy in the bound state.

# Solid-state NMR of biological assemblies and complexes

*David A. Middleton*, School of Biological Sciences, University of Liverpool, Crown Street, Liverpool L69 7ZB; e-mail: d.a.middleton@liverpool.ac.uk

High-resolution solid-state NMR (SSNMR) is a versatile method for the structural analysis of a wide variety of biomolecules and biomaterials that are not readily accessible to other, more conventional, techniques. One advantage of SSNMR is its ability to report on the structural features of biomolecular assemblies and complexes, irrespective of the size or environment of the assembly. These assemblies include the aberrant protein aggregates associated with disease and receptor-ligand complexes of importance to pharmaceutical drug discovery. This talk will give an overview of our recent work in which SSNMR has been used to examine the architectures of amyloid fibril assemblies and to resolve the structural features of small molecule ligands when bound to receptors, either in crystalline form or when embedded in cell membranes. First it will be shown how structural information about the fibrils formed by the protein α-synuclein has been exploited to design novel peptide inhibitors of protein aggregation as potential therapies for Parkinson's disease and Lewy body disorders. Second, recent high-field SSNMR spectra of dimeric complexes formed by the ligand binding domain (LBD) of an ionotropic glutamate receptor will be presented. It is shown that crystallisation of mixed $^{15}N/^{13}C$ labelled LBD dimers enables SSNMR methods to select signals from residues only at the dimer interface, which forms the binding site for a class of drugs called allosteric modulators. Finally it will be shown how a combination of SSNMR measurements of interatomic distances and angles have determined, for the first time, the structure of the ATP substrate in the high-affinity binding site of an ion pump, the Na,K-ATPase. These results demonstrate that SSNMR can provide useful information for drug discovery.

# Chemical shifts – little changes tell us big things

*Prof Mike Williamson*, Dept of Molecular Biology and Biotechnology, University of Sheffield

Chemical shifts are the easiest parameter to measure from an NMR spectrum, and one of the most accurate, and yet we still have very little idea what to do with them. Here I present an outline of the origins of chemical shifts, and show that *changes* in shifts can tell us a lot, even if the exact value of the shift cannot be calculated accurately.

Chemical shifts depend on the shielding of the nucleus by the electrons around it. For $^{1}$H (which only has a maximum of 2 electrons around it), conformation-dependent shifts therefore depend mainly on through-space effects, such as ring currents and hydrogen bonds. For $^{13}$C and $^{15}$N, they depend mainly on bond and dihedral angles. However, in proteins, backbone $^{13}$C=O and $^{15}$NH shifts depend on hydrogen bonding, both to themselves and to the other half of the amide group they are in. Thus the shifts of different nuclei provide information on different things.

One of the simplest applications of shifts is to measure binding affinity. This is easy to do and can be very informative. I take a short detour into proline-rich peptides and tannins to show what binding-induced shifts tell us about the molecular origin of astringency (the dry sensation arising from drinking black tea or red wine).

Aromatic ring currents are easy to calculate and vary in an obvious way with geometry. They can be used either as structure restraints or as structure validation: I illustrate this with a study of DNA intercalation.

The small change in shift induced by high pressure can be used to calculate how a protein structure changes at high pressure. I show how this can be used to calculate high-pressure structures of proteins, and what they tell us.

Finally, pH-induced changes in shift can be very informative about the origins of chemical shifts in proteins. I use this to probe the $^{1}$H and $^{15}$N shift changes in HSQC spectra, and suggest more quantitative ways of using chemical shift perturbation data.

For details and references to this work, see my web site at http://www.nmr.group.shef.ac.uk/MPW.html.