Use of F_2-Coupled-HSQC Experiments and Computer-Assisted Structural Elucidation (CASE) Programs in Structural Determination Studies

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Abstract: The structural elucidation of organic compounds relies heavily on the determination of NMR ^{1}H and ^{13}C chemical shifts and ^{1}H-^{1}H couplings. Carbohydrates occupy an unusual niche in this general process. The structures of 6-membered ring carbohydrates, which exist largely as *chair* conformers, can be determined relatively easily or with great difficulty, depending upon the degree of overlap of proton NMR signals. When severe signal overlap does *not* occur, TOCSY experiments are made for carbohydrates, permitting the identification of entire proton spin systems, and COSY experiments can then be used to determine the structural position of each hydrogen signal.

In cases of moderate to severe ^{1}H NMR signal overlap, however, determination of the spin couplings, and thus orientations, of both (i) aglycone hydrogens and (ii) *non-anomeric* ring hydrogens in sugar units can be exceedingly difficult. Nevertheless, 2-dimensional NMR experiments now exist that permit the relief of spectral congestion by taking advantage of signal dispersion *via* C-13 satellites.[1-3]

CASE programs are also very useful in the determination of structure for compounds whose NMR spectra still do not yield definitive results. Two sets of ^{13}C chemical shifts, approximate and then refined, are calculated and ranked as two types of statistics (HOSE and Neural Network). Correct structures can be distinguished for compounds having very similar structures, and correct assignments can even be made for those with certain identical chemical shift assignments.[4]

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The Sensitivity Advantages of Non-uniform Sampling: theorems and applications

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Abstract: Multi-dimensional NMR of small molecules is particularly well suited for using non-uniform sampling (NUS) to improve the sensitivity of the data. This presentation will briefly review the exact theory for how NUS improves sensitivity, and by how much. Although there has been increasing interest in NUS. some major questions have remained on the scope of the sensitivity enhancement offered by NUS. Strict theorems have been devised in this work to explain (i) when NUS will always have equal or greater sensitivity than conventional uniform sampling, and (ii) how NUS overcomes a major limitation on the sensitivity of uniformly sampled data."

The Role of NMR in Discovery Drug Metabolism: Practical Examples and Sensitivity Limits

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Abstract: The pharmacological and metabolic properties of a drug metabolite are important factors in the selection of a compound during drug discovery. In order to assess these parameters an authentic standard of the metabolite is usually required. For a variety of reasons metabolites can frequently be difficult to synthesize by normal chemical protocols. As an alternative to chemical synthesis, small amounts of these metabolites can be biologically generated, chromatographically isolated and structurally characterized using MS and NMR. The concentration of these isolates can then be quantified using qNMR. Once characterized and quantified these biologically derived isolates can be used in a variety of ways, including pharmacological assays, standards for *in vitro* work to help establish clearance pathways and/or as analytical standards for bioanalytical work to ascertain exposure, among others. While this process circumvents the difficulties of complex chemical synthesis, it provides challenges of its own. The most notable limitations in this process are the small amounts of metabolite generated coupled with the relative insensitivity of NMR. In this presentation the qualitative and quantitative limits of NMR relative to isolated metabolites are defined. Additionally several practical examples of this process will be presented.

Tips and Tricks to Obtain Ultra High Resolution NMR Spectra

Damodaran Krishnan Achary
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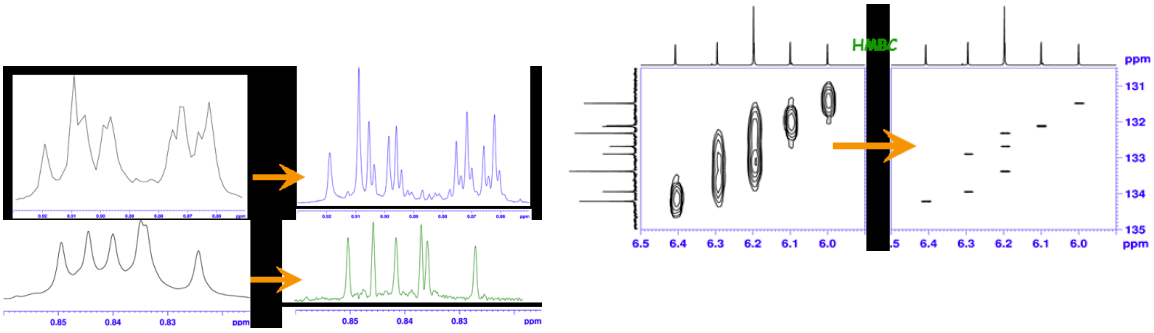
Abstract: Ultra-high magnetic fields and advances in hardware and software technologies have enabled high resolution NMR spectroscopy. Also smarter pulses sequences and NMR methods have provided us with the ability to resolve previously unresolved resonances. But still, obtaining routine high resolution NMR spectra is not quite straight forward and requires understanding basic (mostly overlooked) tricks of the trade. In this lecture, we will walk through these simple tricks for obtaining the highest possible resolution for 1D and 2D NMR of small molecules with full examples. Some of the topics that will be discussed are shimming, tweaking acquisition and processing parameters, covariance, selective excitation, Non-Uniform sampling and Pure Shift 1D and 2D.



Small Molecule Structure Characterization: New and Improved NMR Methods

Thomas Williamson
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Abstract: The structural chemistry landscape highlights a well-established need for a technique that is complementary to standard NMR methods with comparable sensitivity and greater molecular "reach". In addition, there has long been a need for an NMR experiment that allows the facile extraction of structurally and conformationally relevant quantitative values for these long-range heteronuclear connectivities. The Merck NMR group has successfully addressed both of these issues with a new family of pulse sequences. We have recently reported the development of the LR-HSQMBC experiment that combines refocusing and broadband heteronuclear decoupling to enhance the ability to routinely probe long-range $^{4}J_{CH}$, $^{5}J_{CH}$ and under favorable conditions even $^{6}J_{CH}$ correlations and we have quantitatively demonstrated the impact of this approach on the structure elucidation of proton deficient systems. Two other sequences, the Perfect Echo HSQC and the PIP-HSQMBC experiment have also been developed for the quantitative measurement of $^{1}J_{CH}$ and $^{n}J_{CH}$ couplings respectively. The results of these studies will be presented along with some additional techniques developed in our lab towards the goal of dealing with the challenge of molecules with a dearth of molecular ^{1}H "handles".

Using Carbon Detected Experiments with Very High Resolution for More Complete Assignment of Fatty Acid Esters

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Abstract: Updated ^{13}C detected 2D NMR experiments were used with a large number of points in the directly detected dimension to gain a more complete assignment of several fatty acid esters that are of interest as contaminants in processed edible oils. [1] Assigning carbon and proton NMR resonances of molecules containing long hydrocarbon chains has been known to be problematic due to extreme overlap in the proton spectrum. The carbon spectra are less crowded but are difficult to assign without sufficient data on correlations with protons and other carbons. The standard proton detected experiments for obtaining carbon-proton correlations (HSQC, HMBC, HMQC, etc.) all have limitations that make it difficult or impractical to get the higher resolution needed for assignment of such crowded spectra. Older carbon detected experiments, i.e. short and long range HETCOR, are not used as much because of their much lower sensitivity and the difficulty in handling the very large data sets the high resolution versions produce. However, these older experiments offer the possibility of much higher resolution then their proton detected counterparts and long-range couplings can be established through an updated FLOCK experiment. [2,3] Over the past 20 years carbon optimized probes have become more sensitive and computing power has increased making the processing of quite large data sets routine and therefore not an obstacle or time limiting step. Carbon detection becomes more practical with high sensitivity carbon-13 probes and in samples not limited in amount or solubility. While still challenging, the much higher resolution makes it possible to identify resonance that differ by only a few hertz in both the carbon and proton dimension.

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Fun with Small Molecule Glycoconjugate NMR: Nucleosides to Glycopeptides

Joseph Barchi

Head, Structural Glycoconjugate Chemistry and NMR group, Senior Scientist, Center for Cancer Research National Cancer Institute

Abstract: Glycoconjugates come in many flavors, from cell surface and cellular glycans, to glycolipids and nucleic acids. We have examined the conformations of a variety of different sugar conjugates, concentrating on glycopeptides and nucleoside analogues as both anticancer and antiviral drug candidates. This talk will highlight some of our recent work on glycopeptides as well as concentrate on previous published research to determine the structures of conformationally biased nucleosides by NMR spectroscopy. The relationship between analogue conformation and interaction with their biological partners (e.g., enzymes) will be discussed.

NMR in Anisotropic Media and its Applications to the Structural Analysis of Small Molecules

Roberto Gil

Research Professor and NMR Director, Department of Chemistry, Carnegie Mellon University

Abstract: This part of the workshop wants to illustrate how, Residual Dipolar Couplings (RDCs) may provide extremely powerful structural information in the analysis of small molecules, particularly when traditional NMR experiments, such as ^{3}J coupling constant analysis and Nuclear Overhauser Enhancement (NOE) fail to provide a unique configuration and/or conformation. The information obtained by the later methods is of local character and is restricted to structural information in the local environment of a molecule. However, RDCs provide information of non-local character and it is possible to determine the relative configuration of stereocenters no matter how far they are located one from each other. These NMR parameters help to lift the local information limitations provided by traditional methods.

Since RDCs are not directly observed in conventional liquid state NMR experiment (isotropic conditions), the sample needs to be exposed to an anisotropic medium to reveal their values. Anisotropy can be induced in the NMR sample either by using stretched or compressed cross-linked polymer gels or liquid crystal solutions of homopolypeptides such as PBLG or PELG in $CDCl_3$.

Along the two hours of this part of the workshop, we will cover theoretical aspects and the features and limitation of the use of RDCs for the stereochemical analysis of small molecules in organic solvents. We will cover the topics of sample preparation and NMR experiments used for the acquisition of RDCs in aligned media, as well as computationally based RDC data analysis procedures to determine relative configuration and conformations of small molecules.

We have selected few real problems as examples to demonstrate how to determine the configuration and/or conformation of small molecules.

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