BE.342/442 Tuesday, October 4th, 2005
Topic: Guest Lecture on NMR by Peter Carr (Research Scientist, MIT Media Lab)

Background of speaker: Used NMR as a tool to study complex protein dynamics. Currently utilizes little NMR; studies molecular biology/protein engineering to build genes "from scratch" for protein synthesis.

NMR can be look at biomolecules, as well as polymers and inorganic compounds. In biology, used to study proteins, pieces of DNA, and other large systems. NMR can give atomic-level resolution of these large molecules. Secondary structures (alpha helices, beta-sheets), tertiary structures (overall fold of a protein), and quaternary structures (binding and interfaces between multiple molecules) can be resolved as well.

NMR can be performed dynamically, from picoseconds to milliseconds (folding/unfolding of DNA, concerted motions of entire molecules, binding of a small molecule or drug to DNA)

Solution State vs. Solid State NMR:
Samples can be liquid, including simple or complex solutions, or solids, including crystals, colloids, or disordered powders. Sometimes, samples can even be in a gas phase!

NMR Periodic Table:
NMR can detect an isotopes of atoms. Common atoms such as hydrogen, oxygen, nitrogen, etc. have at least one isotope that can be distinguished by NMR. Relative sensitivity is determined by the natural abundance of the isotope multiplied by magnetic sensitivity of that isotope. *The most convenient of all are the spin-½ nuclei, including hydrogen, carbon, nitrogen, and phosphorus.* Carbon and nitrogen are present in every protein and nucleotide base, and are so common to use in NMR that companies dedicate themselves to production of these isotopes! Phosphorus, in the backbone of every oligonucleotide, provides a strong NMR signal.

The NMR Spectrometer
The "guts" of the NMR spectrometer is a huge permanent magnet, superconducting, sitting in a bath of liquid helium, which itself is sitting in liquid nitrogen to keep a cool shell around the helium. The solenoids are capable of generating fields of up to 10 Tesla. The "strengths" of the magnet are measured in megahertz, which relates to the strength of the hydrogen spectrum. Older, more common spectrophotometers have strength of 300-500 MHz. More modern high-field spectrophotometers can have strengths of 750-800+ MHz, and look a bit like spaceships! Despite shielding, these tend to have a high stray field, and must be kept in rooms separate from other experiments—such as one such machine, which is kept in the bottom of an old U. Penn swimming pool!

Principles of NMR Spectroscopy
Particles with nonzero nuclear spins can *split* their energy levels in a magnetic field. By Boltzmann's Law, the two states will be populated with a slightly higher population in the lower-energy state. This difference in population leads to an overall magnetic moment. The difference

in the energies of the two states, which dictates the difference in the populations, depends on the strength of the magnetic field:

$$\Delta E = h\nu = \frac{\gamma \hbar B_0}{2\pi}$$

Where

ΔE is the difference in energy,
h is Planck's constant,
ν is the frequency,
γ is the gyromagnetic ratio of the nucleus,
B_0 is the external magnetic field, and
m is the magnetic quantum number of the electron.

In a simple, 1-pulse NMR experiment, the sample experiences a short, radio-frequency magnetic pulse, and responds with a damped sinusoidal oscillation:

$$s(t) = e^{-iRt} \cos(\nu t)$$

with a frequency that is determined by the type of nucleus, but also the chemical environment around that nucleus. Here, t is time and R is a relaxation constant that characterizing the damping of the oscillation. A particle with a spin responds by oscillating in 3-D like a slightly tilted spinning top, which wobbles as it spins. The dipole points mostly around the z-axis, but precesses around the z-axis so that it also has components in x and y. In the moment vector is projected onto the x-y plane, the circular rotation represents the precession that is measured in NMR.

A Fourier transform of this oscillation shows the frequency of the oscillation plotted against its strength, or amplitude. The quantity corresponding to that frequency is then calculated by:

parts per million = frequency of signal (Hz) / frequency of spectrometer (MHz)

The NMR spectrum of hydrogen in a typical protein (e.g., ubiquitin) gives information about the side groups and secondary structures present, but does not give detailed information about the exact chemistry and bonds in the molecule. Peaks that are similar in frequency overlap and become indistinguishable, so this detailed data becomes inaccessible.

However, additional interpretation allows us to decouple the structures of the molecule from the subtle differences in each atom's environment. Protons that are close to another tend to oscillate coherently in what is called "simple coherence transfer." For example, a series of free induction decays can be measured and Fourier-transformed while changing the delay time between sequential pulses. The heights of the peaks in the Fourier transform, as a function of the delay time, can themselves be Fourier transformed. This yields a two-dimensional contour map. Now the complex, highly overlapped peaks from the simple 1-pulse experiment can be spread out and distinguished from each other!

In more complex experiments, the data can be spread out in 3, 4, or 5 dimensions to further distinguish peaks with differences in the details of the chemical environment: for example, the nearest-neighbor and next-nearest-neighbor atoms in a molecule.

Stuctures can be determined with atomic resolution by beginning with a model of atoms under vague constraints, and then increasing the constraints on these atoms based on NMR data. The categories of information obtained from NMR include:

chemical shift: chemical environment and conformation
J-coupling/splitting: chemical connectivity, conformation/dihedral angles
exchange rates: micro- to millisecond dynamics, stability, folding
dipolar effects: interatomic distances, structure, dipolar bond orientations
relaxation rates: picosecond to millisecond dynamics of folding/unfolding, aggregation

Examples of Peter Carr's work: S
Studying the proteins that link viruses to cell membranes, and exploring drugs that inhibit that fusion (anti-HIV drugs now on the market).
Entropic transitions and atomic-scale free energy of folding of DNA and a protein binds to the major groove of DNA. Entropy and enthalpy of folding reported on a residue-by-residue basis!

Solid-State NMR

A single peak for a labeled residue contains hundreds or thousands of hertz due to the high concentration of matter (ppm) in solids. The peaks have a broad shape, "smearing out" the individual peaks that give information about the sample. In a randomly oriented powder, the sample must be spun to average out the orientations of particles, mimicking the tumbling of molecules in solution NMR. Only recently have these methods allowed people to study proteins in the solid state. (*The reason for these broad peaks is complex and not covered here.*)

Solid state NMR has been used to study…
The organization of fibrils in Alzheimer's beta-amyloid. Various models of organization were proposed, but only the parallel organization model was consisten with NMR data.
Hydroxyapatite organization in the development of teeth.
Secondary structure of silk proteins spun into silk fibers.
MicroNMR with small solenoids.

Limitations of NMR

Size limit: the method is much easier for small macromolecules, under 30,000 Da, although molecules over 100,000 Da have been characterized. Isotopic labeling is required for complex proteins. High solubility is required (1mM is typical for macromolecules—that's an awful lot of protein!).

Solids also often require isotopic labeling, and the high-energy experimental conditions can damage the sample.