NMR Hits the Big Time

High-field magnets and other advancements propel NMR into the structural genomics mainstream | By Aileen Constans

When it comes to structural biology, bigger really is better. Most biological processes are performed by enormous multicomponent complexes, such as the ribosome. To solve the structures of these monsters, biologists traditionally have used two different but complementary techniques, nuclear magnetic resonance (NMR) and X-ray crystallography. They have had considerable success using the latter technique for protein structure determination, but the former technique has lagged behind, in part because it has not been possible to apply NMR to proteins larger than about 30,000 daltons (Da).

But now NMR is catching up. New procedures and a procession of ever-larger magnets have helped researchers chip away at the size ceiling that has constrained them. The biggest of the big: ultrahigh-field 900-MHz instruments. These systems improve peak resolution, enabling spectroscopists to focus on ever-larger molecules. And because the instrument’s sensitivity increases with the magnetic field strength, stronger magnets permit experiments to be run more quickly or with lower sample concentrations--a plus for investigators plagued by...
sample concentrations—a plus for investigators plagued by unstable samples or proteins that aggregate at high concentrations.

Two companies, Billerica, Mass.-based Bruker BioSpin and Palo Alto, Calif.-based Varian, currently supply 900-MHz systems. Representatives from both companies acknowledge that while interest in the multimillion-dollar instruments has been high, actual placement in academic and pharmaceutical labs has been slow owing to the expense of the systems and the difficulty of shipping and setting up the magnets.

Those who do have access to the 900-MHz instruments have seen promising results. "The experiments that we’ve been able to do at 900 MHz on some high-molecular-weight systems using triple resonance, we literally could not do at 800," says Peter Wright, professor and chairman of the Department of Molecular Biology, The Scripps Research Institute, La Jolla, Calif. "We couldn’t get signals, we just couldn’t pull it out of the noise. It really is an amazing technology for high-molecular-weight systems."

It’s an amazing time to be a spectroscopist, too. By coupling these magnets’ strength with new experimental approaches, researchers can now divine the structures of biomolecules that previously were beyond their reach.

**EVERYTHING’S COMING UP TROSY** One new approach in the spectroscopist’s playbook is TROSY, or transverse relaxation-optimized spectroscopy, developed by 2002 Nobel laureate Kurt Wüthrich of the Swiss Federal Institute of Technology, Zurich, and colleagues.

**900-MHz GORILLA:** Structural biologists using NMR have been traditionally limited to working with small biomolecules. But new ultrahigh-field
NMR instruments, like this 900-MHz spectrometer at the Scripps Research Institute, have shattered this size ceiling, making the technique a viable alternative to X-ray crystallography.

TROSY is actually a variant of $^{1}\text{H}-^{15}\text{N}$ correlation spectroscopy, or HSQC (heteronuclear single quantum correlation), a widely used technique that identifies protons bound to nitrogen atoms. In an HSQC experiment, each spin-pair generates a quartet of resonances comprising sharp and broad components; scientists running these experiments generally decouple the proton from the nitrogen to collapse the multiplet into a single sharp peak. This approach improves sensitivity for proteins less than 30 kDa, but for larger molecules, decoupled peaks become too broad for structure determination.

Wüthrich’s team reasoned that for large molecules at very high field strength, it might be useful to skip the decoupling altogether. They could then examine the line width of each individual member of the multiplet and select the narrowest component, thus increasing sensitivity and resolution for large proteins.$^1$

Using TROSY, Wüthrich’s group has successfully tackled the 900 kDa GroEL-GroES complex,$^2$ while Lewis Kay, professor of chemistry and biochemistry, University of Toronto, assigned the backbone chemical shifts of a 723-residue protein, the largest monomeric biomolecule to be assigned to date.$^3$

**POINTING PROTEINS IN THE RIGHT DIRECTION**

Another important contribution to NMR technology in recent years is the application of residual dipolar couplings to protein structural determination.$^4$ Dipolar coupling is a through-space interaction between a pair of nuclear spins, but in NMR protein studies, these interactions are normally not detected, explains Jim Prestegard, professor of chemistry, biochemistry, and molecular biology, University of Georgia, because the molecules tumble in space.

If, however, the proteins are partially aligned through the introduction of a field-oriented medium—such as phospholipid bicelles that interact with the proteins in solution—then residual dipolar coupling, which provides information about angular orientation, can be observed. And, because some aspects of the phenomenon are accentuated in high magnetic fields, the
combination of residual dipolar coupling with ultrahigh-field NMR offers a powerful tool for the study of large systems, giving scientists a new series of constraints for deciphering protein structure.

Courtesy of Vitali Tugarinov

HEAVYWEIGHT CHAMP: In 2002 Lewis Kay and colleagues assigned the backbone resonances of malate synthase G (82 kDa, 723 residues), the largest monomeric protein assigned by NMR spectroscopy to date.

NMR GOES ULTRAFAST The chemical shifts of small organic molecules are relatively straightforward to analyze, as the spectral peaks are well-resolved. In large biomolecules, however, the peaks tend to overlap. Multidimensional techniques can solve this problem, but these experiments require long acquisition times, which increase exponentially with each dimension. A four-dimensional spectrum, for instance, might take weeks to acquire, tying up the instrument and limiting NMR’s use in high-throughput research. More importantly, proteins aren’t usually stable for that long.

Recently several groups have addressed this problem by developing methods for rapid or ultrafast multidimensional NMR. Thomas Szyperski, associate professor of chemistry, and former postdoc Seho Kim of the University of Buffalo, for example, developed a method called GFT NMR, or G-matrix Fourier transform NMR, in which a five-dimensional spectrum can be acquired in slightly more than an hour.

In GFT NMR, data is acquired from many one-dimensional
scans simultaneously, rather than sequentially, significantly accelerating the experiment. Instead of one peak representing an individual chemical shift, the GFT NMR experiment generates multiplets that represent linear combinations of the chemical shifts. This results in a complex spectrum that can be resolved into component frequencies by applying a battery of linear equations, or G-matrices, and Fourier transforms, says Szyperski.

Such data are more difficult to analyze manually than data obtained through standard multidimensional methods, but Szyperski says the condensed nature of the experiment simplifies computational analysis. "Data processing is greatly facilitated because while a 4-D is a huge data set, in the several gigabyte range, we stay in the megabyte range. Even with supercomputers, a 5-D is hardly something that you can or want to transform," Szyperski notes.

Lucio Frydman, professor of chemistry, and colleagues at the Weizmann Institute of Science, Rehovot, Israel, developed an alternate approach. Their technique uses an imaging gradient to partition the sample into tens or hundreds of spatially localized portions, or slices. Each slice is subjected to a single NMR scan, and the signals from all the slices are monitored simultaneously, effectively compressing a complete multidimensional experiment into one scan. "In principle we can therefore carry out an acquisition that normally takes several hours within a fraction of a second," Frydman says.

Such accelerated methods make NMR a more attractive approach to solving large protein structures and monitoring highly dynamic systems, such as folding proteins. But Frydman cautions that single-scan techniques work only for highly sensitive instruments. "This is by no means a replacement for the traditional way of doing NMR because you do pay a price, and the price comes in terms of signal-to-noise," he says. In a traditional multidimensional NMR experiment, signal gets averaged over many scans, but in ultrafast multidimensional NMR the signal is collected in just one. "Within that single scan, you must have enough signal in your system to find the peaks you are looking for," says Frydman.

Courtesy of Y. Shrot and L. Frydman
ACCELERATED NMR: This 3-D TOCSY-HSQC NMR spectrum of glycerol/D₂O was acquired in a single scan. Total experimental time: 141 ms.

PROTEINS ON THE MOVE The structural information obtained in many NMR experiments provides a three-dimensional, static picture of a protein, averaged over time. But proteins are not static—they move. "Proteins aren’t rigid entities. If you look at an NMR structure ... what you’re basically getting is a snapshot. That’s not how proteins function," says Lewis Kay. "Without dynamics we wouldn’t have catalysis, we wouldn’t have binding, we wouldn’t have many of the recognition processes which are absolutely critical for biological function. NMR as a technique allows you to look at these."

NMR relaxation methods sensitive to motions on pico- to nanosecond time scales are widely used to study protein flexibility, particularly in molecular recognition. But until recently, NMR could not be applied to protein dynamics in the microsecond to millisecond range, in which many biologically relevant processes take place. Now, says Arthur Palmer, professor of biochemistry and molecular biophysics, Columbia University, thanks to larger magnets, optimized experimental procedures, new theoretical analyses, and TROSY, researchers have overcome these limitations.

Dorothee Kern, assistant professor of biochemistry, Brandeis University, and colleagues recently investigated the dynamics of the enzyme cyclophilin A during catalysis and detected
motions on the microsecond time scale using NMR spin-relaxation experiments. "We can not only qualitatively determine which part of the proteins are dynamic on the [micro- to millisecond] time scale, but also exactly the frequencies of motion," says Kern. "That was actually very interesting because we saw that these frequencies of motion on the enzymes are actually very similar to the frequencies of the chemical steps of enzyme turnover."

Now the challenge is to study protein dynamics in the midnanosecond to low-microsecond time scale. Several researchers are exploring the use of residual dipolar couplings to probe this range. "The advantage of looking at residual dipolar couplings is that they’re sensitive to a whole range of time scales that run from pico- to milliseconds and tens of milliseconds," explains Prestegard. "They also allow estimates of amplitudes of motion, as opposed to just timescales." These studies are currently in preliminary stages, he says.

Aileen Constans (aconstans@the-scientist.com) is a freelance writer in Pittman, NJ.

References


UNDERSTANDING NMR

Atomic nuclei with spin quantum numbers of 1/2, such as $^1$H, $^{13}$C, and $^{15}$N, exhibit the physical property of a spinning electrical charge—that is, they act as tiny spinning magnets. When placed in an external magnetic field, these nuclear magnets precess about the external magnetic field in two directions: parallel and antiparallel, with one direction being lower energy and, thus, more populated than the other. The vector summation of all these individual nuclear spins produces an observable macroscopic magnetization in the direction of the external magnetic field (the $z$ direction).

In an NMR experiment, the sample is irradiated with a short pulse of radio frequency (RF) energy at the precession frequency of the nuclei. The macroscopic result of this is to rotate the observable magnetization away from the equilibrium $z$ direction and to produce an induced voltage in a receiver coil (the signal). This signal gradually decays as the magnetization slowly returns to its equilibrium value.

The name nuclear magnetic resonance arises from the requirement that the applied RF pulse be at the same frequency (called the Lamor frequency) as the nuclear precession. These frequencies are characteristic of both the nuclear identity (e.g., $^1$H or $^{13}$C) and the local magnetic environment (i.e., the chemical structure of the molecule) of each NMR-active nucleus. It is the minute differences in frequency, which arise from subtle molecular structure factors, that allow NMR to provide structural information.

The Lamor frequency, or chemical shift, provides information about the chemical nature of the nucleus. Spin-spin coupling provides information about the numbers and identities of neighboring nuclei connected through bonds, while the Nuclear Overhauser Effect provides through-space distance...
information. The rate of signal decay provides information about the motion of the portion of the molecule where the observed nucleus resides.