

## Propelled by Recent Advances, NMR Moves Into the Fast Lane

A speedy new NMR technique could finally help structural genomics groups achieve their goal of devising factory-style approaches to mapping protein structures at high speeds

As a tool for mapping the atoms in proteins, nuclear magnetic resonance (NMR) arrived on the scene decades after the workhorse mapping technique, x-ray crystallography. So perhaps it's no surprise that protein structures solved with x-ray crystallography make up 80% of the deposits in the Protein Data Bank, the international protein structure repository. But NMR, which accounts for the other 20%, may be poised to expand its turf.

In the 29 January issue of the *Journal of the American Chemical Society*, two chemists—Thomas Szyperski of the State University of New York, Buffalo, and his former postdoc Seho Kim, now at Rutgers University in Piscataway, New Jersey—report a new way to collect and interpret NMR data, which promises to slash the data-collection time of a typical protein-mapping experiment from days to hours. “This is an important method,” says Cheryl Arrowsmith, an NMR expert at the University of Toronto in Canada. The speed boost, Arrowsmith says, will be particularly useful for structural genomics groups working to devise factory-style approaches to mapping protein structures at high speeds.

Slow speeds have long hobbled NMR. The technique determines molecular structures with the help of powerful magnetic fields, which cause protons in particular atoms to precess like spinning tops, each with a characteristic frequency that depends on its chemical identity. To spot these atomic tops, researchers bombard vials containing millions of copies of a protein in solution with trains of radio-frequency (RF) pulses. The pulses nudge the spinning nuclei, effectively causing tops of the same chemical flavor to whirl in unison. Detectors then record the frequency at which different nuclei are spinning. Nearby atoms also affect the spins in ways that depend on their distance from the original atom and on their chemical identity. A proton in a carbon atom, for example, spins at a different rate depending on whether it sits near another carbon atom or a nitrogen atom. NMR experts use this “chemical shift” to help them solve their giant jigsaw puzzle of how neighboring atoms fit together to make up the protein.

That's relatively straightforward as long as the number of nuclei being looked at is

small. But things get complicated when researchers start trying to map large proteins that harbor dozens of amino acids. In part, that's because the NMR data come out as a series of peaks, each one representing the frequency of rotation of a particular nucleus. When nuclei abound, the peaks in such a spectrum crowd together so tightly that it's impossible for researchers to sort out which peaks correspond to which atoms.



**Speed demon.** Thomas Szyperski, shown perched beside a 750-megahertz NMR machine, says his new method would slash the amount of time needed to map proteins.

Technology has eased matters somewhat, as more-powerful NMR magnets and advances such as cryogenically cooling the RF electronics provide better resolution and sharper signals. In recent years, researchers have also devised “multidimensional NMR” techniques to analyze the interactions not just between pairs of atomic neighbors but among threesomes and foursomes as well—extra clues to the giant molecular puzzle. But that information comes at a price: Each time researchers increase the number of neighbors they evaluate—known in NMR parlance as adding dimensions—they must collect an exponentially greater number of spectra. (Analyzing one dimension requires just a single spectrum. For two, researchers typically collect 64 spectra. For three, it's  $64 \times 64$ , or 4096 spectra, and for four it's  $64 \times 64 \times 64$ , or 262,144 spectra.) “The minimum measurement times explode when the dimensions

are increased,” Szyperski says. A standard 4D NMR experiment, Arrowsmith says, can take up to a week just to collect data.

Szyperski and Kim solved the problem by inventing a technique called G-matrix Fourier Transform NMR, or GFT NMR. Among other things, the technique not only controls the spacing of the RF pulses that bombard the protein sample, as the more conventional approach does, but it also tracks their phase—that is, whether the RF waves begin at their peaks, troughs, or somewhere in between. “Sampling different phases allows you to look at sums and differences of chemical shifts, and from those you can extract all the chemical-shift information you are collecting with even higher precision than with conventional techniques,” Szyperski says. The upshot,

he says, is that researchers can get all the information from a 4D NMR experiment by collecting even fewer spectra than are now needed for 3D experiments. That improvement, he says, should cut data-collection times for larger proteins from days or weeks to hours.

GFT NMR, Szyperski adds, is likely to prove especially useful in conjunction with new high-field NMR magnets, such as the 7-meter-high machines that operate at 900 megahertz. Those machines, particularly when equipped with low-temperature electronics, have more than enough resolution to solve structures for large proteins. But such experiments still take days to collect enough spectra to solve the protein structure. By cutting the number of spectra needed, GFT NMR “will allow scientists to take full advantage of the highest-field NMR machines,” Szyperski says.

GFT NMR data are also easier for computers to analyze, an advantage that should make it easier to automate the jigsaw-solving process once data collection is complete, says Gaetano Montelione, an NMR expert who heads the Northeast Structural Genomics Consortium, of which Szyperski's group is a member. “I'm convinced that this is the way to go for automated NMR data analysis,” Montelione says. If so, NMR may yet give x-ray crystallography a run for its money

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