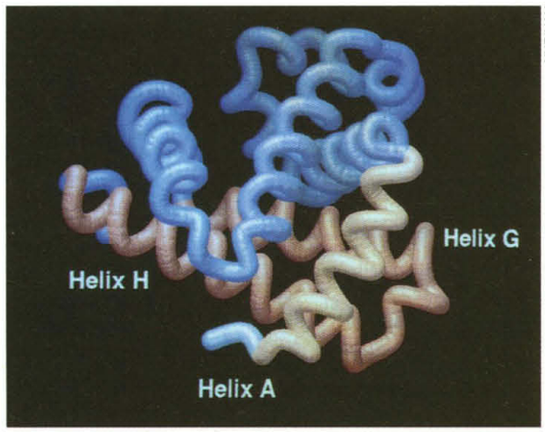
find its own way through the various stages in the folding process: "Each molecule does something differently."

Molecular motion. While a protein in its native conformation usually has a regular and predictable structure, the molecule still has a considerable amount of give. "No protein is totally rigid," says Peter Wright of Scripps. "The emerging view of proteins is that there is significant internal motion." Working with a bacterial enzyme called dihydrofolate reductase (DHFR), Wright has used NMR to investigate how these internal motions might affect the protein's biological function. DHFR catalyzes the conversion of a compound called dihydrofolate to tetrahydrofolate, a coenzyme that enables cells to make numerous organic molecules, including some amino acids. The reaction takes place in a large cleft running along the center of the DHFR enzyme, and also requires a reducing compound called NADPH.



KURIYAN

Fast folders. Helices A, G, and H form first as apomyoglobin folds from a linear chain to an active protein.

Wright and his co-workers exposed the enzyme to nonreactive analogs of dihydrofolate and NADPH to "freeze" the reaction at various steps in the catalytic cycle, and then looked at the enzyme's internal motions at each step. The team found that several regions in the molecule became much less mobile once the inhibitor molecules were bound to the active site. For example, in the unbound state a region called loop 1—which acts as a "cap" over the active site—fluctuates between two slightly different conformations about 30 times per second. But when the inhibitors were bound to the enzyme, these fluctuations stopped. Another highly mobile region near the active site, centered on a glycine molecule in the polypeptide chain, also became much more rigid in the bound state.

"Wright was able to map the changes in protein motion at various steps of the reaction," says Desiree Tsao of the Genetics Institute in Cambridge, Massachusetts. To take things a step further, Wright's collaborator Stephen Benkovic and his colleagues at Pennsylvania State University in University Park made mutant versions of DHFR in which the highly mobile glycine was either replaced with another amino acid or deleted entirely. These changes, Benkovic found, greatly decreased the enzyme's catalytic power—implying that these internal motions play an important role in its biological function.

Exactly what this role might be remains to be determined. But NMR aficionados are confident that their technique will help point the way to solving this mystery, as it has done with the Levinthal paradox. Says Dobson: "NMR is allowing these questions not only to be posed, but to be answered."

–Michael Balter

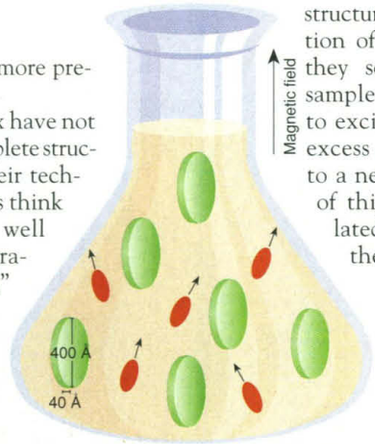
PROTEIN STRUCTURE

Lining Up Proteins for NMR

The tried-and-tested method for mapping the three-dimensional (3D) structure of proteins, x-ray crystallography, has a troublesome shortfall. The technique can pinpoint the location of atoms with extreme accuracy by bouncing off innumerable copies of the protein stacked in a crystal. But many proteins don't readily form such regular assemblies. A rival technique, known as nuclear magnetic resonance (NMR) spectroscopy, can map proteins in their native solution environment and therefore doesn't suffer this problem. But it has never been able to match the peak precision of its rival.

That may soon change. On page 1111, NMR experts Nico Tjandra and Ad Bax of the National Institutes of Health in Bethesda, Maryland, report modifying a long-known NMR technique to dramatically improve NMR's protein-mapping skills. Conventional NMR maps protein structure by determining the identity of atoms in a molecule as well as the distance between given pairs of atoms. With the new technique, which gently aligns the protein molecules in a bath of liquid crystals, researchers can also determine how each bond between neighboring atoms is oriented with respect to the rest of the molecule. By methodically building up a list of such orientations for all neighboring pairs of atoms, researchers should be able to complete a far more precise map of a protein.

So far, Tjandra and Bax have not reported mapping the complete structure of a protein using their technique. But they and others think it is likely to stack up well next to x-ray crystallography. "It's quite impressive," says Stephen Fesik, an NMR spectroscopist at Abbott Laboratories in Chicago, Illinois. "I think this is going to have a big effect on the field." One obvious area of impact, he says, could be in drug design, because designers need detailed structures of proteins so that they can tailor their drugs to interact with them.



Getting oriented. By aligning proteins (red), liquid crystals (green) sharpen NMR's atomic mapping.

All NMR techniques rely on the fact that some atomic nuclei act like tiny bar magnets. When these nuclear magnets are placed in an external magnetic field, they align along the magnetic field lines. If excited by a burst of radio-frequency photons, their magnetic axes precess like wobbling tops around about these lines. As they relax back, they give off a signal at a frequency that betrays their elemental identity. Researchers solve the structure of proteins using a variation of the technique, in which they send radio waves into a sample at frequencies designed to excite particular nuclei. This excess energy can be transferred to a neighboring atom. The rate of this energy exchange is related to the distance between the two atoms.

After painstakingly building up distance information between many hundreds of pairs of nuclei, researchers can calculate a 3D structure of the molecule that fits the data. Extracting that information is not easy, however. In practice, the energy transfer between two atoms is also affected by their constant motion with respect to one another. And there has been no direct way to learn the orientation of the pairs of atoms relative to the rest of the molecule. The resulting structures tend to be fuzzy and imprecise.

Knowing how the bonds are oriented would sharpen the structures considerably. To tease out this extra information, Tjandra and Bax rely on another effect that NMR researchers have known about for decades but have never been able to use to solve

protein structures. When radio waves probe a nuclear magnet, the presence of another close by can cause a signal to emerge that is split between two frequencies because of a magnetic interaction called dipolar coupling. Just how different these two frequencies are from each other, a measure known as "splitting," is very sensitive to the orientation of the bond between the atoms with respect to the external magnetic field. The splitting reaches a peak when the bond is parallel to the external magnetic field. By comparing the splitting seen in many different pairs of atoms, researchers can map the bond orientations and greatly sharpen the structure.

"The problem is that in solution we normally can't see dipolar coupling," says Tjandra. Proteins in solution normally tumble in all directions, thereby washing out the signal. Researchers have tried different techniques to get their proteins to line up in solution, including orienting them with magnetic fields and wedging them among self-aligning molecules known as liquid crystals. But the magnetic fields only produced a small effect, while the liquid crystals had too big an effect. They confined the proteins so firmly that they lined them up perfectly. When the proteins were held in such a regular pattern, dipolar coupling could be seen not just between near neighbor atoms, but between hundreds of atoms in a molecule, swamping researchers with signals. "For anything but small molecules, the data are uninterpretable," says Bax.

That's where Tjandra and Bax made their advance. They diluted a type of fat-based liquid crystal, so that its molecules align themselves in solution with plenty of space between them. That gives the proteins room to move between the liquid crystalline walls, only occasionally bumping into a wall. The proteins themselves are slightly oblong, so repeated bumps cause them to align more or less in the direction of the liquid crystals. The liquid crystals "align their molecules enough so that they can measure something but not so much that the data are uninterpretable," says Lewis Kay, an NMR expert at the University of Toronto. "That's the beauty of this method."

Tjandra and Bax tested the technique's ability to chart the orientation of atom pairs in a protein called ubiquitin. They found that their measurements agreed precisely with the picture provided from a high-resolution x-ray crystal structure. Since then, says Tjandra, they have gone on to sharpen the focus of other NMR structures, although they are not yet ready to reveal those results. If the new structures manage to match the sharpness of x-ray crystal structures, NMR could be in for a whole new focus.

–Robert F. Service

ECOLOGY

Rain Forest Fragments Fare Poorly

The massive clearing of tropical rain forests over recent decades is having a profound effect on Earth's atmosphere—adding carbon dioxide and exacerbating other human causes of global warming. Now it seems that the fragments of forest left when tracts of rain forest are cut are also making their own, unsuspected contribution to the carbon dioxide equation. On page 1117, William Laurance of Brazil's National Institute for Research in the Amazon in Manaus reports on a 17-year study suggesting that, once separated from the bulk of the forest, fragments below a certain size are unable to maintain the structure of the original forest. They lose considerable amounts of biomass as large trees, exposed to wind and weather extremes, are killed or damaged—reducing the amount of biological material in the fragment able to absorb carbon dioxide during growth.

FREDERICK C. ENGLE

Small is vulnerable. Isolated fragments of rain forest soon suffer from exposure to the elements.

"There are so few long-term studies, and this tells us what is actually happening today," says tropical rain forest expert Ghillean Prance, director of Kew Gardens in London. "And the findings are crucial if we are to plan for the future." The results suggest that forestry plans that require patches of forest to be preserved should set a minimum size. They also suggest that climate modelers will need to consider the effects of biomass loss not just in isolated forest patches but also near the edges of intact forest, where the same processes should be at work.

Between 10 and 17 years ago, Laurance's team selected a series of forest patches of 1, 10, and 100 hectares in size that were recently isolated when the forest around them was cleared for cattle pastures. The researchers also marked out a number of identically sized control patches in native forest. The team then estimated the amount of biomass in the different patches by measuring the diameter of all trees along sections within the patches. The original measurements, of more than 50,000 trees, were repeated several times, with the latest measurements taken earlier this year. "The long-term nature of this study is the great thing about it," says ecologist Roger Leakey at the Institute for Terrestrial Ecology in Edinburgh, U.K.

Using a theoretical model, the team converted the diameter measurements into an estimate of the total changes in biomass since the start of the study. The team found that within the patches there was a substantial loss of biomass among the trees up to 100 meters from a forest edge. More than a third of biomass was lost in these regions over the study period compared with control patches, and there was no evidence of recovery. The biomass loss occurred rapidly in the 4 years following clearance of the surrounding forest as trees were killed or damaged by exposure to wind and other changes in microclimate, then stabilized at the lower level.

The team does not yet know whether, over longer time scales, the patches will recover to levels found in the original forest, but they think it unlikely because wind damage will be an ever-present danger to trees near the forest edge. "Original complex forest will more likely be replaced by shorter, scrubby forest with less volume and biomass," says team member Thomas Lovejoy, director of the Smithsonian Institute for Conservation Biology in Washington, D.C.

For climate modelers, the results put another gloomy figure into their calculations. Not only does the carbon capacity from felled trees need to be considered, but also the massive loss of biomass within the edges of all remaining forest and forest fragments. "If you're thinking about forests in the carbon cycle, then these results are important," says Prance.

–Nigel Williams