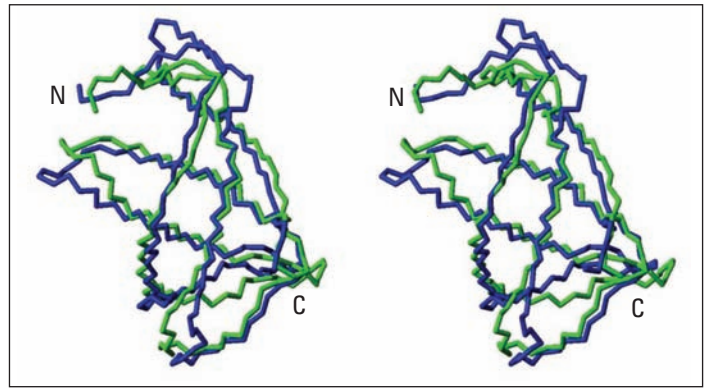
ANALYTICAL CURRENTS

An NMR structure from micrograms of protein

Gaetano Montelione and colleagues at Rutgers University, Bruker BioSpin Corp., and the Robert Wood Johnson Medical School have reported the first complete NMR structure of <100 μg of protein. The researchers used a recently developed microcoil NMR probe to determine the structure. Microcoil probes (1-mm diam) require just a few microliters of sample and offer many advantages over traditional probes (5-mm diam), including enhanced solvent suppression, improved tolerance of salt, simplified shimming, and improved field homogeneity.

The researchers first tested the performance of the microcoil probe on proteins with a range of masses. For proteins up to ~20 kDa, they could obtain ^{1}H–^{15}N heteronuclear single quantum correlation spectral data that was of similar quality to data from traditional probes.

To test the mass detection limits of the microcoil probe, the researchers used the 68-residue TRAM domain protein from *Methanosarcina mazei*.



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Stereo view of the superposition of the lowest-energy conventional solution structures of the TRAM domain from conventional (blue) and microprobe (green) experiments.

They dissolved 72 μg of protein into 6 μL total sample volume and performed a range of NMR experiments, including 3D ^{13}C–^{13}C correlation spectroscopy, 3D ^{13}C–^{13}C total correlation spectroscopy, and 3D ^{13}C-edited nuclear Overhauser enhancement spectroscopy, to obtain structural information. For comparison, they obtained the same NMR data for 1600 μg of the same protein with a traditional probe.

Although the microcoil-probe structural data took approximately twice as much time to collect as with the traditional probe, the researchers found that the backbone of their structure deviated from the traditional structure by an average of only ~0.73 Å.

The researchers note that this microcoil-probe technology could be used in a variety of high-throughput screening systems and will allow analysis of proteins that are difficult to produce in high quantities, such as those from cell-free, insect cell, and eukaryotic expression systems. (*Nat. Methods* **2007**, *4*, 491–493)

Watching a transcription factor in action

Students of genetics learn about the *lac* operon in *E. coli* as a case study in gene regulation. The two genes that are regulated (concurrently) encode permease and β-galactosidase, both of which are involved in lactose metabolism. When a transcription factor called the *lac* repressor binds to the operator—a stretch of DNA that precedes those genes—less enzyme is expressed. The dynamics of this system were worked out with biochemical methods by François Jacob and Jacques Monod in the 1950s. Now, Johan Elf, Gene-Wei Li, and X. Sunney Xie at Harvard University show that they can watch these dynamics in a live bacterium at the single-molecule level.

The researchers expressed the *lac* repressor as a C-terminal fusion with a yellow fluorescent protein. (The DNA-binding domain is at the N terminus.) To simplify the image analysis, the researchers expressed fewer copies of the protein per cell than normal. With a fluorescence microscope and a CCD camera, the Harvard team measured the kinetics as the *lac* repressor bound to and dissociated from the operator in the presence and absence of a synthetic inducer. (The inducer binds the repressor and allows normal or greater-than-normal expression of the enzymes.)

In addition, the researchers followed the repressor as it traveled along the DNA. They calculated that the protein spends ~90% of its time searching for the operator and diffuses along the DNA with a residence time of <5 ms. (*Science* **2007**, *316*, 1191–1194)