Peeking into living eukaryotic cells with high-resolution NMR

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For a biological chemist, possessing atomic-level knowledge of all of the molecules inside a living cell would be nirvana. Until recently, however, biological macromolecules were almost always removed from cells before study at the atomic level. Such reductionism has taught us a lot—just try lifting a general biochemistry text—but the approach is inherently limiting because information about biological context is lost. With the advent of in-cell NMR, it is now possible to study atoms in biological macromolecules while they remain in living cells (1). An article by Selenko *et al.* (2) in this issue of PNAS marks an important advance in these endeavors by providing the first high-resolution glimpse into how the cytoplasm of a higher eukaryotic cell can affect the properties of a folded protein.

Unraveling the effects of biological context is important only if the intracellular environment can impact the properties of proteins. The most obvious difference between a typical *in vitro* sample and the inside of the cell is the solute concentration. Macromolecular solutes reach concentrations of hundreds of grams per liter in cells (3) and other biological fluids, but most *in vitro* studies are performed in buffered solution with <1% of the cellular macromolecule concentration. These conditions give optimal signals but may lack biological relevance.

Theories of macromolecular crowding have been exquisitely crafted (4) to address diffusion, stability, and structure, but let us focus here on a simple analogy, umbrellas. When it rains, many people open their umbrellas. What happens as a crowd builds up? Individuals have less space, and the fraction of closed umbrellas is likely to increase. Now consider those closed umbrellas as folded proteins and the open umbrellas as denatured proteins, and you can visualize why macromolecular crowding should stabilize proteins. In short, crowded conditions generally favor species that minimize their surface area.

NMR spectroscopy is the only routine method that provides atomic-level information about proteins in solution. The hydrogen nucleus is very NMR-sensitive, and, of course, proteins are chock full of

Fig. 1. Heteronuclear single quantum correlation spectra of ^{15}N -enriched GB1 acquired in dilute solution (Top), egg extracts (Middle), and oocytes (Bottom). An example of the frog, *Xenopus laevis*, is shown in the background. (Bottom) An NMR tube filled with 200 oocytes injected with the ^{15}N -enriched protein is shown on the left, and a single oocyte is shown on the right.

hydrogen atoms. This surfeit of hydrogens is also a problem, making it difficult to distinguish individual hydrogens. The problem was solved over the last 30 years by developing experiments that spread the information across several independent dimensions and by enriching the protein with other NMR-active nuclei, for instance ^{15}N . Isotopic enrichment allows sorting of the protons because there is only one backbone nitrogen in each amino acid residue.

Isotopic enrichment is also the key to in-cell NMR. The atoms of the protein

being studied are enriched in the isotope relative to the atoms in other molecules in the cell, thereby separating their signal from those of the unenriched molecules. The first in-cell protein NMR experiments were performed in yeast by enriching with ^{19}F -labeled amino acids (5). Volker Dötsch and colleagues (6) then did the seminal work to bring high-resolution in-cell ^{15}N -NMR and ^{13}C -NMR to the workhorse bacterium *Escherichia coli* (1). Their work opened the door to an exciting new world where pH effects (7), metal binding (8), structure formation (9), protein-protein interactions (10), and dynamics (11) can be measured inside cells. This is but the beginning of the story.

There remains a lot that in-cell NMR will tell us about the environment in prokaryotes such as *E. coli*, but the next goal is to apply in-cell NMR to higher eukaryotic cells. Selenko *et al.* (2) take this step by using oocytes, which are immature eggs. Frog oocytes are used extensively in biological research because, for cells, they are gigantic. Their $\approx 1\text{-mm}$ diameter is 10 times that of a human oocyte, 50 times that of a typical somatic cell, and 200 times longer than rod-shaped *E. coli* cells.

Their significant size makes frog oocytes an easy target for injecting exogenous compounds. Instead of producing their ^{15}N -enriched protein inside the oocytes, Selenko *et al.* used a robot to inject ≈ 200 cells with a ^{15}N -enriched recombinant protein purified from *E. coli*. Such treatment eliminates any background caused by the incorporating of the isotope into other molecules. Fig. 1 shows an NMR sample tube containing enough oocytes for an in-cell NMR experiment. The cells fill $\approx 1\text{ cm}$ of the tube. Next to the tube is the heteronuclear single quantum correlation spectrum obtained from the sample.

The subject of the study is a small (7 kDa) stable protein called GB1. This protein is an excellent choice because we know a lot about its structure,

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