New technique allows study of protein folding, dynamics in living cells

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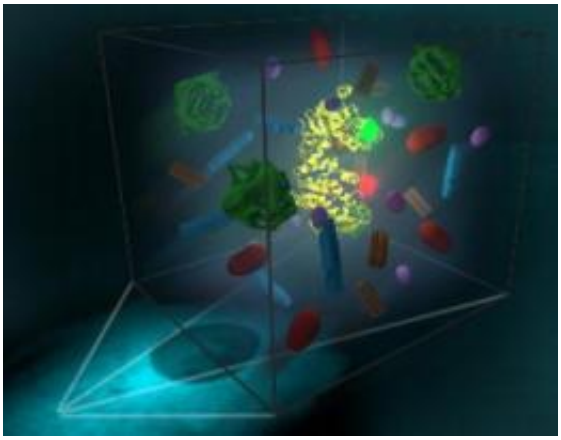

A new technique to study protein dynamics in living cells has been created by a team of University of Illinois scientists, and evidence yielded from the new method indicates that an in vivo environment strongly modulates a protein's stability and folding rate. Credit: Simon Ebbinghaus

A new technique to study protein dynamics in living cells has been created by a team of University of Illinois scientists, and evidence yielded from the new method indicates that an in vivo environment strongly modulates a protein's stability and folding rate, according to research accepted for publication in the journal *Nature Methods* and posted on the journal's Web site Feb. 28.

Martin Gruebele, the James R. Eiszner Professor of Chemistry at Illinois and corresponding author of the paper, says the method that he and his

team of co-researchers engineered marks the first time anyone has been able to follow the real-time folding and unfolding of proteins outside of a test tube.

"This is the first experiment that allows us to observe the dynamics of a protein folding in a live cell," Gruebele said. "Now we have the capability of looking at how fast biological processes occur as a function of time."

To study the biomolecular dynamics inside of a single living cell, Gruebele and his team pioneered a hybrid method they've dubbed "Fast Relaxation Imaging," a technique that combines fluorescence microscopy and fast temperature jumps.

"It's a tool that combines two worlds: chemical dynamics, and the ability to study reactions as they occur; and biological environments, where cell biologists observe how reactions occur in cells," Gruebele said.

To achieve both a fast upward and downward temperature jump, programmed laser pulses are used to pre-heat, spike, plateau, cool and then finally stabilize the temperature in the cell and its aqueous medium at the final value. An inverted fluorescence microscope is used to observe and record what happens inside the cell, all of which takes place in the span of a few milliseconds.

The cells are usually heated to between 96 and 100 degrees Fahrenheit.

"It's like we give them a little bit of a fever," Gruebele said.

Gruebele says that although temperature jumps have been used for some time to study the kinetics of chemical reactions in vitro, that method is limited by what he calls "homogenous kinetics," or an inability to see the dynamics in different areas of the cell.

"We haven't really been able to study dynamics, to see if a chemical reaction like protein folding varies inside of a living cell," he said. "With temperature jumps and pressure jumps, you can do those experiments very quickly, but you don't get any imagery that lets you see if proteins fold faster in one region and slower in another," Gruebele said.

On the other hand, fluorescence microscopy allows researchers to see inside of cells, but it precludes them from studying cell dynamics and kinetics.

"With fluorescence microscopy, we're able to take images of cells and see inside them, but we can't observe how anything rapidly changes or adapts with time, so you can't look at any but the slowest dynamics. This experiment puts those two aspects together," he said.

Since biomolecular dynamics are predominantly studied in vitro, with the results extrapolated to explain how the same processes would function in a living cell, Gruebele says the new technique has yielded some interesting data that could change standard thinking in the field.

"If you perform experiments only in an artificial environment such as a test tube and not in a living cell, you only get one answer," Gruebele said. "It's a reproducible environment; therefore, it always gives you the same answer. If you do it in a cell, we find we get very different answers in different parts of the cell."

According to Gruebele, the proteins studied in vivo using the new technique were more stable, their thermal denaturation was more gradual and their folding kinetics were slower than the same proteins studied in vitro.

Gruebele said that in living cells, "You really expect a lot of heterogeneity, that there would be a lot of differences among different

areas of the cell, that there might be areas of the cell where the protein might be very stable, and other places where it's very unstable. There might be places where it folds very quickly, and other places where it folds very slowly."

The reason for this heterogeneity is that proteins have to thread their way through whatever channel happens to be available, Gruebele said. And, as opposed to the expansive environment of a test tube, there's a lot of cellular furniture for proteins to bump into in living cells.

"You have a very simple, very homogenous environment when you study proteins in vitro," he said. "In a living cell, 30 to 40 percent of the contents are solids of some kind. There are big ones, like ribosomes, and walls, like cell membranes, all the way down to very small parts like other proteins or sugars. So there's really a huge distribution of all these different sizes that a protein has to wend its way around that may hinder it from freely expanding and contracting, as it would do when it unfolds and refolds in an artificial environment.

"So it's the environment, and not just an intrinsic property of the protein, that causes all these variations that we observed."

In addition to revealing the inner working of cell dynamics, Gruebele, who is also a researcher at the Beckman Institute, says Fast Relaxation Imaging will have practical, human-scale applications as well.

"With this new technique, we now have the capability of looking at how fast biological processes occur as a function of time, including potentially interesting disease processes, especially with neurological disorders and diseases that cause dementia such as Alzheimer's, Huntington's, Creutzfeldt-Jakob disease," he said.

There's also the potential to induce disease processes, and study the

dynamics of those processes in a live animal study.

"We can take these proteins that cause these diseases, actually put them into the kind of cells where they cause these diseases, give them a heat shock and actually see if they bind differently to the membranes, if they cause the membrane to puncture," Gruebele said. "We'll be able to follow these events in real time and give researchers an idea of if this is a possible pathway through which disease could occur."

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