UCSD chemists find a better way to trap molecules

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In a new twist of the inventor's perpetual quest to find a better mouse trap, a team of San Diego chemists has devised a better way to trap individual molecules--this time, under conditions resembling the inside of a living cell.

As described in today's issue of the journal Science, the trap is a porous gel whose matrix is bathed in water at room temperature. Once isolated and contained inside this framework, individual light-emitting dyes and fluorescently labeled protein molecules are imaged, thus allowing scientists to follow the activities of the protein under a microscope.

"What we are doing with these techniques is to put fluorescent molecules on selected proteins and use them as light sources to tell us something about how the protein functions," said W. E. Moerner, professor of chemistry and biochemistry at the University of California, San Diego (UCSD), and the study's principal investigator.

"We are doing this in an environment which allows the protein to retain its native structure and its functions to remain intact."

In recent years, researchers have been trying to get a better understanding of proteins and how they work in the body, to gain new insights into disease processes and ultimately to design new drugs to combat these disorders.

Much has been learned about protein structure during this time, with the aid of sophisticated imaging techniques including X-ray crystallography and nuclear magnetic resonance. Using high-performance computers and visualization software, three- dimensional structures of proteins have been captured in atomic detail, with their unique contours and folds dictating how each binds and unbinds to other molecules to sustain life.

But no protein is static. They're constantly vibrating, bobbing and weaving, opening and closing.

"We know that to do their job, proteins somehow must change their shapes," said Moerner, who holds the Distinguished Chair in Physical Chemistry at UCSD. "Some part of the protein must open. Some part must close. That's the part that's been hard to understand, because we don't have a clear picture of that right now, of exactly how that happens."

Seeing a protein moving about in its native environment has proved elusive, primarily because in solution, proteins are continually buffeted by forces that push them around so fast they can't be carefully observed. This buffeting activity, called Brownian motion, is triggered by an uneven number of water molecules pressing against a particle's surface, creating a net force that propels it through the solution in a random, zigzag fashion.

As a result, even today's most sophisticated imaging devices --including confocal microscopy and scanning tunneling microscopy--have experienced limited success in viewing proteins doing critical tasks.

In the Science article, the researchers describe a technique for observing single molecules and proteins in a water-filled environment that builds on a method called single-molecule spectroscopy, developed in 1991 at IBM by Moerner and colleagues. Here, a single molecule is trapped inside a crystal lattice which is cooled to extremely low temperatures near absolute zero. A laser tuned to a specific wavelength then causes the molecule to fluoresce, emitting light. By carefully monitoring the light given off by the trapped molecule, the researchers are able to make direct observations of sometimes extremely subtle and sometimes bizarre motions of the molecule within the crystal.

The technique was a vast improvement over conventional spectroscopic techniques, which by collecting and analyzing light absorbed or emitted by large ensembles of molecules, washed out the subtle, but sometimes significant actions, of individual molecules.

For studying protein function, however, the technique proved virtually useless since very low temperatures, rigid crystals, and dry polymers are incompatible with observations of the motions necessary for biological function.

What was needed, the researchers theorized, was a different way of confining fluorescent molecules, one that was water-based, could work at room temperature, and would allow proteins some motion--although limited enough so they could be studied.

Robert M. Dickson, a postdoctoral researcher in Moerner's laboratory, quickly homed in on a porous gel called polyacrylamide (PAA), routinely used as a sieve in the laboratory to separate proteins by molecular weight. The biologically friendly gel also is transparent to visible and ultraviolet light (useful for microscopic studies), contains tiny pores filled with water, and forms a generally useful media for the study of single biomolecules.

"We made gels containing single small fluorescent molecules in very dilute solutions," Dickson explained. "The gel then polymerized, was excited by laser to fluoresce, and we looked at them under the microscope.

"Eventually, after figuring out how to look for them, where to look for them, we began to see individual molecules that were moving around in the gel throughout its porous structure."

Moerner and Dickson then calculated that the gel matrix successfully restricted the movement of individual protein molecules by a factor of 100. This restriction of motion allowed the observation and study of these individual small molecules in biological environments.

The researchers subsequently employed these techniques to observe single proteins tagged with fluorescent molecules. They were able to observe these proteins for long periods of time, thus enabling future studies designed to better understand protein function.

Aside from learning more about individual proteins, the technique also could be used as a probe to analyze the structure of the surrounding gel. "This could have applications in the study of lots of other types of separation materials," said Moerner. "You could learn something about the pore sizes, their distribution and connectivity, and how easily molecules move from one region to another."

Also participating in the study were D. J. Norris, a postdoctoral researcher at UCSD, and Yin-Ling Tzeng, a researcher with The Scripps Research Institute.

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