Behavior of single protein observed in unprecedented detail by Stanford chemists

March 10 2010, By Louis Bergeron

(PhysOrg.com) -- For the first time, researchers have been able to confine and study an individual protein, one that plays a key role in photosynthesis, without having to pin it down so tightly as to alter its fundamental behavior.

In the first practical application to proteins of a recently developed technique, two Stanford chemists were able to make detailed observations of the dynamic behavior of the molecule for more than one second, a 50- to 100-fold increase in viewing time compared to other methods, and thereby "set a new standard in single-molecule spectroscopy," according to a commentary in the March issue of *Nature Chemistry*.

The groundbreaking study is described in a paper by Stanford chemists W. E. Moerner and Randall Goldsmith in that issue, now available online. The commentary was written by researchers not involved in the study.

Observing molecules one at a time is valuable because it lets researchers get a clear picture of that molecule's changing behavior over time, without the picture being confused by the presence of other molecules.

Up until now, researchers have had to remove a molecule from its normal environment - typically a solution such as the bloodstream or the fluids inside a cell - and "basically staple it to some surface such as a glass slide or a large plastic bead, or imbed it in a synthetic polymer to

observe it," said Goldsmith, a postdoctoral researcher in chemistry.

The result, he said, is like trying to discern how a tiger behaves in the wild by watching it pace back and forth in a cage at the zoo. "You have every reason to be suspicious that you might profoundly alter the behavior of the molecule by binding it to a surface," Goldsmith said.

That perspective is buttressed by the results of their study, in which they "trapped" in solution a molecule of a fluorescent photosynthetic protein called allophycocyanin, which is found in red algae and cyanobacteria (formerly known as blue-green algae). Both algae hold promise as key components in next-generation solar technologies and biofuels.

Moerner, a professor of chemistry, and Goldsmith used a device developed in Moerner's lab several years ago by former graduate student and postdoctoral scholar Adam Cohen, called an Anti-Brownian Electrokinetic (ABEL) trap. In his thesis work Cohen used the trap to precisely measure shape fluctuations in single DNA molecules before starting his own academic career at Harvard.

Brownian motion is the random movement of small particles in a gas or liquid. The movement arises from the particles being bumped by molecules of the fluid; the trap works by cancelling out a molecule's Brownian motion. Goldsmith described the method this way: "If the molecule moves east, we give it a kick west. If it moves west, we give it a kick back east. And we have that process going about 40,000 times a second."

The "kicks" are produced by controlled flows of the solution in which the molecule is placed for observation. The flows are driven by four electrodes evenly spaced around the perimeter of the trap. Although the molecule is actually tumbling around slightly in the solution in response to the many little kicks it receives, it tumbles in such a confined area that

for practical purposes, it is being held in suspension and is stable enough for extended viewing by the researchers. In the case of the protein in this study, Goldsmith said they were often able to hold onto a molecule and view it for more than an entire second.

"That may not sound like very much, but if you don't have the trap and you don't want to staple your molecule to a surface, you are basically limited to 10 or 20 milliseconds," he said.

All the current single-molecule techniques - whether the older, more confining ones or Moerner and Goldsmith's comparatively free-range method - involve fluorescence microscopy, which employs a laser to excite the molecule of interest into emitting photons.

Not all parts of a protein fluoresce, only certain subgroups within the structure, but the brightness levels of the fluorescence tell the researchers something about how the fluorescent subgroups are interacting with each other.

Goldsmith said one of the previous studies had observed three brightness levels, whereas he saw four or more distinct levels in their experiments. "That doesn't sound like it makes a big difference, but for these particular molecules it speaks to a fundamentally different type of behavior," he said.

"We saw that these proteins were undergoing dynamics that would have been more or less impossible to see, had you had them confined," Goldsmith said. "What we think is happening is that the protein that encompasses these fluorescent groups is actually changing shape."

Currently there are only two ABEL traps in the world besides the one in Moerner's lab. "It is a complex scientific instrument. It is not something trivial to build," Goldsmith said. "But whenever we take this story on the

road and talk to other researchers about what the ABEL trap can do, we always see everyone's eyes get really wide, when they see the sort of unperturbed behavior you can get."

"It is not perfect," Goldsmith said. "But we have moved a lot closer to the ideal." "And the ABEL trap is being used for other exciting experiments on single molecules," Moerner said.

More information: 'Watching Conformational- and Photodynamics of Single Fluorescent Proteins in Solution,' Nature Chemistry, March 2010, Vol. 2, No. 3. www.nature.com/nchem/journal/v …/full/nchem.545.html

Provided by Stanford University