Advances in single-molecule research 'revolutionary, not evolutionary'

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Not long ago, the idea of conducting an experiment on a single strand of DNA seemed far beyond the realm of science. But thanks to rapid advances in microscopy in the last decade, researchers can now watch a single gene being transcribed from DNA—one atom at a time—or observe the activity of a protein molecule as it moves inside a living cell.

This emerging revolution in nanomedical research was the focus of two scientific panels titled "Frontiers in Single-Molecule Biophysical Chemistry and Imaging" at the 2006 national meeting of the American Chemical Society (ACS) in San Francisco. Panelists included two Stanford University scientists at the forefront of single-molecule microscopy—W. E. Moerner, the Harry S. Mosher Professor of Chemistry, and Steven M. Block, professor of biological sciences and of applied physics.

"The whole field continues to explode in many different directions," said Moerner, who spoke at the Sept. 12 session. "One frontier involves single-molecule experiments with living cells. We can now identify and observe an individual molecule in action inside a living bacterium. This is a growing area of research that's only a few years old."

Let there be light

One of the biggest challenges for Moerner and his co-workers is how to make an individual molecule visible without harming the live cell. "You

have to be noninvasive," he explained. "You can't blast the organism with X-rays, for example, so we use light instead."

In 1989, Moerner and his fellow researchers at IBM were the first to show that a single organic molecule could be detected with optics. Since joining the Stanford Department of Chemistry in 1998, he has expanded his research to include proteins and other biomolecules. In a study published in the July 18 *Proceedings of the National Academy of Sciences* (PNAS), he and his Stanford colleagues successfully tagged individual molecules of a recently discovered protein called MreB and monitored its movements inside living bacteria.

"MreB is so new we didn't know how it works or what its function was," Moerner said.

Using a very sensitive microscope, graduate student So Yeon Kim, lead author of the PNAS study, produced real-time videos of single molecules in action. "She found that MreBs move around the cell randomly," Moerner said. "But more of a surprise was that some MreBs were part of a filament of many MreBs that moved like a treadmill inside the cell."

The researchers discovered that a single MreB molecule joins one end of the filament and treadmills across its entire length—a distance of approximately 390 nanometers—in about one minute. "The molecule then falls off the other end of the filament and starts zooming around the cell," Moerner said.

In bacteria, MreBs are critical for chromosome replication and maintaining the shape of the cell. They also are closely related to actin, a protein that's essential for healthy cells in plants and animals, including humans. Therefore, understanding the function of a single MreB protein in a bacterium could provide important insights for biomedical research,

Moerner noted.

"This knowledge may lead to the discovery of antibiotics and become targets for new drugs," Moerner said. "The exciting thing is to observe new processes inside the living cell at the single-molecule level."

The PNAS study was co-authored by Stanford researchers Lucy Shapiro, the Virginia and D. K. Ludwig Professor of Developmental Biology; former postdoctoral fellow Zemer Gitai; and graduate student Anika Kinkhabwala. The research was funded by the Department of Energy and the National Institutes of Health.

Nature's nanotechnology

Advances in single-molecule research in the last 15 years have been "revolutionary, not evolutionary," said biophysicist Steven Block, who addressed the ACS meeting on Sept. 10.

"When I was an undergraduate in the early 1970s, the idea of recording data from a single molecule was a pipe dream," he recalled. "No one would ever believe you could do that. It was beyond possibility. Now it is reality. It is literally possible to study the output of a single enzyme at atomic-level resolution."

In 1993, Block and his colleagues were the first to observe the movement of an individual molecule of kinesin, a tiny protein that carries chromosomes, neurotransmitters and other vital cargo along minute tracks called microtubules in living cells. Using a sensitive microscope-based instrument known as an optical trap, the Block team observed that kinesin moves along microtubules in discrete steps that are a mere 8 nanometers long. "Kinesin and other motor molecules are really nature's nanotechnology," he said.

By 2005, he and his colleagues had made dramatic improvements in the optical trap that enabled them to measure a single enzyme moving along a strand of DNA to within a distance of one-tenth of a nanometer, which is equivalent to the diameter of a single hydrogen atom.

"Between 1993 and 2005, the resolution improved from 8 nanometers to 3.4 angstroms," Block said. "That means we were able to make observations that are 25 times smaller in a period of about 10 years. The ability to observe single molecules with this degree of precision opens new windows onto what molecules do and allows you to report their individual behavior rather than their ensemble average—and that's powerful."

As an example, he pointed to a paper he and graduate student William Greenleaf co-authored in the Aug. 11 issue of the journal Science that presents a new method for sequencing DNA using the single-molecule technology. DNA consists of a chain of molecules, or bases, known by the abbreviation A, T, G and C that occur in a specific order that is usually determined chemically. But Greenleaf and Block showed that DNA sequencing also can be done by measuring how long a single enzyme, called RNA polymerase, pauses at each base's location.

"This process is unique because it uses the motion of RNA polymerase, not chemistry, to sequence DNA," Block said. Motion-based sequencing may prove commercially viable one day, he added, noting that Stanford is planning to license the technique.

"Studying individual molecules allows you to discover how each one differs," Block noted. "They're not all exactly the same, the way all hydrogen atoms are the same. Biomolecules have character and subtle differences that we don't fully understand. A lot of diseases are due to genetic defects that cause aberrant behavior in molecules. We'd like to learn what those aberrant behaviors are—how do they work and how do

they fail to work? To answer those questions, we now have these new tools with astonishing precision—the single-molecule methods that allow us to ask new questions about molecular mechanisms."

Source: Stanford University, BY MARK SHWARTZ