New microscope allows scientists to track a functioning protein with atomic-level precision

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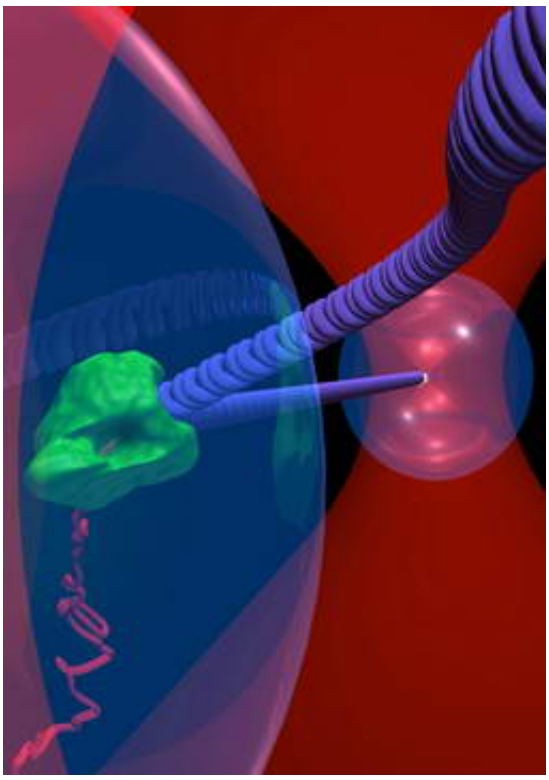

A Stanford University research team has designed the first microscope sensitive enough to track the real-time motion of a single protein down to the level of its individual atoms. Writing in the Nov. 13 online issue of the journal *Nature*, the Stanford researchers explain how the new

instrument allowed them to settle long-standing scientific debates about the way genes are copied from DNA--a biochemical process that's essential to life.

In a second paper published in the Nov. 8 online issue of the journal *Physical Review Letters*, the scientists offer a detailed description of their novel device, an advanced version of the "optical trap," which uses infrared light to trap and control the forces on a functional protein, allowing researchers to monitor the molecule's every move in real time.

"In the Nature experiment, we carried out the highest-resolution measurement ever made of an individual protein," says Steven Block, professor of applied physics and of biological sciences. "We obtained measurements accurate to one angstrom, or one-tenth of a nanometer. That's a distance equivalent to the diameter of a single hydrogen atom, and about 10 times finer than any previous such measurement."

Block co-authored the papers in Nature and Physical Review Letters with three current members of his Stanford Lab--graduate students Elio Abbondanzieri and William Greenleaf and postdoctoral fellow Michael Woodside--together with former graduate student Joshua Shaevitz, now at the University of California-Berkeley, and longtime collaborator Robert Landick at the University of Wisconsin.

Central dogma

In the Nature study, Block and his colleagues tackled a fundamental principal of biology known as the central dogma, which states that in living organisms, genetic information flows from DNA to RNA to proteins.

The process begins with DNA, the famous double helix, which stores genetic data. DNA is often compared to a twisted ladder consisting of

two strands connected by molecular rungs called "bases," which are known by the abbreviations A, T, G and C. Lengthier DNA sequences code for genes, which contain explicit instructions for building a specific protein.

A typical DNA ladder carries thousands of genes that encode thousands of proteins, which keep the organism alive and functioning. A single misplaced letter in gene's DNA sequence--a G substituted for a T, for example--can produce a defective protein that may cause a serious disease.

Transcription

The Block team focused on a crucial step in the central dogma, a process known as "transcription," where each gene is copied from DNA onto RNA. Transcription begins when an enzyme called RNA polymerase (RNAP) latches onto the DNA ladder and pulls a small section apart lengthwise. The RNAP enzyme then builds a new, complementary strand of RNA by chemically copying each base in one of the exposed DNA strands. RNAP continues moving down the DNA strand until the gene is fully copied.

For the Nature experiment, Block and his colleagues used DNA and RNAP extracted from E. coli bacteria, which is remarkably similar to RNAP in more complex organisms, including humans. "RNAP is one of the most important enzymes in nature," Block says. "Without it there would be no RNA messages, no proteins and no life."

Inchworms and scrunching

Exactly how transcription works at the molecular level has been intensely debated among scientists.

"People for years have known that RNA is made one base at a time," Block says. "But that has left open the question of whether the RNAP enzyme actually climbs up the DNA ladder one rung at a time, or does it move instead in chunks--for example, does it add three bases of RNA, then jump along and add another three bases." The latter process, called discontinuous elongation, is like reading a book, he explains: "When you read, you don't advance your eyes one letter at a time. You 'chunk': You read it in pieces."

Two basic hypotheses have been proposed for discontinuous elongation:

-- Ihe inchworm model, in which RNAP moves along DNA like an inchworm, with the front end of the enzyme always ahead of the rear.
-- The scrunching model, whereby RNAP pulls in ("scrunches") a loop of DNA, copies each base in the loop, then grabs another loop farther up the ladder.

Determining which model is correct has been a difficult challenge, because until now, no instrument was sensitive enough to track each microscopic step taken by RNAP along DNA during transcription. That's because conventional optical traps can't measure anything smaller than about 10 angstroms (1 nanometer). However, each base in the DNA ladder--A, T, G or C--is only separated by about 3.4 angstroms. "My lab has been working very hard for the better part of a decade to break the nanometer barrier and attain angstrom-level resolution," Block says.

Light and motion

To achieve that goal, the Block team had to overcome two inherent problems with conventional force clamps: fluctuating signals and bending light waves.

"When you shine a laser through the air, the light beam wiggles around a

bit, for the same reason that stars twinkle in the sky," Block explains. "But we want to use that beam to measure the position of something to within the size of an atom, so if the beam moves just 1 angstrom, that's the end of the story. We took all the optics external to the microscope, enclosed them in a sealed box and replaced the air with helium gas, which has a refractive index that's 10 times closer to that of a vacuum than air. So you get, roughly speaking, 10 times less twinkling and an instrument with angstrom-level stability."

In addition to stabilizing the light, the researchers also had to improve the method for detecting force and displacement. Optical force clamps use tiny forces from an infrared laser beam to trap DNA and other molecules. In a conventional force clamp experiment, microscopic beads are attached near the opposite ends of a long DNA molecule--an arrangement that resembles a weight lifter's dumbbell. A single RNAP enzyme attached to the surface of one bead then moves along the DNA and churns out a complementary strand of RNA, drawing the ends of the dumbbell closer together as it advances. The two beads that form the dumbbell are usually held near the center two separate optical traps. But graduate student William Greenleaf discovered that if one of the two beads in the dumbbell was placed near the outer edge of its trap, the force on it would remain constant, allowing angstrom-level measurements to be made quickly and efficiently.

"That's just what you want--a clamp that allows RNAP to move with impunity, but the force itself doesn't change," Block says. "Normally the bead is inside the trap in the center, but right at the edge of the trap we have this magical property where the force is constant."

Unlike conventional instruments, the new force clamp requires no time-consuming computer computations to correct for competing forces. "This new technique is entirely passive, like a thermos that just sits there and keeps something cool," Block says. "All we have to do is shine light

on the system and everything takes care of itself. As a result, we were finally able to resolve the minuscule, 3.4-angstrom steps taken by E. coli RNAP as it transcribes a bacterial gene."

Settling the debates

With these innovations in place, the research team appears to have settled some of the fundamental arguments over DNA-RNA transcription. "Quite simply, our experiment rules out both discontinuous-location models," Block says. "Neither the inchworm nor the scrunching model is consistent with our data, and the idea that some have held all along--that RNAP climbs the DNA ladder one base pair at a time--is probably the right answer."

The Stanford group also weighed in on another controversy concerning the actual mechanism that allows RNAP to advance. "RNAP is a molecular motor that starts at one end of the DNA and walks down to the other end," Block explains. "It gets its energy from the chemical reaction that occurs when it copies A, T, G or C. It's as if a machine that lays down asphalt could somehow be powered by the asphalt itself."

Scientists have come up with two different models to explain what drives this molecular motor:

-- The power stroke model, in which pent up energy thrusts the enzyme forward--like a loaded spring that's periodically released.
-- The Brownian (or thermal) ratchet model, whereby random thermal energy causes the RNAP enzyme to jiggle back and forth. Each incoming DNA base then locks the enzyme into the forward position so that it cannot jiggle backwards. "It would be as if you were repeatedly bouncing off a wall, and every time you happened to bounce a bit farther away, somebody came in and moved the wall up behind you, so you couldn't bounce so far back. You'd wind up drifting forwards, even

though your own motion was mostly random," Block explains.

In the Nature study, Block and his colleagues concluded that the Brownian ratchet model is probably correct for RNAP, even though several other motor proteins are believed to move instead by the power stroke mechanism. "We've certainly come down hard in favor of the Brownian ratchet camp and against the power stroke camp," Block says. "But does that mean all power stroke models have been ruled out and that all Brownian ratchet models are acceptable? No."

Molecular folding

The Block team also applied the new force clamp technology to one the hottest fields in biomedical research--molecular folding. For a protein to function properly, it has to fold into a specific, intricate three-dimensional shape. Diseases such as Alzheimer's, Mad Cow and Parkinson's may result when proteins do not fold into their correct 3-D conformation. Medical researchers are trying to solve the mystery of how proteins fold in hopes of some day curing these and other diseases.

In the experiment published in Physical Review Letters, the Block group addressed certain aspects of the general folding problem on a simpler scale by focusing on single DNA hairpins--folded structures that can form when a single strand of DNA pairs with itself instead of with the opposite strand. "Hairpins are wonderful models," Block says. "By keeping the force constant, we were able to measure the folding and unfolding transitions of a single DNA hairpin at the angstrom scale. In the future, this may help us understand and predict what shape a more complex linear protein will assume in three-dimensional space."

Major advance

The development of an ultra-stable optical trapping system with angstrom resolution is "a major advance," says Charles Yanofsky, the Morris Herzstein Professor of Biological Sciences at Stanford and a pioneer of modern molecular genetics. The new device is like "adding movies to stills in understanding enzyme action," he says.

"This technical achievement will no doubt lead to new information about the molecular machinery that carries out basic cellular processes, particularly those related to replication, transcription and translation," adds Catherine Lewis, a program director in biophysics at the National Institute of General Medical Sciences (NIGMS).

"If I look in my crystal ball and see where this is going, I think this blows open the field of single-molecule biophysics," Block says. "We have achieved a resolution for a single molecule comparable to what a crystallographer typically achieves in a millimeter-sized crystal, which has 1,000 trillion molecules in it. Not only are we doing all this with one molecule at one-angstrom resolution, we're doing it in real time while the molecule is moving at room temperature in an aqueous solution."

Block notes that it took "years of careful instrument development, sponsored by the National Institutes of Health, and the construction of a special laboratory built by Stanford University to make this possible, along with the simply outstanding efforts of some incredibly bright and hard-working graduate students and postdocs here at Stanford. I am especially proud of this work."

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Source: Stanford University