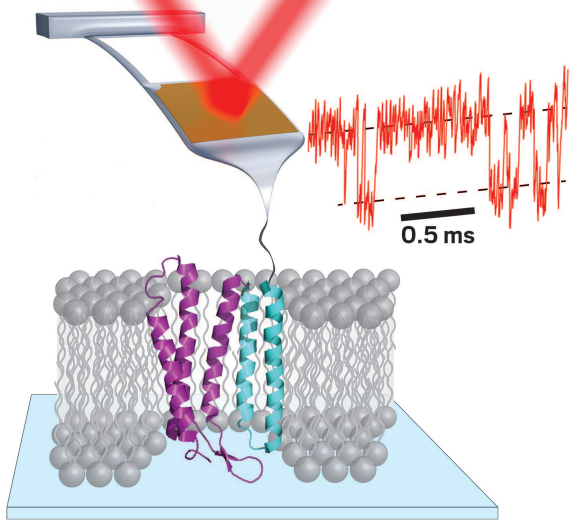
PROTEIN FOLDING

Technique reveals never-before-seen states as protein unfolds

Modified force spectroscopy method captures protein unraveling with improved time resolution



A laser beam (top) detects forces while an AFM cantilever pulls on two bacteriorhodopsin helices (light blue). The SMFS system plots force (vertical axis) versus time as three amino acids reversibly unfold and refold between two intermediate states (dashed lines).

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An improved version of single-molecule force spectroscopy (SMFS) has enabled researchers to study the unfolding of a membrane protein with much higher time resolution and force precision than ever before, revealing a multitude of new details.

Proteins must adopt specific three-dimensional shapes to work properly. If they don't, they can malfunction or cause protein-misfolding conditions such as Alzheimer's disease. Scientists would therefore like to better understand how proteins fold and unfold, including all the intermediate states they adopt along the way.

One type of SMFS technique probes protein-folding intermediates by using an atomic force microscopy (AFM) cantilever to pull on a single protein molecule, unraveling it a little at a time. Most unfolding steps are reversible, so studying unfolding reveals a protein's folding behavior as well.

In the past, SMFS studies found two intermediate states in the unfolding of two helices in the membrane protein bacteriorhodopsin. But molecular dynamics simulations predicted that this process is much more complex, with 10 unfolding intermediates.

Thomas T. Perkins of JILA in Boulder, Colo., and coworkers shortened, reshaped, and surface-modified AFM cantilevers to develop an SMFS system that analyzes proteins 100 times as fast as conventional AFM-based SMFS and with 10 times better force precision. The researchers have now used it to reveal experimentally that two of bacteriorhodopsin's helices unfold with 14 intermediates (*Science* 2017, DOI: 10.1126/science.aah7124).

The findings show that the molecular dynamics results were not far off. "Without sufficiently high time resolution, many intermediate states are masked by instrumental limitations," Perkins says.

The modified system can observe the unfolding of as few as two amino acids over microseconds, whereas conventional AFM-based SMFS typically observes the unfolding of six to 60 amino acids over milliseconds. Another technique called optical trapping can analyze protein unfolding with microsecond time resolution and higher stability than AFM-based SMFS, but it isn't well suited for studying membrane proteins.

The new system's time-resolution and force-precision improvements "represent a tour de force, and I use the pun intentionally," comments Steven Block of Stanford University, an expert on nanoscale biomolecular motions. "The study shows that, with the right instrumentation, you can begin to resolve all the intermediates in protein unfolding," bringing experiment and theory closer together. "The work is a true breakthrough that will in the future lead to all kinds of new insights," Block says.

Perkins notes that the modifications have not been patented in hopes that AFM instrument manufacturers will adopt them. "The improvements in data quality are so compelling that we essentially do not take any AFM-based data with a standard commercial cantilever anymore," he says.—STU BORMAN

SYNTHESIS

Flow process speeds up peptide synthesis

Nature makes amide bonds—the key linkages that string together amino acids in a polypeptide—like a knitting whiz. The ribosome in *Escherichia coli* bacteria, for example, can make eight amide bonds in a second. Chemists are novices by comparison, taking minutes to hours to form an amide bond in a flask.

These scientists are stepping it up, though; a group led by Bradley L. Pentelute of MIT has developed a fully automated flow approach to solid-phase peptide synthesis that can make an amide bond in seven seconds and assemble a peptide at a rate of 40 seconds per amino acid (*Nat. Chem. Biol.* 2017, DOI: 10.1038/nchembio.2318).

In solid-phase peptide synthesis, chemists grow a polypeptide chain on a polymer bead one amino acid at a time through cycles of amide-forming reactions. Pentelute and colleagues automated a 2014 manual flow system for such a process, resulting in an apparatus nicknamed "the Amidator." It consists of a buffet of 50 solutions of different reagents, including natural and unnatural amino acids and chemical activators, hooked up to three pumps. The apparatus coordinates the reagents' stoichiometry and controls when they are heated, all while the reagents are flowing along at 80 mL per minute.

The flow system is 10 to 100 times as fast as batch-based solid-phase peptide synthesis methods currently used, Pentelute says. If the Amidator worked nonstop for a year, it could create tens of thousands of peptides 30 amino acids long. "This really removes one of the bottlenecks to pushing chemical research forward," Pentelute points out, "which is just the time it takes to get your hands on molecules."—BETHANY HALFORD