



▲ **Klaus Schulten.** “The Pittsburgh Supercomputing Center has helped us enormously in doing our calculations.”

## The Rude Mechanicals

As with people, some proteins are more well known than others. Among the established stars, for instance, are the enzymes. Less well known are the mechanical proteins—the brawny, hard-hat, working-class heroes. Their job, in some respects, is simple: exert force.

“Enzymes have been studied for many decades,” says physicist Klaus Schulten, “but for mechanical proteins there wasn’t a good method. Today we have techniques—atomic force microscopy (AFM) and optical tweezers—that allow you to take individual proteins, stretch them and measure their responses.”

Schulten directs the Theoretical Biophysics Group at the University of Illinois Beckman Institute for Advanced Science and Technology. In 1999, he and his colleagues carried out a series of molecular dynamics studies of a mechanical protein called titin. Working hand-in-hand with AFM studies, the computer simulations provide a detailed picture of how titin stretches.

### Tugging on Titin

A long filament of roughly 30,000 amino acids, titin is the largest known protein. In muscle, it helps to form the sarcomere, the integral unit of muscle fiber. When muscle is stretched, titin extends—holding the sarcomere together and providing passive force, like a stretched rubber band, that pulls it back to its unstretched state.

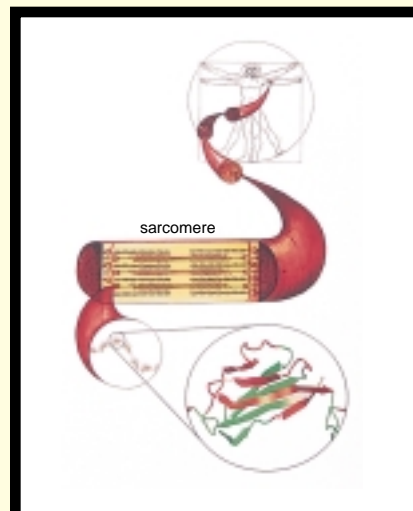
While many large proteins are an aggregate of separate component proteins, titin is a single strand of about

300 tightly-folded domains, each like a molecular coiled spring. Variant types of these spring-like domains are spaced along titin’s length, and the Schulten group’s recent simulations focus on one called I27, related in structure to the antibody immunoglobulin.

AFM experiments with I27 show a phenomenon called pre-stretch. With a weak force (about 50 picoNewton), the I27 extends slightly (5 Angstrom). With more force (above 100 pN), I27 stretches further (to about 15 Angstrom). Only with further added force (about 200 pN) will I27 let go and unfold all the way (about 300 Angstrom).

This shows, says Schulten, that I27 comes with built-in protection against unfolding. While the AFM experiments provide key information, they don’t show how the protein’s molecular architecture provides this protection.

“Without the simulations,” says Schulten, “you have a black box. You know what’s happening but not why or how.” To fill in the missing atom-by-atom details, Schulten and colleagues used a technique developed by his group called steered molecular dynamics.



*The titin I27 domain in muscle fiber*

### Opening a Black Box

If you think of the structure of a protein as a photograph that records the spatial relationships among all the atoms in the molecule, a molecular dynamics simulation is the movie version—it records how the atoms move from one tiny moment to the next. Steered molecular dynamics is a novel approach that can induce molecular changes to occur rapidly that would otherwise take too long to simulate, and it’s especially useful for mimicking AFM experiments.

Using SMD, a series of numerical experiments with I27 showed that I27’s resistance to unfolding arises from a patch of six hydrogen bonds that bridge between two of the protein’s folded strands. With enough force, all six of these bonds rupture simultaneously—the critical event that allows I27 to fully unravel.

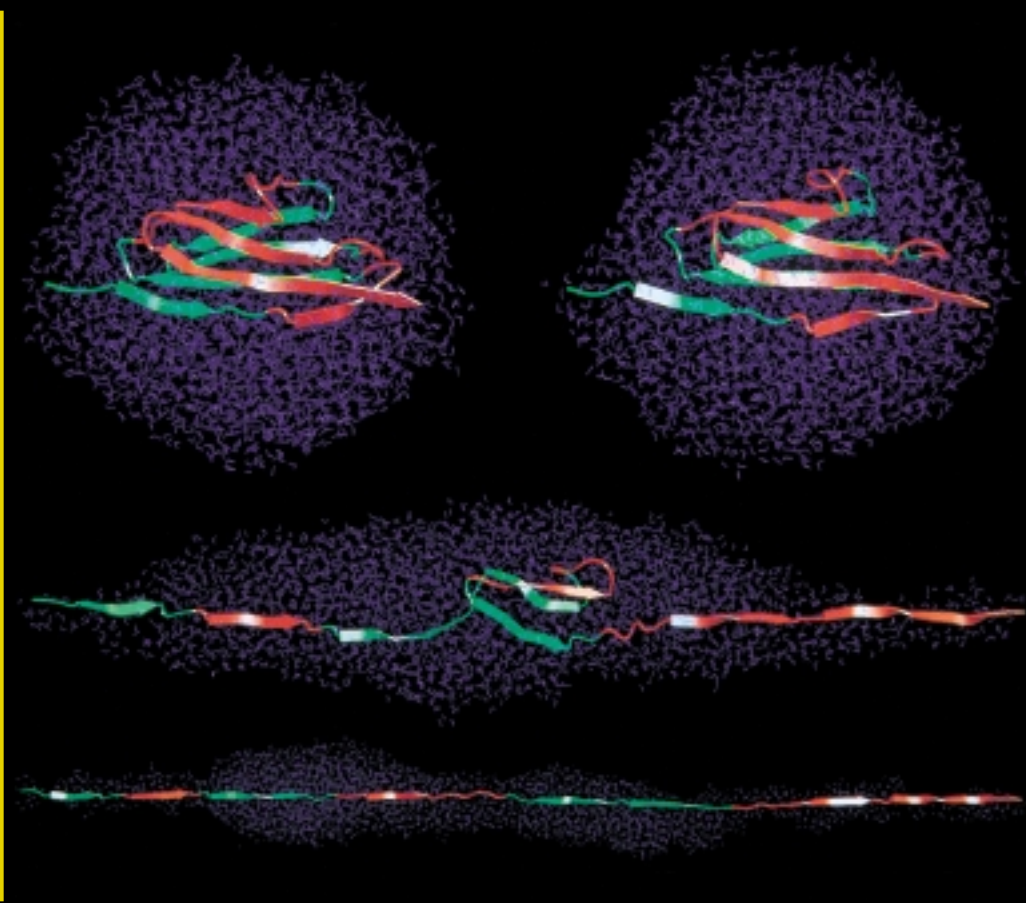
These six hydrogen bonds, explains Schulten, can be thought of as an energy barrier between folded and unfolded I27. With access to Pittsburgh’s CRAY T3E, the researchers did further simulations—up to 18 for each numerical experiment—to investigate this barrier and compare the computational results quantitatively with AFM data. The spread in results among individual simulations corresponded within 10 percent to the experimentally observed positions and height of the energy barrier, giving the researchers a high degree of confidence.

The simulations also showed that two other hydrogen bonds, between two different folded strands, break before the six-bond rupture that precipitates unfolding, contributing to a small energy barrier at an extension of about 5 Angstrom. Does this account for the pre-stretch from AFM experiments?

To clinch whether this intermediate I27 state corresponds to the pre-stretch, Schulten’s collaborator, J. Fernandez at the Mayo Clinic in Rochester, Minnesota,

### Simulations of I27 in Water

The I27 domain is shown as simulated in its unstretched state (upper left), pre-stretch state (upper right) and, at smaller scale, after unfolding and fully extended (bottom). In simulations, the domain is fixed at one end (left) and a stretching force applied at the other end. I27 is a structural motif known as a beta-sandwich: two sheets of ribbon-like strands (orange & green), one on top of the other.



cloned a mutant I27 with a changed amino-acid that eliminated the two hydrogen bonds. AFM experiments showed that this mutant I27 unfolds without an intermediate state.

Compared to enzymes it's a late start, but titin and another mechanical protein, fibronectin, which Schulten and his collaborators have also studied, are

gaining celebrity status. Research shows these proteins play several crucial roles: organizing chromosomes in the nucleus, cell-to-cell communication, and movement of cells relative to each other. SMD simulations have proven themselves as a partner with AFM experiments in learning about these proteins. "Together these research techniques open the door to an

aspect of the cell, protein mechanics, which couldn't be investigated before," says Schulten. "This is an exciting and rich field."

#### More information:

<http://www.psc.edu/science/schulten.html>

### Hydrogen Bonds

The I27 domain in its native state (left), pre-stretch at 10 Angstrom, and unfolded to 25 Angstrom (right). The simulations reveal that unfolding occurs when six hydrogen bonds (dotted lines) between two of I27's strands (A' and G) rupture simultaneously. Two hydrogen bonds (between A & B strands) break at pre-stretch.

