PROTEIN STRUCTURE

Stretching the Limits

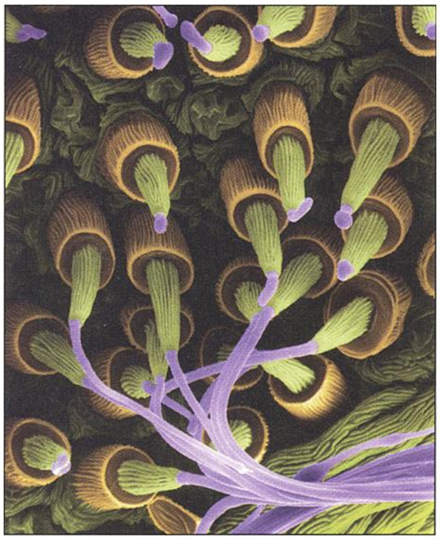
Stretchy proteins perform a variety of critical functions for many organisms. Researchers are now finding out how they work and are beginning to apply that knowledge to new products

Every protein has some wiggle in it. But a very few have taken wiggling to an extreme: They are so flexible that they can expand and contract at will, like molecular springs, withstanding repeated deformations that would destroy other structures. Although only a handful of such elastic proteins are known, they appear in a wide range of biological systems and are critical for their survival.

Take elastin, a rubberlike protein found in blood vessels, lungs, and skin. It absorbs the energy in each pulse sent from the heart to keep peak blood pressure from rupturing delicate capillaries, then releases it to maintain blood pressure between pulses. Insect wings rely on resilin, which stretches in response to each wing beat and releases the stored energy to power the return stroke. Mussels use byssal fiber as a molecular shock-and-spring system to withstand the push and pull of ocean waves and hungry crabs. And a mix of stretchy spider proteins gives webs the ability to catch flying prey without breaking and allows spiders to hang safely from delicate draglines.

How nature performs these feats of contortion has been something of a mystery until recently. But researchers are now discovering that although these proteins exhibit a range of elastic properties, they appear to share a common set of design principles. The scientists have learned this mainly by studying the proteins' gene sequences. (The proteins themselves are difficult to purify, let alone crystallize.) "What's really striking about these proteins is that they have little or no amino acid sequence homology as a whole," explains Arthur Tatham, a protein biochemist at the University of Bristol in Long Ashton, U.K. Yet, he adds, they are similar in that they all contain blocks of repeated sequences that form springs. The springiness, notes Dan Urry, a materials scientist at the University of Minnesota, Twin Cities, really comes from "a single fundamental mechanism," one that involves otherwise loopy protein chains taking on a highly ordered shape when stretched.

As researchers identify the basic properties that underlie these bouncy molecules, some are adapting them for use in medicine, materials science, and even military projects. Artificial silks have the potential to make lightweight yet strong-as-steel protective clothing, for example, and polymers based on elastin are being developed to serve as arterial grafts or even energy-absorbing soundproofing materials. "We can now craft materials with very specific elastic properties based on the structural rules that nature uses," says John Gosline, who studies comparative biomechanics at the University of British Columbia in Vancouver.



Natural strength. Proteins in strands of silk emerging from a spider's spinnerets combine resilience with extreme toughness.

Design equals function

In the 17th century, British physicist Robert Hooke defined elasticity as the measure of an object's ability to deform in proportion to the amount of an applied force and then return to its original state when the force is removed. An important corollary, says Gosline, is that an elastic protein stores energy when it is deformed and releases energy when it assumes its original shape, a property known as resilience. By that definition, nature has achieved near perfection in elastin and resilin, two "elastomer" proteins that release 90% of the energy they absorb. In contrast, one of the elastic regions of byssal thread protein important for mussels is only 28% resilient. It dissipates most of the energy it absorbs as heat.

The basic architecture of these proteins is quite different from that of rubber, the prototypical elastic material. Rubber gets its stretchiness from a complete lack of order in its molecular structure. Its long, randomly coiled chains of protein interact little with each other. "Rubber's elasticity is a simple matter compared to [that of other] proteins," says Gosline, "and the theory to explain rubber's elasticity doesn't apply" to molecules such as resilin.

But elasticity is not the only property that makes these proteins interesting to researchers. Spider dragline silk, for example, is only 35% resilient, but it is nearly as strong as high-tensile steel. Better yet, from a spider's perspective, it takes three times as much energy to break a single thread of spider silk as a strand of Kevlar. This combination of strength and limited resilience allows the single threads in a spider's web to stop a flying insect—just as a Kevlar vest stops a bullet—and then relax slowly so as not to fling the prey free from its trap. "It's the variety of properties that makes these proteins interesting from a materials perspective," says Urry. "Their unique structures impart a range of elasticity, strength, toughness, and durability to suit a particular biological function. These proteins have a great deal to teach us about the design of new materials."

Byssal thread fiber is perhaps the most extreme example of mixing and matching structural features for survival. Mussels secrete byssal threads to anchor themselves to rocks in ocean tidal zones. Each wave that passes pulls a mussel away from its home, and the elastic threads absorb this energy by stretching as much as twofold in length. A highly resilient fiber would spell doom to the mussel, for the protein would release all of its energy, dashing the mussel against its anchoring rock. "But instant recoil doesn't occur because byssal threads, like spider silk, act like a spring-loaded piston that relaxes slowly. Unlike spider silk, however, the two ends of each byssal thread have very different elastic properties," explains Herbert Waite, a marine biochemist at the University of California, Santa Barbara. "The end closest to the mussel is like a soft, extensible spring, while the end closest to the rock is less extensible and stiffer."

The juncture between the two dissimilar materials could create a weak point, however. The mussel solves this problem by combining three different types of structures seen in other elastic proteins to create a smooth gradient rather than a sharp transition in each thread. Rugged, collagenlike domains are scattered throughout each thread. Although biological systems usually make use of collagen's stiffness, it is, in fact, an elastic protein whose resilience equals that of elastin and resilin. At the soft end of each thread near the mussel, the collagen domains are fused with glycine-rich, elastinlike sequences. At the opposite extreme near the rock, the thread con-

Recoil action. Mussels attach to rocks with byssal thread fiber that reacts to motion with a slow give-and-take.

tains collagen domains fused to regions resembling spider web silk. Sequences rich in the amino acid histidine are dotted throughout, providing crosslinks between neighboring strands. The result is a material that is twice as resilient close to the mussel compared to the region attached to the rock. Says Emily Carrington, an integrative marine biologist at the University of Rhode Island, Kingston, who studies mussels living in a variety of ecological niches: "It's an elegant solution to a complex materials problem."

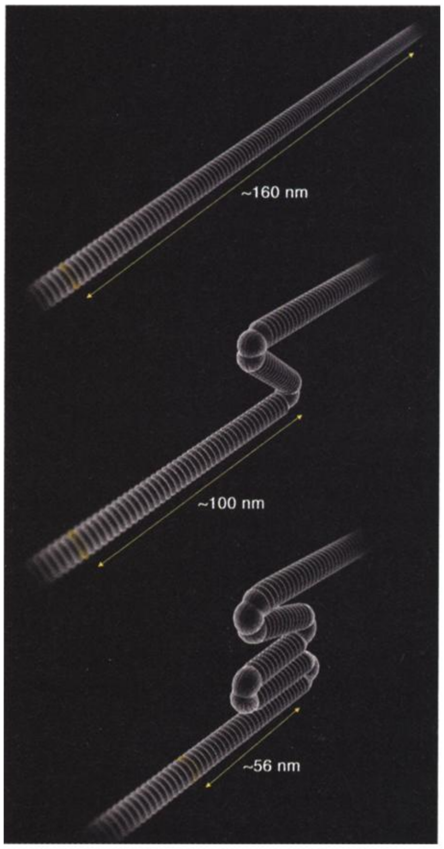
Twists and turns

The ultimate secrets of protein elasticity are still a mystery, largely because these proteins are so big that they have been difficult to isolate and purify. To analyze byssal thread, for example, researchers painstakingly isolated individual fibers and cut them into 2-millimeter-long segments. And even when researchers obtain gene sequences for these elastic proteins, they have had difficulty getting standard recombinant in vitro systems to express them. Nonetheless, they have identified some flexible features using new techniques.

Consider fibrillin, probably the most ubiquitous elastic protein in nature. It appeared early in the evolution of multicellular organisms and can now be found in virtually all dynamic connective tissues, including arteries, skin, and elastic cartilage. Cay Kielty, an extracellular matrix biologist at the University of Manchester, U.K., and her colleagues used automated electron tomography, antibody bonding, and low-resolution x-ray diffraction to examine purified fibrillin microfibrils. They developed the first three-dimensional reconstruction of this protein in both its relaxed and stretched states. This work, published last year in the *Journal of Cell Biology*, shows that unstretched fibrillin looks like a beaded necklace, with the beads spaced 56 nanometers apart (see figure at right). When stretched, the beads appear approximately 160 nanometers apart, which is about as long as a single fibrillin molecule would be, based on amino acid sequence derived from its gene sequence.

The amino acid sequence reveals that fibrillin contains two types of domains: one that binds calcium and would probably be rigid as a result, and a second that can adopt a pattern found in many other elastic proteins, known as a folded β-sheet conformation. Kielty proposes that the β-sheet domains are able to fold upon themselves accordion-style in the unstretched state, and hingelike proline residues appear to connect the two domains. This folding would create the beads seen at intervals of 56 nanometers. The structure could then unfold when the protein is stretched and refold as the protein relaxed.

Other well-studied elastic proteins, such as elastin and spider silk, seem to rely on different structural motifs for their particular combination of elasticity and strength. But all these proteins seem to have flexible domains in common—mostly patterns known as β turns and α helices, in addition to β sheets, connected to other repeated regions by proline- and alanine-rich sequences. Stretching these proteins reorders their three-dimensional structures, adding energy. That change acts as an energy-storage mechanism that can later drive the protein to resume its unstretched conformation, says Tatham.



Spring structure. Using x-ray diffraction and other methods, researchers have outlined the coiled and extended states of fibrillin, a common stretchy protein.

Applied elasticity

This fundamental understanding of protein elasticity is driving the development of novel elastomeric polymers. Urry's work on elastin, for example, has pinpointed specific amino acid sequences that can be polymerized to form workable synthetic materials. Their elastic properties can be tailored by changing the amino acid composition according to a model that Urry has developed based on their water-avoiding quality (hydrophobicity). For example, changing glycine to alanine in the lead position of a five-part sequence that gives elastin its stretchiness produces a durable elastic thermoplastic that can be melted and shaped. Similarly, adding two glutamic acids per 100 amino acids produces a polymer that is soluble at physiological pH but turns into an elastic solid in a more acid environment (at pH3). In proprietary work for the U.S. Navy, Urry's company, Bioelastic Research in Birmingham, Alabama, has developed a series of elastin-derived polymers that efficiently absorb vibrational energy—sound—produced by submarine machinery.

Cardiovascular scientist Fred Keeley and his colleagues at the Hospital for Sick Children in Toronto have also created elastin-derived synthetic polymers that they can form into membranes and tubes. Cells will grow onto this material, raising the possibility that it could be used as a scaffold for arterial grafts or skin growth. Keeley and his colleagues have recently formed a company to commercialize this research.

Perhaps furthest along is work with spider silk. In January, researchers from Nexia Biotechnologies in Montreal and the U.S. Army reported in *Science* (18 January, p. 472) that they had produced recombinant spider silk proteins and successfully spun them into fibers. Since then, the company has begun work with an unnamed medical textile company to develop a commercial spinning process and produce enough of the synthetic silk for clinical development of medical devices such as synthetic ligaments and sutures. In addition, the Army is trying to develop the synthetic silk for soft body armor.

"It used to be only biologists who cared about these proteins," says Keeley. "I think the really elegant work being done today in this field is starting to get the attention of materials researchers."

Although it is still a stretch to expect elastic proteins to make a commercial impact any time soon, most researchers in the field say that they are finally starting to get a handle on one of nature's biggest secrets.

–JOE ALPER

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