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Tight in Titin

The mechanics of a protein joint, gluing two elastic titin molecules together in muscle, are explored using computer simulation in this issue of *Structure* by Lee et al. (2006). Maybe the glue itself also has another, more subtle, sensory role?

Details are coming to light of the structure and mechanics of a fascinating, and possibly bifunctional, molecular anchor located in the sarcomeric Z-disc region of muscle fibers. The 4 MDa megaprotein titin runs between the Z-disc and M-line, and contains elastic elements that sequentially extend as muscle is stretched, generating a passive force that pulls the sarcomere back toward its unstretched length. For this spring to work, something strong must tether titin to the Z-disc. The required anchoring may well be supplied by another protein, telethonin, binding to two N-terminal titin immunoglobulin-like domains named Z1 and Z2.

A very recent crystal structure of the telethonin/Z1Z2 complex has revealed a novel, palindromic, antiparallel assembly of two titin molecules with telethonin wedged in between (Zou et al., 2006). Binding and imaging data suggest that telethonin binds tightly to Z1 and Z2 (Gregorio et al., 1998; Mues et al., 1998; Zou et al., 2006), and this impression is also given by the crystal structure, in which the proteins interact with an extensive network of hydrogen bonds that crosslinks β strands. But anchors must resist pulling, and for telethonin to hold the titin molecules together the complex must withstand the stresses on titin. To probe this directly, one has to try to pull the complex apart, and this is what is undertaken, using "steered molecular dynamics" (SMD) computer simulations, in a report in this issue of *Structure* (Lee et al., 2006).

SMD, also known as "force-probe molecular dynamics" (Heymann & Grubmueller, 1999), involves performing standard molecular dynamics simulations with the application of an additional pulling force. This technique has been used in many computer experiments in the recent past, and notably for the interpretation of atomic force microscopy (AFM) experiments on titin filaments themselves (Paci & Karplus, 2000; Gao et al., 2002; Graeter et al., 2005).

The SMD method does have its drawbacks. One of these is that there is not enough computer power to enable the simulations to be performed for as long as one would wish. Thus, whereas AFM, or in vivo titin stretching, takes place typically on about the millisecond timescale or slower, simulations are on about the 10 ns timescale or faster, and the computational pulling must be correspondingly rather more vigorous. As resistance to stretching varies with the pulling force, this complicates comparison with experiment. Furthermore, other errors may arise from general simulation problems such as imprecision in potential energy functions (e.g., in the representation of the solvent) and various approximations required by the simulation methodology. However, if the limitations are taken into account appropriately, a simulation can be a mine of useful information.

The calculations of Lee et al. (2006) confirm that the β strand crosslinking in the telethonin/Z1Z2 complex confers unusually strong resistance to mechanical force. In control simulations apoZ1Z2 (i.e., without the telethonin) is easily teased apart, giving way when put under only about half as much stress, and the individual Z1 and Z2 domains by themselves also unfold more readily than the complex dissociates. Telethonin is found to distribute the forces between its two joined titin Z2 domains so as to protect the proximal Z1 domains from bearing too much stress. Further, the simulations reveal a fascinating dynamic picture of the main unbinding process, involving the concurrent rupture of no fewer than seven interstrand hydrogen bonds. This is reminiscent of mechanisms found in previous SMD work by the same group on individual titin domains, which also release in an all-or-nothing way via the simultaneous breaking of several β strand-bridging hydrogen bonds (Gao et al., 2002).

Thus, the crystal structure of Zou et al. (2006) and the SMD results of Lee et al. (2006) have shown how nature can glue proteins together. However, it seems that there may be more to the telethonin/titin interaction than mere brawny, stolid resistance. Indeed, the sarcomere needs to sense when it is being exercised, and to react by triggering downstream signals for muscle growth and survival. Titin, being intrinsically sensitive to variable levels of muscle stretch, is well placed to furnish the information needed for stress-response processes. This idea is supported by evidence that titin engages in multiple protein interactions with functionally diverse proteins including distinct biomolecular sensing complexes and signaling molecules (Miller et al., 2004). The sensitivity of this giant protein is exemplified by its C-terminal region, where titin has a lone kinase domain, which, according to recent force-pulling MD simulations, may be tugged into an active conformation by the application of force (Graeter et al., 2005).

Protein complexes in the Z-disc also function as titin stretch sensors, and there is functional evidence that the interaction between telethonin and one or more of a number of Z-disc proteins triggers stretch-activated downstream effector pathways (Miller et al., 2004). The information now available thus evokes an image of telethonin glued tight in titin, steadfastly resisting stretching while feeding the force level back to the Z-disc sensor machinery which converts it into biochemical signals. Further crystal structures of titin, telethonin, and their accomplices in relevant states, combined with dynamic and conformational pathway calculations of the type recently reported for another famous muscle protein, myosin (Fischer et al., 2005), would allow a detailed chemomechanical picture to be obtained as to how this feedback is effected.

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Selected Reading

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Molecular Mechanics of Single Molecules

The mechanics of single molecules of bacteriorhodopsin interacting with purple membrane have been investigated from both sides of the membrane by Kessler and Gaub (2006) in this issue of *Structure*. Remarkably, barriers can be associated with specific amino acid sequences to an accuracy of ±3 amino acids.

Who would have imagined just 25 years ago that it would be possible not only to remove molecules one at a time from membranes, but also to measure, in detail, the interaction forces between the molecule and the membrane as the molecule was being extracted? Yet, surprisingly, this can be done, and, as Kessler and Gaub show, it can be done from both sides of the membrane!

What led to this amazing accomplishment? It took the genius of Gerd Binnig to realize that it was possible to make a macroscopic cantilever with a spring constant softer than the spring constant between two atoms. He used this information to build the first atomic force microscope (AFM) (Binnig et al., 1986). It took the genius of Hermann Gaub to realize that a still softer cantilever could be used to measure the mechanics of a single molecule (Rief et al., 1997b). Building on this breakthrough, Rief, Gautel, Osterhelt, Fernandez, and Gaub demonstrated that it's possible to understand the single-molecule mechanics of unfolding individual protein domains (Rief et al., 1997a). The present work advances the study of single-molecule mechanics by presenting the first, to my knowledge, data on pulling a membrane protein from both the extracellular and the cytoplasmic sides. With this advance, the authors are able to access formerly hidden unfolding barriers in bacteriorhodopsin.

These breakthroughs, of course, themselves were dependent on earlier work. For example, the invention of the AFM built on the Nobel Prize-winning research of a few years earlier in building the first scanning tunneling microscope (Binnig et al., 1982). The work on measuring individual molecule mechanics depended on the invention of AFMs that could operate with the sample in water (Drake et al., 1989) and on the development and techniques to measure force versus distance curves with the AFMs (Weisenhorn et al., 1989). Similarly, the present paper built on the background of Andreas Engel's group's elegant studies of membrane proteins with the AFM (Schabert and Engel, 1994) and the demonstration by Daniel Muller and others (Muller et al., 2002) that these molecules could be pulled from membranes with the AFM.

By pulling from one side of the membrane and then the other, Kessler and Gaub were able to determine the major barriers to extraction and unfolding (Kessler and Gaub, 2006). The two-stage approach provided more complete coverage of the protein length. Although many barriers were observed only when the protein was pulled from one direction, in a few cases, identical positions of resistance were encountered (measured to within three amino acids), suggesting these areas as local traps in the unfolding process.

Looking toward the future, it seems clear that single-molecule mechanics is destined to become an important field. It impacts fields from physics to chemistry and from biology to medicine. As an example, evidence is accumulating that amyloid ion channels formed in cell membranes may be responsible for destabilizing cellular ionic homeostasis, leading to cellular pathophysiology and degeneration in protein misfolding diseases such as Alzheimer's disease. Beautiful images of these amyloid ion channels have been obtained by the group of Ratnesh Lal (Quist et al., 2005). These amyloid ion channels have not, however, been crystallized into two-dimensional crystals. Thus, diffraction techniques cannot be used to study the details of their structure. In Kessler and Gaub's paper, bacteriorhodopsin was studied, for which diffraction data is available (Kessler and