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MECHANOBIOLOGY: USING BLUE WATERS TO DECIPHER THE PHYSICAL PRINCIPLES OF PROTEIN MECHANICS

Allocation: NSF PRAC/9,700 Knh
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EXECUTIVE SUMMARY

At the heart of many cellular processes, mechanoactive proteins are responsible for converting mechanical cues into biochemical signals. Protein mechanics are also crucial in holding large macromolecular complexes together during mechanical stress, while permitting assembly and disassembly of these complexes to continue to take place at physiologically needed rates. Employing state-of-the-art computational tools and in a close collaboration with experimentalists, the research team has shown how specific protein complexes can be "activated" under shear force to withstand high mechanical loads. They have discovered that protein mechanostability is highly dependent on the direction of the force application. They have also shown that mechanical stability can be achieved by a protein architecture that directs molecular deformation along paths that run perpendicular to the pulling axis. These mechanisms of protein mechanical stabilization have potential applications in biotechnology for the development of systems exhibiting shear-enhanced adhesion or tunable mechanics.

RESEARCH CHALLENGE

Interfacing biology, physics, and engineering, mechanobiology studies how mechanoactive proteins sense and respond to mechanical cues in different ways. Guided by advances in single-molecule force spectroscopy, researchers are acquiring a new level of understanding of mechanoactive proteins. However, protein mechanics is a challenging topic to study experimentally because molecular-level mechanical properties remain hidden to ensemble-averaging methods. They require techniques such as molecular dynamics that offer simultaneous high spatial (atomic) and temporal (femtosecond) resolutions.

The research team has employed Blue Waters to investigate how cellulosomes, large molecular machines that can efficiently degrade plant fibers, can be mechanically activated to withstand high-force loads. Understanding how cellulosomes work can have a considerable impact on rational design of more efficient enzymatic complexes for biotechnology and bioreactors. Also, cellulosome-containing bacteria were recently found in the human gut, a paradigm-shifting discovery that shows that at least some humans are able to fully digest plant fibers.

METHODS & CODES

The research group has employed a combination of *in silico* and *in vivo* single-molecule force spectroscopy to elucidate, with atomic resolution, protein mechanics at high-force loads. Using a wide sampling approach, steered molecular dynamics simulations were performed with many replicas, allowing the team to analyze experiments and simulations in the same statistical framework. Wide sampling combined with dynamic network analysis allowed them to visualize most probable deformation pathways through the protein architecture and understand how resistance to mechanical stress arises at the level of individual protein complexes. Simulation results were validated using single-molecule force spectroscopy experiments carried out with an atomic force microscope over a range of loading rates. By recording the force required to break the protein complexes for thousands of individual interactions, the team collected sufficient statistics to analyze the interactions and unfolding pathways thoroughly. They achieved remarkable agreement between simulations and experiments, demonstrating that they probe fundamentally the same molecular process.

RESULTS & IMPACT

The chemistry at the interface between biomolecules affected by mechanical forces is still largely unexplored. Even the best-studied mechanically stable biomolecular complex, namely the streptavidin:biotin complex, has mechanical properties that have only recently been revealed. By using Blue Waters, the research team has shown that a wide range of rupture forces previously reported for the separation of the complex are due to unspecific tethering of streptavidin [1]. In fact, different anchoring points in the same protein can lead to unfolding or unbinding of the complex [2]. In cellulosomes, which are usually found in harsh environments, mechanical stability at specific pulling geometries is crucial for their high efficiency. The team had previously shown that a cohesin:dockerin (two major components of a cellulosome) complex could withstand forces up to four times that of the streptavidin:biotin complex [3], which was previously considered to be the strongest protein complex.

In this project, the researchers discovered a cohesin:dockerin complex that is significantly stronger than the one they previously reported [4]. The scaffold B (ScaB) is found within the same cellulosome as the previously reported CttA complex. The role of the ScaB complex is to connect the large protein machinery of the cellulosome to the cell wall via high-affinity binding to the same cohesin of the CttA complex. This cohesin is itself covalently linked to the peptidoglycan cell wall (Fig. 1). The team's single-molecule experiments show that the ScaB complex, which is among the most mechanically stable complexes known [4], withstands forces up to a nanonewton. The team's simulation results reveal that the mechanical stability is achieved by an architecture that directs the force along pathways running perpendicular to the pulling axis (Fig. 2).

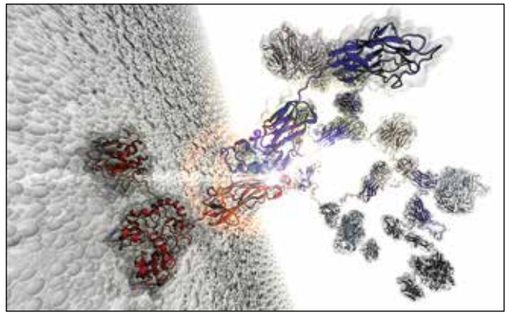

Figure 1: Illustration of a cellulosome, a multienzyme complex found in many plant-fiber degrading bacteria. Cellulosomes are usually attached to the outside of the bacterial cell wall (white surface). Like LEGO bricks, cellulosomes can be assembled in many different shapes by highly stable protein–protein (cohesin:dockerin) interactions. One such interaction is highlighted at the center.

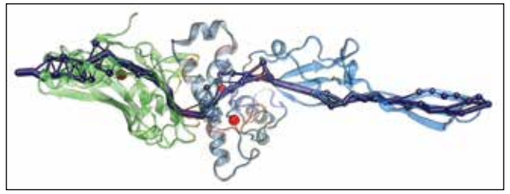

Figure 2: Illustration of an ultrastable cohesin (green):dockerin (blue) interface found in rumen bacteria's cellulosomes. Using a correlation-based network analysis, the researchers calculated the force propagation pathway at the interface. The mechanical stability is achieved by a protein architecture that directs force along paths running perpendicular to the pulling axis at the interface.

Taking advantage of Blue Waters' size and power, the team has also investigated how different cohesin domains found in a single cellulosome scaffold withstand shear forces. The results show that in the same scaffold, different cohesins can have a large (up to a factor of four) difference in mechanical stability, depending on the cohesin's position in that scaffold [5]. Remarkably, all seven cohesins in the same scaffold share high sequence identity. A striking agreement was observed between simulation and experiments, motivating the researchers to computationally explore how point mutations could affect the mechanical stability of cohesins. In an intelligent design strategy, simulations revealed that a single mutation in an alanine residue, replacing it by a glycine residue, could make one of the weaker cohesins much stronger. The experiments also revealed that this mutation, which replaces a methyl group by a single H, was responsible for a 2.6-fold increase in the mechanical stability of that cohesin [5]. The team's results show the vast potential offered by large supercomputers for molecular engineering and biotechnology.

WHY BLUE WATERS

Fast turnover time is fundamental when working synergistically with experimentalists. Blue Waters' sheer size allowed the researchers to obtain many simulation replicas at about the same speed experiments can be performed. Without Blue Waters, testing new hypotheses computationally would not have been feasible.

PUBLICATIONS & DATA SETS

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