modules with known inhibitors of this class of enzymes, showing that their inactivation recapitulates the StrH null phenotype. An interesting question remaining is the mutual contribution of each of the GH20 domains to this function; the data presented suggest a more dominant role for GH20A. The authors also investigate a proposed additional role of StrH in infection: evasion of the immune system (inactivating the security alarm) by blocking binding of complement factor C3 to its activating protease, convertase. Even though the StrH structure was found to have a three-helix bundle domain with similarity to a complement inhibitory protein SCIN, the results reported here indicate that the inhibitors of GH activity reduce the immune protection of bacteria to a similar extent as the StrH null mutants. Thus, it is the activity of the enzyme, rather than its structural features, that is responsible for immune evasion. Perhaps the bacteria are using the cleaved branches as camouflage. A fuller understanding of this observation will have to await further study. What is evident from these results is that being able to clear a path through the security hedge of N-glycans on the host cell surface has been a significant selection force in the evolution of pathogenic bacteria.

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Fibrinogen Unfolding Mechanisms Are Not Too Much of a Stretch

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Molecular explanations for the extraordinary elasticity and extensibility of fibrin fibers are still lacking. Now, Zhmurov et al. (2011) use force spectroscopy experiments, and innovative simulations that match the time and force scales of these experiments, to study fibrinogen behavior under an applied force providing deeper insights into this process.

Fibrinogen is a hexameric plasma protein composed of a pair of three peptide chains designated Aα, Bβ, and γ. At each end of the protein are the globular D regions comprised of the β and γ nodules. Triple α-helical coiled coils connect the D regions to the central, globular E region, which contains the two pairs of fibrinopeptides A and B. Not visible in the crystal structure are the 389 residues long, flexible α-C regions (Figure 1A) (Kollman et al., 2009).

In hemostasis, activated thrombin removes fibrinopeptides A and B, thereby exposing the A and B knobs in the central E region and converting fibrinogen to fibrin. Fibrin then assembles spontaneously into two-stranded, half-staggered protofibrils (Figure 1B). The key interaction that directs this assembly is the A:a interaction between the A knob and the a pocket in the γ nodule. The protofibrils then assemble radially into about 100 nm wide fibrin fibers that comprise the major structural component of a blood clot. The key interactions of protofibril assembly are thought to be the B:b interaction between the B knob and b pocket in the β nodule. Additionally, there is increasing evidence that the α-C region plays a critical role in protofibril assembly (Ping et al., 2011).

Fibrin fibers are among the most elastic and extensible protein fibers (Liu et al., 2006, 2010). They can be stretched elastically to nearly twice their length and to 2.5 times their lengths before rupturing. This large, elastic extensibility was unexpected because fibrin fibers assemble in a regular, near-crystalline fashion, and most protein fibers that have this regular structure, such as collagen fibrils, actin filaments, and microtubules, are far less extensible. The apparent contradiction between large, elastic extensibility and regular, crystalline structure could be resolved by identifying flexible linkers between fibrin molecules and protofibrils, or regions on the fibrinogen molecule that could stretch or unfold.

The origin of this elastic extensibility has been a topic of debate over the past few years, and experimental and computational studies implicate the following possible sources: (1) unfolding of the α-helical coiled coils into β strands (Brown et al., 2007; Lim et al., 2008); (2) unfolding of the γ nodules (Averett et al., 2008); and (3) unfolding of the α-C region (Houser et al., 2010) (Figure 2). A complete understanding of the mechanical

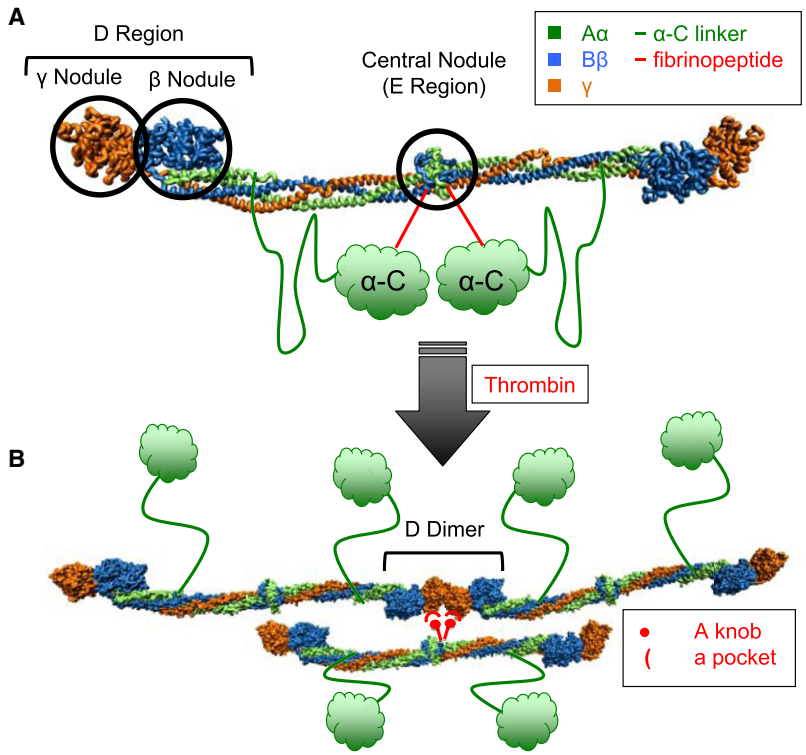

Figure 1. Fibrin Assembly
Molecular structures of human (A) fibrinogen and (B) fibrin fibers. The molecular structures were based on the X-ray structures of the human fibrinogen (PDB: 1GHG; Kollman et al., 2009) and D dimer (PDB: 1FZE). The unstructured α-C regions are shown as schematics. The fibrin structure was constructed by performing a best fit of each end of fibrinogen to each D region of the D dimer.

properties of fibrin will improve our understanding and treatment of thrombotic events, such as heart attacks, ischemic strokes, and pulmonary embolisms.

To address these outstanding issues, Zhmurov et al. (2011) [in this issue of *Structure*] employed atomic force microscopy (AFM)-based force spectroscopy and molecular dynamics (MD) simulations to identity the underlying molecular mechanism of fibrin's extensibility and elasticity. A main reason why their study is of particular note is because simulating relevant biomolecules on timescales that can be directly compared with experiments remains an extraordinary challenge. The major bottleneck for simulating these processes is the force evaluation involved in these complex systems that must be computed for each pair of interacting particles.

As such, detailed atomistic resolution folding simulations are restricted to very small (~50 amino acids long), fast-folding (<μs) proteins at atomistic detail, even though biologically relevant biomolecules are much larger and can fold much slower. Indeed, a single protein chain is, on average, 400 amino acids long and takes considerably longer than 1 ms to fold. Mechanical unfolding also occurs on a similar timescale when a modest force is applied to the system in experiments. For atomistic simulations to observe unfolding events under mechanical force, the only recourse is to apply unnaturally high forces to observe unfolding (Lim et al., 2008). Coarse-grained simulations increase the timescales of simulations, while still accurately capturing the folding mechanisms of biologically relevant biomolecules (Cho et al., 2009; Hills and Brooks, 2009). In these classes of simulations, groups of atoms, such as residues, are represented by a bead, thereby simplifying the calculations. Unfortunately, the computational demands of even coarse-grained simulations require very high forces to observe unfolding events.

Another approach to optimize MD simulations is to perform them on graphics processing unit (GPU) hardware, which readily lends itself to a high degree of parallelization and the very fast floating-point calculations. GPUs are specialized video cards that were originally designed to perform floating-point calculations that are characteristic of operations for rendering to a graphical display. In this parallel architecture many processors independently execute the same set of instructions on large sets of data. GPUs are well suited to perform MD simulations because the general algorithm is to compute the same set of force calculations (i.e., instructions) on the coordinates and velocities of each of the individual beads (i.e., large set of data).

The present study by Zhmurov et al. (2011) used a combination of AFM-based single molecule unfolding and GPU-based coarse-grained force unfolding simulations to study fibrinogen

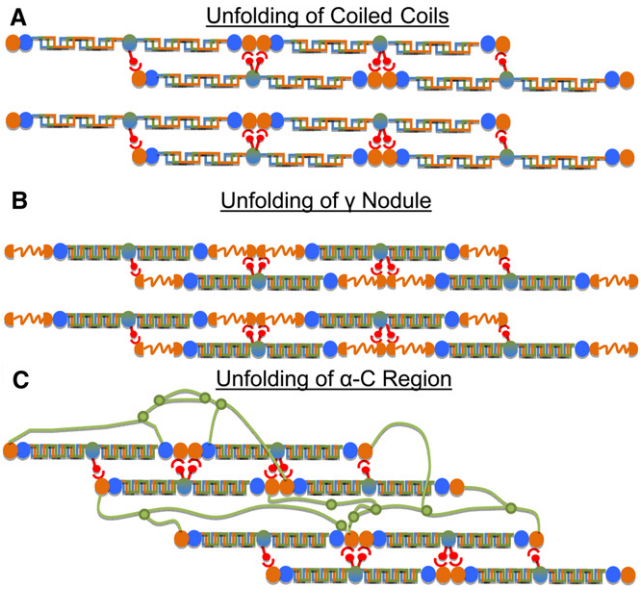

Figure 2. Schematics of Three Possible Mechanisms of Fibrin Fiber Extensibility
(A) Unfolding of the coiled chains.
(B) Unfolding of the γ nodules.
(C) Unfolding of the α-C region.

monomers and oligomers. The result of their tour de force is that the peak-to-peak distances in the force extension curves from simulations are nearly identical to those observed in the experiments, strongly supporting that the simple coarse-grained model accurately captures the mechanical unfolding mechanisms observed in experiments. The simulations revealed that when force is applied to fibrinogen, the γ nodule partially unfolds, and the coiled coils reversibly convert from an α-helical to an extended β strand structure. The authors then suggest that these two mechanisms may explain the extraordinary elasticity and extensibility of fibrin fibers. These mechanisms have been proposed before, but a clear verification was still missing, and the authors provide convincing evidence that these mechanisms are indeed feasible.

A limitation of the experimental and simulation designs is that they could not probe the role of the flexible, unstructured α-C region. This is because single fibrinogen molecules or short chains of abutting monomers were tested, rather than double-stranded, half-staggered protofibrils, or even complete fibers. There is emerging evidence that the α-C region plays a significant role in fibrin fiber formation, especially in protofibril assembly, and it has been suggested that the stretching and unfolding of the α-C region may be the major molecular mechanism that endows fibrin fibers with their large extensibility (Houser et al., 2010).

It is also entirely possible that all three mechanisms (Figure 2) could be going on in parallel. Thus, in the future it would be exciting to perform the forced unfolding experiments and simulations with protofibrils.

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Tackling the Legs of Mannan-Binding Lectin

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The recognition of pathogen surfaces by mannan-binding lectin activates MASP proteases, leading to complement activation. A crystal structure by Gingras et al. (2011) in this issue of *Structure* now shows how the collagen-like stems of mannan-binding lectin bind MASP-1 through a minimalist set of interactions.

Collagen is the most abundant protein in vertebrates. Its basic structure was worked out over 50 years ago and consists of three polypeptide chains with characteristic Gly-X-Y repeats that are intertwined to form a left-handed triple helix (Brodsky and Persikov, 2005). Gly-X-Y repeats are not confined to collagens, however, and short stretches of the motif are also found in a number of proteins of the immune system, such as the macrophage scavenger receptor A, the complement protein C1q, and mannan-binding lectin (MBL). MBL and the closely-related ficolins play an important role in innate immunity; their binding to carbohydrate arrays on the surface of pathogens triggers the activation of complement (Wallis et al., 2010). This process critically involves the MBL-associated serine proteases (MASPs), which, upon binding to a pathogen surface, are converted from inactive zymogens to active proteases. How this happens is still unclear, but an important piece of the puzzle is now provided by Gingras et al. (2011) in this issue of *Structure*, who have determined a crystal structure of the MBL-MASP contact region.

The basic unit of MBL is a homotrimer consisting of a collagen-like stem, an α-helical-coiled coil neck region, and three globular carbohydrate recognition domains. Circulating MBL contains two or more of these homotrimers covalently linked at their N-termini by a number of disulphide bonds (Figure 1). This structure is commonly likened to a bouquet of flowers. MASPs are homodimers with a modular architecture; their N-terminal CUB-EGF-CUB regions mediate binding to MBL, and their C-terminal regions contain the serine proteases that autoactivate upon pathogen recognition. A previous elegant study by Wallis et al. (2004) mapped the MASP binding site to