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Protein Mechanics: From Single Molecules to Functional Biomaterials

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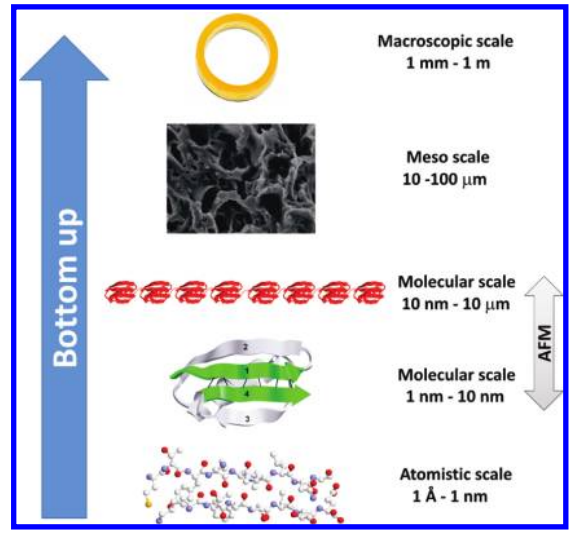

Elastomeric proteins act as the essential functional units in a wide variety of biomechanical machinery and serve as the basic building blocks for biological materials that exhibit superb mechanical properties. These proteins provide the desired elasticity, mechanical strength, resilience, and toughness within these materials. Understanding the mechanical properties of elastomeric protein-based biomaterials is a multiscale problem spanning from the atomistic/molecular level to the macroscopic level. Uncovering the design principles of individual elastomeric building blocks is critical both for the scientific understanding of multiscale mechanics of biomaterials and for the rational engineering of novel biomaterials with desirable mechanical properties.

The development of single-molecule force spectroscopy techniques has provided methods for characterizing mechanical properties of elastomeric proteins one molecule at a time. Single-molecule atomic force microscopy (AFM) is uniquely suited to this purpose. Molecular dynamic simulations, protein engineering techniques, and single-molecule AFM study have collectively revealed tremendous insights into the molecular design of single elastomeric proteins, which can guide the design and engineering of elastomeric proteins with tailored mechanical properties. Researchers are focusing experimental efforts toward engineering artificial elastomeric proteins with mechanical properties that mimic or even surpass those of natural elastomeric proteins.

In this Account, we summarize our recent experimental efforts to engineer novel artificial elastomeric proteins and develop general and rational methodologies to tune the nanomechanical properties of elastomeric proteins at the single-molecule level. We focus on general design principles used for enhancing the mechanical stability of proteins. These principles include the development of metal-chelation-based general methodology, strategies to control the unfolding hierarchy of multidomain elastomeric proteins, and the design of novel elastomeric proteins that exhibit stimuli-responsive mechanical properties.

Moving forward, we are now exploring the use of these artificial elastomeric proteins as building blocks of protein-based biomaterials. Ultimately, we would like to rationally tailor mechanical properties of elastomeric protein-based materials by programming the molecular sequence, and thus nanomechanical properties, of elastomeric proteins at the single-molecule level. This step would help bridge the gap between single protein mechanics and material biomechanics, revealing how the mechanical properties of individual elastomeric proteins are translated into the properties of macroscopic materials.

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Introduction

Elastomeric proteins are molecular springs subject to stretching forces under their natural environments and play many important biological roles in diverse processes ranging from cell adhesion, muscle contraction, to insect flight.[1] They also constitute biomaterials with superb mechanical properties. In order to understand the design of natural elastomeric proteins and aid the engineering of their biomimetics, considerable efforts have been made to characterize elastomeric protein-based biomaterials in various aspects, from their chemical components, structures, to mechanical properties.[1] However, most of these efforts were carried out on bulk materials. Investigating the mechanical properties of elastomeric proteins at the single-molecule level was not possible until the late 1990s. The development of single-molecule force spectroscopy techniques,[2−4] in particular the atomic force microscopy (AFM), has made it possible to stretch and measure the mechanical properties of elastomeric proteins one molecule at a time, fostering the development of the field of single protein mechanics and engineering.[5,6]

Elastomeric proteins can be roughly classified into two categories: entropic spring-like[7] and shock-absorber-like elastomeric proteins.[3,5,8] Entropic spring-like elastomeric proteins are made of flexible, nonglobular, and often unstructured proteins. Typical examples include elastin, abductin, and resilin.[1] In contrast, shock-absorber-like elastomeric proteins consist of individually folded globular domains arranged in tandem. The giant muscle protein titin is representative of this class.[9] Under a stretching force, folded globular domains may unfold into random coil-like structures, leading to energy dissipation.[3] Over the past decade, the development of single-molecule force spectroscopy techniques has enabled the characterization of mechanical properties of shock-absorber-like elastomeric proteins in great detail.[5,6,10−12] In combination with protein engineering and molecular dynamics (MD) simulation,[13,14] these force spectroscopy studies have unveiled some design principles employed by nature to construct such complex elastic/mechanical elements, shedding light on the working mechanism of some elastomeric proteins within their biological setting. Based on such insights, protein mechanics efforts have been extended to proteins that do not exhibit mechanical functions under their natural settings. These efforts help expand the toolbox of elastomeric proteins, and also open possibilities toward engineering artificial elastomeric proteins with mechanical features that are comparable or even superior to those of natural ones (refs 6, 10, and 15 and references therein).

Single-Molecule AFM: A Powerful Tool to Investigate the Mechanical Properties of Elastomeric Proteins One Molecule at a Time

Due to its high spatial resolution and force sensitivity (∼5 pN), AFM has evolved into a powerful technique to manipulate individual molecules and measure their mechanical properties at the single-molecule level[5,6,10,12] (Figure 1A). Stretching tandem modular proteins at constant pulling speeds results in characteristic sawtooth-like force−extension curves, where each force peak corresponds to mechanical unfolding of individual domains in the tandem modular protein (Figure 1B). Rich information about the mechanical properties of proteins can be obtained from force−extension measurements and be used to reconstruct the free energy profile for mechanical unfolding and folding reactions. Mechanical stability can be defined as the most probable unfolding force at a given pulling speed, which can be readily determined from an unfolding force histogram built from force−extension curves. The mechanical stability of proteins is determined by the mechanical unfolding energy barrier $\Delta G_{T\text{-}N}$ and the unfolding distance Δx_u between the native state and mechanical unfolding transition state (Figure 1C). Using Monte Carlo simulations[3] and the Bell−Evans model,[16,17] both $\Delta G_{T\text{-}N}$ and Δx_u can be determined from the unfolding force histogram and the pulling speed dependence of the unfolding force. It is of note that mechanical and chemical/thermal unfolding generally follows different reaction pathways; thus, the mechanical unfolding energy barrier does not necessarily correlate with that for chemical/thermal unfolding. Moreover, mechanical stability, which is determined by $\Delta G_{T\text{-}N}$, generally does not correlate with thermodynamic stability $\Delta G_{U\text{-}N}$.

From Natural Elastomeric Proteins to Their Biomimetics

Natural elastomeric proteins have been one of the focuses of protein mechanics studies.[5,12] Among these proteins, the muscle protein titin, which is responsible for the passive elasticity of muscles, is the most extensively studied one. Considerable effort has been dedicated to understanding design principles of titin and its constituting domains (mainly the 27th immunoglobulin domain),[3,13,18−22] largely shaping our current understanding of the molecular design of titin-like elastomeric proteins. These experimental and simulation studies revealed that the overall elastic properties of titin are determined by its constitutive domains and can be reconstructed by combining mechanical properties of its individual elements/domains.[21] Thus, investigating the mechanical properties of individual

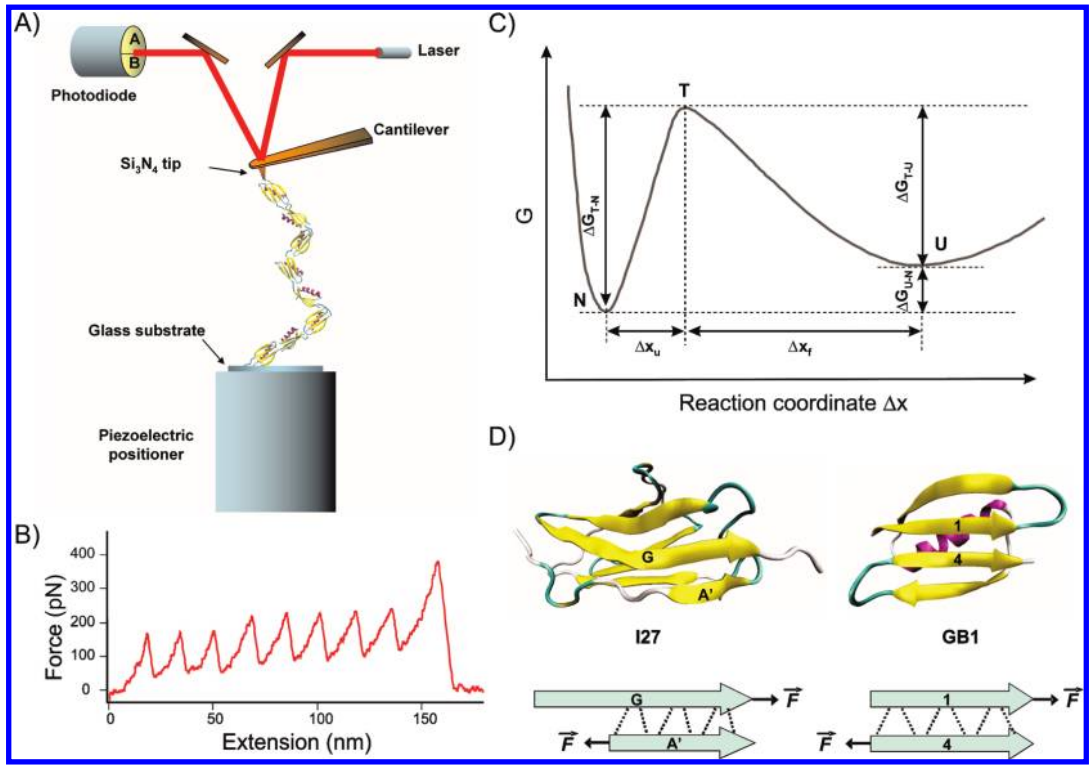

FIGURE 1. Single-molecule AFM experiments on elastomeric proteins. (A) Schematic of the force spectroscopy mode of AFM. The elastomeric protein of interest is stretched between the AFM cantilever and solid substrate to measure its force−extension relationships. (B) Typical force−extension curve of a polyprotein $(GB1)_8$. Stretching a tandem modular protein results in characteristic sawtooth-like force−extension curves, where each sawtooth peak corresponds to the unfolding of individual domains in the polyprotein. The last peak corresponds to the stretching and subsequent detachment of the unfolded polyprotein chain. (C) Free energy diagram for the mechanical unfolding/folding reactions of proteins can be reconstructed from single-molecule AFM experiments. $\Delta G_{T\text{-}N}$ and $\Delta G_{T\text{-}U}$ correspond to the unfolding and folding free energy barriers, respectively. Δx_u and Δx_f denote unfolding distance and folding distance along the reaction coordinate defined by the applied force, respectively. (D) Shear topology is a general feature among most mechanically stable proteins. The A'G-strands in I27 and β-strands 1 and 4 in GB1 constitute representative shear topology.

domains provide molecular level mechanistic insight into the molecular determinants of protein mechanical stability.

During the mechanical unfolding, a stretching force is applied to the protein along a defined direction and only a small number of residues in the protein directly bear the stretching force. Thus, the mechanical unfolding process is largely a local event[13] and depends upon the pulling direction.[23,24] This contrasts with the chemical unfolding processes: during chemical denaturation, all surface residues of the protein are exposed to solvents and chemical unfolding is global in nature. Therefore, protein topology and interactions in the force bearing region are important to the mechanical stability of proteins. It was revealed that the majority of mechanically stable elastomeric proteins exhibit shear topology, in which two force-bearing β-strands are arranged in parallel and form a "mechanical clamp".[6,25] Upon stretching, noncovalent interactions, often backbone hydrogen bonds, between the two force-bearing β-strands need to be ruptured simultaneously in order to extend and unfold the protein (Figure 1D). Thus, such a shear topology underlies the high mechanical stability of a variety of elastomeric protein domains.[13,26−28]

Building upon these insights into the design of natural elastomeric proteins, we have carried out studies to engineer biomimetic artificial elastomeric proteins. In addition to mimicking the mechanical properties of natural elastomeric proteins, such artificial systems may also allow for the engineering of elastomeric proteins with novel functional traits, such as enzymatic activity[29,30] and fluorescence,[31,32] that are not found in naturally occurring elastomeric proteins. As a first step, we focused on designing and engineering biomimetic artificial elastomeric proteins with significant mechanical stability. Using shear topology of two force-bearing β-strands as a screening criterion, we screened and identified a suite of functionally nonmechanical yet mechanically stable proteins[33,34] with GB1, the B1 IgG binding domain of streptococcal protein G, as a representative example (Figure 1D). Using single-molecule AFM, we characterized the mechanical

properties and mechanical unfolding energy landscape of GB1 in detail.[34,35] We found that although GB1 does not have any known mechanical function, polyprotein $(GB1)_8$ shows a unique combination of mechanical properties, including high mechanical stability, very fast folding kinetics, superb folding fidelity, and ability to fold against residual forces. These properties are either comparable or superior to those of natural elastomeric proteins. Based on similar ideas, many nonmechanical proteins were predicted to be mechanically stable,[28,36] and many more artificial elastomeric proteins were constructed and characterized (refs 10 and 15 and reference therein), suggesting a promising future for such biomimetic efforts.

Rational Enhancing of the Mechanical Stability of Proteins: General Considerations

Despite the critical role played by the mechanical clamp in determining protein mechanical stability, interactions mediated by residues distant from the mechanical clamp can also affect mechanical stability.[20,37] In addition, homologous proteins with essentially the same topology are known to display vastly different mechanical stability.[21,38] These results suggest that subtle and delicate interactions are also important in fine-tuning protein mechanical stability. However, completely understanding the molecular determinants of protein mechanical stability remains elusive, and accurate prediction of mechanical stability of proteins from their three-dimensional structures is still not possible. Over the past few years, significant progress was made toward rationally enhancing mechanical stability of proteins using a protein engineering approach.[33,37,39−41] However, these successes remain sparse and cannot be generalized to other proteins. Developing rational and general methodologies to enhance the mechanical stability of proteins has become increasingly important and has been the focus of our design efforts.

Mechanical stability, defined as the most probable unfolding force, does not correlate with the thermodynamic stability of proteins.[22] Instead, it is determined by the mechanical unfolding energy barrier $\Delta G_{T\text{-}N}$ and the unfolding distance Δx_u.[16] A larger $\Delta G_{T\text{-}N}$ and/or a smaller Δx_u lead to a higher mechanical unfolding force. Therefore, to rationally increase the mechanical stability of a given protein, it is necessary to either increase $\Delta G_{T\text{-}N}$ and/or reduce Δx_u. Increasing $\Delta G_{T\text{-}N}$ leads to a decrease in the unfolding rate, while reducing Δx_u makes the mechanical unfolding transition state highly native-like and the protein strong yet brittle.

To increase $\Delta G_{T\text{-}N}$ along the mechanical unfolding pathway, it is essential to preferentially stabilize the native state over the mechanical unfolding transition state. If the mechanical unfolding transition state is stabilized to the same extent as to the native state, there is no net change in $\Delta G_{T\text{-}N}$ for mechanical unfolding. Therefore, we can borrow ideas from methods developed to improve thermodynamic stability of enzymes and adapt them for preferentially stabilizing the native state over the transition state.

Protein−ligand interactions are ubiquitous in biology and are used extensively in nature and in enzyme engineering to increase thermodynamic stability of proteins by stabilizing the native state over the unfolded state. Adapting these tricks for mechanical engineering of proteins, we have used engineered ligand binding (i.e., engineered metal chelation) and endogenous ligand binding to tune protein mechanical stability.

Engineered Metal Chelation Is a General and Rational Approach to Enhance the Mechanical Stability of Proteins

Engineered metal chelation is a widely used method to enhance the thermodynamic stability of proteins,[42] in which a bi-histidine motif is engineered into proteins as a ligand (divalent metal ion) binding site. We have successfully adapted and developed this methodology into a general and rational approach toward enhancing protein mechanical stability.[43,44] The binding of divalent metal ions to an engineered bi-histidine metal chelation site can stabilize the native state. The key to enhancing the mechanical stability is to design the metal chelation site such that the engineered bi-histidine site has lower (ideally zero) binding affinity at the mechanical unfolding transition state. Thus, metal chelation can preferentially stabilize the native state over the transition state and increase $\Delta G_{T\text{-}N}$. Proof-of-principle of this methodology was demonstrated using GB1 as a model system.[43] MD simulations predicted that the force-bearing β-strands 1 and 4 of GB1 slightly slide against each other in the mechanical unfolding transition state.[45] Therefore, if we engineer a bi-histidine site across β-strands 1 and 4, the sliding of both strands would distort the metal chelation site in the mechanical unfolding transition state, resulting in lower metal-binding affinity. Using this design principle, we successfully increased the mechanical stability of GB1 via metal chelation (Figure 2). The engineered bi-His mutant G6-53 with histidine substitution at positions 6 and 53 binds Ni^{2+} with high affinity. The binding of Ni^{2+} increased the unfolding force of G6-53 by ~120 pN. It was found that the

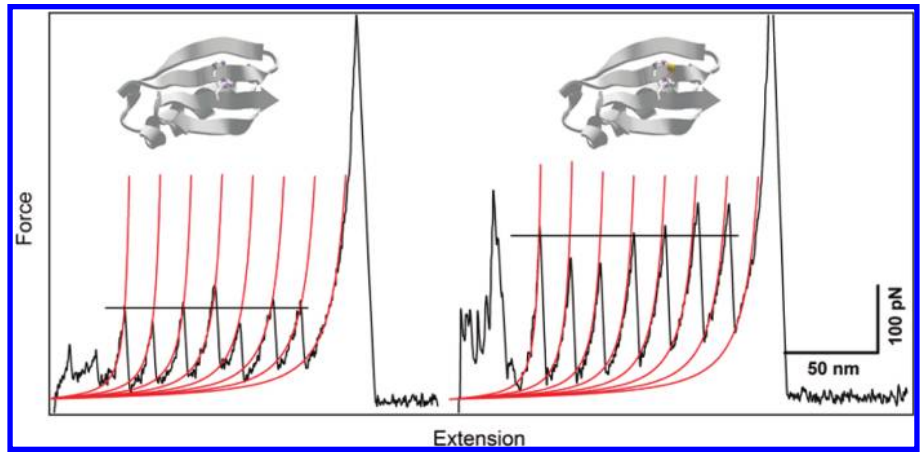

FIGURE 2. Enhancing mechanical stability by engineered metal chelation. Two histidine residues are engineered across β-strands 1 and 4 of GB1 at positions 6 and 53 to form a metal chelation site. The binding of Ni^{2+} significantly increased the unfolding force of GB1 (120 pN for apo-form and ~240 pN for Ni^{2+}-bound form).

metal chelation predominantly increased the mechanical unfolding energy barrier $\Delta G_{T\text{-}N}$, and also slightly rigidifies the structure of G6-53, resulting in a slightly smaller Δx_u.

Engineered metal chelation sites can be easily integrated into different proteins, making this method a general approach to rationally enhance the mechanical stability of a wide range of proteins. However, successful implementation of this method requires detailed knowledge of the mechanical unfolding pathway, especially for proteins that unfold via multiple unfolding pathways and/or unfolding intermediate states (as demonstrated for Fn3 domain of tenascin-C).[44] For this purpose, steered MD simulations are of particular importance.

Enhancing the Mechanical Stability of Proteins by Endogenous Ligand Binding: Trial and Error

Many proteins contain endogenous ligand binding sites, which can potentially be used to enhance mechanical stability of proteins. Since GB1 binds IgG antibody with high affinity, we explored the use of IgG binding to enhance the mechanical stability of GB1.[41,46] GB1 binds Fab and Fc fragments of IgG with different epitopes that are distant from the force bearing region of GB1 (β-strands 1 and 4). Using single-molecule AFM, we found that the binding of Fab and Fc to GB1 leads to an increase in mechanical stability of GB1 by ~80 pN (from ~180 to ~260 pN)[41,46] (Figure 3). Further mutagenesis studies showed that the mechanical stability enhancement of GB1 upon ligand binding does not correlate with binding affinity, as the binding of the Fc fragment to GB1 mutants with reduced binding affinity leads to a similar increase in mechanical unfolding force. Again, the increase in mechanical stability is predominantly due to the increase in $\Delta G_{T\text{-}N}$ upon binding of the IgG fragment, with the Δx_u decreased slightly.

It is of note that although the Fc binding site in GB1 is distant and physically decoupled from the force-bearing region, the binding of the Fc fragment increases the mechanical stability of GB1. We speculated that there is long-range coupling occurring between the Fc binding site and the force-bearing region.[41] Through a possible allosteric mechanism, the stretching of GB1 leads to a somewhat lower binding affinity to Fc at the mechanical unfolding transition state. However, a detailed molecular mechanism still needs to be unveiled.

Compared with the engineered metal chelation approach,[43] enhancing the mechanical stability of proteins via endogenous ligand binding remains largely trial-and-error. For example, the binding of small ligands significantly increases the mechanical stability of dihydrofolate reductase (DHFR) from Chinese hamster,[47] but it had little effect on DHFR from *E. coli*.[48]

Designing Novel Elastomeric Proteins with Tailored Nanomechanical Properties: A Mechanical Chameleon with Dual Mechanical Stability

Natural elastomeric proteins behave as either pure entropic springs or shock-absorbers, depending on their biological roles in tissues and biomaterials.[1,8] Based on the fact that the binding of the Fc fragment can enhance the mechanical stability of GB1 and the Fc binding epitope is physically decoupled from the force-bearing region,[41] we designed a novel elastomeric "chameleon" protein that can switch its mechanical properties between entropic springs and shock absorbers in response to ligand binding (Figure 4).[49] We used proline mutagenesis to disrupt the force bearing

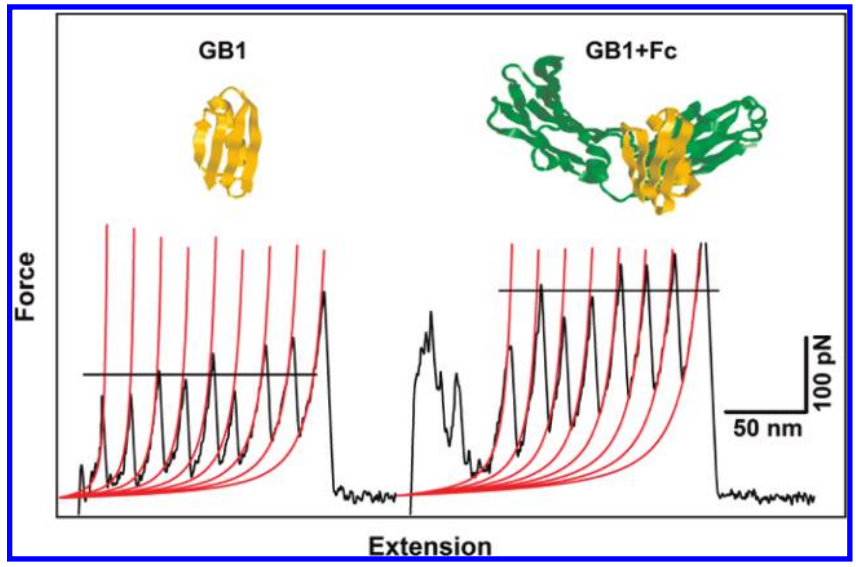

FIGURE 3. Binding of the Fc fragment increases the mechanical stability of GB1. (A) Stretching polyprotein $(GB1)_8$ results in typical sawtooth-like force−extension curves with average unfolding force of ~180 pN. (B) The binding of Fc to GB1 increases the mechanical unfolding force of GB1 to ~260 pN. Insets show the three-dimensional structure of GB1 and GB1/Fc complex.

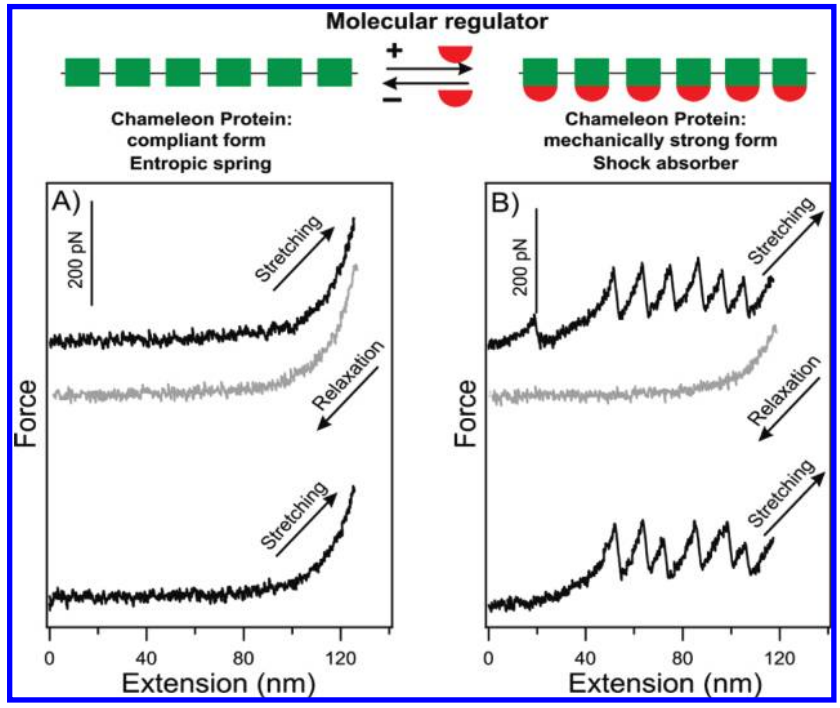

FIGURE 4. Engineered chameleon elastomeric protein $(GT18P)_8$ exhibits dual mechanical stability regulated by ligand binding. (A) Polyprotein $(GT18P)_8$ behaves as an entropic spring, and no hysteresis between stretching and relaxation curves was observed. (B) $(GT18P)_8$ behaves as a shock absorber in the presence of Fc. Stretching $(GT18P)_8$ in 11 μM Fc leads to sawtooth-like force−extension curves. The unfolded GT18P domains can fold back after relaxation and rebind with hFc to regain their mechanical strength. The inset illustrates the reversible switch of $(GT18P)_8$ between the two different mechanical states upon the regulation of hFc.

region of GB1 while preserving its Fc binding ability. Proline mutation in β-strands disrupts backbone hydrogen bonds and creates a bulge in the β-strand. Such a dramatic alternation to the force bearing region significantly reduced the mechanical stability of GB1 when residue 54 or 18 was substituted with a proline. The unfolding force of GB1 mutants GV54P and GT18P decreased from ~180 pN to an almost undetectable level (<30 pN), and altered their unfolding behavior such that both mutants behave as entropic springs. Since these mutations are distant from the Fc binding surface, they did not cause a dramatic change of their binding affinities to Fc. Therefore, these GB1 mutants

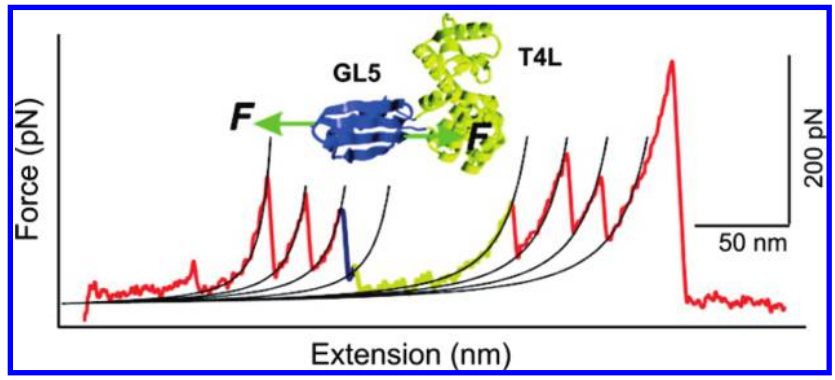

FIGURE 5. Reversed mechanical unfolding hierarchy by domain insertion. The mechanical unfolding force of GL5 is around 130 pN, while T4L unfolds at ~50 pN. Upon insertion of T4L into GL5, a reversed mechanical unfolding hierarchy was observed. The figure shows a typical force−extension curve of $(GL5)_4$-(GL5/T4L)-$(GL5)_4$. Unfolding of the mechanically weaker domain T4L (colored in yellow) was observed to occur only after the unfolding of the host domain GL5 (colored in blue), suggesting a reversed mechanical unfolding hierarchy between GL5 and T4L.

can be converted into a mechanically stable form upon Fc binding and serve as shock absorbers. Moreover, upon removing the molecular regulator Fc, the "chameleon" proteins can reversibly switch back to their mechanically labile state. These chameleon proteins represent prototype "smart" elastomeric proteins whose mechanical features are responsive to external stimuli, and can work as smart mechanical/elastic elements in nanomechanics and material sciences.

Regulating the Mechanical Unfolding Hierarchy of Elastomeric Proteins by Domain Insertion

After gaining insights into how the mechanical properties of individual protein domains may be tuned, we also endeavored to control and regulate the mechanical unfolding hierarchy in tandem modular proteins. Most elastomeric proteins are tandem modular proteins.[50] Upon stretching, the folded domains unfold sequentially, strictly following their mechanical stability order from weakest to strongest.[3,22] Such a design poses challenges for incorporating mechanically labile yet functionally important proteins into artificial elastomeric proteins, as these domains will unfold first upon stretching, resulting in the loss of functionality. To overcome this challenge, we employed a domain insertion strategy to design reversed mechanical unfolding hierarchy to provide mechanical protection to the mechanically labile domain.[51] We designed a domain insertion protein in which the mechanically stable domain GL5 (a loop insertion mutant of GB1)[52] serves as the host and the mechanically labile guest protein T4-lysozyme is inserted into a flexible loop of GL5 (Figure 5). Since the N- and C-termini of T4 lysozyme are proximal, splicing of T4 lysozyme into GL5 did not affect the structure of both domains[52]

As designed, the host protein GL5 provided mechanical protection to the mechanically labile protein T4 lysozyme, with single-molecule AFM experiments showing that the mechanically labile T4 lysozyme unfolds only after the unfolding of the mechanically stronger host domain GL5 (Figure 5). Force clamp studies showed that the lifetime of T4 lysozyme was prolonged by ~1500 fold by its insertion into GL5. The domain insertion approach represents a key step toward incorporating mechanically labile, yet functionally desirable, proteins into multifunctional elastomeric proteins. The resultant proteins can serve as prototype mechanoenzymes whose activity can be switched off by the unfolding of the host domains.

From Well-Characterized Single-Molecule Building Blocks to Functional Biomaterials

Combining single-molecule AFM and protein engineering, we have gained tremendous insights into the design of elastomeric proteins, enabling the rational design and construction of novel elastomeric proteins with tailored nanomechanical properties. We believe that these engineered elastomeric proteins can provide well-defined molecular building blocks for the bottom-up construction of novel elastomeric protein-based biomaterials. Such efforts will help to bridge the gap between understanding single-molecule and biomaterials characteristics and help to achieve rational engineering of biomaterials at the molecular level.

Based on these ideas, we have spearheaded experimental efforts toward utilizing well-characterized elastomeric proteins as building blocks to engineer novel biomaterials.[53,54] One of the direct challenges in doing so is to assemble and incorporate individual elastomeric proteins into macroscopic biomaterials. Naturally occurring elastomeric proteins contain mechanical elements as well as unique sequences that are responsible for assembling individual elastomeric proteins into functional tissues and biomaterials. For example, the A-band part of titin acts as a template and molecular ruler during the assembly of thick filaments and acts to organize titin within sacromeres.[55] For nonstructured elastomeric protein resilin, the formation of a resilin network is through the cross-linking of tyrosine residues into di-tyrosine adducts.[56] Therefore, to engineer elastomeric protein-based biomaterials, it is imperative to develop chemical strategies for efficient cross-linking and assembly of individual elastomeric proteins into three-dimensional networks and macroscopic materials.

Toward this goal, we have designed novel elastomeric protein-based biomaterials to mimic the passive elastic properties of muscles.[54] Passive elastic properties of muscle are largely mediated by titin, which is composed of hundreds of folded Ig domains and some unstructured unique sequences, such as PEVK and N2B sequences.[9] Single-molecule AFM and myofibril studies demonstrated that the passive elastic properties of muscle can be reconstituted by combining mechanical properties of individual mechanical elements of titin.[21] To mimic the passive elastic properties of muscle, we designed novel artificial elastomeric proteins based on GB1 and resilin to serve as mini-titin mimicry (Figure 6A). In these GB1-resilin-based elastomeric proteins, GB1 domains are used to mimic titin Ig domains, and resilin sequences are used to mimic entropic spring-like unique sequences in titin. Single-molecule AFM studies revealed that GB1-resilin-based elastomeric proteins exhibit nanomechanical properties similar to those of individual titin molecules. We then employed a well-characterized photochemical method to cross-link GB1-resilin-based elastomeric proteins via the formation of di-tyrosine adducts and cast them into solid biomaterials (Figure 6D). Tensile tests indicated that, at low strain, these GB1-resilin-based biomaterials behave as rubber-like materials showing high resilience; at high strain, these materials behave as shock-absorber-like materials showing strain-dependent hysteresis and stress relaxation. Our results showed that the resultant materials exhibit a Young's modulus, resilience, and stress relaxation behavior similar to those of myofibrils (Figure 6E, F). Some of these properties can be readily explained by the nanomechanical properties of individual GB1-resilin-based elastomeric proteins. This study provided a unique example about how macroscopic materials can be designed using well-characterized elastomeric protein-based building blocks, and also revealed the great potential of using designed elastomeric proteins to tailor the macroscopic mechanical properties of biomaterials.

Conclusion and Perspective

Significant progress has been made toward the mechanical engineering of elastomeric proteins with tailored nanomechanical properties, ranging from reversed mechanical unfolding hierarchy to well-defined and stimuli-responsive mechanical stability. These efforts are helping to gain complete understanding of the molecular determinants of protein mechanical stability. The ultimate goal of protein mechanics is to achieve de novo design of elastomeric proteins with tailored nanomechanical properties and desirable functionality. Such proteins may serve as novel mechano-elements, such as fluorescence-based force sensors and mechanically controlled enzymes[57] for integration into functional nanomechanical assemblies. Furthermore, such tailored elastomeric proteins may serve as building blocks for designing novel elastomeric protein-based biomaterials. These endeavors will be important for designing new generations of high performance materials that have a myriad of applications within fields such as material science and biomedical engineering. However, the use of tandem modular elastomeric proteins in such applications is still in its nascent stage. Developing efficient cross-linking strategies and assembly methods is important for constructing elastomeric protein-based biomaterials. Such developments can benefit greatly from recent progress in click chemistry[58] and protein science involving incorporation of noncanonical amino acids.[59] Furthermore, how nanomechanical properties of individual elastomeric proteins are translated into macroscopic material properties remains an open question. Simple random three-dimensional networks will serve as the simplest model system to address this question. However, higher order structures frequently seen in natural biomaterials remain to be successfully incorporated into elastomeric protein-based synthetic biomaterials. Moreover, multiscale modeling of mechanical properties of elastomeric protein-based biomaterials will be critical,[60] where the synergistic combination of such modeling with experimental techniques holds the key to fully harnessing the great poten-

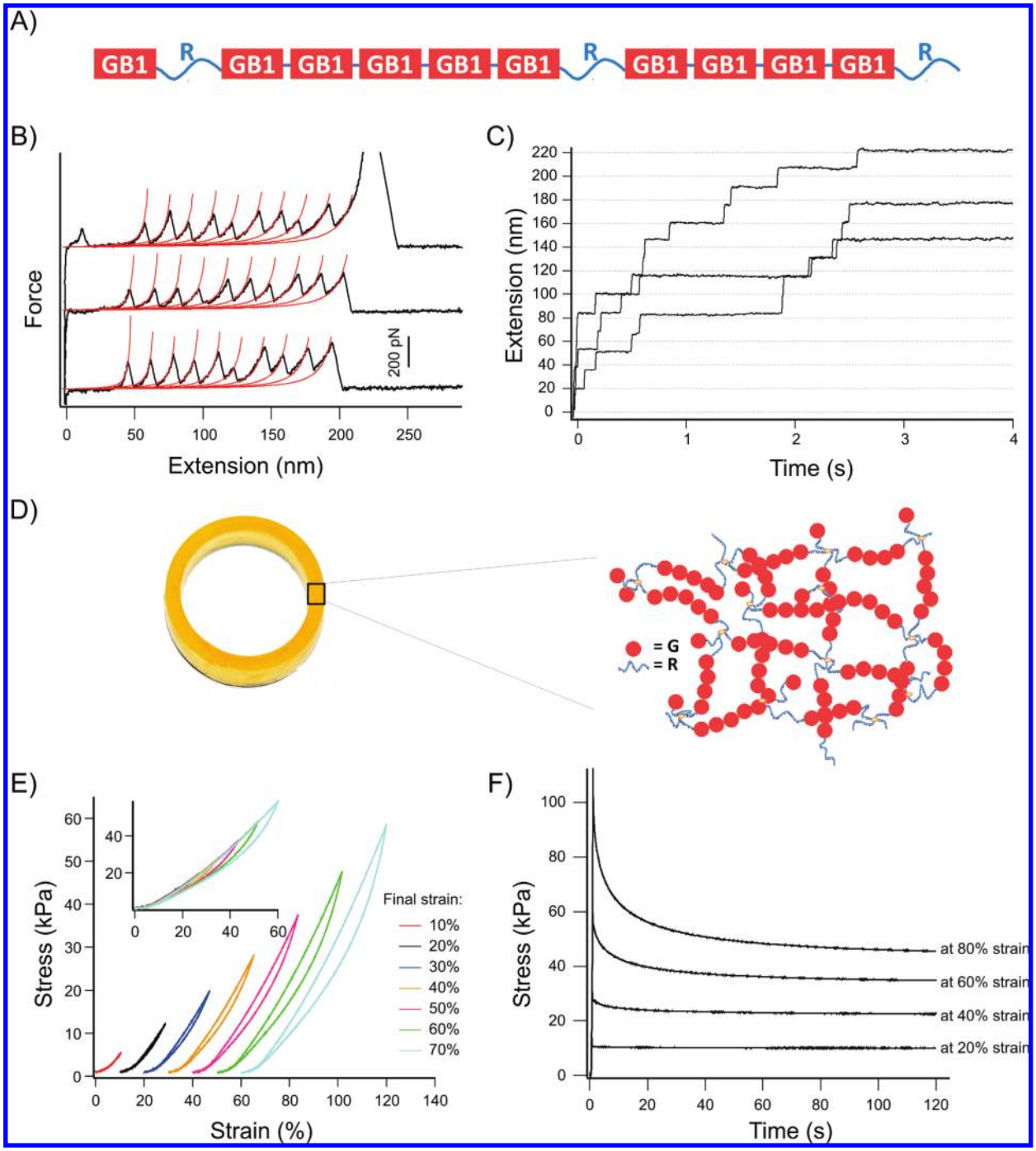

FIGURE 6. Engineering elastomeric protein-based biomaterials via the bottom-up approach. (A) Schematic of miniature-titin-like elastomeric protein GRG_5RG_4R. G represents GB1 domain and R represents resilin consensus sequence. (B) Force−extension curves of GRG_5RG_4R. The initial featureless spacer corresponds to the stretching of unstructured resilin sequences, and the sawtooth peaks correspond to the unfolding of GB1 domains. (C) Stepwise unfolding of GRG_5RG_4R under a constant force. The stepwise elongation resulted from the unfolding of GB1 domains. (D) A photograph of a hydrogel ring constructed from GRG_5RG_4R and the schematic of the network structure. (E) Representative stress−strain and stress-relaxation curves of GRG_5RG_4R-based biomaterials. (F) Stress-relaxation curves of GRG_5RG_4R-based biomaterials at constant strains.

tial offered by elastomeric proteins. It is safe to anticipate that the greater discoveries in the field of protein mechanics are yet to come!

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BIOGRAPHICAL INFORMATION

Hongbin Li received his bachelor's degree (1993) from Tianjin University in China and Ph.D. degree (1998) from Jilin University in China. He then worked as a postdoctoral fellow at the Mayo Foundation and as an Associate Research Scientist at Columbia University. In 2004, he joined the University of British Columbia, where he is currently an Associate Professor in Chemistry and Canada Research Chair in Molecular Nanoscience and Protein Engineering.

Yi Cao obtained his bachelor's degree (2001) and Master's degree (2004) from Nanjing University in China. He joined Dr. Li's group at the University of British Columbia in 2004 and earned his Ph.D. degree in 2009. After a short postdoctoral study in Dr. Li's group, he joined the Department of Physics at Nanjing University as a faculty member in 2010.

FOOTNOTES

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