LETTERS

Designed biomaterials to mimic the mechanical properties of muscles

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The passive elasticity of muscle is largely governed by the I-band part of the giant muscle protein titin[1–4], a complex molecular spring composed of a series of individually folded immunoglobulin-like domains as well as largely unstructured unique sequences[5]. These mechanical elements have distinct mechanical properties, and when combined, they provide the desired passive elastic properties of muscle[6–11], which are a unique combination of strength, extensibility and resilience. Single-molecule atomic force microscopy (AFM) studies demonstrated that the macroscopic behaviour of titin in intact myofibrils can be reconstituted by combining the mechanical properties of these mechanical elements measured at the single-molecule level[8]. Here we report artificial elastomeric proteins that mimic the molecular architecture of titin through the combination of well-characterized protein domains GB1[12] and resilin[13]. We show that these artificial elastomeric proteins can be photochemically crosslinked and cast into solid biomaterials. These biomaterials behave as rubber-like materials showing high resilience at low strain and as shock-absorber-like materials at high strain by effectively dissipating energy. These properties are comparable to the passive elastic properties of muscles within the physiological range of sarcomere length[14] and so these materials represent a new muscle-mimetic biomaterial. The mechanical properties of these biomaterials can be fine-tuned by adjusting the composition of the elastomeric proteins, providing the opportunity to develop biomaterials that are mimetic of different types of muscles. We anticipate that these biomaterials will find applications in tissue engineering[15] as scaffold and matrix for artificial muscles.

The string of folded immunoglobulin domains and unstructured unique sequences constitute two distinct types of entropic springs in titin[7,8]. The string of folded immunoglobulin domains has higher persistence length than the unstructured sequences and extend first during stretching. Only under high stretching forces at the high end of the physiological range of sarcomere length, when the string of immunoglobulin domains are straightened, can a small number of folded immunoglobulin domains unfold to extend the length of titin and dissipate energy, effectively preventing damage due to overstretching[16]. These features are combined to give rise to the passive mechanical properties of muscles at the macroscopic level[1,2,7,17], which manifest as a Young's modulus close to 100 kPa, increasing energy dissipation at higher sarcomere length, and stress relaxation at a constant strain[4,14,16]. To design biomaterials mimicking the fine-tuned passive elastic properties of muscle, it is critical to incorporate these mechanical features at both single-molecule and macroscopic biomaterial levels. Towards this goal, here we first engineered artificial elastomeric proteins that mimic the molecular architecture and nanomechanical properties of individual titin molecules. Then we used these proteins to construct biomaterials that mimic the passive elastic properties of muscle.

To engineer titin-mimicking artificial elastomeric proteins (see Methods and Supplementary Fig. 1), we used the well-characterized GB1 domains[12] to mimic folded titin immunoglobulin domains, because GB1 domains exhibit mechanical properties comparable to those of titin immunoglobulin domains[12], and we used a consensus repeat of the random-coil-like protein resilin[13] to mimic unstructured sequences (such as the N2B sequence in titin), because resilin is a highly elastic and resilient protein[13,18,19] and is also largely unstructured[20] (see Supplementary Fig. 2). With these two building blocks, we constructed artificial elastomeric proteins $(G–R)_4$ and GRG_5RG_4R, where G represents individual GB1 domain, and R represents individual resilin repeat.

We first used AFM techniques[6,21] to characterize their nanomechanical properties at the single-molecule level. Stretching $(G–R)_4$ results in characteristic sawtooth-like force–extension relationships[12] (Fig. 1a), where individual force peaks correspond to the mechanical unfolding of GB1 domains and are characterized by an unfolding force of ~180 pN and a contour length increment ΔL_c of ~18 nm. Owing to the dimerization of $(G–R)_4$ via carboxy-terminal cysteine residues (Supplementary Information), force–extension curves can show as many as eight GB1 unfolding events. The featureless 'spacer', which is of length L_0 and occurs before the GB1 unfolding force peaks, corresponds to the stretching of random-coil-like resilins and folded GB1 domains (Fig. 1a), confirming the entropic spring-nature of resilin repeats. Because the persistence length of GB1 is much larger than that of unstructured resilin, fitting the Worm-like-chain model of polymer elasticity to the spacer yielded a persistence length of 0.49 ± 0.09 nm (average ± s.d., $n = 188$) for resilin, comparable to that of the random-coil-like sequence N2B in titin[8,9,22] and unfolded polyprotein chains[6,21]. Stretching polyprotein GRG_5RG_4R yielded force–extension curves with similar sawtooth patterns but with shorter spacers owing to the fewer resilin domains in GRG_5RG_4R (Fig. 1b and Supplementary Fig. 3). Moreover, the mechanical unfolding of GB1 domains is reversible, because unfolded GB1 domains can refold to regain mechanical resistance upon relaxation[12]. These nanomechanical properties of $(G–R)_4$ and GRG_5RG_4R largely mimic those of individual titin molecules.

We then used these miniature-titin-like elastomeric proteins to construct biomaterials to mimic the passive mechanical properties of muscle. Individual titin molecules are well-aligned and organized in the filament lattice of muscle[3]. However, it remains challenging to mimic such ordered structures in synthetic biomaterials. As an alternative, we created chemically crosslinked GB1–resilin networks to exploit the nanomechanical properties engineered into individual GB1–resilin molecules. We used the well-developed $[Ru(bpy)_3]^{2+}$-mediated photochemical crosslinking strategy[23], which allows the crosslinking of two tyrosine residues in close proximity into dityrosine adducts (Supplementary Fig. 4). This method was used successfully to

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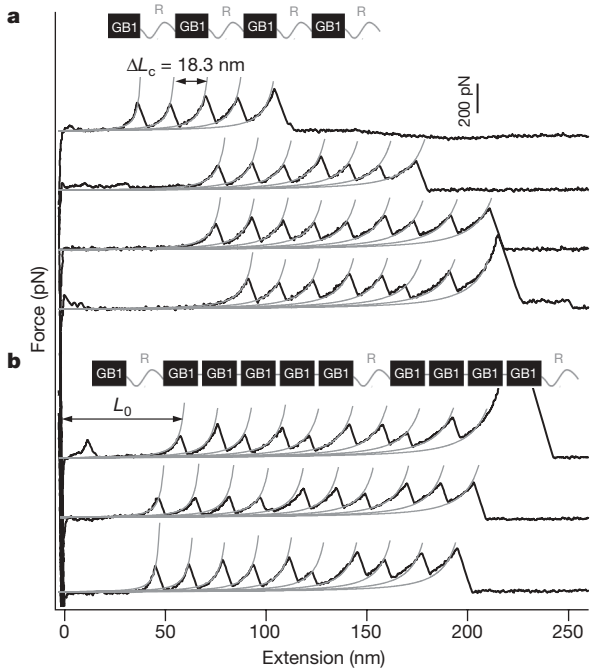

Figure 1 | Force–extension curves of two polyproteins. a, $(G–R)_4$. **b**, GRG_5RG_4R. The force peaks, characterized by a ΔL_c of ~18 nm and an unfolding force of ~180 pN, result from the mechanical unfolding of GB1 domains. Stretching resilins does not result in any unfolding force peaks; instead we see a featureless spacer of length L_0. The notable difference between the force–extension curves of $(G–R)_4$ and GRG_5RG_4R is the shorter featureless spacer of GRG_5RG_4R, which is due to fewer resilin repeats in GRG_5RG_4R. Grey lines correspond to the worm-like chain model fits to the experimental data.

crosslink recombinant resilins into solid biomaterials[13]. The use of resilin repeats, which provide the majority of crosslinking sites in GB1–resilin polyproteins, enables an efficient approach with which to prepare GB1–resilin-based biomaterials. We found that on illumination with white light, GB1–resilin polyproteins can readily be crosslinked into solid and transparent biomaterials at room temperature from their concentrated (>150 mg ml^{-1}) solutions (Supplementary Information). The middle panel of Fig. 2a shows optical photographs of moulded rings of both polyproteins. The formation of dityrosine crosslinks was indicated by their characteristic blue fluorescence upon ultraviolet irradiation[13] (Fig. 2a, top panel).

Protein-based biomaterials, such as those based on elastin[24–28], resilin[13] and abductin[29], are engineered from non-globular elastomeric proteins that behave like entropic springs. To our knowledge, the GB1–resilin-based biomaterial is the first chemically crosslinked biomaterial that incorporates folded, mechanically resistant globular domains in its constituent elastomeric proteins, enabling us to examine their macroscopic mechanical properties and investigate how the microscopic properties of individual proteins are translated into macroscopic ones in biomaterials.

Here we carried out tensile measurements to characterize the mechanical properties of GB1–resilin-based biomaterials in PBS at room temperature. For technical reasons, we used ring-shaped samples for tensile testing[30] (see Supplementary Information). Typical stress–strain curves of $(G–R)_4$ and GRG_5RG_4R-based biomaterials are shown in Fig. 2b-c. It is evident that GB1–resilin-based biomaterials are elastic. GRG_5RG_4R can be stretched to a strain as high as 135% without breaking. The Young's modulus is ~70 kPa for $(G–R)_4$ and ~50 kPa for GRG_5RG_4R (at 15% strain), both close to the Young's modulus measured for myofibrils/myocytes, which is in the range 60–100 kPa within the physiological range of sarcomere length[4,14]. These biomaterials are isotropic (Supplementary Information), so the measured Young's modulus reflects the overall isotropic property of the biomaterials.

Resilience is a measure of a material's ability to deform reversibly without loss of energy[18]. Resilin is known for its superb resilience[19], and resilin-based biomaterials constructed using the same photochemical crosslinking method did not show appreciable hysteresis even at 250% strain[13,31] (Fig. 2d). To examine the influence of folded GB1 domains on the resilience of GB1–resilin-based biomaterials, we measured the resilience of these biomaterials. The stretching and relaxation curves of both $(G–R)_4$ and GRG_5RG_4R at low strain (<15%) were superimposable and no hysteresis was observed (Fig. 2b, c), suggesting high resilience for both materials at low strains. However, the stretching and relaxation curves were no longer superposable at higher strains and hysteresis started to develop, indicating that some of the work done during stretching was dissipated and cannot be recovered upon relaxation. The hysteresis increases with the increase of strain (Fig. 2b, c), indicating that the resilience of GB1–resilin-based materials decreases with the increase of strain (Fig. 2d). This behaviour is similar to that of myofibrils or myocytes[14,16,32], which showed increasing hysteresis between stretching and relaxation at increasing sarcomere lengths, indicating that GB1–resilin-based biomaterials, just like muscles[16], behave like shock-absorbers at higher strains by effectively dissipating energy.

The observed hysteresis, that is, energy dissipation, during cyclic experiments indicates that stretching GB1–resilin-based biomaterials to higher strains involved the breakage of weak non-covalent bonds[33] in the crosslinked network. And the breaking of such bonds is reversible, as the hysteresis observed in GB1–resilin-based biomaterials can be fully recovered upon relaxation. As shown in the insets of Fig. 2b and c, during subsequent stretching, stress–strain curves superpose on one another regardless of the final strain, suggesting a full recovery of the hysteresis. Moreover, the recovery of hysteresis is very fast: during cyclic stretching–relaxation experiments, the stretching–relaxation loops were identical (Supplementary Fig. 6) even when there was no waiting time between consecutive cycles, suggesting that the recovery of hysteresis occurs at a timescale significantly shorter than the dead time of our Instron, which is estimated to be ~1 s. Again, this reversible hysteresis behaviour is similar to that for myofibrils and myocytes[16,32]. It is also interesting to note that the recovery of hysteresis can occur even under residual stress. In partial relaxation experiments (Fig. 2e), when the biomaterial was partially relaxed to a strain above 35%, no recovery of hysteresis was observed. When the biomaterial was relaxed to below 35% strain, partial recovery started to occur. The degree of recovery depends on the residual stress: the lower the residual stress, the higher the percentage of recovery (Fig. 2e).

It is evident that the mechanical behaviours of GB1–resilin-based biomaterials differ significantly from those of resilin-based biomaterials, highlighting the significant roles of folded GB1 domains in determining the mechanical properties of the resultant biomaterials. Given that photochemically crosslinked resilin-based biomaterials show only negligible hysteresis during stretching (Fig. 2d), the observed hysteresis in GB1–resilin-based biomaterials probably resulted from folded GB1 domains and the associated structural changes of the crosslinked network. The hysteresis observed in tensile experiments indicates that the stretching of GB1–resilin-based biomaterials involved breaking of weak non-covalent bonds[33]. It is well known from single-molecule AFM experiments that, on stretching, force-induced rupture of non-covalent bonds can lead to the unfolding of GB1 domains and dissipation of energy[12]. Therefore, the unfolding of some GB1 domains during stretching could provide a plausible molecular mechanism to explain the hysteresis observed in GB1–resilin-based biomaterials at high strains. The fast recovery rate of hysteresis and the ability to recover hysteresis under residual stress are consistent with the fast folding kinetics of GB1 domains and the ability of GB1 domains to refold under residual force observed in single-molecule AFM experiments[12], providing qualitative evidence that the hysteresis observed in biomaterials probably originates from the unfolding of a small number of GB1 domains.

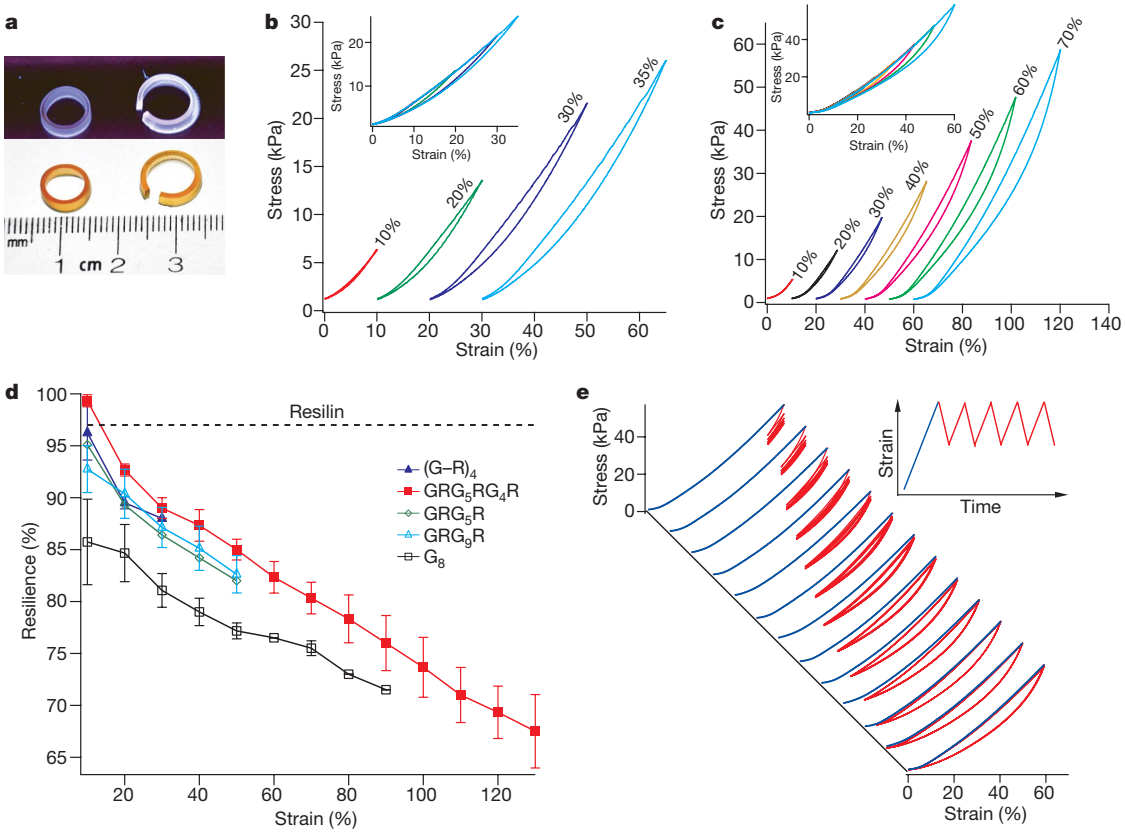

Figure 2 | Mechanical properties of $(G–R)_4$ and GRG_5RG_4R-based biomaterials. **a**, Photographs of moulded rings built from $(G–R)_4$ (left, intact) and GRG_5RG_4R (right, after being loaded to failure in tensile test) under white light (middle panel) and ultraviolet illumination (top panel). **b**, **c**, Representative stress–strain curves of $(G–R)_4$ (**b**) and GRG_5RG_4R (**c**) measured in PBS. For clarity, stress–strain curves are offset relative to one another. Final strains are shown on the curves. Insets show the superposition of the stress–strain curves at different strains. **d**, Resilience of GB1–resilin-based biomaterials decreases with the increase of strain. In contrast, biomaterials constructed from resilin do not show any appreciable hysteresis (data taken from ref. 13). **e**, GRG_5RG_4R-based biomaterials can recover hysteresis under residual stress. During stretching–relaxation experiments, when the biomaterial is partially relaxed to a strain above 35%, no recovery of hysteresis was observed. When the biomaterial was relaxed to below 35% strain, we started to observe partial recovery. The degree of recovery increased with the decrease of residual stress. For clarity, the initial stretching trace is coloured blue. The inset shows the experimental protocol of the partial relaxation experiments. The pulling speed used in the experiments was 25 mm min^{-1}. Error bars indicate standard deviation of the data.

To further compare the energy dissipation behaviours of the designed biomaterials with those of myofibrils/myocytes, we carried out stress-relaxation experiments at constant strains. When GRG_5RG_4R was stretched rapidly to a given strain that was held constant afterwards, we observed clear stress relaxation (Fig. 3a), again suggestive of the existence of energy dissipation processes. The larger the initial strain, the greater the amplitude of stress relaxation. We found that the stress-relaxation behaviours can be described reasonably well by double-exponential fits. The relaxation rates (k_1 and k_2) were observed to increase with the increase of strain, but the increase of fast-phase rate k_1 mainly occurs at higher strain while the increase of slow-phase rate k_2 occurs at lower strain (Fig. 3b).

The stress-relaxation behaviours of GB1–resilin-based biomaterials are qualitatively similar to those of myofibrils[16,17], but they are very different from the behaviour of biomaterials made of resilin, in which negligible stress-relaxation was observed in similar experiments[31]. The unfolding of a few immunoglobulin domains was proposed as a possible molecular mechanism to explain the stress-relaxation behaviours of myofibrils[31]. Similarly, Monte Carlo simulations[6] on force-relaxation behaviour of GRG_5RG_4R at constant extension revealed that the unfolding of some GB1 domains can lead to force-relaxation behaviours similar to those seen in our experiments and similar dependence of the fast-phase relaxation rate on extension (Supplementary Fig. 7). However, the simulated behaviour of the slow-phase relaxation rate differed from the experimental data. It is clear that the relaxation behaviours simulated at the single-molecule level cannot be directly compared with the stress-relaxation behaviours of GB1–resilin-based biomaterials quantitatively, because GB1–resilin molecules are not well-aligned in the photochemically crosslinked three-dimensional network and the force experienced by individual molecules cannot be measured directly. A more detailed model combining the possibility of GB1 unfolding with a three-dimensional network is required to describe the stress-relaxation behaviour at the macroscopic level. Moreover, it is important to note that stress relaxation is considered to be a viscoelastic property macroscopically. Although domain unfolding can lead to stress relaxation, the direct demonstration of domain unfolding in macroscopic materials is yet to be achieved. Therefore, it is possible that other microscopic processes or mechanisms, such as friction experienced by folded domains during stretching, may also contribute to the stress-relaxation behaviours of muscles as well as GB1–resilin-based biomaterials.

Our results demonstrate that the incorporation of folded, mechanically resistant globular domains into elastomeric proteins provides a novel approach with which to construct biomaterials that have unusual macroscopic mechanical properties. Such a bottom-up approach offers the opportunity to tailor the macroscopic properties of biomaterials by fine-tuning the nanomechanical properties of their molecular building blocks at the single-molecule level. To demonstrate such possibilities, we used chemical denaturant to affect the nanomechanical properties of individual GB1 domains in order to modulate the mechanical properties of macroscopic biomaterials.

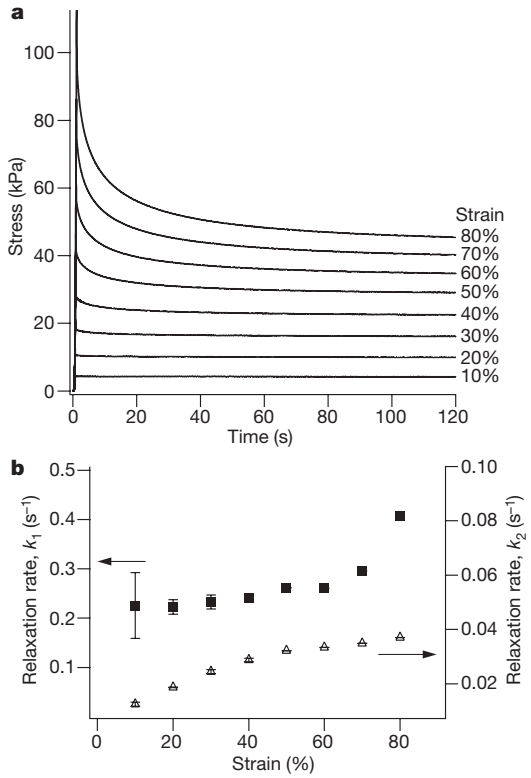

Figure 3 | GB1–resilin-based biomaterials exhibit pronounced stress relaxation behaviours. a, Representative stress-relaxation curves of GRG_5RG_4R at varying strains. **b**, Relaxation rates of GRG_5RG_4R-based biomaterials depend upon the initial stress. The relaxation rates were obtained by fitting the stress-relaxation to a double-exponential equation: $\sigma(t) = \sigma_0 + A_1\exp(-k_1t) + A_2\exp(-k_2t)$, where $\sigma(t)$ is the stress at time t, σ_0 is the offset, A_1 and A_2 are decay amplitudes and k_1 (filled squares) and k_2 (open triangles) are relaxation rates. Error bars indicate fitting errors.

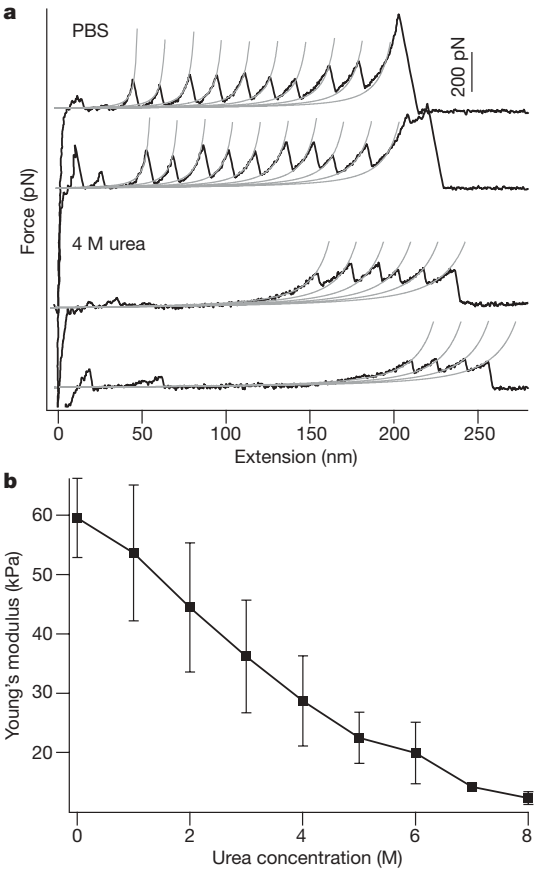

Figure 4 | The macroscopic mechanical properties of GB1–resilin-based biomaterials can be fine-tuned by controlling the nanomechanical properties of the constituting elastomeric proteins at the single-molecule level. a, Force–extension curves of single GRG_5RG_4R molecules in PBS and in 4 M urea. The long featureless spacers observed in force-extension curves of GRG_5RG_4R in 4 M urea largely correspond to the stretching of mechanically labile, unfolded GB1 domains. The unfolding force of GB1 domains that remain folded in 4 M urea is also significantly reduced. Grey lines are WLC fits. **b**, Young's modulus of GRG_5RG_4R-based biomaterial can be modulated by chemical denaturant urea. The conversion of folded GB1 domains into unfolded sequence leads to the dramatic decrease in Young's modulus of the biomaterials in a urea-concentration-dependent manner. Error bars indicate standard deviation of the data.

Folded globular domains are mechanically more resistant than their unfolded conformations, but less extensible. Because chemical denaturants can affect folded states of globular proteins, we used urea to modulate the nanomechanical properties of GB1–resilin-based elastomeric proteins. Figure 4a shows such an example for GRG_5RG_4R. In the presence of 4 M urea, about half of GB1 domains are unfolded, resulting in the loss of their mechanical resistance. Such a change is clearly evident in the force–extension relationships of GRG_5RG_4R (Fig. 4a), which are characterized by long featureless spacers before the unfolding events of the remaining folded GB1 domains. Such long featureless spacers correspond to the stretching of predominantly unfolded GB1 domains. The conversion of folded GB1 into unfolded sequences leads to a dramatic decrease in Young's modulus of the biomaterials in a urea-concentration-dependent fashion: the Young's modulus reduced from ~60 kPa in PBS to ~10 kPa in 8 M urea. We note that this change is fully reversible at both molecular and macroscopic levels. Replacing urea with PBS allowed GB1 domains to refold and thereby regain their mechanical resistance. Macroscopically, the biomaterial can recover its original Young's modulus when replacing urea with PBS. This macroscopic change in mechanical properties of biomaterials can readily be explained with information from the single-molecule level: the conversion of folded GB1 domains into mechanically labile and more extensible sequences effectively increased the length between cross-linking points, leading to the decrease in Young's modulus of the material. Similarly, it is also possible to modulate the mechanical properties of these biomaterials (Supplementary Fig. 8) in other ways, such as adjusting the relative GB1/resilin content, just as the passive elastic properties of different muscles are mediated by different isoforms of titin.

To fulfill their biological functions, different biological tissues possess distinct mechanical properties. For example, mammalian tendon is highly resilient (resilience >90%) but relatively inextensible (breaking strain of ~13%), whereas elastin is resilient (90%) and extensible (breaking strain of ~150%) but lacks toughness[18]. Mimicking the biomechanical properties of different tissues has been an important challenge in biomaterials research. Here we have designed a muscle-mimetic biomaterial, which is highly resilient at low strains, but also extensible and tough at high strain, to mimic the passive elastic properties of muscles. Titin is largely responsible for the passive elastic properties of myofibrils. A hallmark of titin-like elastomeric proteins is their ability to unfold under a stretching force to dissipate energy effectively and prevent damage to tissues by overstretching[6,8,10,11,16]. The hysteresis and stress-relaxation observed in stretching of myofibrils have been explained by force-induced unfolding of a small number of immunoglobulin domains[16].

All these properties have been well reproduced in biomaterials constructed from GB1–resilin-based artificial elastomeric proteins. Therefore, GB1–resilin-based polyproteins mimic the architecture and mechanical properties of titin at the single-molecule level, and biomaterials based on GB1–resilin polyproteins mimic the titin-mediated passive elastic properties of muscles (Supplementary

Information). These designed biomaterials represent a new type of muscle-mimic, which is fully hydrated and biodegradable, and we anticipate that they will find applications in material sciences as well as in tissue engineering by serving as scaffold and matrix for artificial muscles. Moreover, our results indicate that nanomechanical properties engineered into individual polyproteins can be translated into macroscopic properties in materials, a new example of obtaining novel macroscopic mechanical features by designing in such features at the single-molecule level. This method represents a new avenue towards tailoring the macroscopic mechanical properties of biomaterials and can be applied to the design of a wide range of materials.

METHODS SUMMARY

Preparation of GB1–resilin-based polyproteins were performed using previously published protocols[12,21]. We used the 15-amino-acid consensus resilin repetitive sequence (GGRPSDSYGAPGGGN) from the first exon of the *Drosophila melanogaster* CG15920 gene to construct GB1–resilin-based elastomeric proteins[13]. Single-molecule AFM experiments were performed on a custom-designed atomic force microscope as described[12]. Hydrogel-like biomaterials of GB1–resilin was constructed using a photochemical crosslinking strategy as described[13,23]. Tensile tests were performed on an Instron-5500R tensometer with a custom-made force gauge in PBS at constant temperature (22 °C). For technical reasons, ring-shaped biomaterial specimens were used[30] (Supplementary Information). Resilience was calculated from the ratio of the area under the relaxation curve to the area under the extension curve at a given strain using custom-written software in Matlab. The local slope at 15% strain on the extension curve was taken as the modulus at 15% reported in the paper.

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1. Maruyama, K. Connectin/titin, giant elastic protein of muscle. *FASEB J.* **11,** 341–345 (1997).
2. Wang, K. Titin/connectin and nebulin: giant protein rulers of muscle structure and function. *Adv. Biophys.* **33,** 123–134 (1996).
3. Tskhovrebova, L. & Trinick, J. Titin: properties and family relationships. *Nature Rev. Mol. Cell Biol.* **4,** 679–689 (2003).
4. Granzier, H. L. & Irving, T. C. Passive tension in cardiac muscle: contribution of collagen, titin, microtubules, and intermediate filaments. *Biophys. J.* **68,** 1027–1044 (1995).
5. Labeit, S. & Kolmerer, B. Titins: giant proteins in charge of muscle ultrastructure and elasticity. *Science* **270,** 293–296 (1995).
6. Rief, M., Gautel, M., Oesterhelt, F., Fernandez, J. M. & Gaub, H. E. Reversible unfolding of individual titin immunoglobulin domains by AFM. *Science* **276,** 1109–1112 (1997).
7. Linke, W. A. *et al.* I-band titin in cardiac muscle is a three-element molecular spring and is critical for maintaining thin filament structure. *J. Cell Biol.* **146,** 631–644 (1999).
8. Li, H. *et al.* Reverse engineering of the giant muscle protein titin. *Nature* **418,** 998–1002 (2002).
9. Watanabe, K. *et al.* Molecular mechanics of cardiac titin's PEVK and N2B spring elements. *J. Biol. Chem.* **277,** 11549–11558 (2002).
10. Kellermayer, M. S., Smith, S. B., Granzier, H. L. & Bustamante, C. Folding-unfolding transitions in single titin molecules characterized with laser tweezers. *Science* **276,** 1112–1116 (1997).
11. Tskhovrebova, L., Trinick, J., Sleep, J. A. & Simmons, R. M. Elasticity and unfolding of single molecules of the giant muscle protein titin. *Nature* **387,** 308–312 (1997).
12. Cao, Y. & Li, H. Polyprotein of GB1 is an ideal artificial elastomeric protein. *Nature Mater.* **6,** 109–114 (2007).
13. Elvin, C. M. *et al.* Synthesis and properties of crosslinked recombinant pro-resilin. *Nature* **437,** 999–1002 (2005).
14. Linke, W. A., Popov, V. I. & Pollack, G. H. Passive and active tension in single cardiac myofibrils. *Biophys. J.* **67,** 782–792 (1994).
15. Langer, R. & Tirrell, D. A. Designing materials for biology and medicine. *Nature* **428,** 487–492 (2004).
16. Minajeva, A., Kulke, M., Fernandez, J. M. & Linke, W. A. Unfolding of titin domains explains the viscoelastic behavior of skeletal myofibrils. *Biophys. J.* **80,** 1442–1451 (2001).
17. Higuchi, H. Viscoelasticity and function of connectin/titin filaments in skinned muscle fibers. *Adv. Biophys.* **33,** 159–171 (1996).
18. Gosline, J. *et al.* Elastic proteins: biological roles and mechanical properties. *Phil. Trans. R. Soc. Lond. B* **357,** 121–132 (2002).
19. Andersen, S. O. The cross-links in resilin identified as dityrosine and trityrosine. *Biochim. Biophys. Acta* **93,** 213–215 (1964).
20. Nairn, K. M. *et al.* A synthetic resilin is largely unstructured. *Biophys. J.* **95,** 3358–3365 (2008).
21. Carrion-Vazquez, M. *et al.* Mechanical and chemical unfolding of a single protein: a comparison. *Proc. Natl Acad. Sci. USA* **96,** 3694–3699 (1999).
22. Li, H. *et al.* Multiple conformations of PEVK proteins detected by single-molecule techniques. *Proc. Natl Acad. Sci. USA* **98,** 10682–10686 (2001).
23. Fancy, D. A. & Kodadek, T. Chemistry for the analysis of protein-protein interactions: rapid and efficient cross-linking triggered by long wavelength light. *Proc. Natl Acad. Sci. USA* **96,** 6020–6024 (1999).
24. Urry, D. W. *et al.* Elastin: a representative ideal protein elastomer. *Phil. Trans. R. Soc. Lond. B* **357,** 169–184 (2002).
25. Vrhovski, B., Jensen, S. & Weiss, A. S. Coacervation characteristics of recombinant human tropoelastin. *Eur. J. Biochem.* **250,** 92–98 (1997).
26. Bellingham, C. M. *et al.* Recombinant human elastin polypeptides self-assemble into biomaterials with elastin-like properties. *Biopolymers* **70,** 445–455 (2003).
27. Welsh, E. R. & Tirrell, D. A. Engineering the extracellular matrix: a novel approach to polymeric biomaterials. I. Control of the physical properties of artificial protein matrices designed to support adhesion of vascular endothelial cells. *Biomacromolecules* **1,** 23–30 (2000).
28. Nagapudi, K. *et al.* Protein-based thermoplastic elastomers. *Macromolecules* **38,** 345–354 (2005).
29. Bochicchio, B. *et al.* Synthesis of and structural studies on repeating sequences of abductin. *Macromol. Biosci.* **5,** 502–511 (2005).
30. Lillie, M. A., Chalmers, G. W. & Gosline, J. M. The effects of heating on the mechanical properties of arterial elastin. *Connect. Tissue Res.* **31,** 23–35 (1994).
31. Dudek, D. M. *et al.* Dynamic Mechanical Properties of Synthetic Resilin. in *Annual Meeting of the Society for Integrative and Comparative Biology* abstr. S7.7, e50 <http://icb.oxfordjournals.org/cgi/reprint/49/suppl_1/e1> (Oxford University Press, 2009).
32. Helmes, M. *et al.* Mechanically driven contour-length adjustment in rat cardiac titin's unique N2B sequence: titin is an adjustable spring. *Circ. Res.* **84,** 1339–1352 (1999).
33. Amin, A. F. M. S., Lion, A., Sekita, S. & Okui, Y. Nonlinear dependence of viscosity in modeling the rate-dependent response of natural and high damping rubbers in compression and shear: experimental identification and numerical verification. *Int. J. Plast.* **22,** 1610–1657 (2006).

Supplementary Information is linked to the online version of the paper at www.nature.com/nature.

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