Supplementary information for

Conformation-driven strategy for resilient and functional protein materials

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This PDF file includes:

- Supplementary Text
- Figs. S1 to S15
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- Caption for Movie S1
- References (53-94)

Other Supplementary Materials for this manuscript include the following:

Movie S1
Dynamic photo-crosslinking

The dynamic photo-crosslinking is associated with both exposure time and exposure length; the latter is the distance between a light source and the top surface of the precursor solution (Fig. S4). We found that illuminance intensity drastically increased from 28990 lux to 242800 lux when exposure length decreased from 15 cm to 2.5 cm. To maintain consistent and evenly distributed light exposure, we chose 10 cm throughout this work. Yet, shorter exposure length most likely leads to faster crosslinking. We then studied the time required for photo-crosslinking silk fibroin hydrogels, using three characterizations, including fluorescence spectra (Fig. 2A, D, S4), rheology (Fig. S5), and water content (Fig. S6).

Dityrosine crosslinks exhibit characteristic fluorescence primarily in the emission range from 280 nm to 290 nm and in the excitation range from 360 nm to 400 nm (53, 54), distinct from the autofluorescence of Ru(II) and silk fibroin (Fig. S4). The relative fluorescence intensity of dityrosine crosslinks gradually increased as a function of crosslinking time and approximated a plateau at around 180 s (Fig. 2A, D).

Photo-crosslinking is also reflected in viscoelastic properties that are characterized by oscillatory rheological tests (Fig. S5). We first performed an oscillatory strain sweep to determine the linear viscoelastic region (LVR) of strains that roughly ranges from 0.2% to 6%. We chose 3% strain in the following tests. We used an oscillatory time sweep to characterize the stability of the precursor silk fibroin solution in the dark; over 300 s, loss modulus (G") and storage modulus (G') exhibited a slight increase from 27.6 kPa to 34.5 kPa and from 16.2 kPa to 21.2 kPa, respectively. The slight increase may be ascribed to solvent evaporation and exposure to environmental light. In the next oscillatory time sweep, we turned on the light at around 50 s. The light exposure led to the immediate increase of G', G" and damping factor, tan(δ), which is the ratio of G' to G". In particular, tan(δ) reached an almost equilibrium at 180 s after the light exposure. We also tan(δ) to estimate the resilience of the in situ crosslinked silk fibroin hydrogel that turns out to be around 95%, largely consistent with the results obtained by uniaxial tensile tests (98.2% at 0.1 mm/mm) and DMA (95% at 1 Hz).

Water content of silk fibroin hydrogels was inversely proportional to crosslinking times (Fig. S6). From 30 s to 180 s, the water content decreased from 92.6 ± 0.5% to 84.6 ± 0.6%. It is because longer crosslinking time leads to higher crosslinking density, thus restricting swelling. Water content also became almost unchanged after 180 s crosslinking time, consistent with the two above characterizations. For 120 s crosslinking time, swollen hydrogels exhibited a water content of 85.6 ± 0.7%, compared with 75% of as-prepared hydrogels (25 wt%). Although 180 s crosslinking time may lead to a higher crosslinking density, we used 120 s throughout the manuscript to be consistent.

Biomedical potential

We investigated cell encapsulation and in vitro degradation of photo-crosslinked silk fibroin hydrogels to imply the potential utility in biomedical applications (Fig. S7). Mouse embryonic fibroblasts 10T1/2s were encapsulated in the photo-crosslinked hydrogels and cultured for 7 days. The cells demonstrated good cell viability (87.6 ± 3.5% at day 1 and 81.2 ± 9.4% at day 7) and largely maintained the metabolic activity
around 80% of that of the cells cultured on tissue culture plates (TCP). Also, confocal fluorescence images showed the development of the cytoskeleton (F-actin) of 10T1/2s within hydrogels. On day 1, the cytoskeleton resembled a sphere and exhibited blurred F-actin filaments; on day 7, the cytoskeleton became spreading in a polygon shape with anchor points, and the F-actin filaments became more clear and recognizable. These results supported the cytocompatibility of the photo-crosslinking approach and silk fibroin materials.

Besides, the enzymatic degradation of the swollen silk fibroin hydrogel, one of critical merit of protein-based materials, was demonstrated using a non-specific protease, Pronase E (Fig. S7). A cylindrical yellowish swollen hydrogel was degraded into scattered white debris after incubating in 0.1 U/ml Pronase E at 37°C overnight. The short time of degradation may be associated with the random coils-dominated conformation and non-specific activity of Pronase E.

**Conformational characterizations of rigid hydrogels**

β-sheets-dominated rigid hydrogels were obtained by treating random coils-dominated swollen hydrogels with 90 v/v% methanol (Fig. S2). Due to the different predominant conformations, rigid hydrogels exhibited drastically different Raman and FTIR spectral features from swollen hydrogels. The rigid hydrogels exhibit the Raman amide I band at 1663 cm⁻¹, the amide II band at 1231 cm⁻¹, the backbone stretching at 1083 cm⁻¹, in contrast to that of swollen hydrogels at 1667 cm⁻¹, 1251 cm⁻¹, and 1104 cm⁻¹ (Fig. 2B). These band shifts were larger than the 1 cm⁻¹ nominal resolution of Raman spectra and supported the conformational change from random coils to β-sheets. Also, the rigid hydrogel exhibited the disappearance of the broad peak at 942 cm⁻¹ indicated the deviation from a random coil conformation; the peak that emerged at 882 cm⁻¹ indicated the formation of β-sheets (55). The FWHM of the Raman amide I narrowed from 58.9 ± 0.5 cm⁻¹ to 26.9 ± 0.1 cm⁻¹, suggesting the molecular conformations becoming less flexible (56). The Raman Try ratio (I₈₅₀/I₈₃₀) decreased from 3.3 ± 0.2 to 2.0 ± 0, indicating the increased conformational restriction of tyrosine phenol rings (57). The different FWHM and the Try ratio of the rigid hydrogel, compared to solutions and swollen hydrogels, also supported the β-sheets-dominated conformation (Fig. 2E, F).

FTIR spectral results were consistent with the Raman ones. The rigid hydrogels exhibit the FTIR amide I band at 1621 cm⁻¹, a major shift from 1645 cm⁻¹ of swollen hydrogels, suggesting a major conformational change from random coils to β-sheets (Fig. 2C) (58). According to deconvoluted results of FTIR amide I, rigid hydrogels exhibited a notable increase of β-sheets content from 2 ± 1% to 59 ± 2% and a decrease of random coils from 77 ± 7% to 19 ± 1% (Fig. 2G). β-turn is a precursor of β-sheets and slightly increases from 21 ± 6% to 29 ± 0% (Fig. 2G). Raman and FTIR spectral results collectively confirmed the β-sheet-dominated conformation of rigid hydrogels.

**Comparisons of mechanical properties between silk fibroin hydrogels**

The mechanical difference of swollen hydrogels from as-prepared ones is largely due to the swelling of the molecular network and the straightened molecular chains (59). Swelling strengthens the molecular chains, thus reducing chain flexibility and thus
probably leading to reduced resilience (Fig. 3C-E). Swelling also decreases Young’s modulus from 98.5 ± 0.5 kPa to 68.1 ± 1.1 kPa, and the extensibility from around 1 mm/mm to 0.6 mm/mm. We also estimated molecular weight between crosslinks (M<sub>c</sub>) using the fitting results of the classical rubber theory, which gives rise to 26.4 ± 0.5 kDa and 29.8 ± 0.5 kDa for as-prepared and swollen hydrogels, respectively. The slightly increased M<sub>c</sub> supports the swelling molecular network.

The mechanical difference between rigid and swollen hydrogels is largely due to the conformational transition from random coils to β-sheets. The β-sheets are characterized by tightly stacked polypeptide chains via hydrogen bonds (Fig. S2), and have been known to underlie the superior strength of silk materials (60) and to enhance mechanical performance by forming a double-network (61). In contrast to random coils exhibiting highly flexible molecular chains, the β-sheets exhibited low conformational flexibility and thus decreased elastic resilience (Fig. 3D-E, S9-11). In addition to resilience, β-sheets-dominance also reduced the extensibility of the rigid hydrogels from 1.1 ± 0.1 mm/mm to 0.2 ± 0.1 mm/mm, increased tensile strength from 72.4 ± 8.6 kPa to 380.0 ± 31.7 kPa, and increased Young’s modulus from 68.1 ± 1.1 kPa to 1.3 ± 0.3 MPa.

Regenerated silk fibroin, used in this work, exhibits a higher molecular weight (100 kDa) than several recombinant protein elastomers, such as An16 (18.9 kDa), RLP12 (27.5 kDa), MeTro (60 kDa), and Rec1-resilin (28.5 kDa) (Table S2). The higher molecular weight may improve ultimate tensile strength and extensibility (62). The as-prepared and swollen hydrogel exhibited an ultimate tensile strength of 72.4 ± 8.6 and 39.5 ± 0.6 kPa, respectively. The former is higher than An16 (17.1 ± 5.3 kPa), Rec1-resilin (55 ± 10 kPa), and MeTro (52.6 ± 5.2 kPa) (Table S2). The extensibility of as-prepared and swollen hydrogel is 1.1 ± 0.1 mm/mm and 0.7 ± 0.1 mm/mm, respectively, in comparison to RLP12 (0.3 mm/mm) and EP20-24-24 (0.8 mm/mm) (Table S2).

**Directional methanol treatment**

We used two baths of water and 90 v/v% methanol solution, respectively, to transform random coils into β-sheets spatially (Fig. S12). The two baths with distinct compositions led to directional transport of methanol from one end to the other and probably a methanol gradient within the graded hydrogels. The opacity of hydrogels indicates the formation of β-sheets, which increased as a function of the treatment time.
Fig. S1. Experimental measurement of elastic resilience. a) Resilience measured from cyclic strain-stress curves using equation of Resilience (%) = U/(H+U) × 100. Area between a pair of loading-unloading curves (labeled H) indicates mechanical hysteresis or energy dissipation; the area below unloading curve (labeled U) indicates the release of the stored elastic energy; the area below loading curve (H+U) indicates the energy required for the deformation. b) Resilience estimated from damping factor, tan(δ), which is the ratio of loss (E″, G″) to storage (E′, G′) moduli.
Fig. S2. a) Schematics of swollen and rigid hydrogels fabricated from as-prepared hydrogels. Swollen hydrogels are obtained by swelling as-prepared hydrogels in 0.9 w/v% sodium chloride saline for three days, and exhibit random coils-dominated conformation; rigid hydrogels are obtained by treating swollen hydrogels with 90 v/v% methanol for one hour, exhibiting predominantly β-sheets. b) Optical and fluorescent images of swollen and rigid hydrogels. Both hydrogels exhibit characteristic blue fluorescence (excitation at 300 nm) of dityrosine crosslinks. Rigid hydrogels become translucent due to β-sheets-induced microfibrils that scatter incident light.
Fig. S3. Polarized optical images of recoiled and stretched silk fibroin hydrogels. Recoiled or relaxed hydrogels exhibit a magenta background; stretched hydrogels (at 0.3 mm/mm strain) exhibit increased birefringence, approaching $4 \times 10^{-4}$. Increased birefringence is associated with straightened molecular chains and enhanced molecular alignments, thus implying reduced conformational entropy ($\Delta S < 0$). Orange dash lines indicate hydrogel boundary. Double arrows indicate stretching direction. A, P, and S indicate analyzer, polarizer, and the slow axis of the first-order red plate, respectively.
Fig. S4. a) Illuminance (lux) measured as a function of distance from light source to top surface of precursor solutions. b) Fluorescence excitation-emission matrix spectra with 0 s exposure time in the first and 120 s in the second row. Four samples include 1) Ru(II), 2) silk fibroin solution, 3) the mixtures of Ru(II) and silk fibroin, and 4) the mixtures of Ru(II), silk fibroin, and sodium persulfate (SPS). Autofluorescence of silk fibroin and Ru(II) can be distinguished from characteristic fluorescence of dityrosine crosslinks, highlighted by a white dash box.
Fig. S5. Rheological characterization for photo-crosslinking. a) Oscillatory strain sweep determines linear viscoelastic region (LVR) of photo-crosslinking precursor solutions at 1 Hz in the dark. Loss and storage moduli are $G''$ and $G'$, respectively. b) Oscillatory time sweep indicates stability of the solution at 1 Hz and 3% strain in the dark. c) Oscillatory time sweeping of the photo-crosslinking process. $G'$ becomes larger than $G''$ at around 80 s. d) $\tan(\delta)$, derived from Fig. S5c, and resilience estimated by equation (1). Arrows indicate occurrence of light exposure.
**Fig. S6. Swelling of silk fibroin hydrogels.** a) Water content of swollen silk fibroin hydrogels in 0.9 w/v% sodium chloride saline as a function of crosslinking times (30, 60, 90, 120, 180 s). Water content is calculated by the weight of swollen hydrogels before ($M_w$) and after ($M_d$) drying overnight in a 60°C oven. Water content of as-prepared hydrogels is 75%, identical to that of the photo-crosslinking precursor solution, where silk fibroin concentration is 25 wt%. b) Representative optical images of as-prepared, swollen, and oven-dried silk fibroin hydrogels in cylindrical shapes from side view.
Fig. S7. Cell encapsulation and in vitro degradation of photo-crosslinked silk fibroin hydrogels. 

a) Projected confocal fluorescent images of F-actin cytoskeletons of 10T1/2s cells within hydrogel over 7 days. Green and blue fluorescence indicate F-actin and silk fibroin, respectively. 

b) and c) Representative fluorescent images and cell viability at days 1 and 7, respectively, exhibiting non-statistical difference. 

d) Cell metabolic activity maintains around 80% of that of cells culture on tissue culture plates (TCP). 

e) In vitro degradation of a swollen silk fibroin hydrogel in cylindrical shapes by incubation in 0.1 U/ml Pronase E solution at 37°C overnight. Cylindrical swollen hydrogel is degraded into white debris. (n = 3 independent samples, mean ± s.d., n.s. P > 0.1, single-factor ANOVA)
Fig. S8. Raman characterizations of swollen hydrogels. a) Typical Raman spectra of swollen hydrogels, compared with silk fibroin solutions, as-prepared, and rigid hydrogels, suggesting random coils-dominated conformation. b) FWHM of Raman amide I band of as-prepared and swollen hydrogels have no statistical difference. c) Raman Tyr ratio \((I_{830}/I_{850})\) of swollen hydrogels slightly higher than as-prepared ones, probably due to swelled molecular network that leads to void space between molecules, favoring vibration of tyrosine phenol ring. (n = 3 independent spectra, \(P < 0.1\), n.s. \(P > 0.1\), single-factor ANOVA)
Fig. S9. Elastic resilience of silk fibroin hydrogels. a) Consecutive stretch-recoil cycles of swollen hydrogels with final strains from 0.1 mm/mm to 0.7 mm/mm, shifted along x-axis for clarity. Inset shows superimposed curves with recoverability. Red dash curves indicate fitting results with statistical rubber theory, roughly starting to deviate at 0.3 mm/mm strain. b) Consecutive stretch-recoil cycles of rigid hydrogels with final strains from 0.1 mm/mm to 0.3 mm/mm, shifted along x-axis for clarity. Inset shows superimposed curves with bare recoverability and an image of dogbone-shaped rigid hydrogel under testing. c-d) Cyclic stretch-recoil of as-prepared hydrogels at 0.5 or 0.7 mm/mm strain. Inset shows corresponding load force-time curves. At 0.5 mm/mm, resilience and tensile modulus remain largely stable as a function of cycle numbers. At 0.7 mm/mm strain, resilience decreases from 97.3 ± 1.3% to 93.5 ± 1.2%, and tensile modulus increases from 87.1 kPa to 105.8 kPa. Decreased resilience and increased modulus at 0.7 mm/mm strain may be associated with irreversible formations of molecular interactions during cyclic stretching that elongates chains and exposes molecular surface, which needs further experimental examinations.
Fig. S10. Viscoelastic properties of silk fibroin hydrogels by dynamic mechanic tests. **a-c** Typical storage ($E'$) and loss ($E''$) moduli and damping factor, $\tan(\delta)$, tested as a function of frequency. **d** Summary of $\tan(\delta)$ of the three hydrogels for estimating elastic resilience by using equation (1). The range of curves is determined by the instrument.
Fig. S11. Correlation between resilience and conformations. a) Typical cyclic tensile curves of as-prepared, swollen, and rigid hydrogels at 0.1 mm/mm strain. Both as-prepared and swollen hydrogels exhibit exceptional resilience, in contrast to drastically reduced resilience of rigid hydrogels. Inset highlights the curves of as-prepared and swollen hydrogels. b) Typical optical images of swollen hydrogels treated with 90 v/v% methanol for 0, 1, 2, or 3 minutes. Decreased transparency of hydrogels is due to the increased formation of β-sheets that form microfibrils to scatter light. c) and d) Cyclic tensile tests at 0.1 mm/mm strain of swollen hydrogels treated with 90 v/v% methanol for 0, 1, 2, or 3 minutes. Resilience and modulus summarized as a function of methanol treatment time. e) and f) Raman characterization of methanol-treated swollen hydrogels. FWHM of the Raman amide I and Raman Tyr ratio (I850/I830) are linearly correlated (R²=0.99), indicating consistent conformational characterization by two Raman features.
Fig. S12. Directional methanol treatment for graded protein materials. a) Schematic of experimental setup for directional treatment of swollen hydrogels with 90 v/v% methanol. Methanol flow from the methanol bath on the left, through swollen hydrogel, and to the water bath on the right, indicated by white arrows. b) and c) Side and top views of experimental setup. Inset indicates swollen hydrogels placed on the unwrapped tissue paper. d) and e) Typical images and dynamic curves of hydrogel opacity as a function of treatment time. Area in white dash box in d) is used for analysis of transparency.
**Fig. S13. Raman mapping of graded hydrogels.**

a) A distance of around 2.5 mm in the central transition zone of graded hydrogels is characterized by Raman mapping. b) Spectra of Raman mapping, compared to resilient and rigid ends of graded hydrogels. Amide I band and tyrosine doublet (Tyr) are labeled.
**Fig. S14. Mechanical properties of local regions of graded protein hydrogels.**

**a)** Schematics of punched hydrogel samples from three local regions of graded hydrogels, including resilient end, rigid end, and middle transition zone. Dash circles in 6 mm diameter indicate punching positions. **b)** and **c)** Resilience and modulus at three local regions of graded hydrogels measured by cyclic compression tests at 0.3 mm/mm strain. (n = 3 independent measurements, ****P < 0.0001, ***P < 0.001, single-factor ANOVA followed by Scheffe’s post-hoc test)
Fig. S15. Finite element analysis-based simulations of swollen and graded hydrogels. a) Model geometry, material composition, and von Mises stress of simulated graded hydrogels before (first row) and after (second row) 180° rotation. Rigid regions with a higher Young’s modulus are labeled in red. b) Simulation results of swollen hydrogels for comparison.
Table S1. Sequence motif and glycine/proline content.

| Proteins | Amino-acid sequence repeat motif \(^a\) | Glycine content \(^b\) | Proline content \(^b\) |
|----------|----------------------------------------|-----------------------|------------------------|
| Resilin  | Exon-1 GGRPSDSYGAPGGGN                | 39-42 mol%            | 7-12 mol%              |
|          | Exon-3 GYSGGRPGQDLG                   |                       |                        |
| Elastin  | Elastic domain VPGVG PGVGVA           | 33 mol%               | ~12 mol%               |
|          | Crosslinking domain GPGKPLKPV AAAAKAAKA |                      |                        |
| Abductin | GGFGGMGGGX                            | 58 mol%               | <0.1 mol%              |
| Major ampullate silk (Dragline) | GGYGPGS GPGGY | ~40 mol% | 0-17 mol% |
| Flagelliform silk (Viscid) | GPGGX | ~55 mol% | 14-17 mol% |
| Byssal thread | PreCol-D GPGGG | 35 mol% | ~7 mol% |
|          | PreCol-P GAGPG                         |                       |                        |
| Bombyx mori Silk fibroin (Heavy chain) | Hydrophobic domain GAGAGS GAGAGY GAAS | 45.9 mol% | 0.3 mol% |
|          | Hydrophilic domain SGFGPYVANGGYSGYEY-AWSSESDF |                      |                        |

PreCol, pre-pepsinized collagen. \(^a\) Bold letters indicate possible crosslinking sites; \(^b\) Refs, (63-65).
Table S2. Tensile properties of common elastomeric proteins and recombinant hydrogels.

| Proteins and recombinant polypeptides | Molecular weight (kDa) | Concentration (mg/ml) | Crosslinking mechanism | Sample geometry | Ultimate tensile strength (kPa) | Tensile resilience (%) | Refs |
|--------------------------------------|------------------------|-----------------------|------------------------|-----------------|-------------------------------|------------------------|------|
| Resilin                              |                        |                       |                        |                 |                               |                        |      |
| Dragonfly tendon                     | 58.6                   | 400-500 \(^c\)       | Tyrosine/Enzyme        | NA              | 4 (MPa)                       | 92                     | (66-68) |
| Recl1-resilin                        | 28.5                   | 100-300               | Tyrosine/Ru(II)/persulfate | Strip          | 55 ± 10                        | 92-97                  | (69) |
| An16                                 | 18.9                   | 200                   | Lysine/THPP            | Dogbone (film)  | 17.1 ± 5.3                     | 94 ± 3 (\(\varepsilon = 1\)) | (70, 71) |
| RLP12                                | 27.5                   | 200, 250              |                        |                 |                               | 97.7 ± 1 (\(\varepsilon = 0.3\)) | (72, 73) |
| Collagen                             |                        |                       |                        |                 |                               |                        |      |
| Mammalian tendon                     |                        |                       |                        |                 |                               |                        |      |
| Bovine ligament                      | 72                     | 400-500 \(^c\)       | Lysine/Desmosine       | NA              | 2 (MPa)                       | 90                     | (66) |
| MeTro                                | 60                     | 100                   | Methacryloyl/Irgacure 2959 | Cylinder       | 21.7 ± 5.5                     | 73.4 ± 1.8 (\(\varepsilon = 0.6\)) | (74, 75) |
| Elastin                              |                        |                       |                        |                 |                               |                        |      |
| EP30P-30P-30P                        | 11.9                   |                       |                        |                 |                               | 48                     |      |
| EP20-24-60                           | 16.9                   | 10                    | Lysine/Coacervation/Genipin | NA          |                               | 78                     | (76) |
| EP20-24-60P                          | 17.1                   | 2.3                   | Lysine/Coacervation/PQQ | NA             |                               | 81                     |      |
| EP20-(24)\(_i\)                      | 31                     | 3.1                   |                           |                 |                               | 77 ± 10                |      |
| Abductin                             |                        |                       |                        |                 |                               |                        |      |
| Aequipecten                          | 11.3                   |                       |                        | NA              |                               | 96.15 ± 0.25           | (78, 79) |
| Ensis                                |                        |                       |                        | NA              |                               | 81.81 ± 2.15           |      |
| Mussel Byssus                        |                        |                       |                        |                 |                               |                        |      |
| Bathymodiolus thermophilus           | 95 (PreCol-P)          |                       | Metal complexion       | NA              |                               | 26.2 ± 14.1 (MPa)      | 39.4 ± 1.9 |
| Guekenia demissa                     |                        |                       |                        |                 |                               | 20.5 ± 10.4 (MPa)      | 37.7 ± 4.7 |
| Dreissaena polymorpha                |                        |                       |                        |                 |                               | 48.4 ± 26.0 (MPa)      | 24.8 ± 3.5 |
| A. diadematus dragline silk          | 270-320 (Nephila)      | >250 \(^c\)          | Physical Fiber         |                 | 1.1 (GPa)                     | 35                     |      |
| A. diadematus viscid silk            | 360 (Nephila)          | >250 \(^c\)          |                           |                 |                               | 35                     | (66, 82-84) |
| Bombyx mori Silk fibroin             | 390 (Fibroin, heavy chain) | >250 \(^c\)   |                           |                 |                               | 500 (MPa)              | 35    |
| Silk                                 |                        |                       |                        |                 |                               |                        |      |
| Regenerated silk fibroin             |                        |                       |                        |                 |                               |                        |      |
| (As-prepared)                        |                        |                       |                        |                 |                               |                        |      |
| Regenerated silk fibroin             |                        |                       |                        |                 |                               |                        |      |
| (Swollen)                            | ca. 100 (SDS)          | 250 \(^c\)          | Ru(II)/persulfate       | Dogbone         | 39.5 ± 0.6                     | 97.3 ± 1.3 (\(\varepsilon = 0.1\)) | 93.5 ± 1.2 (\(\varepsilon = 0.6\)) |
| Regenerated silk fibroin             |                        |                       |                        |                 |                               |                        |      |
| (Rigid)                              |                        |                       | Ru(II)/persulfate + Methanol |                | 379.5 ± 31.7                   | 86.2 ± 0.9 (\(\varepsilon = 0.1\)) | 70.8 ± 4.4 (\(\varepsilon = 0.2\)) |

\(^a\) Italics indicate native proteins; \(^b\) obtained from cyclic compression tests; \(^c\) measured by drying weight. Abbreviations: \(\varepsilon\), strain (nm/mm); RLP, resilin-like peptides; EP, elastin polypeptides; Ru(II), Tris(2,2'-bipyridyl) ruthenium; THPP, [tris(hydroxymethyl) phosphino] propionic acid; MeTro, methacryloyl-substituted tropoelastin; PQQ, pyrroloquinoline quinone; SDS, sodium dodecyl sulfate polyacrylamide gel electrophoresis; NA, not available. Values are either expressed as mean ± s.d. or as noted in the source.
| Material formats | Concentration (mg/ml) | Crosslinking approach | Tensile test | Resilience (%) | Refs  |
|-----------------|---------------------|-----------------------|--------------|----------------|-------|
| Sponge          | < 40                | Lyophilization        | Yes          | NA             | (85)  |
|                 | 30                  | Lyophilization        | No           | 71.7 ± 1.3 (ε = 0.2) abc | (86)  |
|                 | 50                  | HRP                   | No           | 84.5 (ε = 0.4) ab 63.8 (ε = 0.7) ab | (87)  |
|                 | 10-50               | Riboflavin            | No           | 94.6 ± 0.2 (ε = 0.8) ab | (88)  |
|                 | NA                  | Ru/persulfate & Methanol | No           | 62.8 (ε = 0.2) ab | (89)  |
|                 | 40                  | HRP                   | No           | 78.3 (ε = 0.32) ab | (90)  |
| Hydrogel        | 150                 | Binary solvent        | Yes          | around 40      | (91)  |
|                 | 300                 | Ru/persulfate         | No           | 75.3 (tan(δ)=0.18) d | (53)  |
|                 | 20                  | HRP                   | No           | 94 (ε = 0.3) ae 91 (ε = 0.3, 20% HA) ae | (92)  |
|                 | 250                 | Ru/persulfate         | Yes          | 97.3 ± 1.3 (ε = 0.1) 93.5 ± 1.2 (ε = 0.6) 86.2 ± 0.9 (ε = 0.1) 70.8 ± 4.4 (ε = 0.2) | As-prepared, Swollen, Rigid | This work |

a Compression test; b measured from loading-unloading curves; c treated with methanol and glycerol; d estimated from the damping factor (tan(δ)) in dynamic mechanical tests, according to ref. (63); e measured from published column charts. ε, strain (mm/mm); HRP, horseradish peroxidase; HA, hyaluronic acid; NA, not available.
| Materials                  | Protein components | Gradient                  | Mechanism      | Young’s modulus            | Resilience                      | Refs  |
|----------------------------|--------------------|---------------------------|----------------|-----------------------------|---------------------------------|-------|
| Byssal thread              | PreCol-D & -P      | Composition               |                | 50 MPa to 500 MPa (from proximal to distal) \(^a\) | 53% to 28% (from proximal to distal) \(^a\) | (65)  |
| Squid beak                 | Histidine-rich protein | Composition               |                | 70 MPa to 2 GPa (from untanned to heavily tanned) \(^a\) | NA                              | (93)  |
| Tarsal setae of ladybird beetle | Resilin          | Composition               |                | 1.2 MPa to 6.8 GPa (from tip to base) \(^b\)          | 80% to 61% (from tip to base) \(^b\) | (94)  |
| Graded silk fibroin hydrogels | Silk fibroin       | Conformation              |                | 55.5 ± 1.5 kPa to 732.1 ± 19.5 kPa (from resilient to rigid) \(^c\) | 92.8 ± 3.4% to 53.6 ± 1.2% (from resilient to rigid) \(^c\) | This work |

\(^a\) Uniaxial tensile results; \(^b\) AFM nanoindentation curves; \(^c\) Unconfined compression results. PreCol, pre-pepsinized collagen; NA, not available.
Movie S1.
Uniaxial tensile tests of as-prepared and rigid silk fibroin hydrogels.
53. Whittaker JL, Choudhury NR, Dutta NK, & Zannettino A (2014) Facile and rapid ruthenium mediated photo-crosslinking of Bombyx mori silk fibroin. J. Mater. Chem. B 2(37):6259-6270.
54. Malencik DA, Sprouse JF, Swanson CA, & Anderson SR (1996) Dityrosine: preparation, isolation, and analysis. Anal. Biochem. 242(2):202-213.
55. Rousseau M-E, Lefevre T, Beaulieu L, Asakura T, & Pézolet M (2004) Study of protein conformation and orientation in silkworm and spider silk fibers using Raman microspectroscopy. Biomacromolecules 5(6):2247-2257.
56. Martel A, et al. (2008) Silk fiber assembly studied by synchrotron radiation SAXS/WAXS and Raman spectroscopy. J. Am. Chem. Soc. 130(50):17070-17074.
57. Hernández B, Coïc YM, Pflüger F, Kruglik SG, & Ghomi M (2016) All characteristic Raman markers of tyrosine and tyrosinate originate from phenol ring fundamental vibrations. J. Raman Spectrosc. 47(2):210-220.
58. Yang H, Yang S, Kong J, Dong A, & Yu S (2015) Obtaining information about protein secondary structures in aqueous solution using Fourier transform IR spectroscopy. Nat. Protoc. 10(3):382-396.
59. Kamata H, Akagi Y, Kayasuga-Kariya Y, Chung U-i, & Sakai T (2014) “Nonswellable” hydrogel without mechanical hysteresis. Science 343(6173):873-875.
60. Giesa T, Arslan M, Pugno NM, & Buehler MJ (2011) Nanoconfinement of Spider Silk Fibrils Begets Superior Strength, Extensibility, and Toughness. Nano Lett. 11(11):5038-5046.
61. Su D, et al. (2017) Enhancing mechanical properties of silk fibroin hydrogel through restricting the growth of β-sheet domains. ACS Appl. Mater. Interfaces 9(20):17489-17498.
62. Bowen CH, et al. (2018) Recombinant spidroins fully replicate primary mechanical properties of natural spider silk. Biomacromolecules 19(9):3853-3860.
63. Gosline JM (2018) The structural Origin of Elasticity and Strength. Mechanical design of structural materials in animals, (Princeton University Press), pp 59-73 and 290-313.
64. Rauscher S, Baud S, Miao M, Keeley FW, & Pomes R (2006) Proline and glycine control protein self-organization into elastomeric or amyloid fibrils. Structure 14(11):1667-1676.
65. Waite JH, Vaccaro E, Sun C, & Lucas JM (2002) Elastomeric gradients: a hedge against stress concentration in marine holdfasts? Philos. Trans. R. Soc., B 357(1418):143-153.
66. Gosline J, et al. (2002) Elastic proteins: biological roles and mechanical properties. Philos. Trans. R. Soc., B 357(1418):121-132.
67. Ardell DH & Andersen SO (2001) Tentative identification of a resilin gene in Drosophila melanogaster. Insect Biochem. Mol. Biol. 31(10):965-970.
68. Weis-Fogh T (1960) A rubber-like protein in insect cuticle. J. Exp. Biol. 37(4):889-907.
69. Elvin CM, et al. (2005) Synthesis and properties of crosslinked recombinant pro-resilin. Nature 437(7061):999-1002.
70. Lyons RE, et al. (2009) Comparisons of recombinant resilin-like proteins: repetitive domains are sufficient to confer resilin-like properties. Biomacromolecules 10(11):3009-3014.
71. Lyons RE, et al. (2007) Design and facile production of recombinant resilin-like polypeptides: gene construction and a rapid protein purification method. Protein Eng., Des. Sel. 20(1):25-32.
72. Li L, Teller S, Clifton RJ, Jia X, & Kiick KL (2011) Tunable mechanical stability and deformation response of a resilin-based elastomer. Biomacromolecules 12(6):2302-2310.
73. Charati MB, Ifkovits JL, Burdick JA, Linhardt JG, & Kiick KL (2009) Hydrophilic elastomeric biomaterials based on resilin-like polypeptides. Soft Matter 5(18):3412-3416.
74. Annabi N, et al. (2013) Engineered cell-laden human protein-based elastomer. Biomaterials 34(22):5496-5505.
75. Annabi N, et al. (2017) Engineering a highly elastic human protein–based sealant for surgical applications. Sci. Transl. Med. 9(410):eaai7466.
76. Muiznieks LD, Reichheld SE, Sitarz EE, Miao M, & Keeley FW (2015) Proline-poor hydrophobic domains modulate the assembly and material properties of polymeric elastin. Biopolymers 103(10):563-573.
77. Bellingham CM, et al. (2003) Recombinant human elastin polypeptides self-assemble into biomaterials with elastin-like properties. Biopolymers 70(4):445-455.
78. Kahler GA, Fisher Jr FM, & Sass RL (1976) The chemical composition and mechanical properties of the hinge ligament in bivalve molluscs. The Biological Bulletin 151(1):161-181.
79. Cao Q, Wang Y, & Bayley H (1997) Sequence of abductin, the molluscan ‘rubber’ protein. Curr. Biol. 7(11):R677-R678.
80. Brazee SL & Carrington E (2006) Interspecific comparison of the mechanical properties of mussel byssus. The Biological Bulletin 211(3):263-274.
81. Deming TJ (1999) Mussel byssus and biomolecular materials. Curr. Opin. Chem. Biol. 3(1):100-105.
82. Zhou CZ, et al. (2001) Silk fibroin: structural implications of a remarkable amino acid sequence. Proteins: Structure, Function, and Bioinformatics 44(2):119-122.
83. Omenetto FG & Kaplan DL (2010) New opportunities for an ancient material. Science 329(5991):528-531.
84. Vollrath F & Knight DP (2001) Liquid crystalline spinning of spider silk. Nature 410(6828):541.
85. Rnjak-Kovacina J, et al. (2015) Lyophilized Silk Sponges: A Versatile Biomaterial Platform for Soft Tissue Engineering. ACS Biomater. Sci. Eng. 1(4):260-270.
86. Brown JE, et al. (2017) Shape memory silk protein sponges for minimally invasive tissue regeneration. Adv. Healthcare Mater. 6(2):1600762.
87. Partlow BP, et al. (2014) Highly tunable elastomeric silk biomaterials. Adv. Funct. Mater. 24(29):4615-4624.
88. Kuang D, et al. (2019) Highly elastomeric photocurable silk hydrogels. Int. J. Biol. Macromol. 134:838-845.
89. Kim CS, Yang YJ, Bahn SY, & Cha HJ (2017) A bioinspired dual-crosslinked tough silk protein hydrogel as a protective biocatalytic matrix for carbon sequestration. NPG Asia Mat. 9(6):e391-e391.
90. Stoppel WL, et al. (2016) Elastic, silk-cardiac extracellular matrix hydrogels exhibit time-dependent stiffening that modulates cardiac fibroblast response. J Biomed Mater Res., Part A 104(12):3058-3072.
91. Zhu Z, et al. (2018) High-Strength, Durable All-Silk Fibroin Hydrogels with Versatile Processability toward Multifunctional Applications. *Adv. Funct. Mater.* 28(10):1704757.
92. Raia NR, et al. (2017) Enzymatically crosslinked silk-hyaluronic acid hydrogels. *Biomaterials* 131:58-67.
93. Miserez A, Schneberk T, Sun C, Zok FW, & Waite JH (2008) The transition from stiff to compliant materials in squid beaks. *Science* 319(5871):1816-1819.
94. Peisker H, Michels J, & Gorb SN (2013) Evidence for a material gradient in the adhesive tarsal setae of the ladybird beetle Coccinella septempunctata. *Nat. Commun.* 4(1):1661.