Supporting Information

Strain Stiffening in Dynamic Supramolecular Fiber Networks

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1. Materials

All solvents used were of reagent grade quality or better and purchased from Biosolve, Sigma-Aldrich or Actu-All Chemicals. DCM was dried using molecular sieves (3 Å) prior use. Diethylenetriaminepentaacetic dianhydride (DTPA) was obtained from Sigma-Aldrich (Benzotriazol-1-yloxy)tripyrrolidinophosphonium hexafluorophosphate (PyBOP) was obtained from Bachem, 1-Azido-4,7,10-trioxa-13-tridecanamine (TOTA-N_3) was obtained from Iris Biotech. The synthesis of DA, DA-AC and DA-N_3 has been performed according to literature procedures.[1,2]

2. General methods

The mechanical properties of the hydrogels were tested by oscillatory rheometry. Dynamic viscoelastic measurements were performed on a stress-controlled Anton Paar and a Physicia MCR 501 Discovery HR-3, TA Instruments rheometers equipped with a 25-mm stainless steel sand-blasted plate-plate geometry to prevent slippage of the sample in a temperature-controlled environment. Measurements were performed by placing mineral oil around the sample to minimize evaporation at a fixed plate-to-plate gap of 500 µm. The temperature was fixed to 25 °C. After addition of the catalyst, gelation was monitored under small-amplitude oscillatory strain (1 %) and an angular frequency of 6.28 rad s^{-1}. To probe the elasticity of the gels, a steady pre-stress, σ was applied on which an oscillatory stress, $\delta\sigma(t) = \delta\sigma e^{iwt}$ was superimposed with an amplitude of at most 10% of σ and an angular frequency of ω = 6.28 rad s^{-1}.

HDX-MS measurements were carried out using a Xevo G2 QTof mass spectrometer (Waters) with a capillary voltage of 2.7 kV and a cone voltage of 20 V. The source temperature was set at 100 °C, the desolvation temperature at 400 °C, and the gas flow at 500 L h^{-1}. The sample solutions subjected to HDX were introduced into the mass spectrometer using a Harvard syringe pump (11 Plus, Harvard Apparatus) at a flow rate of 50 µL min^{-1}.

^{1}H-NMR and ^{13}C-NMR spectra were recorded on a Varian Mercury Vx 400MHz (100MHz for ^{13}C), a Varian Mercury Plus 200MHz (50MHz for ^{13}C), or a Bruker 400MHz NMR spectrometer. Chemical shifts are given in ppm. (d) values relative to residual solvent or tetramethylsilane. Splitting patterns are labelled as s, singlet; d, doublet; dd, double doublet; t, triplet; q, quartet; quin, quintet; m, multiplet and b stands for broad.

MALDI-TOF-MS spectra were obtained using a PerSeptive Biosystems Voyager-DE PRO spectrometer with a-cyano-4-hydroxycinnamic acid (CHCA) and 2-[(2E)-3-(4-tert-butylphenyl)-2-methylprop-2-enylidene]malononitrile (DCTB) as matrices.

SAXS profiles were recorded on SAXLAB GANESHA 300 XL SAXS equipped with a GeniX 3D Cu Ultra Low Divergence micro focus sealed tube source producing X-rays with a wavelength λ = 1.54 Å at a flux of 1×108 ph s^{-1} and a Pilatus 300 K silicon pixel detector with 487 × 619 pixels of 172 × 172 μm^2 in size placed a three sample-to-detector distances of 113, 713, and 1513 mm respectively to cover a q-range of $0.07 \leq q \leq 3.0$ nm^{-1} with $q = 4\pi/\lambda(\sin \theta/2)$. Silver behenate was used for calibration of the beam center as well as the q-range. Samples were measured within 2 mm quartz capillaries (Hilgenberg GmbH, Germany). The two-dimensional SAXS patterns were brought to an absolute intensity scale using the calibrated detector response function, known sample-to-detector distance, measured incident and transmitted beam intensities, and azimuthally averaged to obtain one-dimensional SAXS profiles. The scattering curves of the fibers were obtained by subtraction of the scattering contribution of the solvent and quartz cell.

Cryo-Electron micrographs were acquired from BA aqueous solutions (18 mg mL^{-1}) placed in a sonication bath for 1 h and subsequently allowed to re-assemble for 2 d in a UV-protected environment unless otherwise noted. Samples were diluted by a factor of 1000 with mili-Q water prior to imaging. Vitrified films were prepared in an automated vitrification robot (VitrobotTM Mark III, FEI) at 20 °C and at a relative humidity of 100%. In the preparation chamber, 3 µL sample was applied on a Quantifoil grid (carbon support film on a copper grid, typeR 2/2, Electron Microscopy Sciences), which was glow discharged prior use (Cressington 208 carbon coater operation at 5 mA for 40 s). Subsequently, the samples were blotted to remove the excess of solution and vitrified in liquid ethane. The vitrified films were transferred to a cryoholder (Gatan 626) and studied in a FEI Tecnai 20 (type Sphera) microscope operating at 200 kV, equipped with a LaB6 filament. The images were recorded using a 1k × 1k Gatan CCD camera. Gatan DigitalMicrograph was used for image analysis. Contour length calculation was done by implementing the Curve Tracing plugin from Image J.

3. Synthetic procedures

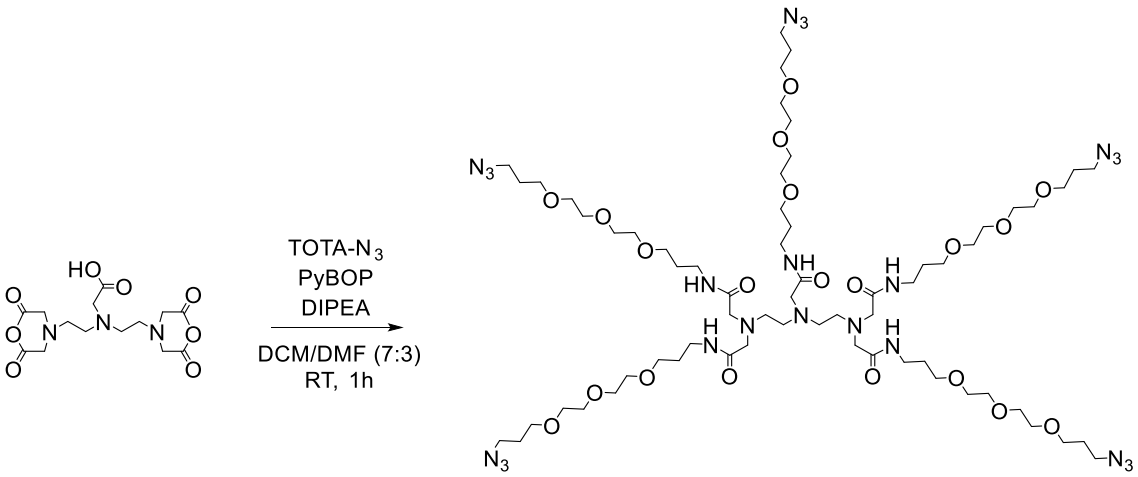

Figure S1. Synthetic route towards 5-N_3

DTPA-anhydride (26 mg, 0.074 mmol) was dissolved in 10 mL of CH_2Cl_2:DMF (7:3, v:v), solid PyBOP (127 mg, 0.24 mmol) and TOTA-N_3 (100 mg, 0.41 mmol) were added. The pH was adjusted to 8 with N,N-Diisopropylethylamine (DIPEA). After 1 h of stirring at room temperature, solvents were removed in vacuo. The resulting crude was dissolved in 10 mL of CH_2Cl_2 and washed with 5% $NaHCO_3$ (10 mL). The organic phase was evaporated, and the resulting crude was transferred to a 15 mL tube while dissolved in CH_2Cl_2 (2 mL) and hexane (12 mL) was added. The mixture was stirred vigorously and centrifuged. The supernatant was discarded and the remaining oily precipitate corresponded to pure compound (93 mg, 82% yield).

^{1}H-NMR (400 MHz, $CDCl_3$): δ = 7.52 (t, 4H, J = 5.50 Hz, N*H*, 7.33 (t, H, J = 5.66 Hz, N*H*), 3.60 (m, 40H, OC*H*$_2$), 3.52 (m, 20H, OC*H*$_2$$CH_2CH_2$), 3.37 (t, 10H, J = 6.70, CH_2CH_2C*H*$_2$$N_3$), 3.32 (m, 10H, NHC*H*$_2$$CH_2CH_2$), 3.14 (s, 8H, NC*H*$_2$CO), 3.05 (s, 2H, NC*H*$_2$CO), 2.63 (m, 4H, NC*H*$_2$$CH_2$N), 2.57 (m, 4H, NCH_2C*H*$_2$N), 1.83 (m, 10 H, CH_2C*H*$_2$$CH_2N_3$), 1.77 ($NHCH_2$C*H*$_2$$CH_2$).

^{13}C-NMR (100 MHz, $CDCl_3$): δ = 170.46, 70.56, 70.49, 70.35, 70.16, 69.56, 67.94, 58.45, 53.29, 48.52, 37.64, 29.16.

MS: Theoretical mass for $[C_{64}H_{123}N_{23}O_{18}+H]^+$: 1533.93. Experimental mass detected by LC-MS: 768.08 (M+2)/2.

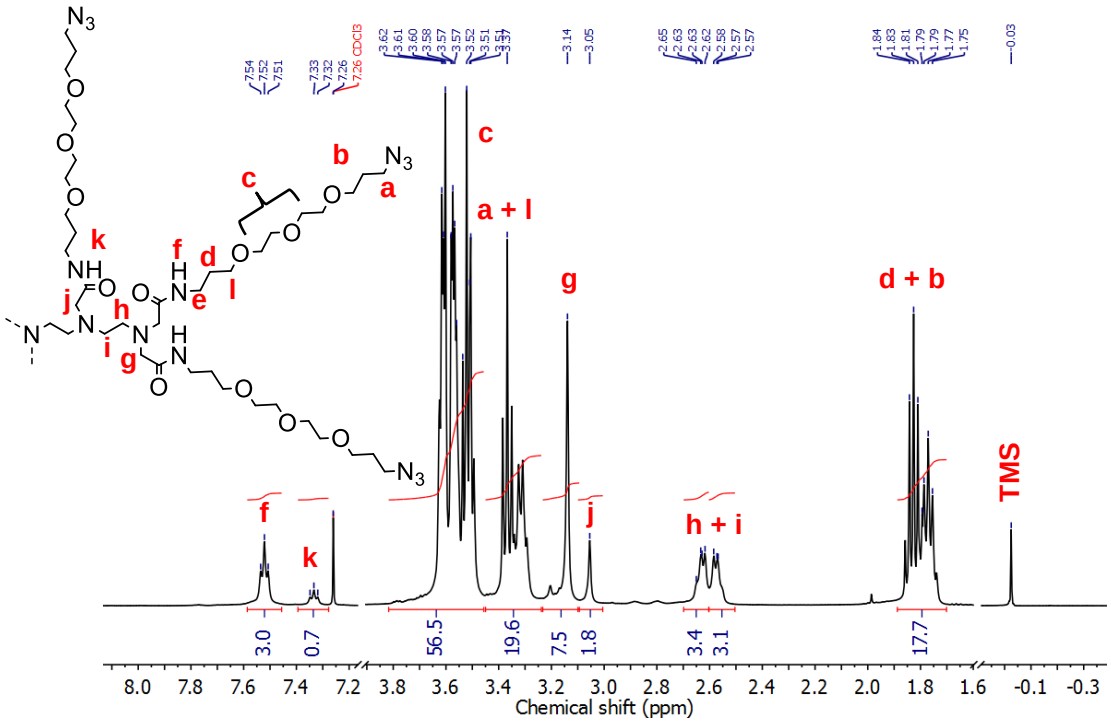

Figure S2. ^{1}H-NMR ($CDCl_3$, 25 °C) spectrum of 5-N_3

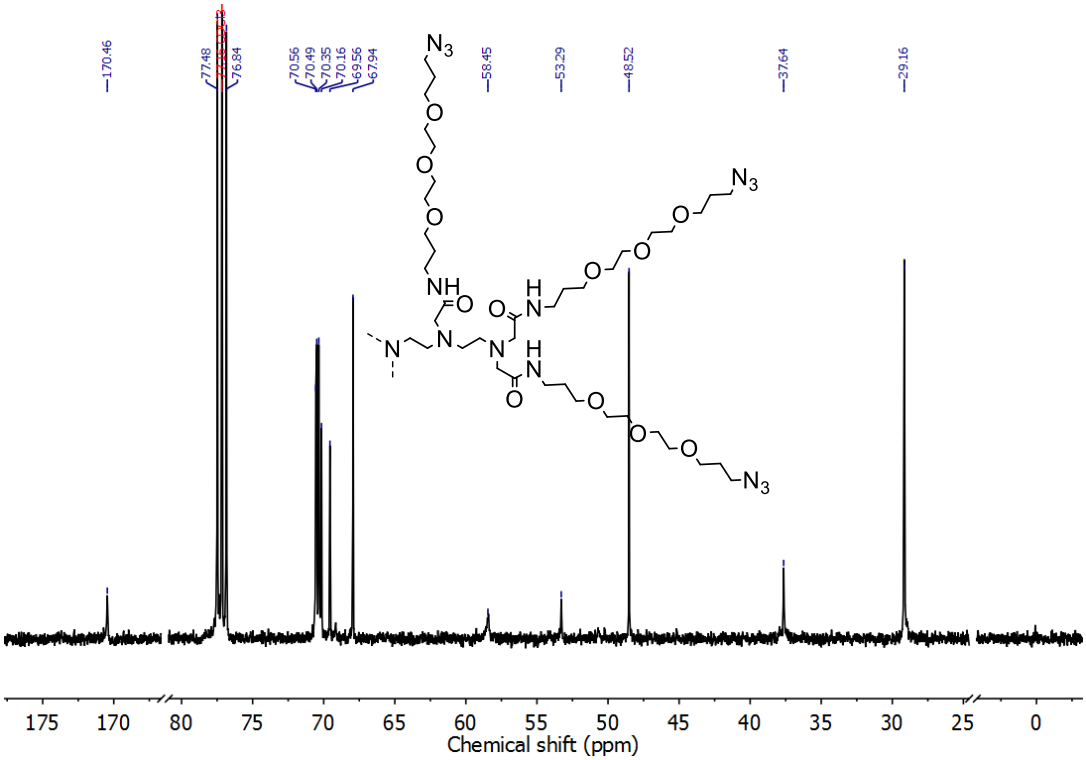

Figure S3. ^{13}C-NMR ($CDCl_3$, 25 °C) spectrum of 5-N_3.

4. Gel preparation method

BA/BA-AC and BA/BA-N_3 mixtures were weighed in the solid state to the desired molar ratio and added into separate vials. The analogues were co-assembled by adding ca. 300 μL chloroform and allowing the solvent to evaporate overnight. The remaining solids were subsequently dissolved in mili-Q water to a concentration of 50 mg mL^{-1} by placing the vials in a sonication bath assisted by vigorous vortexing until the full dissolution of the solid material. The resultant viscous solutions were left to equilibrate at ambient temperature in a UV-protected environment for a minimum of 2 d. Selectively crosslinked samples were prepared by mixing equal volumes of BA/BA-AC and BA/BA-N_3 solutions and the concentration was adjusted by adding mili-Q water. Non-selectively crosslinked samples were prepared by adding 5-N_3 from a concentrated aqueous stock solution (20 mg mL^{-1}) to a solution of BA/BA-AC fibers ensuring an azide/alkyne stoichiometric molar ratio followed by dilution to the desired concentration.

PDA samples were prepared in analogous manner to BA hydrogels. Covalent fixation was performed by transferring the aqueous solutions containing BA fibers to quartz cuvettes (1×1 cm) and irradiating at 254 nm for 15 min under continuous stirring using a Luzchem photoreactor (model LCZ 4V) equipped with 7.2 W UV-C lamps prior to crosslinking.

Crosslinking was done by following a slightly modified standardized protocol,[3] in which $CuSO_4$ and Tris(3-hydroxypropyltriazolylmethyl)amine (THPTA) were premixed in water in a 1 to 5 molar ratio and added to the reaction mixture ensuring a final concentration of 0.1 and 0.5 mM respectively. The reaction was started by adding an aqueous solution of sodium ascorbate to a final concentration of 10 mM.

5. Fitting of SAXS profiles

Small angle X-Ray scattering was used to determine the cross-sectional radius of BA fibers in solution and the gel state. Density measurements of water and DA aqueous solutions were performed using an Anton Paar DMA 5000M.

First, the specific volume (υ) of BA rods at a concentration of 15 mg mL^{-1} was determined by measuring the density (ρ) of BA aqueous solutions over the concentration range indicated in Figure S4.

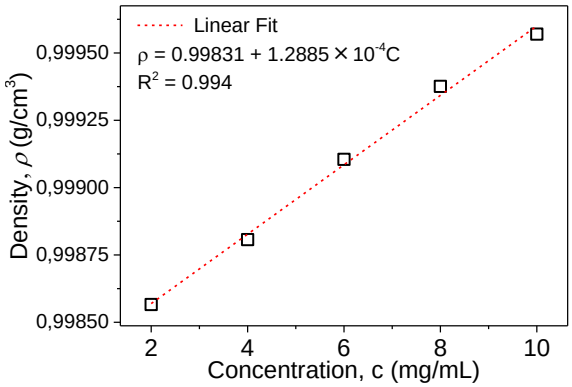

Figure S4. Plot of ρ vs c of BA in mili-Q water measured at 20 °C including linear regression.

Thus, the density of a 0.015 g cm^{-3} BA aqueous solution was calculated by extrapolation of the linear fit in Figure S4, D_{BA} = 1.0002 g cm^{-3}. The specific volume was then calculated using the following equation:

$$\upsilon = \frac{1}{D_{water}}\left(1-\frac{D_{BA}-D_{water}}{c}\right) = \frac{1}{0.9982\ g\ cm^{-3}}\left(1-\frac{1.0002-0.9982\ g\ cm^{-3}}{0.015\ g\ cm^{-3}}\right) =$$

$$= 0.8682\ cm^3 g^{-1}$$

To fit the experimental data with a non-linear least squares procedure implemented in Igor Pro, we employed a model that calculates the form factor of a flexible cylinder with a uniform scattering length density (ρ_{cyl}) and cross-sectional radial polydispersity which is averaged over a Schultz distribution of cylinder radii. The non-negligible diameter of the cylinder is included by accounting for excluded volume interactions within the walk of a single cylinder. Inter-cylinder interactions are not included.

We then proceeded to fix the values of volume fraction (ϕ = 0.00868) and use the values of contour length (L_c = 157 nm) and Kuhn length (b = 2×l_p = 560 nm) as derived from cryo-EM. The tabulated value of ρ_{water} = 9.37 × 10^{10} cm^{-2} was used.

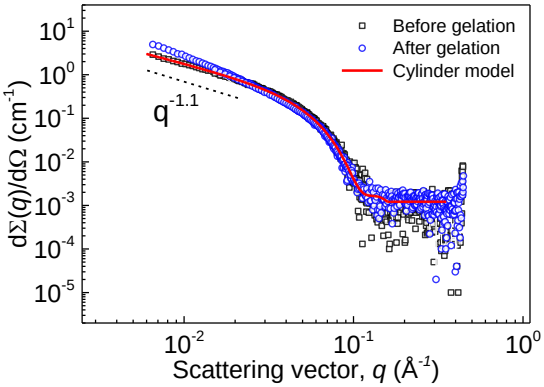

Figure S5. SAXS profiles recorded before and 24 h after crosslinking of 15 mg mL^{-1} BA/BA-AC (20 mol% BA-AC) solutions with 5-N_3.

Fitting of the scattering data with the model (Figure S5, red line) gave a value of 3.3 ± 0.2 nm for the radius, consistent with the value of 3.3 nm measured in cryo-EM. The fitting procedure also provided a value for ρ_{cyl} = (10.69 ± 0.02) × 10^{10} cm^{-2} that agrees well with the fitting results reported in previous work[4], namely ρ_{cyl} = (10.43 ± 0.01) × 10^{10} cm^{-2}.

6. BA exchange dynamics measured by rheology

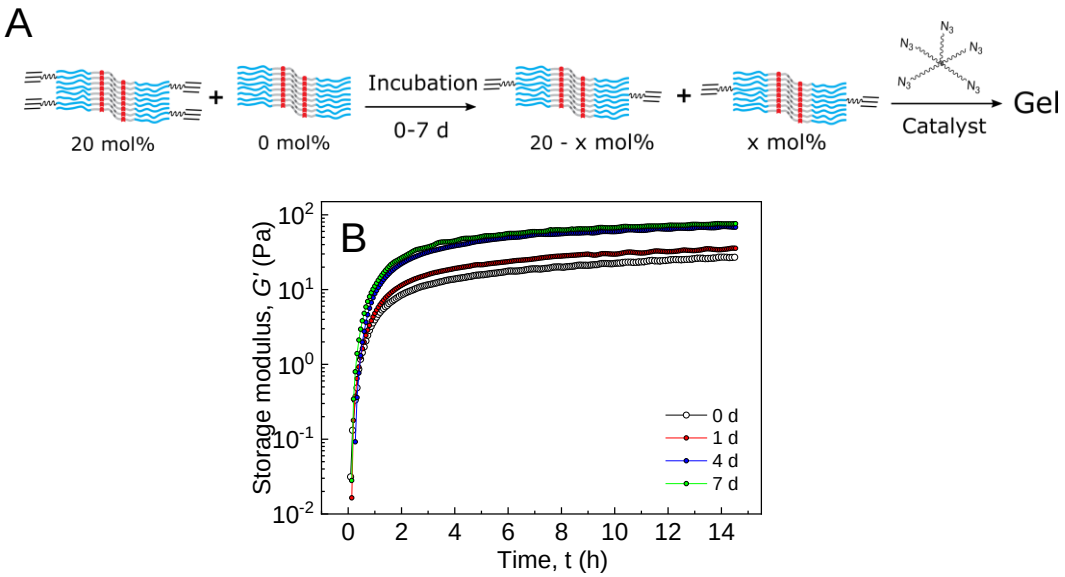

Figure S6. Reversibility of BA fibers in water measured by rheology. (A) Monomer exchange between labeled (BA/BA-AC) and unlabeled (BA) fibers followed by gelation via covalent crosslinking-fixation with 5-N_3. (B) Time course of storage modulus G' measured by applying oscillatory shear γ = 1% and ω = 6.28 rad s^{-1} during Cu-catalyzed click reaction of 16 mg mL^{-1} BA/BA-AC (20 mol% BA-AC) mixed with 16 mg mL^{-1} BA plotted as a function of incubation time.

7. Critical stress and critical strain calculation

The calculation of the critical stress σ_c was performed by normalizing the differential modulus K' to its value in the linear regime G_0. K'/G_0 was then plotted against the applied pre-stress σ and the critical stress was graphically determined as the intersection between linear and nonlinear regimes (Figure S9).

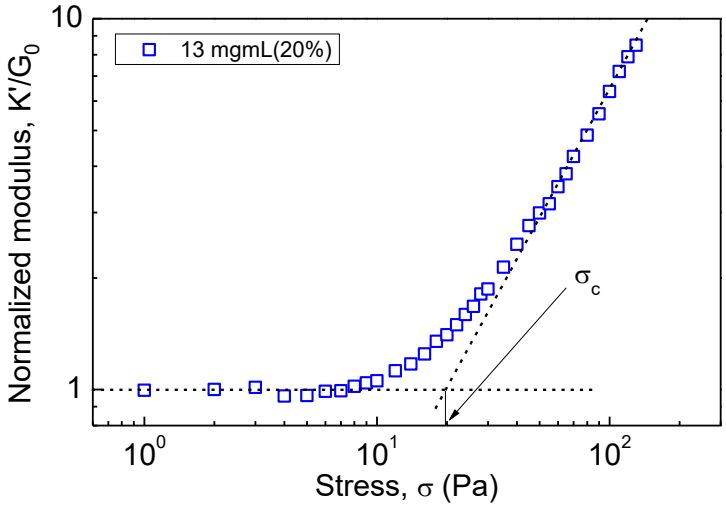

Figure S7. Graphical method to calculate σ_c. Plot of K' vs stress σ with K' normalized to G_0 of a BA (13 mg mL^{-1}; 20 mol% BA-AC) hydrogel obtained using multiarm crosslinking.

The critical strain was then calculated by dividing σ_c by the plateau modulus G_0, such that:

$$\gamma_C = \frac{\sigma_c}{G_0}$$

8. Self-healing experiment

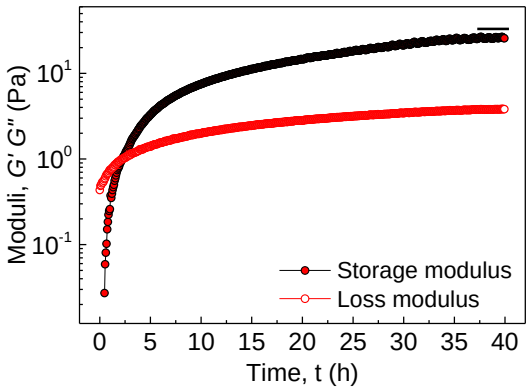

Figure S8. Time course of storage *G'* and loss *G''* moduli of a directly crosslinked BA (30 mg mL^{-1}; 2.5 mol% crosslinkable BA) hydrogel recorded at a constant strain amplitude of γ = 1% and ω = 6,28 rad s^{-1} during Cu-catalyzed click reaction.

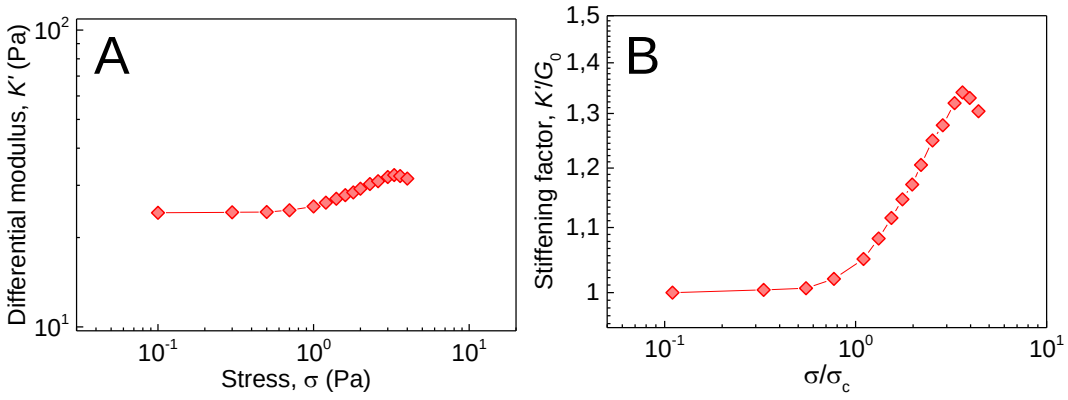

Figure S9. Nonlinear mechanics of a BA (30 mg mL^{-1}; 2.5 mol% crosslinkable BA) hydrogel measured 18 h after rupture. (A) Differential modulus *K'* vs stress σ. (B) Plot of *K'* vs stress *σ* with *K'* normalized to G_0 and *σ* normalized to σ_c with $K'_{max}/G_0 = 1.34$.

9. References

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(3) Hong, V.; Presolski, S. I.; Ma, C.; Finn, M. G. Analysis and Optimization of Copper-Catalyzed Azide–Alkyne Cycloaddition for Bioconjugation. *Angew. Chem. Int. Ed.* **2009**, *48* (52), 9879–9883. https://doi.org/10.1002/anie.200905087.