Electronic Supplementary Material (ESI) for Polymer Chemistry.

Modulating Stiffness with Reversibly Photo-Switchable Hydrogels

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S.1 Materials and Methods

Chemicals and Reagents

All starting materials were purchased from Sigma Aldrich and used as received unless stated otherwise. All other solvents were purchased from commercial sources and were used without any further purification unless otherwise noted. ^{1}H-NMR (500 MHz) and ^{13}C-NMR (125 MHz) spectra were recorded in D_2O, $(CD_3)_2SO$ or $CDCl_3$ as stated and recorded using Bruker 500 MHz DCH Cryoprobe Spectrometer. Dialysis of the polymers was carried out by placing the reaction solutions into a dialysis tube (Spectrum Labs, Spectra/Por, standard grade regenerated cellulose dialysis membrane 6, MWCO 15,000 Da) which was subsequently submerged in specified aqueous solutions. The external solutions were stirred at room temperature and replaced periodically over a 5 d period (ca. 4-5 times daily). The dialyzed polymer solution was then transferred into a round bottomed flask, frozen in a dry ice/acetone bath and lyophilized on a VirTis BenchTop Pro Freeze Drier to yield fluffy solid materials.

7-ethoxycarbonylmethoxycoumarin

In darkness, 7-hydroxycoumarin (10.0 g, 61.7 mmol) was added to a solution containing ethyl bromoacetate (12.4 g, 73.9 mmol) and potassium carbonate (12.5 g, 90 mmol) in

anhydrous acetone (450 mL). The solution was refluxed for three h, cooled to room temperature and the salt filtrated. The product was recrystallized from ethanol with an isolated yield of 63%.

Yield = 9.58 g, 63%; ^{1}H-NMR (DMSO-d6, 500 MHz) δ (ppm) = 7.99 (1H, d, J = 9.5 Hz, Ar-H), 7.64 (1H, d, J = 8.4 Hz, Ar-H), 6.97 (2H, m, Ar-H), 6.31 (1H, d, J = 9.5 Hz, Ar-H), 4.92 (2H, s, CH_2), 4.18 (2H, q, J = 7.1 Hz, CH_2), 1.21 (3H, t, J = 7.1, CH_3); ^{13}C-NMR (DMSO-d6, 125 MHz) δ (ppm) = 168.29 (R(C=O)OR), 160.76 (R(C=O)OR), 160.31 (Ar-C), 155.24 (Ar-C), 144.33 (Ar-CH), 129.64 (Ar-CH), 113.01 (Ar-CH), 112.97 (Ar-C), 112.74 (Ar-CH), 101.60 (Ar-CH), 65.03 (CH_2), 60.94 (CH_2), 14.11 (CH_3); FT-IR (ATR) v (cm^{-1}) = 3077 (w), 1708 (s), 1610 (s), 1155 (m), 1068 (s), 1017 (m), 840 (s); Anal. Calcd for $C_{13}H_{12}O_5$: C, 62.90; H, 4.87; O, 32.23. Found: C, 62.67; H, 4.88; O, 32.45.

7-carboxymethoxycoumarin

In darkness, 7-ethoxycarbonylmethoxycoumarin (7.05 g, 30.4 mmol) was subsequently hydrolyzed for 18 h in a mixture of 1,4-dioxane (280 mL), water (400 mL) and sodium hydroxide (15.8 g, 395 mmol). The resulting product was extracted with 3:1 chloroform / methanol mixture, acidified with hydrochloric acid and recrystallized from ethanol with an isolated yield of 94%.

Yield = 7.97 g, 94%; ^{1}H-NMR (DMSO-d6, 500 MHz) δ (ppm) = 7.98 (1H, d, J = 9.5 Hz, Ar-H), 7.63 (1H, dd, J = 9.3 Hz, 4.1 Hz, Ar-H), 6.95 (2H, m, Ar-H), 6.30 (1H, d, J = 9.5 Hz, Ar-H), 4.82 (2H, s, CH2); ^{13}C-NMR (DMSO-d6, 125 MHz) δ (ppm) = 169.73 (R(C=O)OH), 160.94 (R(C=O)OR), 160.34 (Ar-C), 155.25 (Ar-C), 144.35 (Ar-CH), 129.60 (Ar-CH), 112.88 (Ar-CH), 112.83 (Ar-C), 112.69 (Ar-CH), 101.54 (Ar-CH), 64.89 (CH_2); FT-IR (ATR) v (cm^{-1}) = 3079 (w), 1711 (m), 1616 (m), 1251 (m), 1156 (w), 1124 (m), 1073 (m), 998 (w), 833 (m); HRMS = 220.0368 (found), 220.0372 (calculated for [$C_{11}H_8O_5$]+). Anal. Calcd for $C_{11}H_8O_5$: C, 60.01; H, 3.66; O, 36.33. Found: C, 59.89; H, 3.59; O, 36.52.

General procedure for functionalisation of polysaccharides with 7-carboxymethoxycoumarin

7-carboxymethoxycoumarin (250 mg) was dissolved in anhydrous thionyl chloride (2.5 mL) and refluxed in darkness for 3 h. Excess solvent was removed under vacuum and redissolved in the minimum amount of anhydrous *N*-methyl-2-pyrrolidone. The 7 chlorocarbonylmethoxycoumarin intermediate solution was then injected into a solution of polysaccharide (1.5 g) in anhydrous *N*-methyl-2-pyrrolidone and triethylamine (238 µL, 173 mg, 1.74 mmol) at 0 °C. The reaction was left to come to 25 °C and stir overnight in an inert atmosphere in darkness. The mixture was then dialysed versus water for 3 d, twice precipitated in cold acetone, and dried under vacuum to yield coumarin functionalised polysaccharides.

7-carboxymethoxycoumarin functionalised hydroxyethyl cellulose

2-hydroxyethyl cellulose (Average MW = 720 kDa and 1.3 MDa) was purchased from Sigma Aldrich. The product was isolated using the method described above, to yield a white solid precipitate.

720 kDa 7-carboxymethoxycoumarin functionalised hydroxyethyl cellulose

Yield = 1.42 g, 93%; degree of COU functionalisation = 6%; ^{1}H-NMR (D2O, 500 MHz) δ (ppm) = 8.05 - 7.72 (1H, Ar-H), 7.68 - 7.38 (1H, Ar-H), 7.09 - 6.66 (2H, Ar-H), 6.41 - 6.07 (1H, Ar-H), 4.54 - 2.69 (263H, polymer backbone); FT-IR (ATR) ν (cm^{-1}) = 3415 (br, w), 2885 (w), 1740 (w), 1615 (w), 1379 (w), 1060 (s).

1.3 MDa 7-carboxymethoxycoumarin functionalised hydroxyethyl cellulose

Yield = 1.37 g, 91%; degree of COU functionalisation = 5%; ^{1}H-NMR (D2O, 500 MHz) δ (ppm) = 8.05 - 7.72 (1H, Ar-H), 7.68 - 7.38 (1H, Ar-H), 7.09 - 6.66 (2H, Ar-H), 6.41 - 6.07 (1H, Ar-H), 4.54 - 2.69 (263H, polymer backbone); FT-IR (ATR) ν (cm^{-1}) = 3415 (br, w), 2885 (w), 1740 (w), 1615 (w), 1380 (w), 1060 (s).

7-carboxymethoxycoumarin functionalised hyaluronic acid

Hyaluronic acid sodium salt from Streptococcus zooepidemicus (1.5-1.8 MDa) was purchased. In order to solubilise the polysaccharide in organic solvents, an ion exchange was carried out using Dowex® 50WX8 hydrogen form resin. The beads were filtered out *via* two passes through Grade 1 filter paper.

Yield = 1.31 g, 87%; degree of COU functionalization = 5%; ^{1}H-NMR $(CD_3)_2SO$, 500 MHz) δ (ppm) = 8.05 - 7.72 (1H, Ar-H), 7.68 - 7.38 (1H, Ar-H), 7.09 - 6.66 (2H, Ar-H), 6.41 - 6.07 (1H, Ar-H), 3.20 - 2.65 and 2.05 - 0.85 (65H, polysaccharide backbone), 2.07 (15H, s, -NHCOCH3); FT-IR (ATR) v (cm^{-1}) = 3352 (br, w), 2900 (w), 1740 (w), 1615 (w), 1360 (w), 1044 (s).

Gel Formation

Dried powders of functionalised polymers and CB[8] were added to a 6 mL screw top vial in darkness. Milli-Q H_2O (18 mΩ) was added to bring the gel concentration to 2% wt/wt functional polymer. While wrapped in aluminum foil, the polymer/CB[8] solution was stirred at 1000 RPM and 37 °C for 16 h, subsequently the gels were vortexed for 2-3 min and left covered in foil at room temperature for 12 h.

Isothermal Titration Calorimetry (ITC)

All ITC experiments were carried out on a Microcal ITC200 at 298.15 K in water. The host molecule (CB[8]) was held in the sample cell, and guest molecule (COU-OH) was held in the injection syringe. The concentration of CB[8] was calibrated by the ITC titration with a standard aqueous solution of 1-adamantanamine. The concentration of COU-OH was calibrated by NMR using 3-(Trimethylsilyl)-1-propanesulfonic acid sodium salt as internal standard in 90% H_2O and 10% D_2O. In order to avoid bias or arbitrary offsets caused by manual adjustment of baseline, all raw data (thermograms) of ITC were integrated by NITPIC (v.1.2.2), fitted in Sedphat (v.12.1b), and visualized through GUSSI (v.1.2.1).[33]

Rheology

All rheological sweeps were conducted on an AR-G2 Rheometer (TA Instruments, New Castle, DE, USA) with a 20 mm parallel plate geometry between 20.0 – 20.5 °C. Environmental temperature was recorded using the built in platinum resistance thermocouple in standard AR-Series Peltier lower plates. A UV Light Guide lower plate (TA Instruments) was used to irradiate hydrogel samples *in situ*. Zero gap, rotational mapping (precision bearing mapping; 2 iterations), geometrical inertia, friction, and UV irradiation calibrations were done prior to each use of the rheometer. Hydrogel samples were loaded onto the rheometer with a 600-1000 μm loading gap. Water was placed in the 20 mm parallel plate trap to minimize dehydration. Samples were loaded and probed in the dark to prevent uncontrolled photo-dimerisation. Complex moduli were reported as the average of 3 runs at 1 rad/s. Statistical significance and *p*-values were determined using ANOVA with Tukey post-test.

Reversibility Studies

After covalent dimerisation of COU-functionalised polymer hydrogels *in situ*, the samples were carefully taken off the rheometer and irradiated in a photoreactor UV chamber (Luzchem, Gloucester, Ontario, CA) at 254 nm for a specified amount of time. UV intensity was calculated based off the manufacturer's specifications. Samples were removed in the dark and immediately covered with aluminum foil. The samples were re-loaded onto cleaned rheometer plates and various oscillatory time, amplitude, and frequency sweeps were run again. *In situ* curing on the rheometer at 254 nm was not possible with the accessory UV source. Due to these equipment limitations, it was not possible to expose to each hydrogel sample to more than two cycles without sample evaporation and deformation when handling.

S.2 Supporting Figures

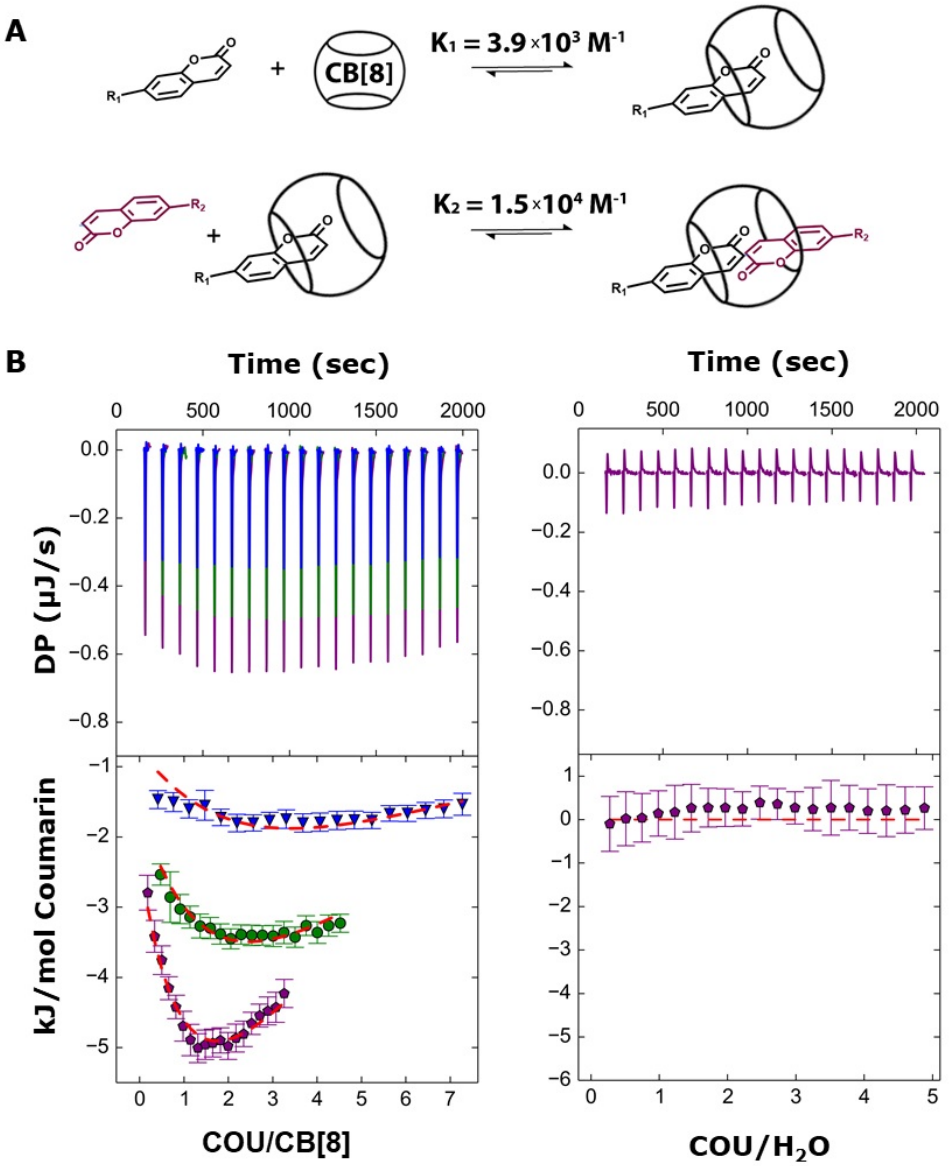

Figure S1: Isothermal titration calorimetry data demonstrating 2:1 homoternary binding of coumarin to cucurbit[8]uril. (A) graphic illustrating 2:1 binding with the 1st and 2nd binding equilibrium constants at 298 K. (B) ITC data with three titration curves (left) obtained using different CB[8] concentrations fit to a sequential model. ITC data of COU-OH (0.46 mM aqueous solution) into water without CB[8], exhibiting negligible background heat (right).

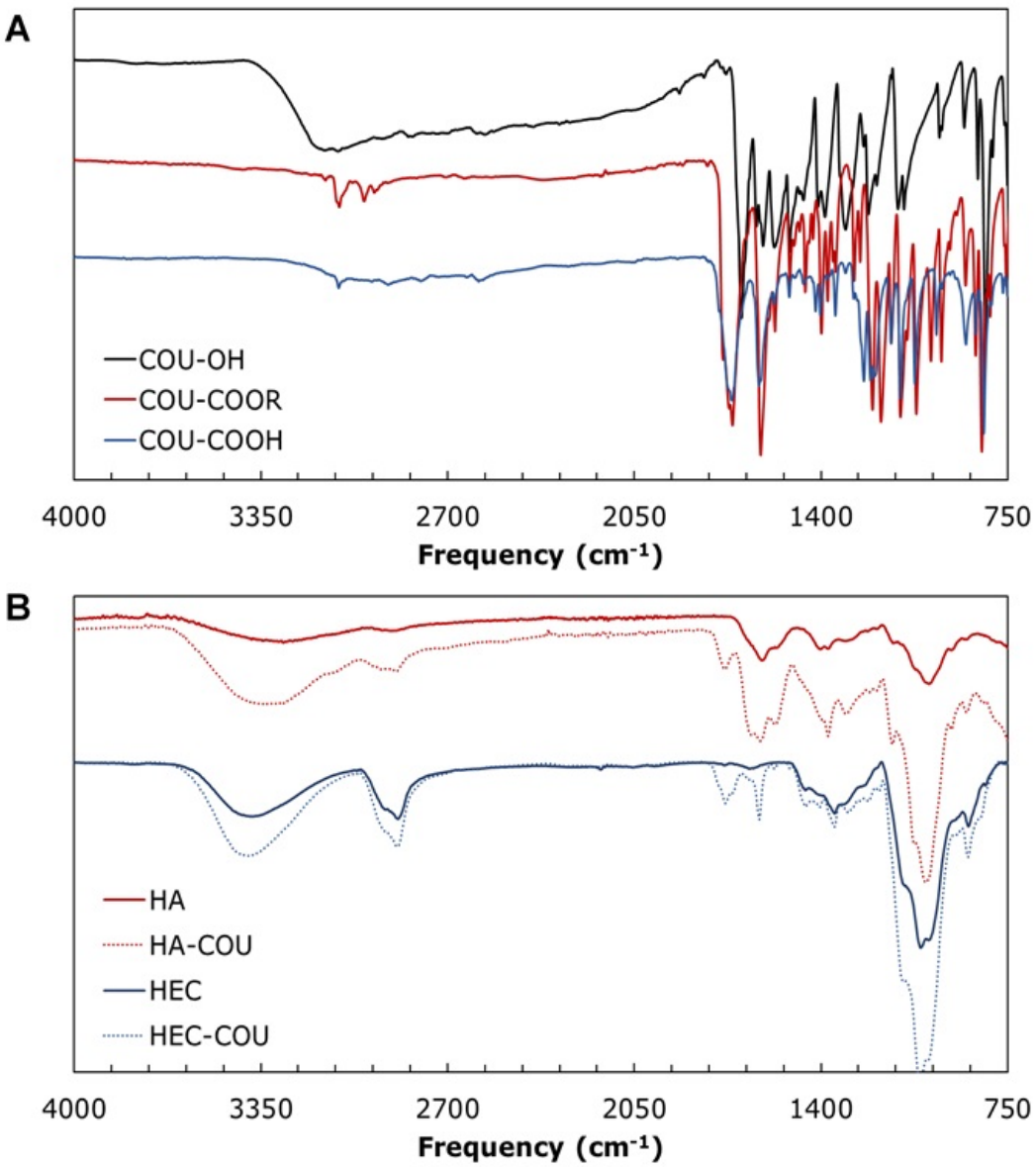

Figure S2: Fourier-transform infrared (FTIR) spectroscopy of COU derivatives and functional polymers. (A) Spectra of COU derivatives: COU-OH, COU-COOR, and COU-COOH with diagnostic stretching frequencies indicative of hydroxyl (3500-3000 cm^{-1}), ester (1725 cm^{-1}) and carboxylic acid (broad 3000-2500 cm^{-1} and 1780-1710 cm^{-1}) functionality. (B) Polysaccharides HA and HEC functionalised with COU. Characteristic ester peak formed (1725 cm^{-1}) suggesting pendent COU functionality to the polysaccharide backbones.

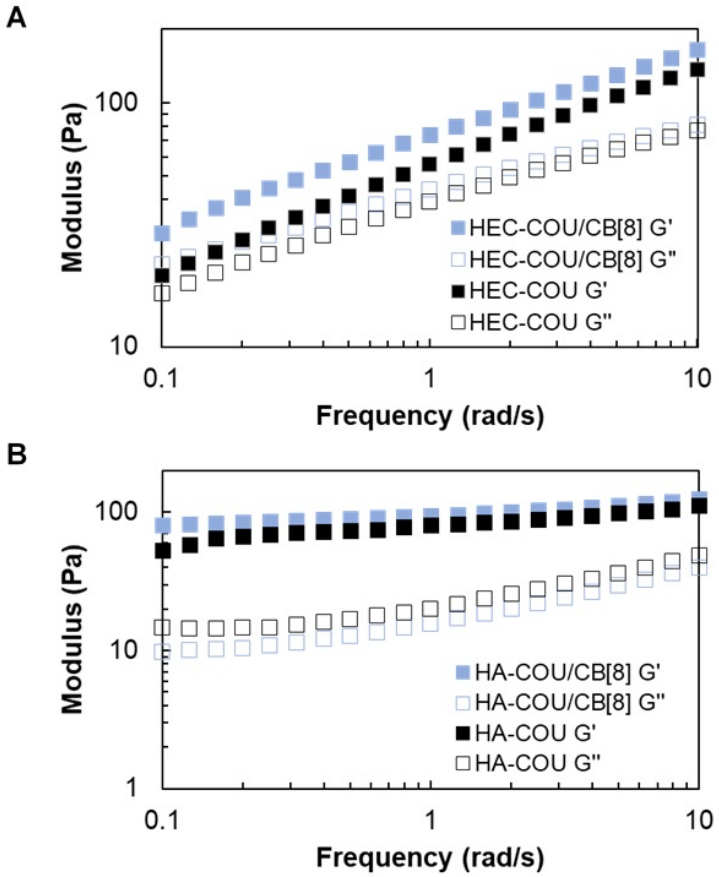

Figure S3: CB[8] thickens gels formed from 1.3 MDa HEC and 1.5-1.8 MDa HA-based gels. (A) Oscillatory frequency sweeps showing the storage (G′) and loss (G″) moduli vs. frequency of HEC-COU and HEC-COU/CB[8] systems with a 1.3 MDa HEC backbone. (B) Oscillatory frequency sweeps showing G′ and G″ vs. frequency of HA-COU and HA-COU/CB[8] systems with a 1.5-1.8 MDa HA backbone.

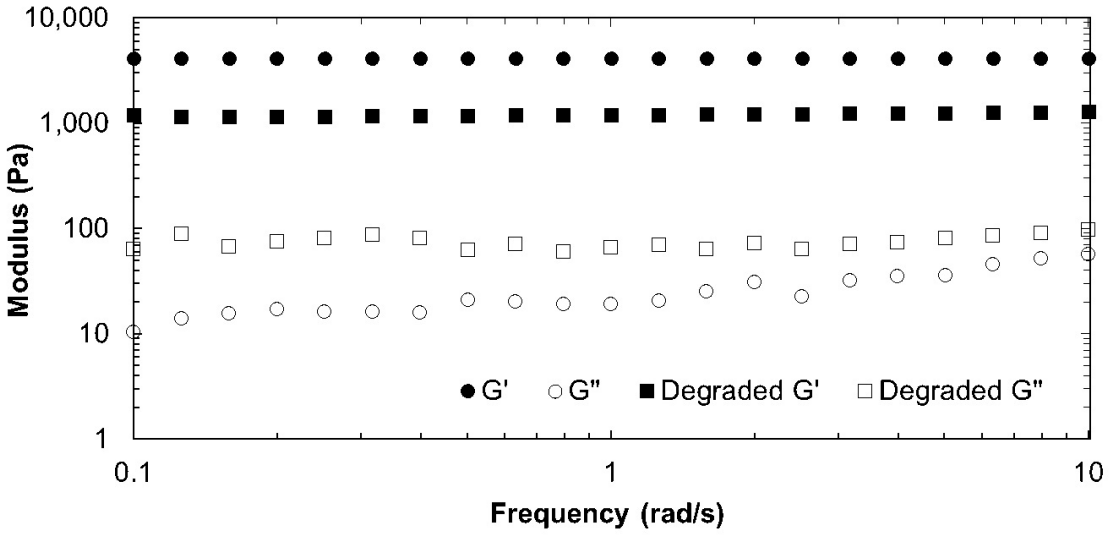

Figure S4: Oscillatory frequency sweeps of HEC-COU/CB[8] gels before and after chain scission via irradiation at light with wavelength λ = 254 nm.

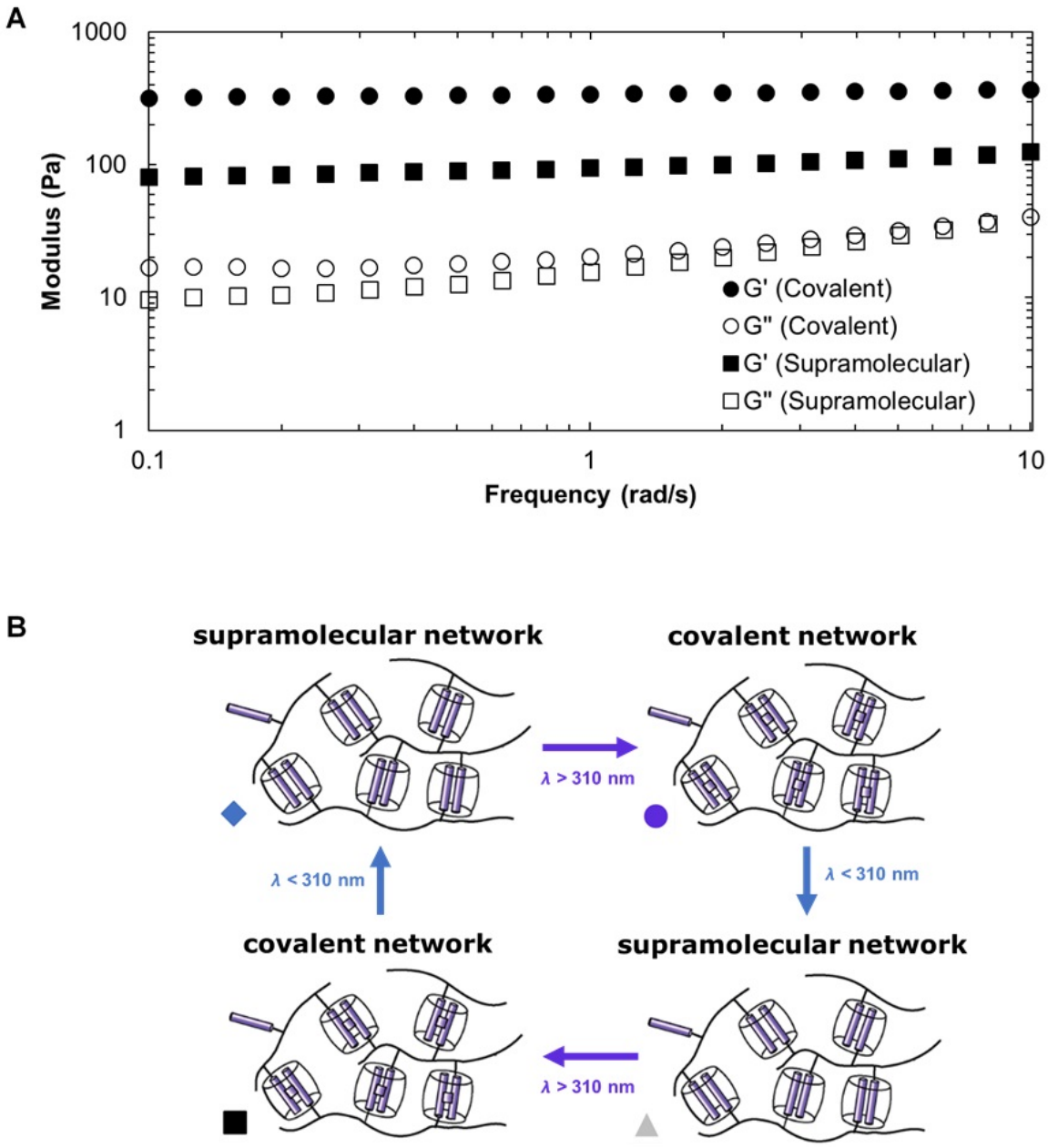

Figure S5: Rheological analysis of HA-COU/CB[8] in physical and covalent states. (A) Oscillatory frequency sweeps of supramolecular and covalent HA-COU/CB[8] gels.(B) Cartoon illustrating photo-mediated toggling between supramolecular and covalent networks shown in Fig. 4.

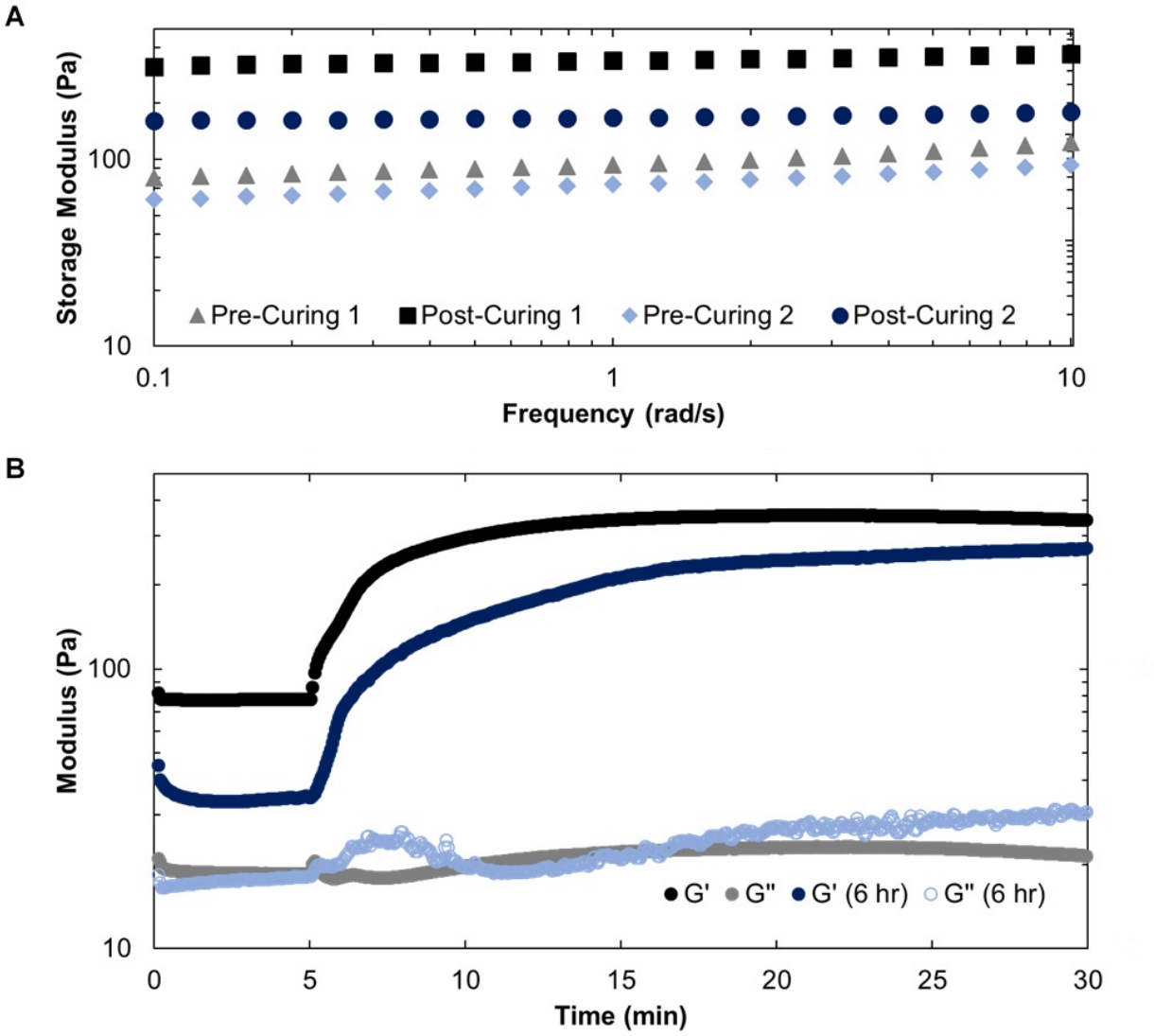

Figure S6: Photo-reversibility and chain scission of HA at 6 h. (A) Oscillatory frequency sweeps showing storage modulus (G′) vs. frequency for HA-COU/CB[8] gels before and after curing (irradiation at 350 nm), and decoupling with irradiation at 254 nm. (B) Oscillatory time sweeps showing that overexposed HA-COU/CB[8] undergoes chain scission but COU-COU interactions can be re-dimerised to yield a covalent network with lower overall matrix stiffness.

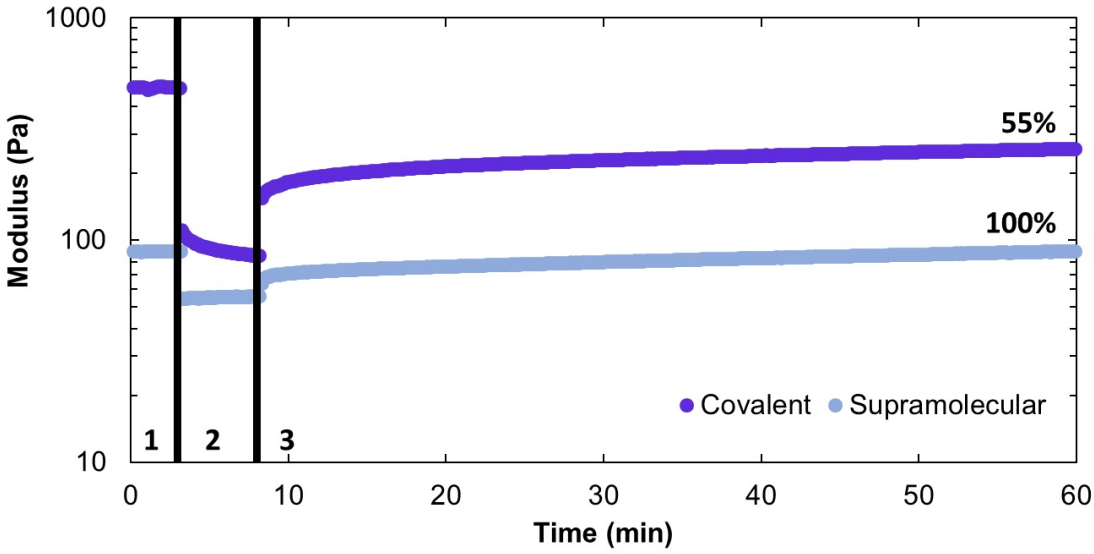

Figure S7: Oscillatory time sweeps of HA-COU/CB[8] systems to measure recovery time after high applied shear. **1**: ω = 1 rad/s, $\hat{\gamma}$ = 1%; **2**: ω = 50 rad/s, $\hat{\gamma}$ = 50%; **3**: ω = 1 rad/s, $\hat{\gamma}$ = 1%.