

Supporting Information

for

Light-Induced Remodeling of Physically Crosslinked Hydrogels Using Near-IR Wavelengths

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Experimental Details

Synthesis of 6-bromo-4-chloromethyl-7-hydroxycoumarin: All reagents were purchased from Sigma-Aldrich (St. Louis, MO, USA) and used as received unless otherwise stated. 4-bromoresorcinol (945 mg, 5 mmol) was combined with ethyl 4-chloroacetoacetate (1 ml, 7.4 mmol) for 40 min. Concentrated sulfuric acid (5 ml) was added dropwise thereafter and the reaction proceeded at room temperature for 6 days. The reaction mixture was precipitated into ice water, filtered, and washed with copious amount of cold water. ^1H NMR (300 MHz, DMSO- d_6) δ = 11.53 (s, 1H), 8.00 (s, 1H), 6.92 (s, 1H), 6.48 (d, J =0.9, 1H), 5.00 (d, J =0.9, 2H).

Synthesis of poly(methacrylic acid)-poly(ethylene glycol)-poly(methacrylic acid) triblock copolymer: Poly(*tert*-butyl methacrylate)-PEG-poly(*tert*-butyl methacrylate) was synthesized using ATRP.⁴¹ Briefly, *tert*-butyl methacrylate (5 ml, 30 mmol) and PEG20000 macroinitiator (6.16 g, 0.3 mmol) were dissolved in 5 ml THF at 40 °C in a round bottom flask and bubbled with N_2 to degas. PMDETA (0.386 ml, 1.8 mmol) was dissolved in 1 ml THF in another flask and flushed with N_2 . CuBr (88 mg, 0.6 mmol) was introduced into a Schlenk flask and purged under N_2 for one hour. Liquid reactants were transferred to a Schlenk flask using degassed syringes.

The flask was immersed in an oil bath at 60 °C for 24 h. The reaction mixture was passed by neutral alumina column to remove copper and precipitated into water/methanol. The PtBMA-PEG-PtBMA product was dried under vacuum at 40 °C for 24 h. PMAA-PEG-PMAA triblock copolymer was prepared by hydrolysis of poly(*tert*-butyl methacrylate) blocks. PtBMA-PEG-PtBMA was dissolved in dichloromethane and 8-fold molar excess of trifluoroacetic acid was added dropwise. The reactants were left at room temperature overnight and the PMAA-PEG-PMAA product gradually precipitated. The crude product was filtered, washed with dichloromethane and dried at room temperature for 24 h to yield the final product.

Synthesis of poly(6-bromo-4-chloromethyl-7-hydroxycoumarin)-based triblock copolymer:

PMAA-PEG20000-PMAA (500 mg, 1.37 mmol with respect to –COOH, average DOP = 36 for each PMAA A block, average DOP = 72 total) was dissolved in 20 ml DMSO and combined with DBU (0.25 ml, 1.65 mmol) and 6-bromo-4-chloromethyl-7-hydroxycoumarin (476.7 mg, 1.65 mmol) in a round bottomed flask. The reaction proceeded at 60 °C for 24 h, after which 1 ml of acetic acid was charged into the flask to neutralize the DBU. The mixture was purified by dialysis (MWCO 3000, Spectrum Laboratories, Rancho Dominguez, CA, USA) against methanol. The precipitate inside dialysis tubing was collected, dried, dissolved in DMSO again and precipitated into cold methanol. The final product was filtered and dried under vacuum at room temperature for 24 h. This overall synthetic strategy is more efficient over direct polymerization of coumarin methacrylate monomers by ATRP because it obviates the (de)protection steps of the hydroxyl group at the 7 position. The degree of esterification was calculated by comparing the peaks assigned to aromatic groups of coumarin (7.79, 6.79 and 6.20 ppm) with those assigned to methylene groups in PEG blocks (3.5 ppm). The latter has a known

number of repeat units via molecular weight calculations. ^1H NMR (300 MHz, DMSO-d_6) δ = 7.79 (s, 1H), 6.79 (s, 1H), 6.20 (s, 1H), 5.14 (s, 2H), 3.5 (s, 50H).

Hydrogel preparation: ABA triblock copolymers were dissolved in a DMSO/methanol solvent mixture (1:4 by weight) to produce polymer solutions (15% w/w). Approximately 200 μl of polymer solution was added drop wise into a ddH₂O reservoir (~15 ml). Spherical hydrogels were formed via solvent exchange. The rate of dilution of the DMSO/methanol carrier solvent governs the assembly process. Hydrogel formation via physical crosslink formation of the A blocks takes place at this time. The newly introduced polymer solution forms a semi-permeable interface that permits gradual solvent exchange and network formation without premature dilution of the polymer into the aqueous environment. Hydrogels are equilibrated in aqueous reservoirs for 24 hr to obtain fully swollen networks. Samples are then sectioned into desired geometries for subsequent characterization. The robustness of this hydrogel fabrication technique is inferred through reproducible values for storage modulus and swelling ratio. The spontaneous self-assembly process is driven by the differential residual free energies of polymer-solvent interactions between the hydrophobic A segments compared to the hydrophilic B segments.

Rheological and spectroscopic characterization of hydrogel network: Rheological measurements were conducted using a rheometer (HR-2, TA Instruments, New Castle, DE, USA) with a UV exposure accessory (30 mW cm^{-2} , Lumen Dynamics, Mississauga, Ontario, Canada) operating at room temperature. Hydrogel samples ($n = 3$) were prepared into disks ($D \times H = 13 \times 0.4 \text{ mm}^2$). Strain amplitudes of 0.5% was used to ensure that the hydrogel remained in the linear viscoelastic regime during measurements. Network disintegration

experiments were restricted to 1000 sec or less to limit analytic complications arising from dehydration. The kinetics of light-induced disintegration of hydrogels was studied using the aforementioned parallel-plate rheometer with a UV exposure accessory. The normalized storage modulus $\bar{G}'$ is defined as $G'(t)/G'_0$ where $G'(t)$ and G'_0 are the time dependent and initial storage moduli of the material, respectively. Single-photon uncaging of hydrogel networks was performed by exposing networks to UV irradiation for prescribed times ($\lambda_{\text{max}} = 365 \text{ nm}$, 10 mW cm^{-2} , Blak-Ray, Upland, CA, USA). The molar extinction coefficient of brominated coumarin was reported to be $14\,800 \text{ M}^{-1} \text{ cm}^{-1}$ at 365 nm wavelength.²⁶ Two-photon uncaging was performed using a 20X objective of laser scanning microscope (LSM510, Zeiss, Thornwood, NY, USA) equipped with a Ti: Sapphire laser (Coherent, Santa Clara, CA, USA). Coumarin serves as a fluorophore that can be imaged using two-photon excitation at 740 nm. Z-stack images were recorded to measure the depth of the grating microstructures. Maskless two-photon uncaging of photolabile networks was performed by raster scanning a pulsed laser beam ($\lambda = 740 \text{ nm}$, 1.8 nJ per pulse) within the gel to induce local network disintegration. Total raster times of approximately one minute were used owing to the large two-photon uncaging cross section of [6-bromo-7-hydroxycoumarin-4-yl]methyl. The proposed mechanism for hydrogel disintegration was further investigated by loading secondary reporter dyes into the networks. Rhodamine B was incorporated into physical crosslinks during hydrogel formation and equilibrated in aqueous environments for 24 h. Exogenous aqueous solutions were collected and characterized by UV-vis spectroscopy. Rhodamine B-loaded hydrogels ($n = 3$) in water baths (100 μl) were each independently raster-scanned using a near-IR laser ($\lambda = 740 \text{ nm}$, 4.3 nJ per pulse) across ten square regions with a nominal area of $900 \mu\text{m}^2$ (10 regions $\times$ 900

μm^2 each = 9000 μm^2 total). The exogenous water was sampled and analyzed with a plate reader (Safire2, Tecan, San Jose, CA, USA). Samples were charged with fresh water (100 μl) after each exposure and incubated in the dark for one hour to achieve intermittent irradiation patterns. Absorbance intensities at $\lambda = 540 \text{ nm}$ (A_{540}) were normalized using absorbance values measured at $\lambda = 650 \text{ nm}$ (A_{650}) to produce a normalized Rhodamine B intensity $I_{\text{max}} = A_{540}/A_{650}$. Fluorescent polystyrene microparticles ($D = 1 \text{ }\mu\text{m}$, Polysciences, Warrington, PA, USA) were incorporated into the apical region of hydrogels during hydrogel preparation and the selective cleaning of polymer surfaces were conducted using UV irradiation and two-photon laser as described above.

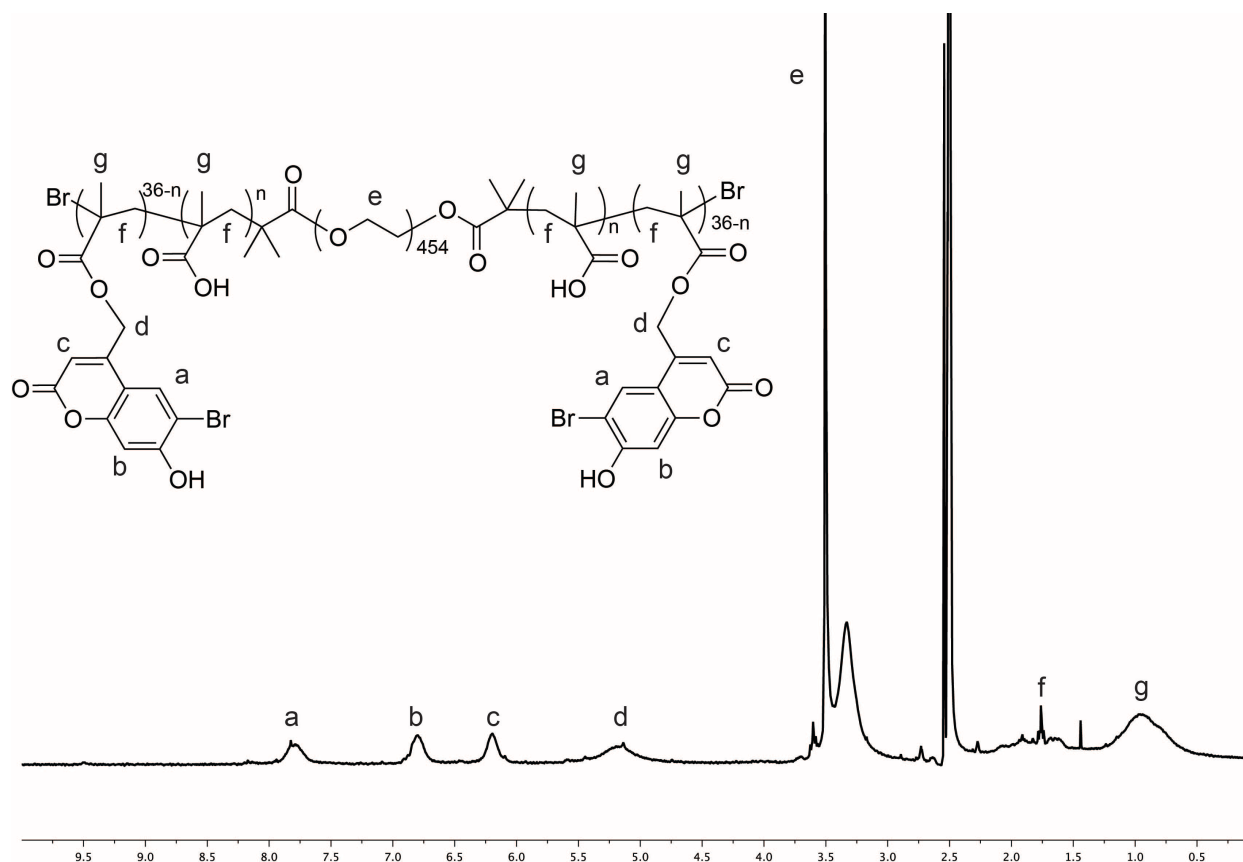


Figure S1. ¹H NMR spectrum of poly(MAA-co-BHCMM)-PEG-poly(MAA-co-BHCMM). The degree of esterification of coumarin was calculated by comparing the peaks assigned to aromatic groups (7.79, 6.79 and 6.20 ppm) with those assigned to methylene groups in PEG blocks (3.5 ppm).

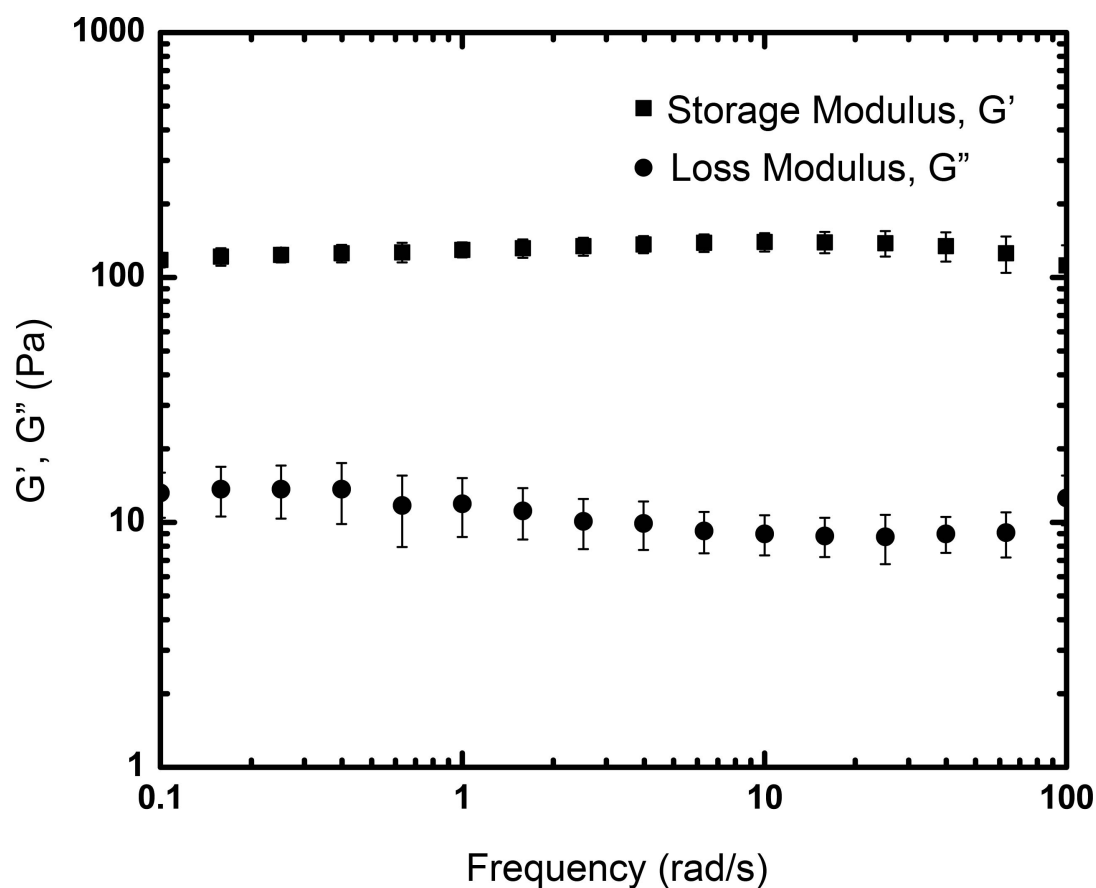


Figure S2. Representative rheology data from poly(MAA-co-BHCMM)-PEG-poly(MAA-co-BHCMM) hydrogels. Values for both the storage (G' ; 139.72 ± 19.3 Pa) and loss (G'' ; 12.24 ± 1.3 Pa) modulus were both relatively frequency-independent in the measured range (0.1 to 100 rad s^{-1}) at strain amplitudes of 0.5%. These data indicate that the self-assembled hydrogels are elastic and solid-like.

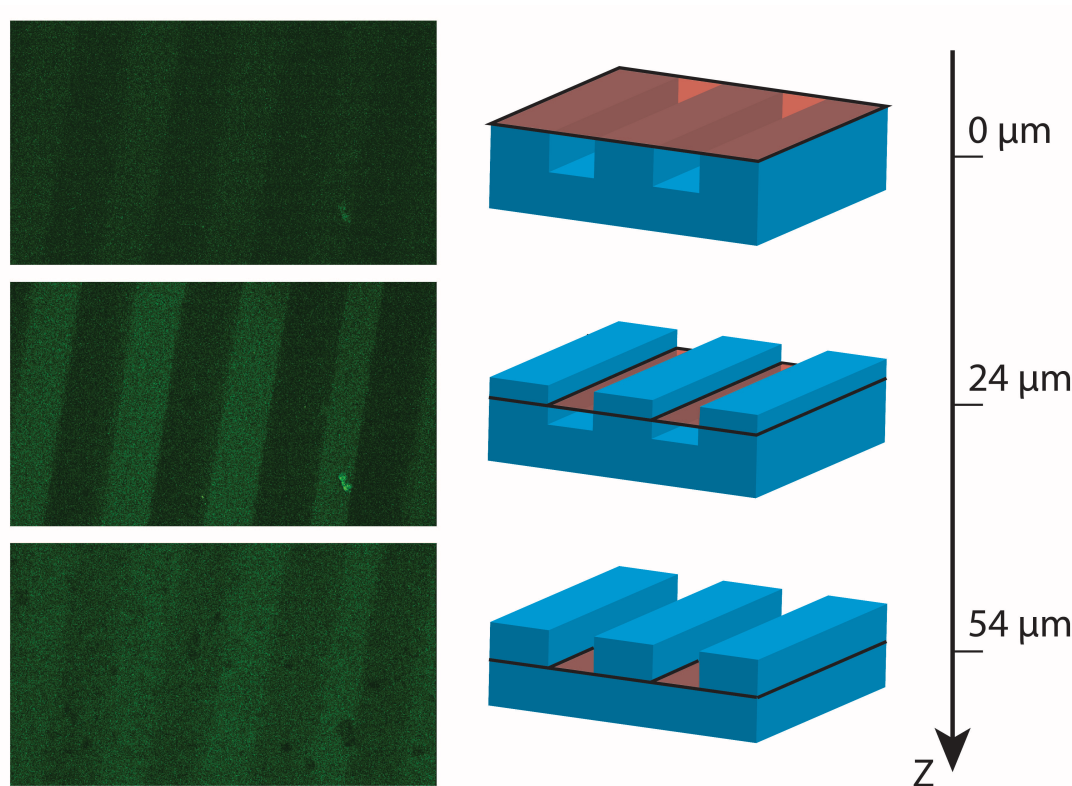


Figure S3. Estimation of UV penetration depth based on Z-stack images recorded using two-photon laser scanning microscopy. Exposing the apical surface of hydrogels for 15 min of continuous UV exposure produces features heights of approximately 50 μm . Photomasks were used to define microstructures in the x-y plane. Fiducial microstructures provide in-plane contrast and permit accurate measurements of feature heights. Representative images were taken at $z = 0, 24$, and $54 \mu\text{m}$, which corresponds to the top surface, feature mid-line, and trough of the microstructure, respectively.

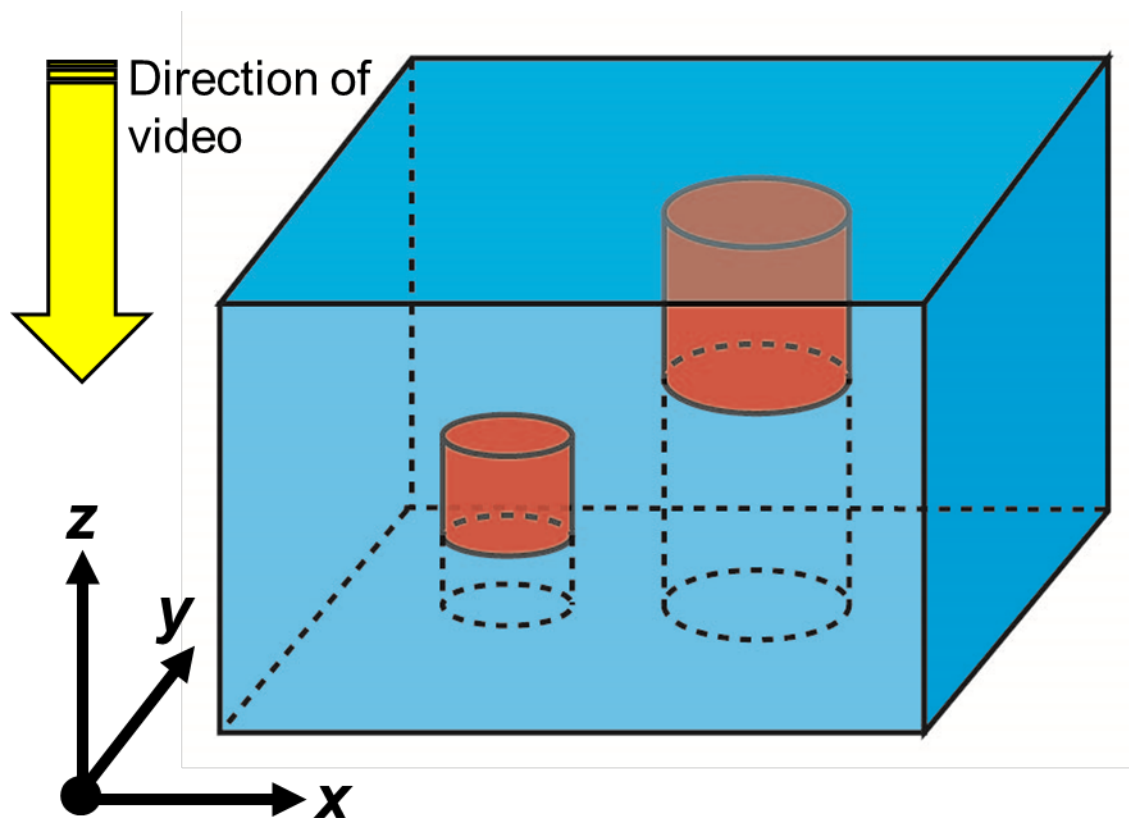


Figure S4. Schematic illustration of 3D cylinder shape fabrication within the hydrogel using two-photon uncaging. Z-stack optical images are shown in **Video S2**.

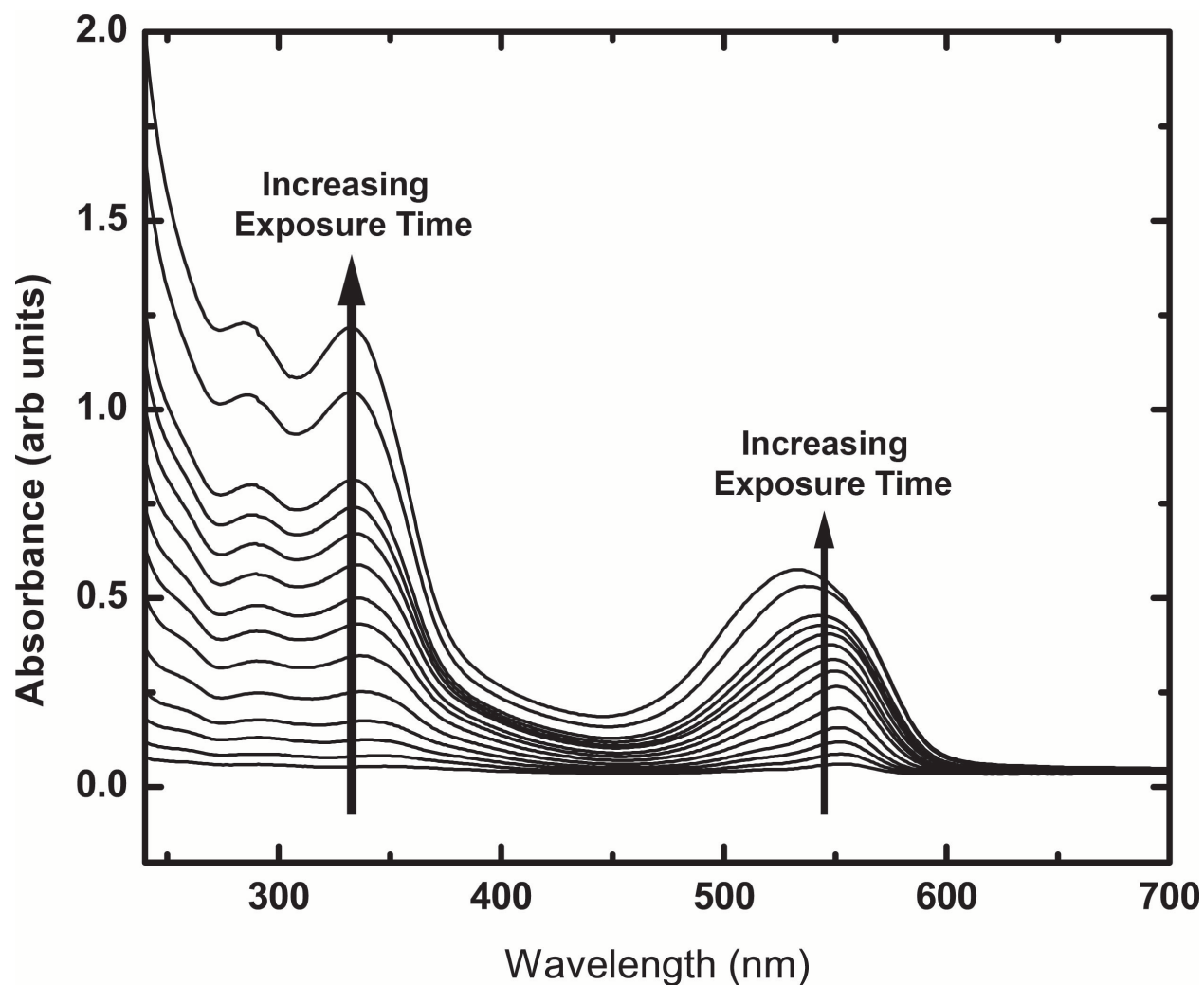


Figure S5. UV-vis absorption spectra of external water solutions after UV exposure as a function of irradiation time. Rhodamine B release is evident by the increasing intensity of absorbance peak at 540 nm. The absorbance peak at 330 nm is attributed to the release of free hydroxycoumarin which is generated as a by-product of light-induced uncaging.

Video Captions

Video S1. Time-lapse video of disintegration of a voxel within a three-dimensional hydrogel. Micrographs were recorded every 1 sec for over 50 sec. Scale bar represents 100 μm .

Video S2. Z-stack fly-through of three-dimensional microstructures that are embedded within a physically crosslinked hydrogel network. The direction of the video path is shown in Figure S4. Scale bar represents 100 μm .