Online Supporting Information

Reversible Hydrogel Dynamics by Physical-Chemical Crosslink Photoswitching using a Supramolecular Macrocycle Template

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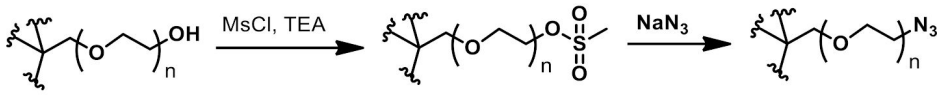
Contents of Supporting Information:

1- Synthetic and Experimental Methods

1.1 Synthesis of 4armPEG10k-azide

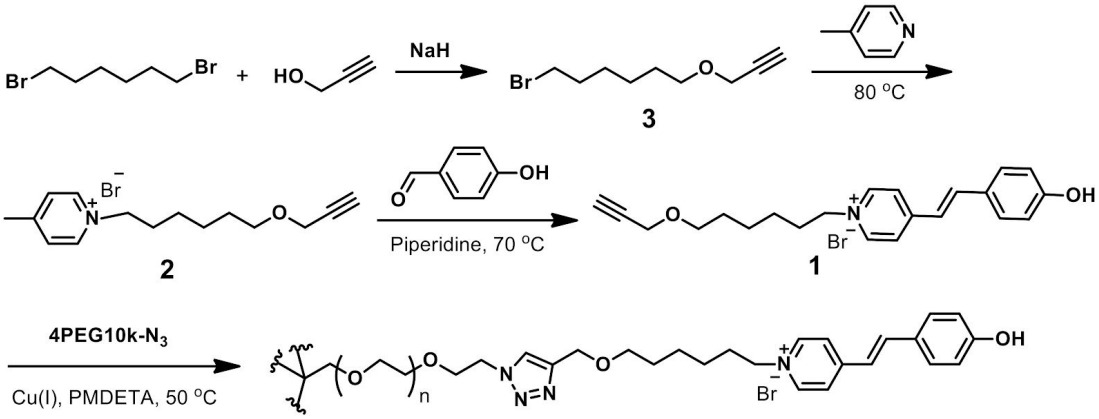


4armPEG10k-Ms **4armPEG10k-N_3**

*Synthesis of 4 arm PEG terminated with mesylate (**4armPEG10k-Ms**)* - In a dry round-bottom flask, hydroxyl-terminated 4-arm PEG (M_n = 9560, 6.31 g, *Creative PEGWorks*) was dissolved in 50 mL DCM with triethylamine (3.50 mL). The solution was cooled to 0 °C in an ice bath and methanesulfonyl chloride (MsCl, 1.9 mL, *Beantown Chemical*) was added slowly. The flask was then removed from the ice bath and the reaction mixture was stirred for 24 h at room temperature. After quenching with a small volume of water, the reaction mixture was diluted into 200 mL of DCM and washed with brine three times (200 mL each). The organic layer was dried over anhydrous Na_2SO_4 and evaporated under reduced pressure into a small volume which was precipitated into cold diethyl ether. The product was obtained as a colorless powder (6.30 g, 97% yield). ^{1}H-NMR (500 MHz Bruker, 25 °C, $CDCl_3$, ***Figure S1***): δ (ppm) = 4.37 (m, 2H), 3.83-3.43 (m, PEG chain), 3.40 (s, 2H), 3.07 (s, 3H).

*Synthesis of 4 arm PEG terminated with azido group (**4armPEG10k-N_3**)* - Sodium azide (0.52 g) and **4armPEG10k-Ms** (2.00 g) were dissolved in DMF (15 mL) and stirred at 60 °C for 2 days. The reaction mixture was then diluted into 200 mL of DCM and washed with brine three times (200 mL each). The organic layer was dried over anhydrous Na_2SO_4 and evaporated under reduced pressure into a small volume which was precipitated into cold diethyl ether. The product was obtained as a colorless powder (1.98 g, 100% yield). ^{1}H-NMR (500 MHz Bruker, 25 °C, $CDCl_3$, ***Figure S1***): δ (ppm) = 3.80-3.40 (m, PEG chain), 3.40 (s, 2H), 3.39 (t, 2H).

1.2 Synthesis of 4-arm PEG terminated with Brooker's Merocyanine (PEG_{4a}-BM)



PEG4a-BM

*Synthesis of compound **3*** - Sodium hydride (2.00 g, 60% dispersion in mineral oil, *Beantown Chemical*) was slowly added to a solution of propargyl alcohol (2.24 g, 99%, *Alfa Aesar*) in dry THF (50 mL). The mixture was stirred for 20 min at room temperature. Then,

1,6-dibromohexane (29.28 g) was added and the reaction mixture was stirred at room temperature for 24 h. After quenching with a small volume of water, the reaction mixture was transferred to a 50 mL centrifuge tube. The supernatant was collected by centrifuge and evaporated under reduced pressure. The residue was purified on a silica column with elution by a mixture of hexane/ethyl acetate (20:1, v/v). The target product was obtained as colorless oil (4.90 g, 56% yield). ^{1}H-NMR (500 MHz Bruker, 25 °C, $CDCl_3$, ***Figure S2***): *δ* (ppm) = 4.13 (d, *J* = 2.4 Hz, 2H), 3.52 (t, *J* = 6.6 Hz, 2H), 3.41 (t, *J* = 6.8 Hz, 2H), 2.42 (t, *J* = 2.4 Hz, 1H), 1.87 (m, 2H), 1.61 (m, 2H), 1.43 (m, 4H).

*Synthesis of compound **2*** - 4-methylpyridine (1.50 g, 98%, *Alfa Aesar*) and compound **3** (2.18 g) were dissolved in isopropyl alcohol (10 mL) and stirred at 80°C for 12 h. The reaction mixture was evaporated under reduced pressure and precipitated into cold diethyl ether. The solid (2.87 g, 92% yield) was collected by filtration and washed two times with cold diethyl ether. ^{1}H-NMR (500 MHz Bruker, 25 °C, $CDCl_3$, ***Figure S2***): *δ* (ppm) = 9.30 (d, *J* = 6.7 Hz, 2H), 7.85 (d, *J* = 6.7 Hz, 2H), 4.92 (t, *J* = 7.4 Hz, 2H), 4.10 (d, *J* = 2.4 Hz, 2H), 3.47 (t, *J* = 6.3 Hz, 2H), 2.67 (s, 3H), 2.44 (t, *J* = 2.4 Hz, 1H), 2.01 (m, 2H), 1.56 (m, 2H), 1.40 (m, 4H).

*Synthesis of compound **1*** - *4*-hydroxybenzaldehyde (1.22 g, 98%, *Beantown Chemical*), piperidine (0.2 mL) and compound **2** (2.87 g) were dissolved in isopropyl alcohol (15 mL) and stirred at 70 °C for 18 h. Upon cooling, a sticky red precipitate formed in the reaction mixture. The precipitate was collected by centrifuge in a 50 mL centrifuge tube and washed several times with isopropyl alcohol. The target product was obtained as a red powder (3.00 g, 78% yield). ^{1}H-NMR (500 MHz Bruker, 25 °C, D_2O, ***Figure S3***): *δ* (ppm) = 8.45 (d, *J* = 5.7 Hz, 2H), 7.87 (d, *J* = 5.7 Hz, 2H), 7.66 (d, *J* = 16.0 Hz, 1H), 7.55 (d, *J* = 7.8 Hz, 2H), 7.05 (d, *J* = 16.0 Hz, 1H), 6.85 (d, *J* = 7.8 Hz, 2H), 4.37 (t, *J* = 7.0 Hz, 2H), 4.13 (s, 2H), 3.54 (t, *J* = 6.4 Hz, 2H), 2.81 (s, 1H), 1.93 (m, 2H), 1.55 (m, 2H), 1.33 (m, 4H).

*Synthesis of **PEG_{4a}-BM*** - Compound **1** (52 mg), **4armPEG10k-N_3** (306 mg), copper(II) sulfate pentahydrate ($CuSO_4 \cdot 5H_2O$, 3.0 mg, *BDH, ACS grade*) and *N,N,N',N'',N''*-Pentamethyldiethylenetriamine (PMDETA, 98%, 2.5 uL, Acros) were dissolved in DMF (10 mL) in a Schlenk flask. The flask was degassed with three freeze-pump-thaw cycles. On the last cycle, the flask was opened to quickly add sodium ascorbate (20 mg) into the flask before re-capping the flask. The flask was vacuumed and backfilled with N_2 over 5 cycles before immersion in a 50 °C oil bath to thaw the solution and initiate the 'click' reaction. After 72 h, the reaction was quenched by exposure to air. The reaction mixture was diluted with 20 mL of DCM and passed through a short Al_2O_3 column. The resulting liquid was was evaporated under reduced pressure, following which the solution was precipitated into cold diethyl ether. The product was obtained as red fine powder (0.34 g, 95% yield). ^{1}H-NMR (500 MHz Bruker, 25 °C, D_2O, ***Figure S3***): *δ* (ppm) = 8.46 (m, 2H), 8.00 (s, 1H), 7.89 (m, 2H), 7.70 (m, 1H), 7.56 (m, 2H), 7.05 (m, 1H), 6.82 (m, 2H), 4.57 (m, 4H), 4.38 (m, 2H), 3.90-3.49 (m, PEG chain), 3.46 (s, 2H), 1.91 (m, 2H), 1.53 (m, 2H), 1.29 (m, 4H).

1.3 Synthesis of linear PEG terminated with Brooker's Merocyanine (PEG_{1a}-BM)

PEG_{1a}-BM was synthesized according to identical methods as described for **PEG_{4a}-BM**. Briefly, mono-methoxy PEG (M_n = 2000, *Creative PEGWorks*) was first converted to a mesylate intermediate, and then to an azide. Subsequently, a 'click' reaction was performed with compound **1** as described for the 4-arm variant. The product was obtained as red fine powder (90% yield). ^{1}H-NMR (500 MHz Bruker, 25 ^{o}C, D_2O, ***Figure S4***): δ (ppm) = 8.51 (d, J = 5.6 Hz, 2H), 8.01 (s, 1H), 7.93 (d, J = 5.6 Hz, 2H), 7.73 (d, J = 16.2 Hz, 1H), 7.61 (d, J = 8.2 Hz, 2H), 7.13 (d, J = 5.6 Hz, 1H), 6.89 (d, J = 8.0 Hz, 2H), 4.57 (m, 4H), 4.41 (m, 2H), 3.92-3.46 (m, PEG chain), 3.34 (s, 3H), 1.93 (m, 2H), 1.53 (m, 2H), 1.29 (m, 4H).

1.4 Synthesis of Cucurbit[8]uril

Cucurbit[8]uril (**CB[8]**) was prepared according to literature reports,[1,2] with minor modifications. Briefly, the crude mixture of CB variants that results from a standard synthesis was precipitated into a large amount of water to reduce the final HCl concentration to 5% and obtain an insoluble fraction that mainly consisted of cucurbit[6]uril (CB[6]) and **CB[8]**. Subsequently, CB[6] was removed through serial extractions with 50% formic acid/water to leave an insoluble solid fraction that was primarily **CB[8]**. Pure **CB[8]** was then obtained by recrystallization with hot 37% HCl in water.

1.5 Preparation of Supramolecular Hydrogels

CB[8] (6.4 mg) and **PEG_{4a}-BM** (27.4 mg) were fully dissolved in water to form a 2 wt% viscous solution. The solution was lyophilized to ensure homogenous mixing, and resuspended at 5 wt% in water. The resulting hydrogels were maintained overnight in the dark to ensure homogeneity prior to mechanical testing or exposure to light to facilitate transformation.

1.6 Transformation of Hydrogel by UV-light Irradiation

Supramolecular hydrogels were placed into a mold prepared by punching an 8 mm hole into a 3.2 mm silicone rubber sheet and sandwiching this between two quartz microscope slides. To induce photodimerization, the gel was irradiated with 365-nm UV (~5 mW/cm^2) light for 4 hours using a Newport Corporation UV-IR light source equipped with 150 W xenon (Xe) arc lamp and UG1 colored glass bandpass filter (50.8 x 50.8 mm). The reverse process was achieved by irradiating with 254-nm UV (~5 mW/cm^2) for 12 hours using an ULTRA-LUM UVC 515 Ultraviolet Multilinker.

1.7 Rheological and Bulk Mechanical Testing

Mechanical properties of the hydrogels were studied using TA Instruments discovery HR-2 rheometer fitted with a Peltier stage set to 25 ^{o}C. Oscillatory frequency sweep measurements from 0.1 rad/s to 200 rad/s were conducted at 10% strain. Step stress relaxation experiments were conducted at 10% strain amplitude and the testing duration was up to 1000 s. All measurements were performed using an 8 mm parallel plate. Self-healing experiments were

conducted on hydrogels within sealed 2 ml glass vials. A blade was used to make a full-thickness cut through 200 µL hydrogels. The cut was monitored visually to determine the time it took for full defect healing. Swelling experiments were also conducted in 2 ml glass vials. with 200 µL hydrogels prepared in the bottom of the vial and 600 µL of DI water carefully layered over the top. Samples were left to sit undisturbed and monitored for gel swelling and dissipation. At 72 hours, vials were inverted to determine whether gels maintained their self-supporting character.

1.8 ^{1}H-NMR Time Studies

Solution of **PEG_{1a}-BM** (11.4 mM, 2.2 wt.%) and **CB[8]** (5.7 mM) in D_2O were irradiated with UV light and monitored serially with increased exposure time. ^{1}H-NMR spectra were acquired on a Bruker 500 MHz spectrometer at 25 °C.

1.9 UV-Vis Spectroscopy

Solutions of **PEG_{1a}-BM** (0.114 mM, 0.022 wt.%) and **CB[8]** (0.057 mM) were combined in a quartz vial (path length: 1 cm) and irradiated according to the procedures and with the equipment described for the hydrogel studies. At serial exposure times, UV/Vis absorption spectra were obtained using an Agilent Cary 60 UV/Vis with Cary WinUV scan application software. The absorbance peak data vs. time was fit to a standard first-order reaction rate equation (Graphpad Prism) to obtain the rate constant and reaction half-life for both the forward (dimerizing) and reverse (monomerizing) reaction.

2- Supporting Data and Characterization

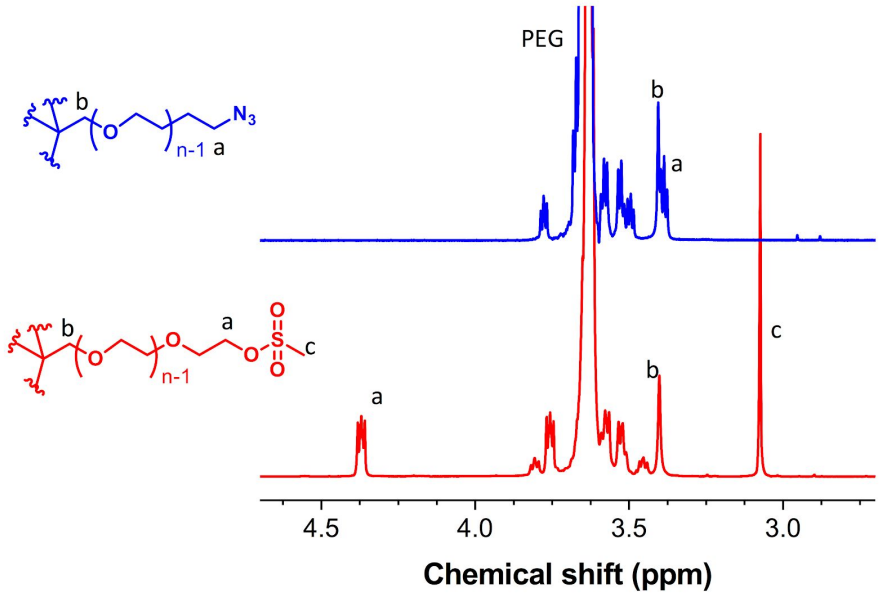


Figure S1: ^{1}H-NMR (500 MHz, 25 ºC, $CDCl_3$) spectra for mesylate-modified (bottom, red) and azide-modified (top, blue) 4-arm PEG used in the synthesis of the BM-modified PEG macromer.

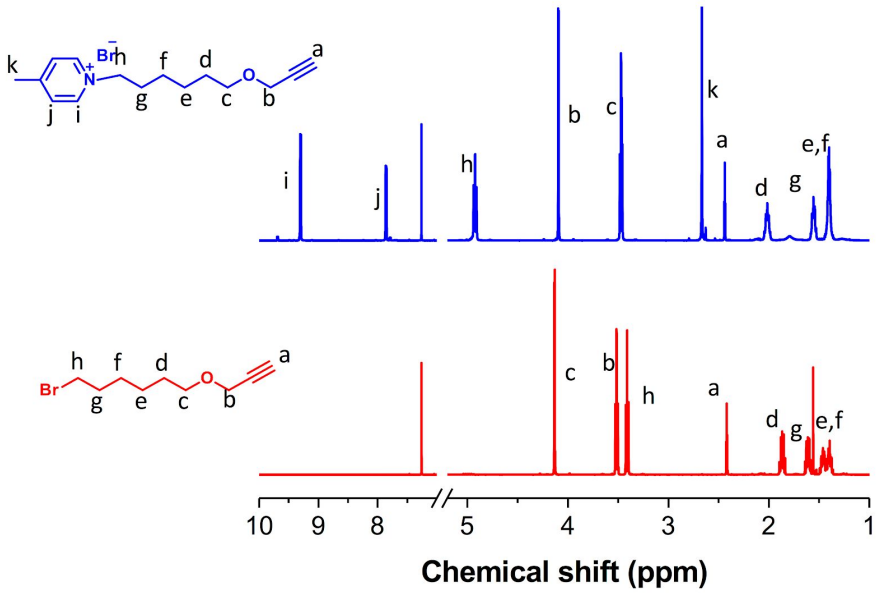


Figure S2: ^{1}H-NMR (500 MHz, 25 ºC, $CDCl_3$) spectra for compound **3** (bottom, red) and compound **2** (top, blue) precursors used in the synthesis of the BM-modified PEG macromer.

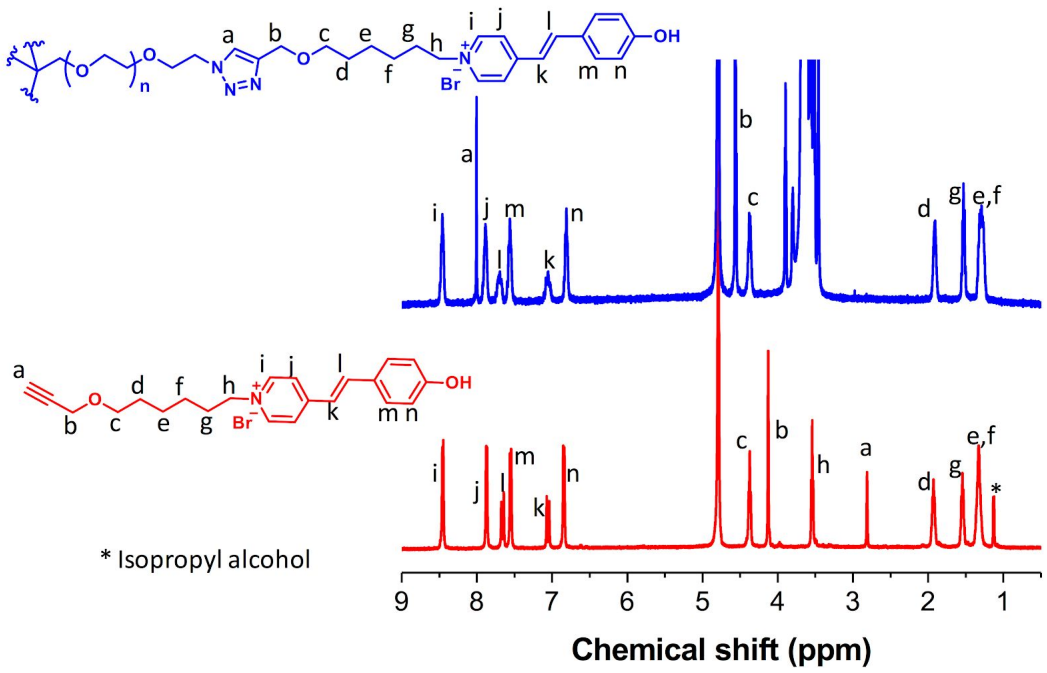


Figure S3: ^{1}H-NMR (500 MHz, 25 °C, D_2O) spectra for compound **1** (bottom, red) and the final macromer product, **PEG_{4a}-BM** (top, blue).

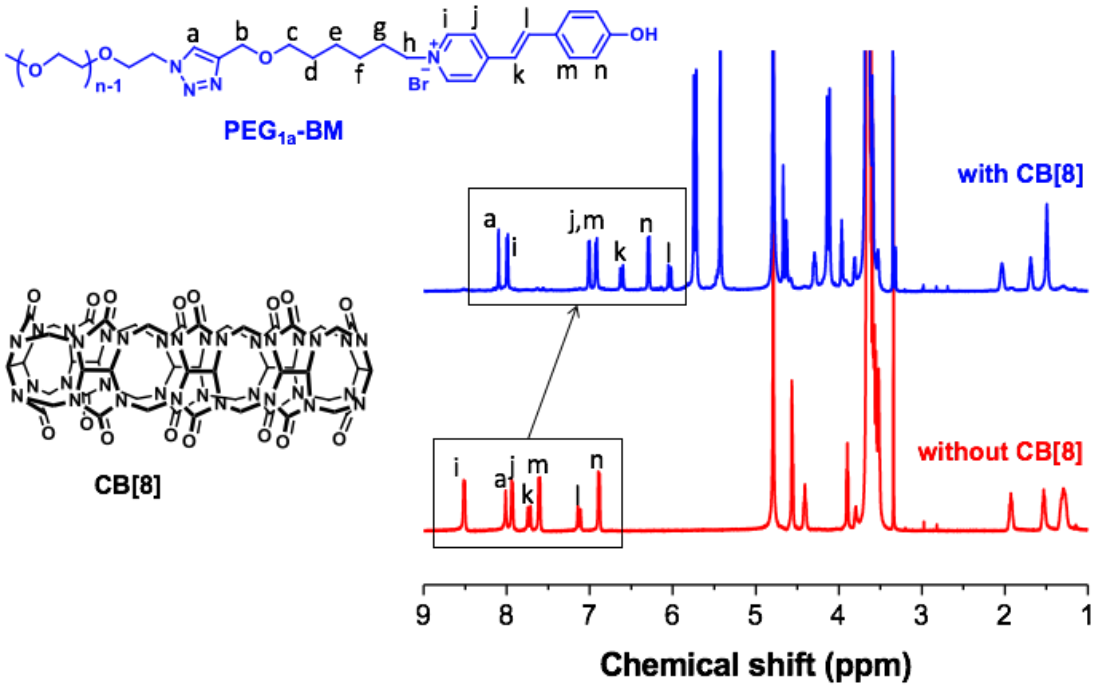


Figure S4: ^{1}H-NMR (500 MHz, 25 °C, D_2O) spectra for **PEG_{1a}-BM** (1 wt% in water) without addition of **CB[8]** (bottom, red) and after addition of **CB[8]** (top, blue), with box denoting shift in CB[8]-included protons.

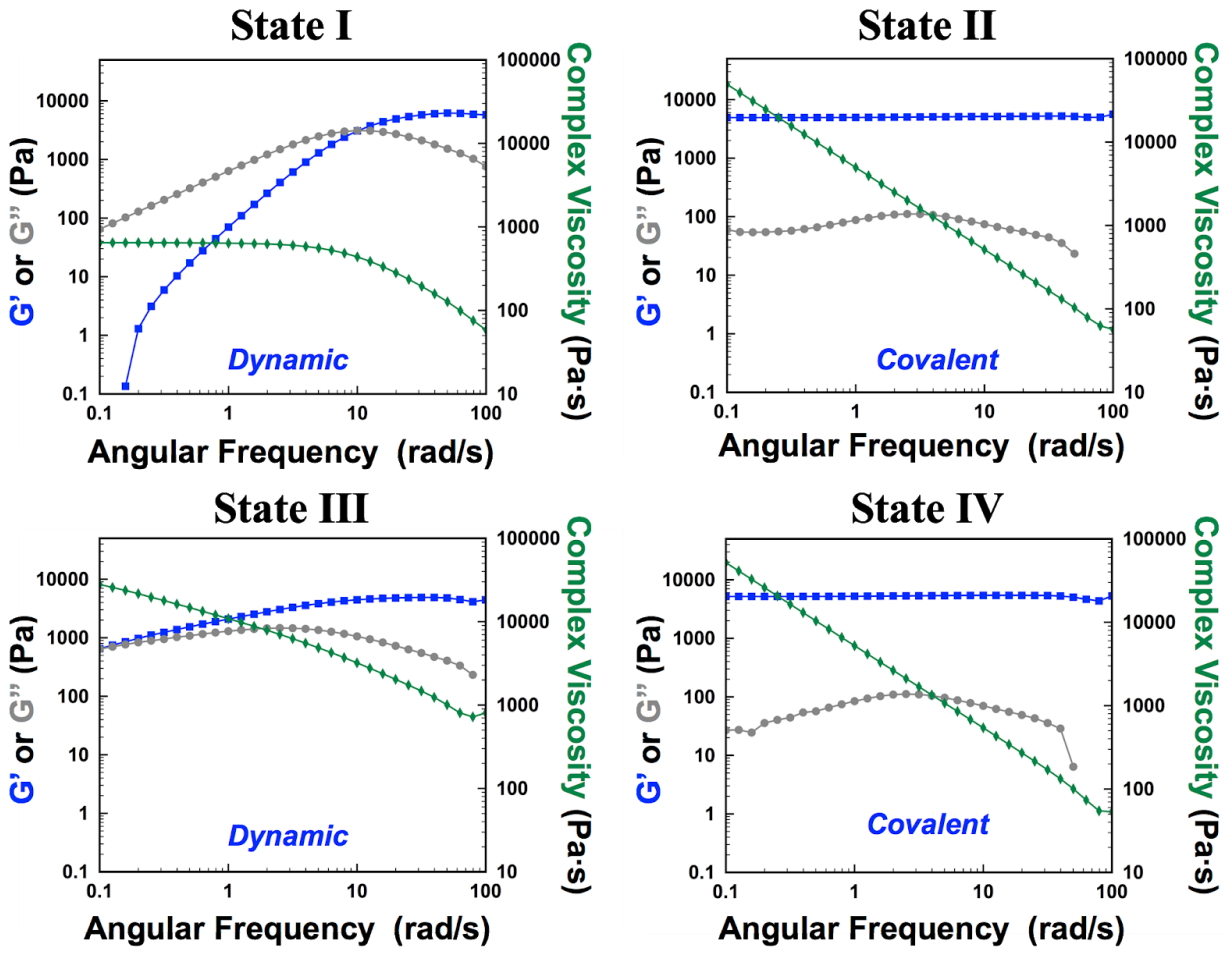
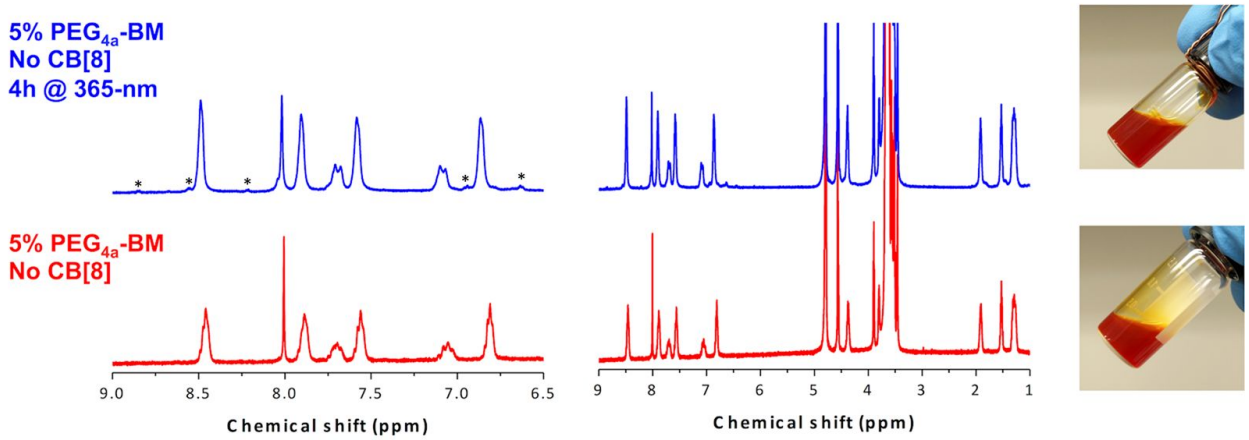


Figure S5: Dynamic osciallatory rheology for hydrogels of all states showing the storage modulus (G', blue), loss modulus (G'', gray) and complex viscosity (green).



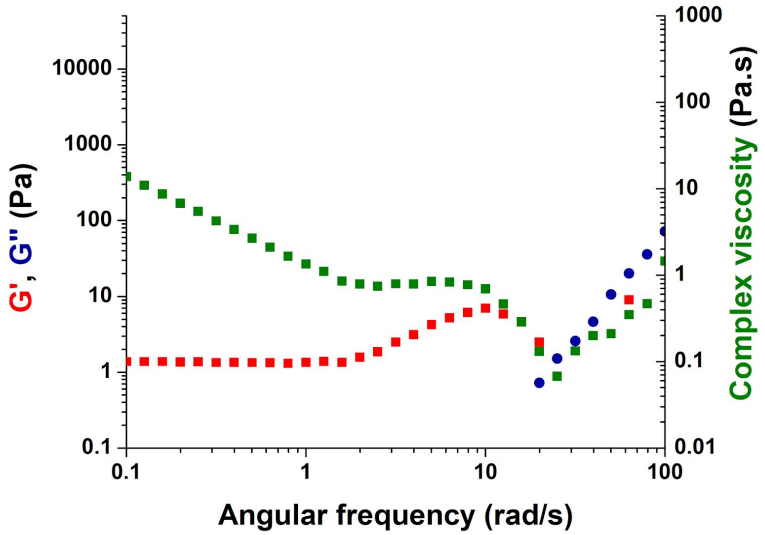


Figure S6: ^{1}H-NMR (500 MHz, 25 °C, D_2O) of **PEG_{4a}-BM** without the addition of CB[8] before and after 365-nm irradiation along with pictures of the 4-arm macromer solution before and after irradiation. Solutions were measured by rheology, with no evidence of crosslinking or increase in viscosity following irradiation.

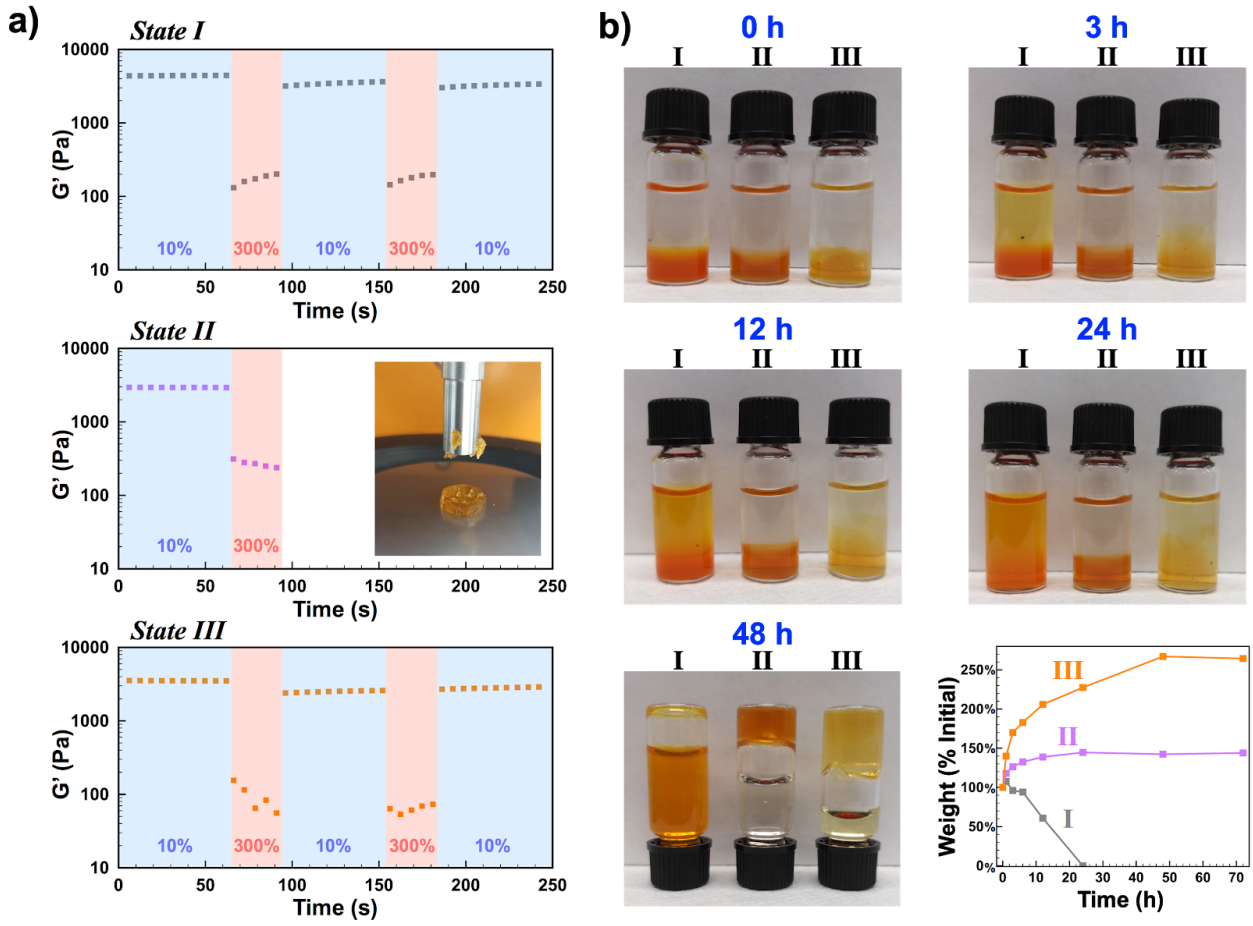


Figure S7: The type and extent of crosslinking dictates the bulk dynamic hydrogel properties. (a) Step-strain rheology alternating between 10% staring and 300% strain for physically crosslinked hydrogels (State I), chemically crosslinked hydrogels (State II), or hydrogels with crosslinks reversed by exposure to 254-nm irradiation (State III). The inset in State II data shows mechanical destruction of the hydrogel at high strain. (b) Hydrogel swelling and dissipation determined by bathing pre-formed hydrogels in water and measuring weight change of the hydrogel as a function of their initial weight, as well as assessing hydrogel stability though vial inversion.

Figure S8: Self-healing studies for hydrogels of State I, II, and III after cutting hydrogel disks and maintaining these in contact at the bottom of a scintillation vial for time indicated in the inset.

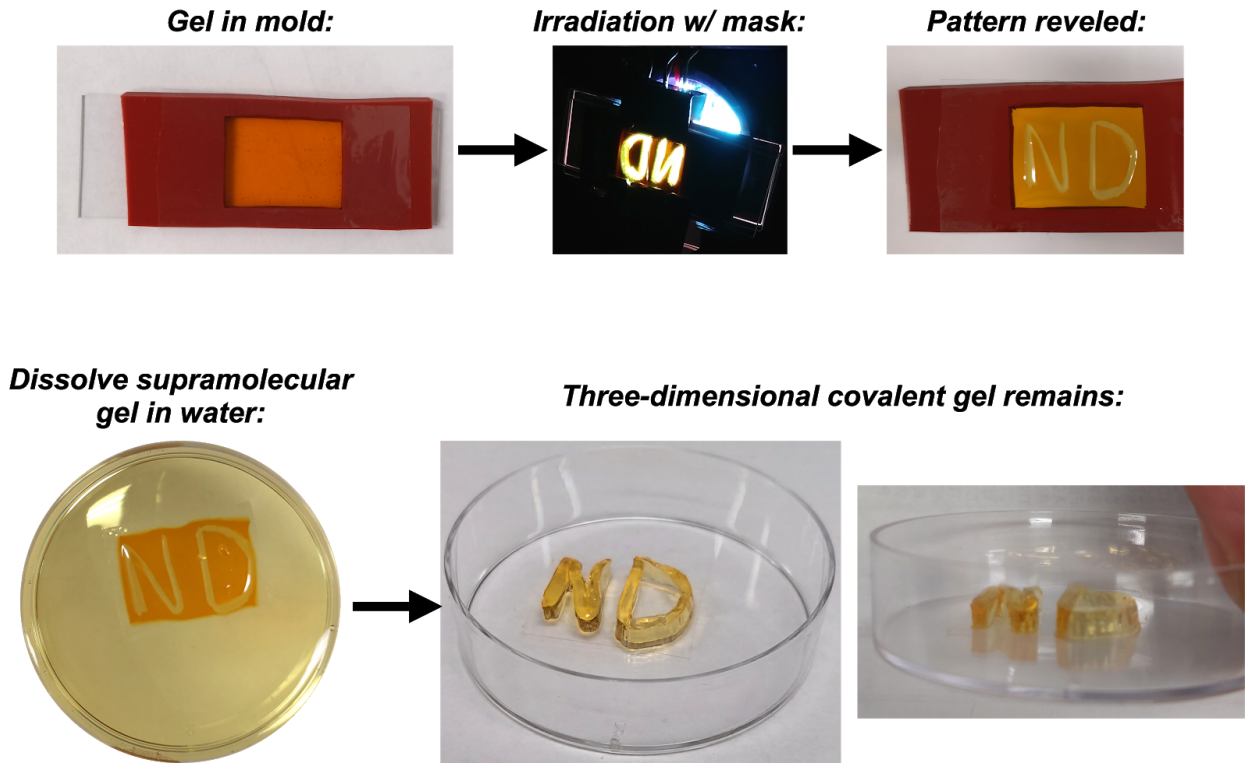


Figure S9: Irradiation of the hydrogel using a mask enables 3-dimensional features to be patterned in the hydrogel. Subsequently placing the hydrogel in a bath of water dissolves the supramolecular network leaving behind a three-dimensional hydrogel with the desired pattern.

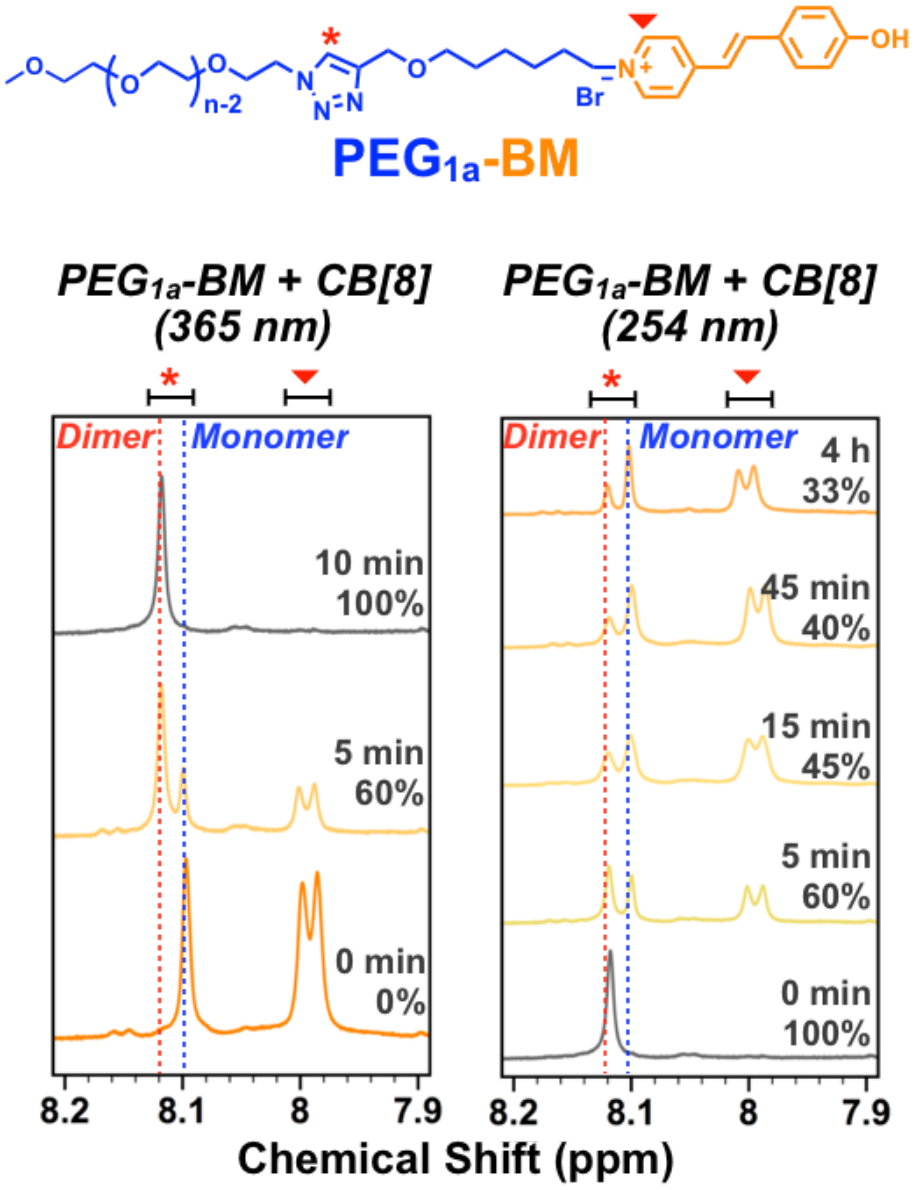


Figure S10: Structure of PEG_{1a}-BM, denoting the protons monitored to determine dimer/monomer content and ^{1}H-NMR used to track the conversion of monomeric PEG_{1a}-BM to dimer upon 365 nm irradiation (left) and reversal of dimer formation upon 254 nm irradiation (right), with the percent dimer shown for each time.

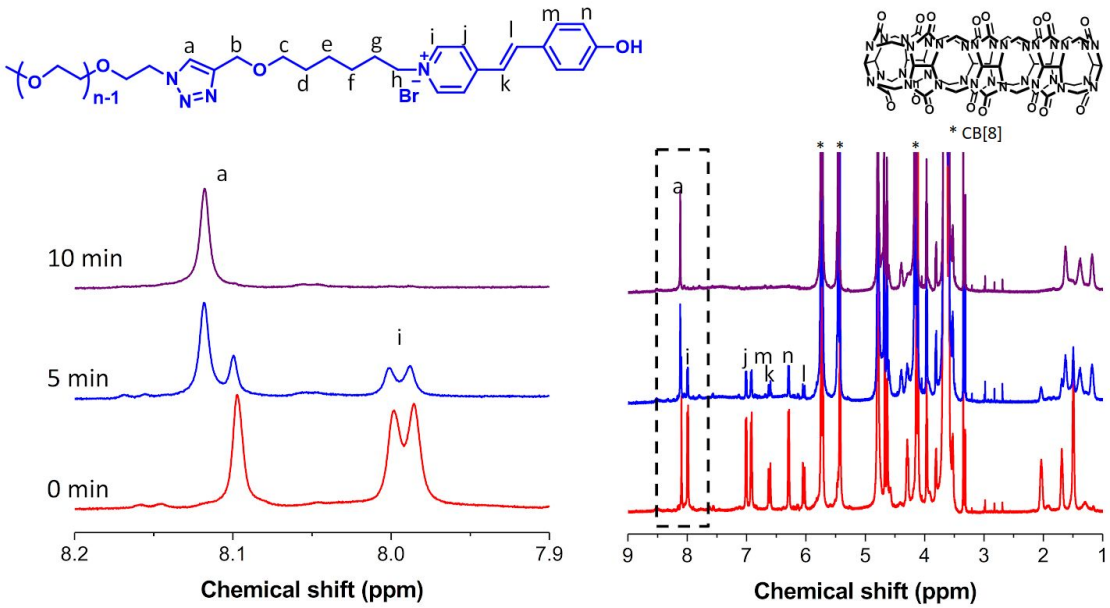


Figure S11: ^{1}H-NMR (500 MHz, 25 °C, D_2O) time-course study for **PEG_{1a}-BM** and **CB[8]** exposed to 365-nm irradiation with the full spectra shown (right) as well as a zoom-in on peaks corresponding to the BM monomer and dimer formation. The seeming disappearance of many guest-associated protons (i, j, k, l, m, n) aligns with previous observation for the dramatic broadening of guest peaks upon photodimerization within CB[8].[3]

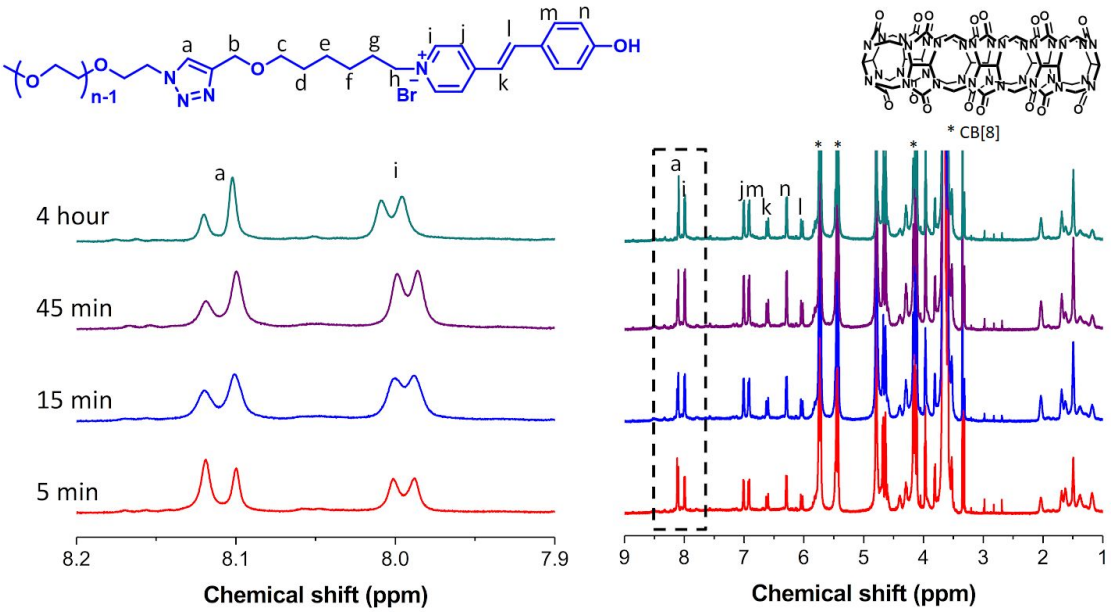


Figure S12: ^{1}H-NMR (500 MHz, 25 °C, D_2O) timecourse study for **PEG_{1a}-BM** and **CB[8]** exposed to 254-nm irradiation with the full spectra shown (right) as well as a zoom-in on peaks corresponding to the BM monomer and dimer. Guest protons that disappear upon dimerization (i, j, k, l, m, n) re-emerge upon monomer formation. 0-minute spectra not shown as it is identical to 10 min spectra from Figure S7.

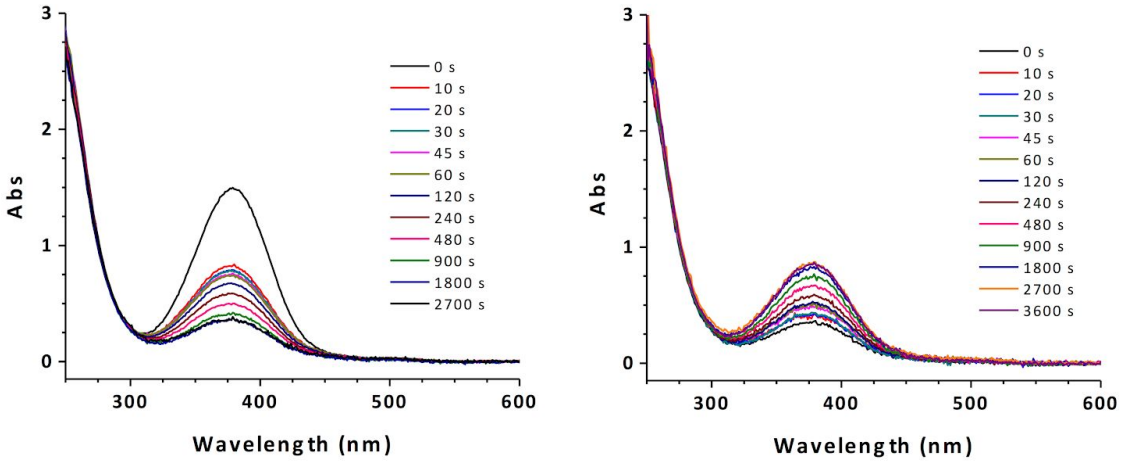


Figure S13: UV/Vis of **PEG_{1a}-BM** (0.114 mM) with sub-stoichiometric (10%) **CB[8]** (0.0057 mM) exposed to 365-nm irradiation (left) followed by 254-nm irradiation (right).

3- Supplemental References:

[1] J. Kim, I.-S. Jung, S.-Y. Kim, E. Lee, J.-K. Kang, S. Sakamoto, K. Yamaguchi, K. Kim, *J. Am. Chem. Soc.* **2000**, *122*, 540–541.
[2] D. Bardelang, K. A. Udachin, D. M. Leek, J. C. Margeson, G. Chan, C. I. Ratcliffe, J. A. Ripmeester, *Cryst. Growth Des.* **2011**, *11*, 5598–5614.
[3] S. P. Gromov, A. I. Vedernikov, L. G. Kuz'mina, D. V. Kondratuk, S. K. Sazonov, Y. A. Strelenko, M. V. Alfimov, J. A. K. Howard, *Eur. J. Org. Chem.* **2010**, *2010*, 2587–2599.