Supplementary Information

Red-Light Induced Folding of Single Polymer Chains

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Contents

1 General

1.1 1D NMR Spectroscopy Measurements

^{1}H- and ^{13}C-spectra were recorded on a *Bruker* Avance III HD 600 MHz spectrometer, equipped with a BBO-Probe (5 mm) with z-gradient (^{1}H 600.13 MHz, ^{13}C: 150.90 MHz). All measurements were carried out in deuterated solvents. The chemical shift (δ) is reported in parts per million (ppm) relative to the residual solvent protons. The measured coupling constants were calculated in Hertz (Hz). MESTRENOVA 11.0 software was used to analyze the spectra. The signals were designated as follows: s = singlet, bs = broad singlet, d = doublet, t = triplet, dd = doublet of doublets, q=quartet and m = multiplet.

1.2 UV-Vis Spectroscopy

UV-vis absorbance spectra were recorded on a *Shimadzu* UV-2700 spectrophotometer equipped with a CPS-100 electronic temperature control cell positioner. Samples were measured at ambient temperature in *Hellma Analytics* quartz high precision cells with a path length of 10 mm.

## 1.3 Size Exclusion Chromatography

The SEC measurements were conducted on a *PSS* SECurity^2 system consisting of a *PSS* SECurity Degasser, *PSS* SECurity TCC6000 Column Oven (35 °C), *PSS* SDV Column Set (8x150 mm 5 µm Precolumn, 8x300 mm 5 µm Analytical Columns, 100000 Å, 1000 Å and 100 Å) and an *Agilent* 1260 Infinity Isocratic Pump, *Agilent* 1260 Infinity Standard Autosampler, *Agilent* 1260 Infinity Diode Array and Multiple Wavelength Detector (A: 254 nm, B: 360 nm), *Agilent* 1260 Infinity Refractive Index Detector (35 °C). HPLC grade THF, stabilized with BHT, is used as eluent at a flow rate of 1 mL·min^{-1}. Narrow disperse linear poly(styrene) (M_n: 266 g·mol^{-1} to 2.52x10^6 g·mol^{-1}) and poly(methyl methacrylate) (M_n: 202 g·mol^{-1} to 2.2x10^6 g·mol^{-1}) standards (*PSS* ReadyCal) were used as calibrants. All samples were passed over 0.22 µm PTFE membrane filters. Molecular weight and dispersity analysis was performed in *PSS* WinGPC UniChrom software (version 8.2).

1.4 LC-MS Measurements[1]

LC-MS measurements were performed on an UltiMate 3000 UHPLC system (Dionex, Sunnyvale, CA, USA) consisting of a pump (LPG 3400SZ, autosampler WPS 3000TSL) and a temperature-controlled column compartment (TCC 3000). Separation was performed on a C18 HPLC-column (Phenomenex Luna 5µm, 100 Å, 250 × 2.0 mm) operating at 40 °C. A gradient of ACN:H2O 10:90 → 80:20 v/v (additive 10 mmol L^{-1} NH$_4$CH$_3$CO$_2$) at a flow rate of 0.20 mL min^{-1} during 15 min was used as the eluent. The flow was split in a 9:1 ratio, where 90 % (0.18 mL min^{-1}) of the eluent was directed through the UV-detector (VWD 3400, Dionex, detector wavelengths 215, 254, 280, 360 nm) and 10 % (0.02 mL min^{-1}) was infused into the electrospray source. Spectra were recorded on a LTQ Orbitrap Elite mass spectrometer (Thermo Fisher Scientific, San Jose, CA, USA) equipped with an HESI II probe. The instrument was calibrated in the *m/z* range 74-1822 using premixed calibration solutions (Thermo Scientific). A constant spray voltage of 3.5 kV, a dimensionless sheath gas and a dimensionless auxiliary gas flow rate of 5 and 2 were applied, respectively. The capillary temperature was set to 300 °C, the S-lens RF level was set to 68, and the aux gas heater temperature was set to 125 °C.

1.5 Tunable Laser Studies[2]

All laser experiments were conducted using the apparatus shown in **Figure S1**. The light source was an Opotek Opolette 355 OPO, producing 7 ns, 20 Hz pulses. The output beam was initially passed through a beam expander (-50 mm and 100 mm lens combination) to ensure it was large enough to uniformly irradiate the entire sample volume. The beam then passed through an electronic shutter and was directed upwards using a UV silica right angle prism. Finally, the beam entered the sample, suspended in an aluminum block, from below. The laser energy deposited into the sample was measured above the aluminum block before and after experiments using a Coherent EnergyMax thermopile sensor (J-25MB-LE) to account for any power fluctuations during irradiation.
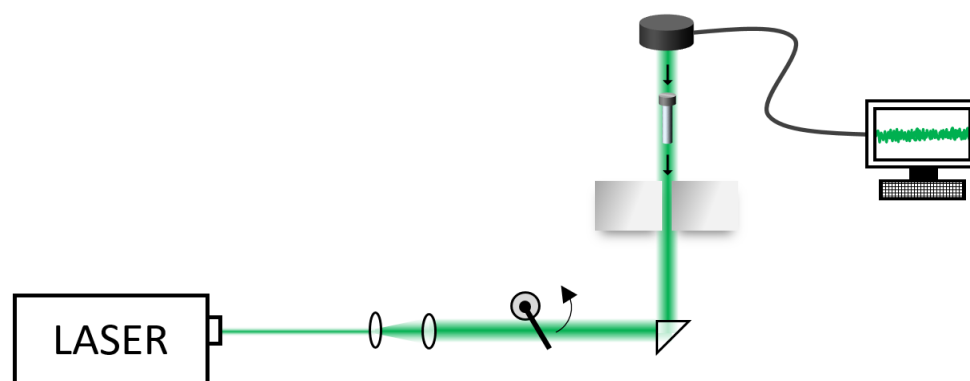
Figure S1. Schematic diagram of apparatus used for laser experiments.
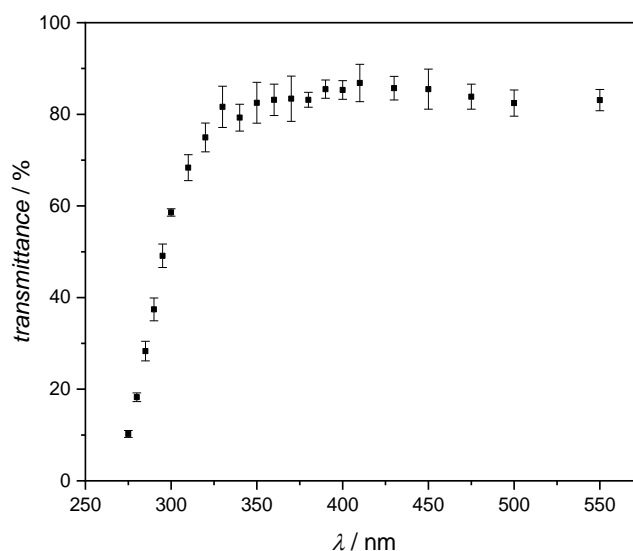
Figure S2. Transmittance of the bottom of the glass vials used in this study. The transmittance values shown and used here were obtained analogously to a method reported previously.[3] The glass vials were cut at a height of 3 mm.

For laser measurements, all samples were prepared in a 0.7 mL glass crimp vials (ID 6.2 mm) capped with a rubber/PTFE septum. The wavelength dependent glass transmittance, essential for quantitative

measurements, is presented in **Figure S2**. Precise photons numbers were determined from the laser pulse energy using the following relation

$$N_p = \frac{E_{pulse} \lambda f_{rep} t}{hc \, [T_\lambda/100]} \tag{1}$$

where E_{pulse} is the measured pulse energy above the aluminum block, λ is the wavelength of the incident radiation, f_{rep} is the laser repetition rate, t is the irradiation time, h is Planck's constant, c is the speed of light and T_λ is the wavelength dependent glass transmittance presented in **Figure S2.** Once an initial measurement was completed and the photon number was known, the required energies at other wavelengths can be found by rearranging Equation 1 to give

$$E_{pulse} = \frac{N_p hc [T_\lambda/100]}{\lambda f_{rep} t} \tag{2}$$
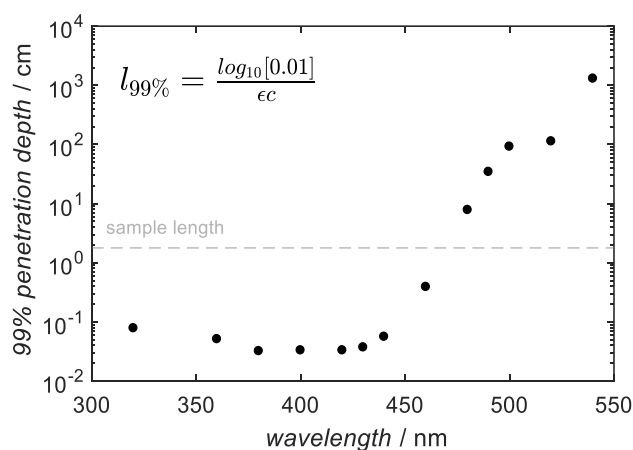
Figure S3. Wavelength dependent penetration depth, at which 99% of the incident light is absorbed. Calculated using the molar absorptivities at each wavelength investigated and a sample concentration of 1 mmol L^{-1}.

1.6 Emission Spectrum of 625 nm LED
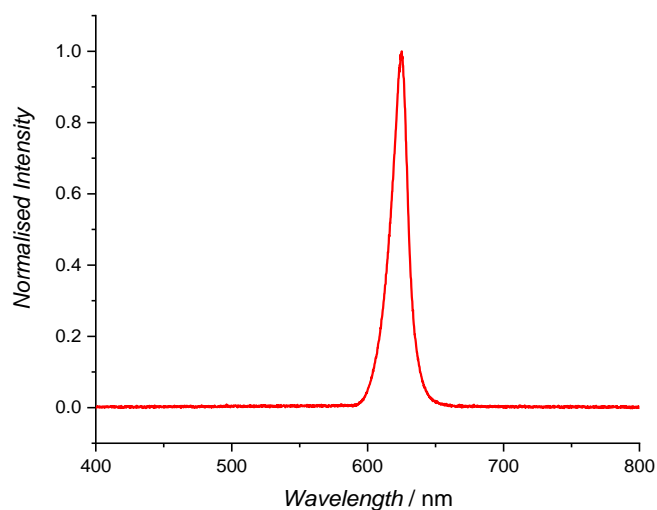
Figure S4. Emission spectrum of the commercially available 625 nm LED (10 W) from *ELE Doctor* used for irradiation experiments.

1.7 Kinetic Data from UV-Vis Spectra
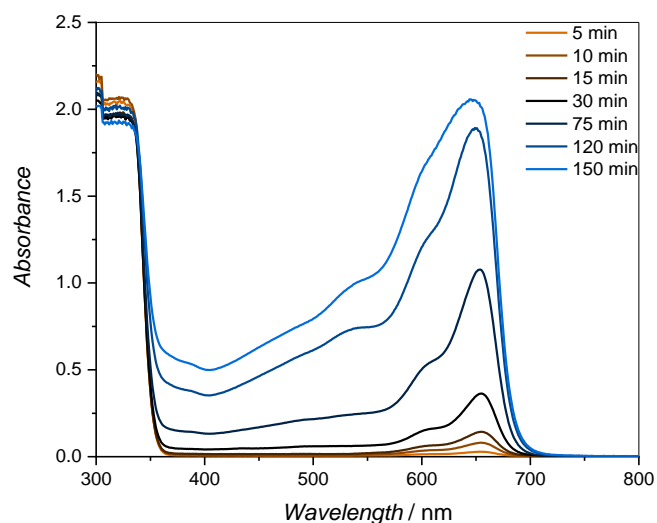
Figure S5. UV-Vis spectrum of the product mixture after irradiating **MB-MMA** with a 625 nm LED (10 W) at regular time intervals.

The irradiation experiments at λ_{max} = 625 nm and subsequent UV-vis data collection were conducted as follows: 2.03 mg of **MB-MMA** was dissolved in 4 mL ACM (1.12·mmol L^{-1}) and 2 mL of the solution were placed in a quartz cuvette before it was irradiated with a red 10W LED (λ_{max}=625 nm), and the UV-Vis absorbance was measured at regular intervals during the irradiation period.

2   Experimental Data

2.1   M1 (trichloromethyl 3,7-bis(dimethylamino)-10H-phenothiazine-10-carboxylate)
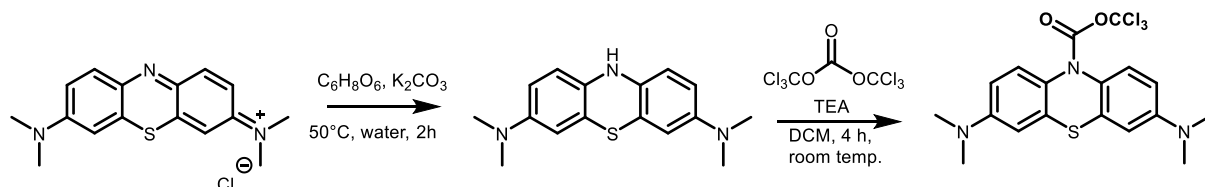

Methylene blue (2 g, 6.25 mmol) was dissolved in water (100 mL). To this solution, K_2CO_3 (3.45 g, 26 mmol) was added, follow by ascorbic acid (1.76 g, 10 mmol). The solution was stirred at 50 °C for 2 h upon which the solution turns clear brown. The mixture was extracted with dichloromethane (50 mL x 3), dry ($MgSO_4$) and concentrated to ca. 50 mL. Triphosgene (0.9 g, 3.12 mmol) and triethylamine (0.9 g, 9 mmol) were added. The solution was stirred for 4 h at ambient temperature and concentrated onto silica gel. The mixture was purified by column chromatography running with dichloromethane as the eluent, yielding a white powder as the pure product. The compound is highly hygroscopic and slowly turns blue under ambient atmosphere. It was used directly in the next step (yield= 40% (1.10 g))

2.2   MB-MMA (3-(3,7-bis(dimethylamino)-10H-phenothiazine-10-carboxamido)propyl methacrylate)
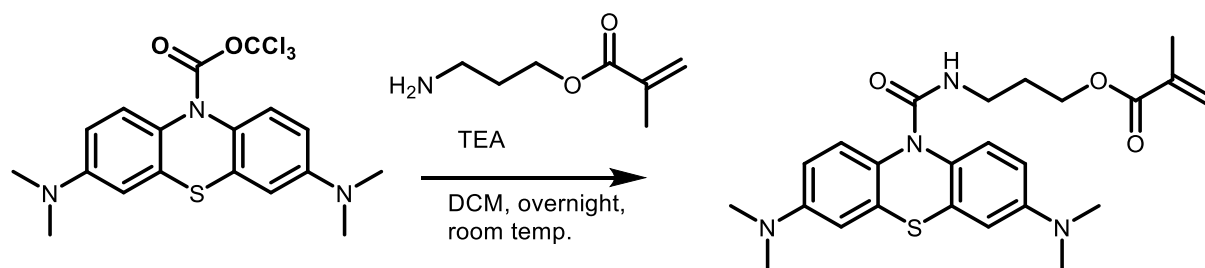
M1 was dissolved in DCM (50 mL) and to this solution was added N-(3-Aminopropyl)methacrylamide hydrochloride (0.71 g, 4 mmol), followed by triethylamine (1 g, 10 mL). The solution was stirred at ambient temperature overnight, washed with HCl 1 M (50 mL), water (50 mL), brine (50 mL), and dried (MgSO4). The crude product was purified by column chromatography eluting with DCM→DCM: MeOH (v/v = 10/0.5), to afford the product as white powder (yield= 81% (0.91 g)).

^{1}H NMR (600 MHz, Acetonitrile-d_3) δ 7.29 (d, J = 8.8 Hz, 2H), 6.96 (s, 1H), 6.75 (d, J = 2.8 Hz, 2H), 6.69 (dd, J = 8.9, 2.9 Hz, 2H), 5.64 – 5.58 (m, 2H), 5.28 (p, J = 1.6 Hz, 1H), 3.22 – 3.16 (m, 2H), 3.14 (q, J = 6.2 Hz, 2H), 2.92 (s, 12H), 1.87 (dd, J = 1.6, 1.0 Hz, 3H), 1.59 – 1.48 (m, 2H).

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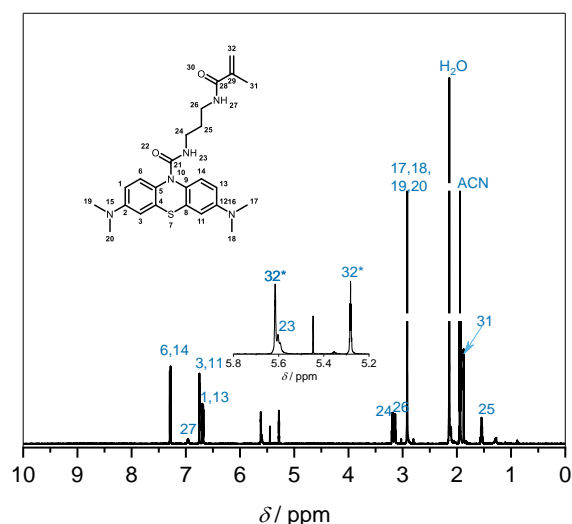
**Figure S6**. ^{1}H NMR spectrum of **MB-MMA** in deuterated ACN (600 MHz)

Table S1. Summary of experimental and theoretical m/z values for **MB-MMA** from LC-MS data

Symbol	m/z_{exp}	m/z_{theor}	Δ_{ppm}	Composition
[M +H]$^+$	454.2264	454.2271	1.54	$C_{24}H_{31}N_5O_2SH^+$
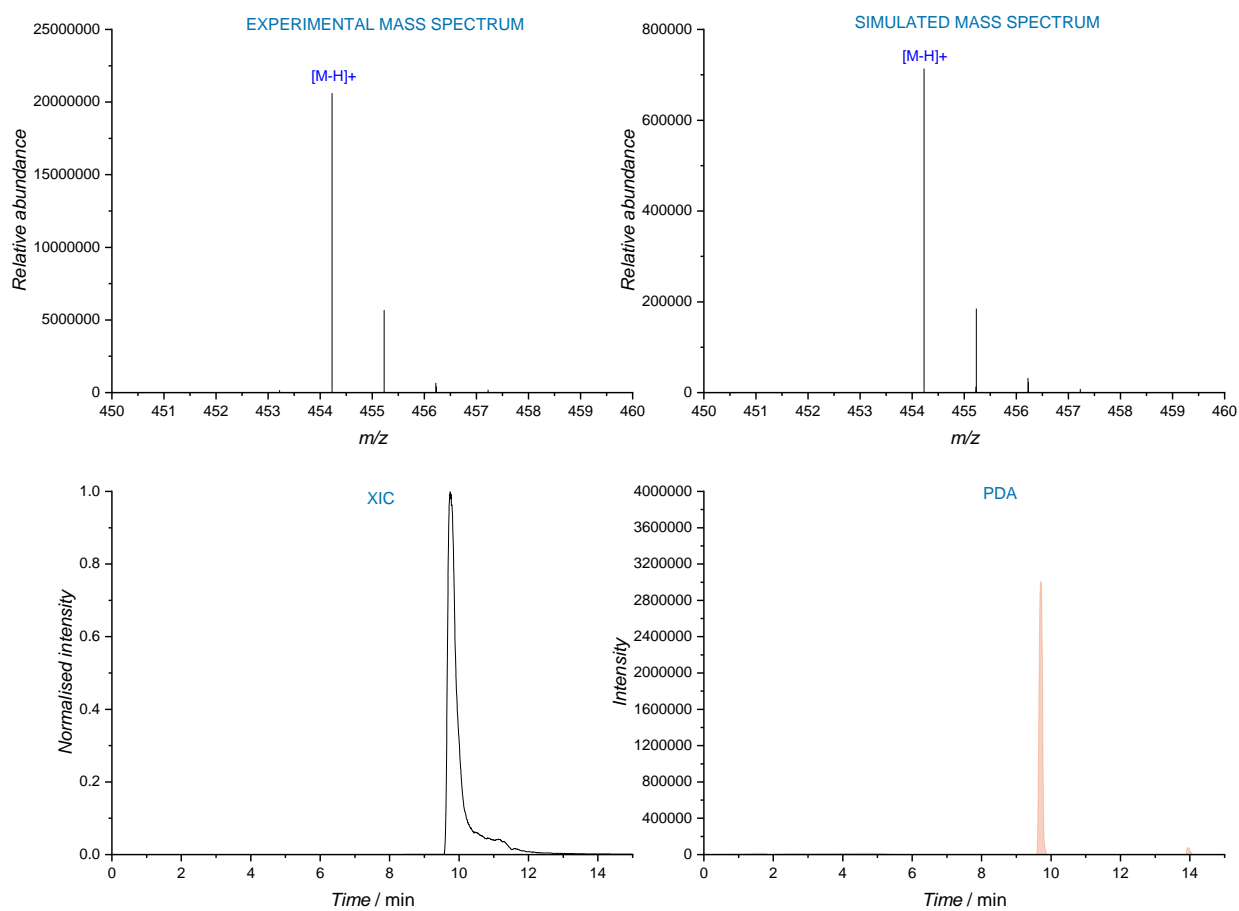
Figure S7. Experimental mass spectrum [Top left], simulated mass spectrum [top right], extracted ion chromatogram (XIC) [Bottom left] and relative absorbance intensity on the photodiode array (PDA) over time of **MB-MMA**.

2.3 P1
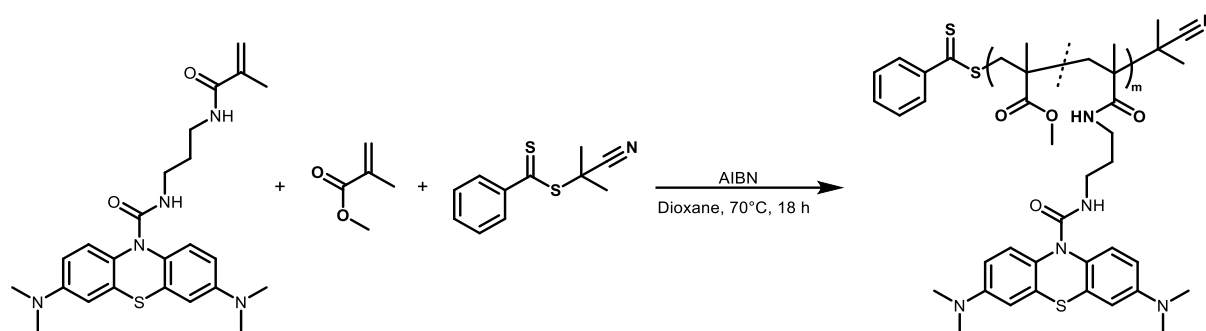
MMA was passed through a short alumina column to remove the inhibitor before being utilized in the polymerization. MMA (962 µL, 200 eq., 9 mmol), **MB-MMA** (614 mg, 30 eq., 1.36 mmol), 2-cyano-2-propyl benzodithioate (10 mg, 1 eq., 45 µmol) and AIBN (1.4 mg, 0.2 eq., 9 µmol) were dissolved in 5.5 mL dioxane and deoxygenated via sparging with argon for 20 min in a crimped vial. The mixture was subsequently heated at 70 °C for 18 h, before being quenched by exposure to ambient air. The polymer was purified by precipitation into cold methanol thrice over and drying overnight in a vacuum oven at 40°C. The theoretical M_n of **P1** is 34000 g·mol^{-1} given 100% conversion of the monomers, reacted in a feed ratio of 3:20 (**MB-MMA**:MMA). The observed monomer conversion is 58%, with **MB-MMA** contributing to approximately 9.8% of the molar mass, which equates to approx. 13 **MB-MMA** units per polymer chain.

^{1}H NMR (600 MHz, Chloroform-*d*) δ 7.38 – 7.30 (m, 2H), 6.85 (s, 1H), 6.68 (d, *J* = 40.3 Hz, 4H), 5.17 – 5.07 (m, 1H), 3.59 (s, 27H), 3.34 – 3.11 (m, 3H), 2.94 (d, *J* = 10.1 Hz, 12H), 2.10 – 1.75 (m, 20H), 1.49 – 1.35 (m, 3H), 1.02 (s, 12H), 0.84 (d, *J* = 10.9 Hz, 22H).

SEC (THF) M_n= 19.6 kg·mol^{-1} |M_w =23.5 kg·mol^{-1} | Đ=1.2
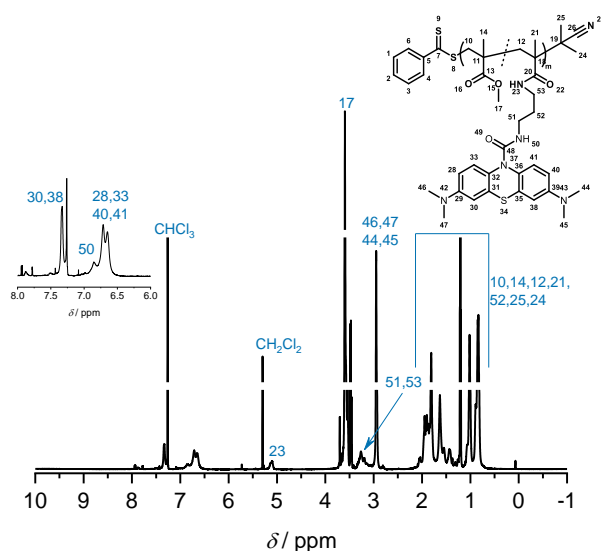
Figure S8. ^{1}H NMR spectrum of **P1** in deuterated ACN (600 MHz)

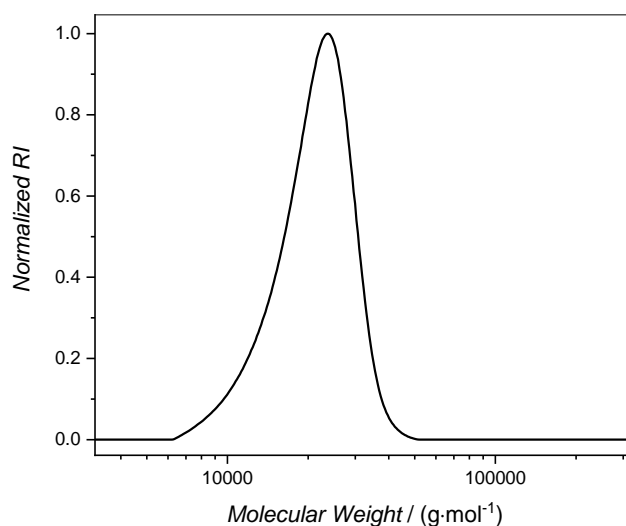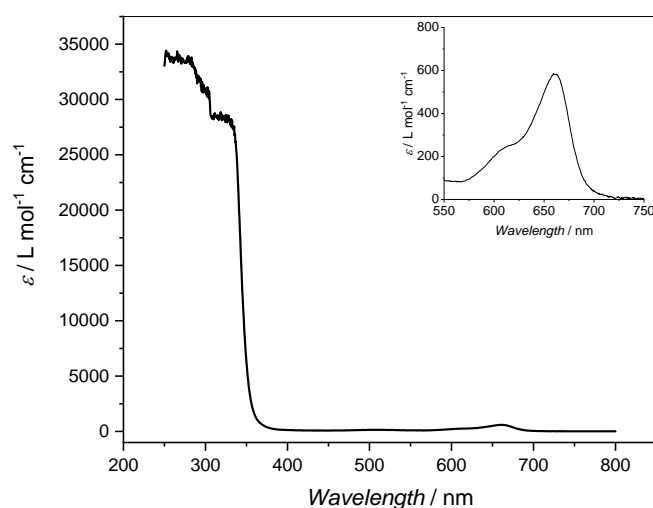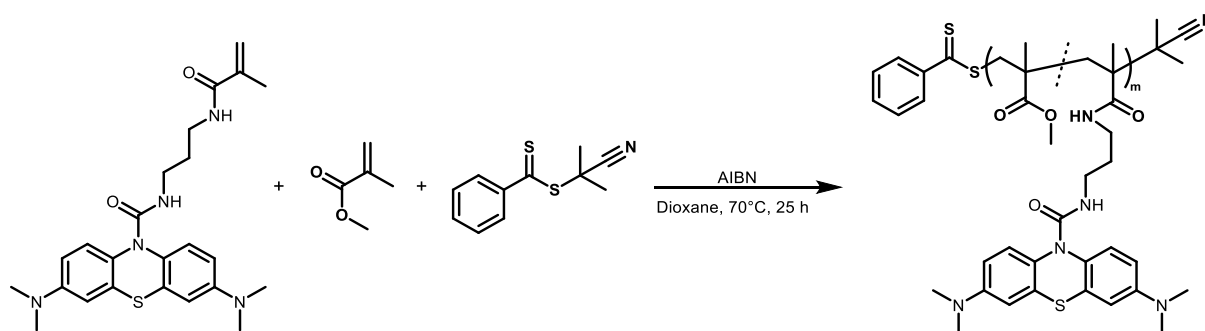**Figure S9.** SEC of **P1** in THF

Figure S10. Molar extinction coefficient of **P1** in ACN

2.4   P2

MMA was deinhibited by passing through a short alumina column before being utilized in the polymerization. MMA (385 μL, 200 eq., 3.6 mmol), **MB-MMA** (245 mg, 30 eq., 542 μmol), 2-cyano-2-propyl benzodithioate (2 mg, 0.5 eq., 9 μmol) and AIBN (0.3 mg, 0.1 eq., 2 μmol) were dissolved in

3 mL dioxane and deoxygenated via sparging with Argon for 20 minutes in a crimped vial. The mixture was subsequently heated to 70 °C and stirred for 25 h before being quenched via exposure to ambient air. The polymer was purified by precipitating in cold diethyl ether thrice over and drying overnight in a vacuum oven at 40 °C. The theoretical M_n of **P2** is 67000 g·mol^{-1} given 100% conversion of the monomers, reacted in a feed ratio of 3:20 (**MB-MMA**:MMA). The observed monomer conversion is 18%, with **MB-MMA** contributing to approximately 6.5% of the molar mass, which equates to close to 6 **MB-MMA** units per chain.

^{1}H NMR (400 MHz, Acetonitrile-d_3) δ 7.50 – 7.21 (m, 2H), 7.05 (d, J = 55.0 Hz, 1H), 6.74 (d, J = 26.3 Hz, 4H), 5.61 (s, 1H), 3.57 (s, 43H), 2.95 (s, 12H), 2.14 (s, 16H), 1.89 (s, 8H), 1.81 (s, 12H), 1.51 (s, 5H), 1.36 – 1.26 (m, 2H), 1.20 (s, 2H), 1.08 – 0.91 (m, 16H), 0.80 (s, 27H).

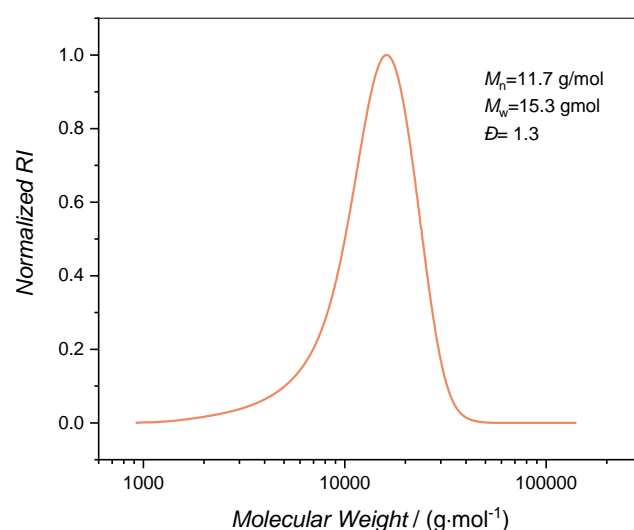SEC (THF) M_n= 11.7 kg·mol^{-1} |M_w =15.3 kg·mol^{-1} | Đ=1.3

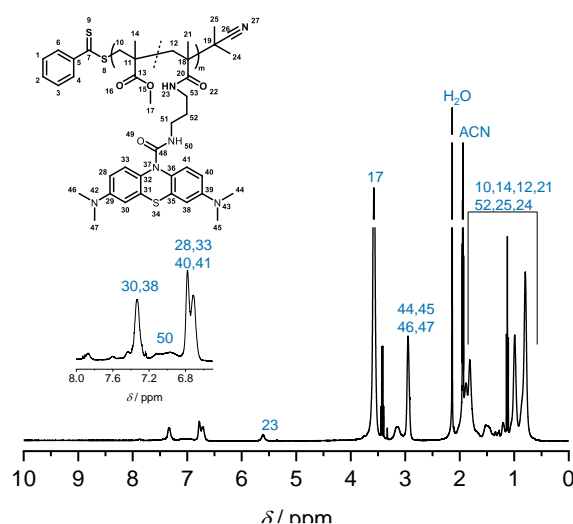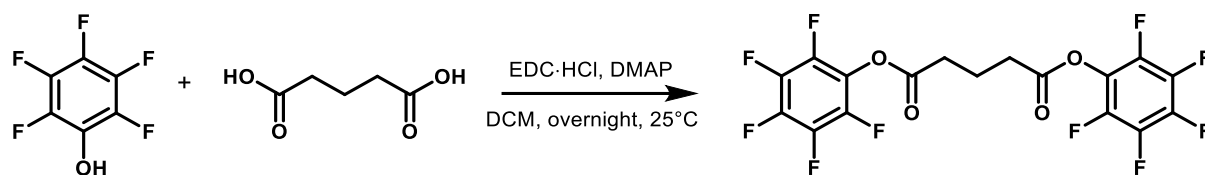**Figure S11.** SEC of **P2** in THF

Figure S12. ^{1}H NMR spectrum of **P2** in deuterated ACN (400 MHz)

2.5   M2 (PFP-glutaric acid crosslinker)

Pentafluorophenol (2.93 g, 2.1 eq., 15.9 mmol), glutaric acid (1.0 g, 1eq., 7.57 mmol) and 4-dimethylaminopyridine (185 mg, 0.2 eq., 1.51 mmol) were weighed into a round bottom flask and dissolved in 50 mL DCM. The reaction mixture was cooled while stirring in an ice bath and 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (3.19 g, 2.20 eq., 16.65 mmol) was added to the chilled mixture. The mixture was allowed to stir overnight and reach room temp gradually.

The mixture was subsequently washed twice with saturated sodium bicarbonate solution and once with saturated brine solution and dried over anhydrous sodium sulfate. The remaining organic solvent was removed under reduced pressure and the product was isolated as a white powder. (Yield=2.72 g, 77%).

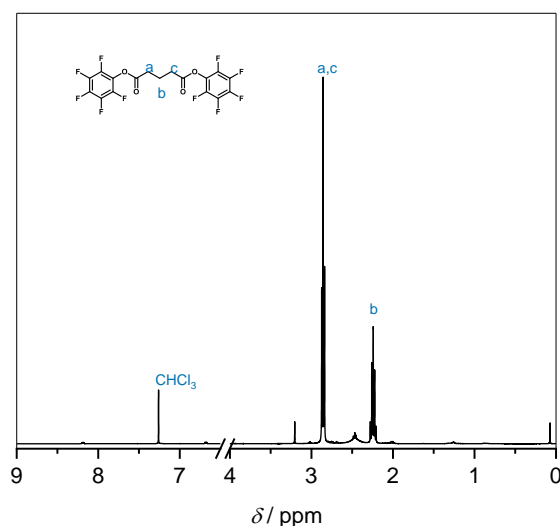^{1}H NMR (600 MHz, Acetonitrile-d_3) δ 2.86 (t, J = 7.4 Hz, 4H), 2.13 (p, J = 7.4 Hz, 2H).

^{1}H NMR (400 MHz, Chloroform-d) δ 2.86 (t, J = 7.2 Hz, 4H), 2.24 (p, J = 7.2 Hz, 2H).[4]

^{19}F NMR (565 MHz, Acetonitrile-d_3) δ -154.73 − -154.92 (m), -160.38 (t, J = 21.0 Hz), -164.52 − -164.74 (m).
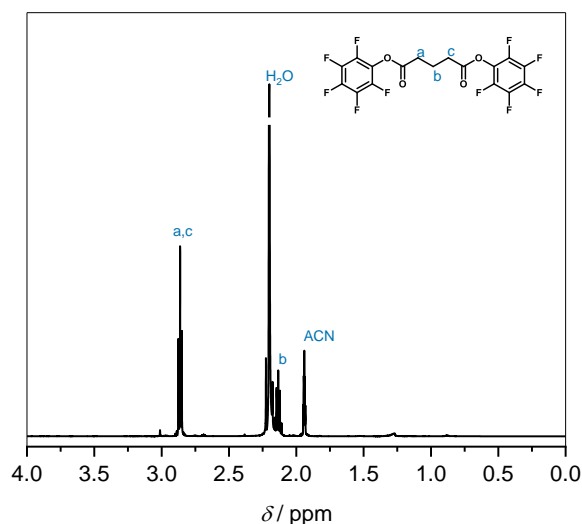
Figure S13. ^{1}H NMR spectrum of **M2** in deuterated ACN (600 MHz)

Figure S14. ^{1}H NMR spectrum of **M2** in deuterated Chloroform (400 MHz)
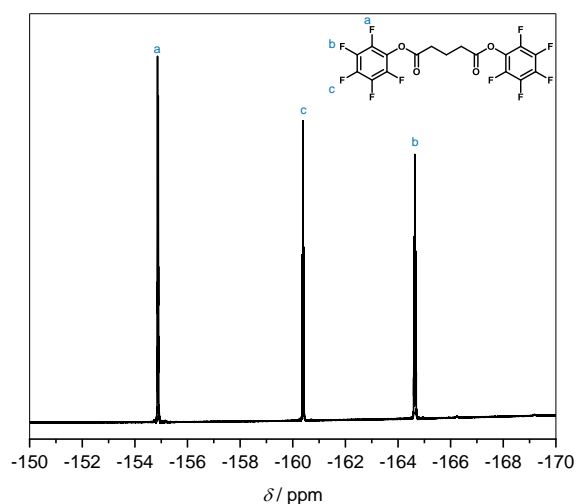
Figure S15. ^{19}F NMR spectrum of **M2** in deuterated ACN (600 MHz)

2.6 Crosslinker test reaction
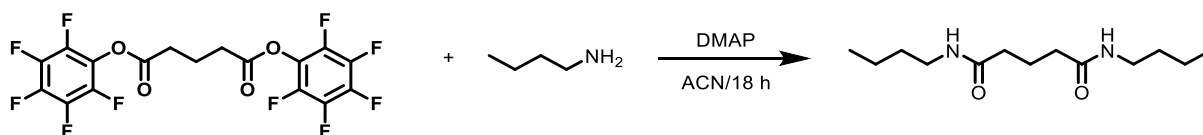
M2 (6.0 mg, 1 eq., 12.9 µmol), butylamine (2.55 µL, 2.0 eq., 25.6 µmol) and 4-dimethylaminopyridine (0.3 mg, 0.2 eq., 2.6 µmol) were dissolved in 3 mL acetonitrile and allowed to stir overnight at ambient temperature.

^{1}H NMR (600 MHz, Acetonitrile-d_3) δ 6.36 (s, 2H), 3.19 − 3.03 (m, 6H), 2.91 − 2.81 (m, 1H), 2.09 (t, J = 7.4 Hz, 4H), 1.81 − 1.74 (m, 2H), 1.62 − 1.53 (m, 1H), 1.47 − 1.38 (m, 4H), 1.38 − 1.27 (m, 5H), 0.91 (td, J = 7.3, 6.3 Hz, 7H).

Figure S16. ^{1}H NMR spectrum of N^1,N^5-dibutylglutaramide in deuterated ACN (600 MHz)

3   Irradiation experiments

3.1   Photocleavage of MB-MMA

0.61 mg of **MB-MMA** was dissolved in 4 mL ACN and irradiated with a red-light LED (λ_{max}=625 nm) for 3 hours. The product mixture was then analyzed via LC-MS and compared to the starting material.

Table S2. Summary of the experimental and theoretical *m/z* values of **MB-MMA** and its photodegradation products

Symbol	m/z_{exp}	m/z_{theor}	Δ_{ppm}	Composition
[PP1 +H]$^+$	143.1179	143.1177	1.40	$C_7H_{14}N_2OH^+$
[PP2]$^+$	284.1212	284.1216	1.41	$C_{16}H_{18}N_3S^+$
[PP3]$^+$	270.1055	270.1059	1.48	$C_{15}H_{16}N_3S^+$

Figure S17. Mass spectrum of the photodegradation products of **MB-MMA**

The general reaction for the photodeprotection of the polymers and subsequent intramolecular crosslinking to form SCNPs is as follows:
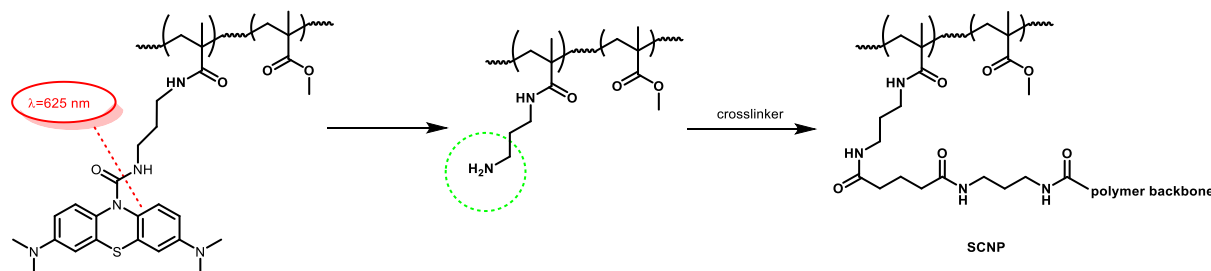
All samples utilized the red-light LED described in section 1.6 and were irradiated in 20 mL clear glass headspace vials (LLG-Headspace Crimp Neck Vial 22.5x75.5mm, clear, flat bottom, flat DIN crimp neck) while placed at a distance of 1 cm from the LED.

3.2   P1'': Folding of P1 at 0.25 mg mL^{-1} (13.9 µmol·dm^{-3})

P1 (1.5 mg, 0.08 µmol, 1 eq.), **M2** (1.5 mg, 3.3 µmol, 39 eq.) and 4-dimethylaminopyridine (0.1 mg, 0.8 µmol, 10 eq.) were dissolved in 6 mL acetonitrile and subsequently irradiated with a red light LED (λ_{max}=625 nm) for 1.5 h (placed 1 cm away from the sample) and allowed to stir in the dark for 3 days. (**P1''**)

Table S3. Summary of M_p and compactions of the deprotected polymer **P1'** and SCNP **P1''** relative to the parent polymer **P1**

	M_p / kg·mol^{-1}	% Compaction
P1	26.5	0
P1'	22.4	15.5
P1''	18.7	29.4

## 3.3   P2'': Folding of P2 at 0.25 mg mL^{-1}

P2 (1.3 mg, 0.11 µmol, 1 eq.), **M2** (1.4 mg, 3.0 µmol, 27 eq.) and 4-dimethylaminopyridine (0.1 mg, 1.1 µmol, 10 eq.) were dissolved in 6 mL acetonitrile and then irradiated with a red light LED (λ_{max}=625 nm) for 1.5 h (placed 1 cm away from the sample) and allowed to stir in the dark for 3 days.

Table S4. Summary of M_p and compactions of the deprotected polymer **P2'** and SCNP **P2''** relative to the parent polymer **P2**

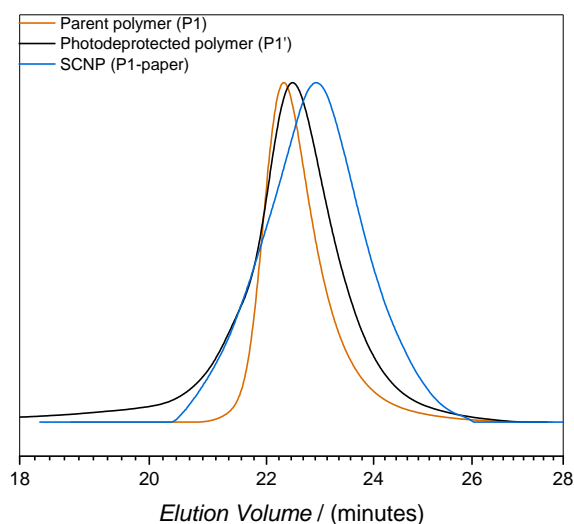| Polymer	M_p / kg·mol^{-1}	% Compaction
P2	15.6	0
P2'	14.8	5.1
P2''	11.3	27.6

3.4 Folding of P1 with a paper barrier

A single sheet of bright white (CIE whiteness: 150) 80 gsm copy paper from Planet Ark, Australia (acid free, 100% recycled) was used as the barrier. The paper was cut such that only single sheet wrapped around the sample vial without any overlapping regions (experimental setup shown below).
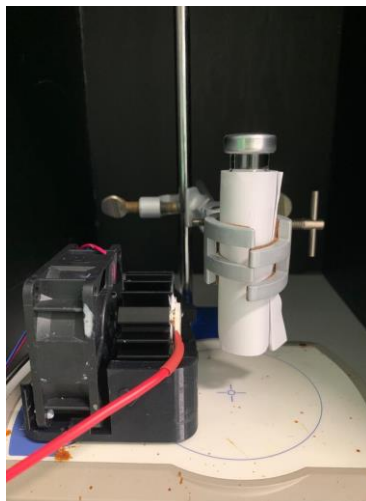
Figure S18. Set-up of the photoreaction and folding of **P1** through a paper barrier. The sample vial is wrapped with a single sheet of bright white 80 gsm paper and irradiated with a red light LED (λ_{max}=625 nm).

P1 (1.5 mg, 0.08 µmol, 1 eq.), **M2** (1.5 mg, 3.3 µmol, 39 eq.) and 4-dimethylaminopyridine (0.1 mg, 0.8 µmol, 10 eq.) were dissolved in 6 mL acetonitrile and the sample of **P1** was irradiated with a red light LED (λ_{max}=625 nm) for 4 h (placed 1 cm away from the sample) and allowed to stir in the dark for 3 days.

Figure S19. SEC elugrams of **P1**, **P1'** and **P1**-paper

Table S5. Summary of M_p and compactions of the deprotected polymer **P1'** and SCNP **P1-paper** relative to the parent polymer **P1**

Polymer	M_p / kg·mol^{-1}	% Compaction
P1	26.5	0
P1'	22.4	15.5
P1-paper	16.2	38.9

3.5   Folding of P1 with a tissue barrier (chicken breast)

A slice of deli style chicken breast from the brand Bërg, Aldi Australia, was used as the tissue barrier. The slice of tissue was 1.1 mm thick on average as measured by a pair of vernier callipers. According to the nutritional information supplied on the food packaging, per 100 g of chicken breast, the tissue contains 13.1 g of protein, 3.2 g of fat and 0.8 g of carbohydrates.

P1 (1.5 mg, 0.08 μmol, 1 eq.), **M2** (1.5 mg, 3.3 μmol, 39 eq.) and 4-dimethylaminopyridine (0.1 mg, 0.8 μmol, 10 eq.) were dissolved in 6 mL acetonitrile A single slice of chicken breast was wrapped around the sample vial (setup shown below) and the sample of **P1** was irradiated with a red light LED (λ_{max}=625 nm) for 6 h (placed 1 cm away from the sample) and allowed to stir in the dark for 3 days.

Figure S20. Setup of the photoreaction and folding of **P1** through a tissue barrier. The sample vial is wrapped with a single slice of deli style chicken breast and irradiated with a red light LED (λ_{max}=625 nm)
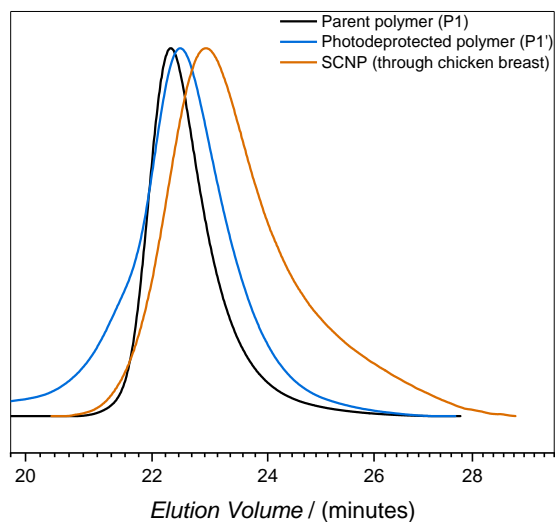
Figure S21. Elugram of **P1** relative to the SCNP formed by irradiation across a tissue barrier (chicken breast) with a red light LED (λ_{max}=625 nm)

Table S6. Summary of M_p and compactions of the deprotected polymer **P1'** and SCNP **P1-chicken** relative to the parent polymer **P1**

Polymer	M_p / kg·mol^{-1}	% Compaction
P1	26.5	0
P1'	22.4	15.5
P1-chicken	16.3	38.5

4   Supplementary Results

4.1 ^{1}H NMR Spectra for The Action Plot of MB-MMA
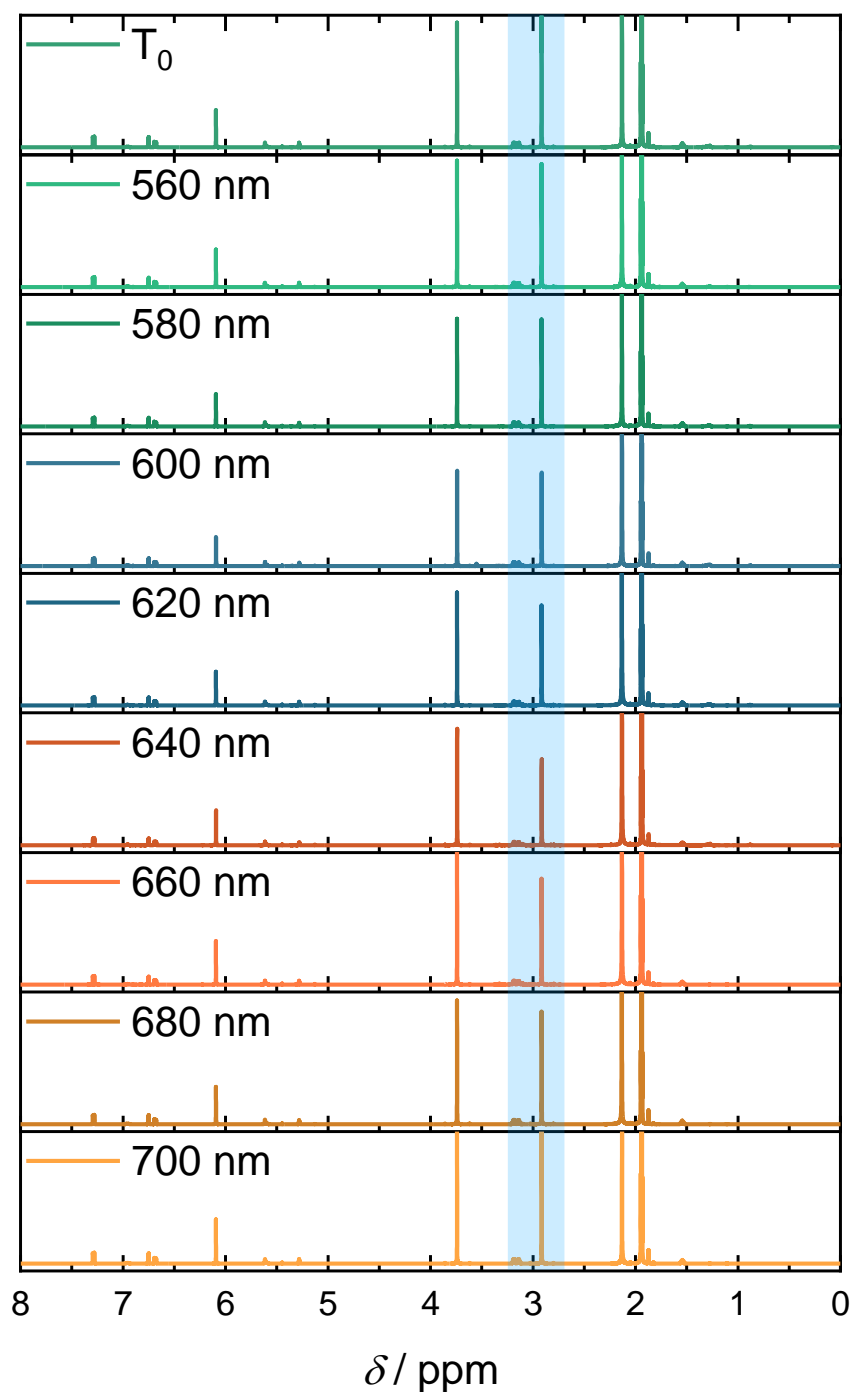
Figure S22. ^{1}H NMR spectra of the **MB-MMA** and photodegradation products in deuterated acetonitrile after irradiation at each wavelength with a tunable laser (630 µmol of photons at each wavelength) at a concentration of 1.1 mmol·dm^{-3}.

5   References

1.   Gruendling, T.; Guilhaus, M.; Barner-Kowollik, C., Quantitative LC–MS of Polymers: Determining Accurate Molecular Weight Distributions by Combined Size Exclusion Chromatography and Electrospray Mass Spectrometry with Maximum Entropy Data Processing. *Analytical Chemistry* **2008,** *80* (18), 6915-6927.

2.   Irshadeen, I. M.; Walden, S. L.; Wegener, M.; Truong, V. X.; Frisch, H.; Blinco, J. P.; Barner-Kowollik, C., Action Plots in Action: In-Depth Insights into Photochemical Reactivity. *Journal of the American Chemical Society* **2021,** *143* (50), 21113-21126.

3.   Menzel, J. P.; Noble, B. B.; Lauer, A.; Coote, M. L.; Blinco, J. P.; Barner-Kowollik, C., Wavelength Dependence of Light-Induced Cycloadditions. *J. Am. Chem. Soc.* **2017,** *139* (44), 15812-15820.

4.   Imming, P.; Jung, M.-H., Pentafluorophenyl Esters of Dicarboxylic Acids. *Arch. Pharm.* **1995,** *328* (1), 87-91.