

Supporting Information

Reactive Thermoresponsive Copolymer Scaffolds

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General Experimental

Oligo(ethyleneglycol) methyl ether methacrylate (OEGMA₃₀₀, $M_n \sim 300$ g/mol, Aldrich) was dissolved in CH₂Cl₂, passed over a basic aluminium oxide column and dried under vacuum prior to use. All other chemicals, including *O*-(carboxymethyl)hydroxylamine (**2**), were purchased from Sigma-Aldrich or Alfa Aesar and were used as received without further purification. ¹H and ¹³C NMR spectra were recorded on a Jeol ECS-400 spectrometer at 400 and 100 MHz, or a Bruker Avance 300 spectrometer at 300 and 75 MHz, respectively, with the residual solvent signal as an internal standard. Mass spectrometry was performed on a Waters LCT premier mass spectrometer (Waters Inc.). Gel permeation chromatography (GPC) was conducted on a Varian ProStar instrument (Varian Inc.) equipped with a pair of PL gel 5 μm Mixed D 300 × 7.5 mm columns with guard column (Polymer Laboratories Inc.), a Varian 325 UV-vis dual wavelength detector (254 nm), a Dawn Heleos II multi-angle laser light scattering detector (Wyatt Technology Corp.) and a Viscotek 3580 differential RI detector in series. Near monodisperse polystyrene standards (Polymer Laboratories) were used for calibration. Data collection was performed with Galaxie software (Varian Inc.) and chromatograms analyzed with the Cirrus software (Varian Inc.) and Astra software (Wyatt Technology Corp.). Melting points were determined on a Stuart Melting Point SMP11 apparatus.

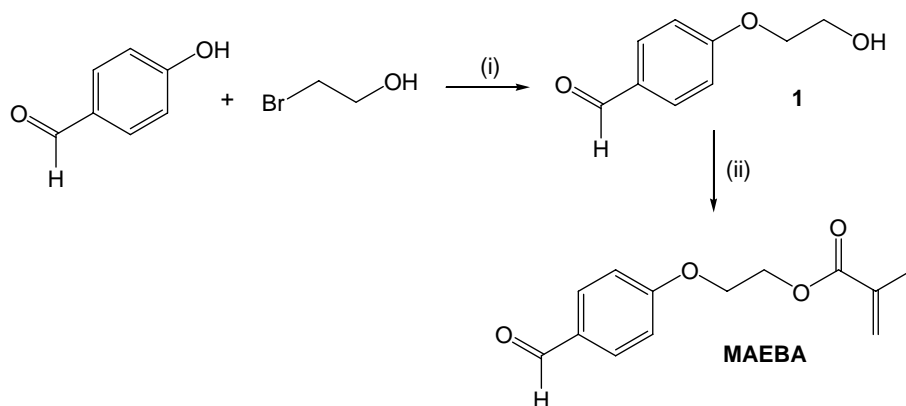
The cloud points of the polymer solutions (concentrations equal to 2 mg ml⁻¹ of the parent polymer) in 0.1 M NaCl solution, at specified pH values, were measured on a Cary 100 Bio UV-Vis spectrophotometer (Varian Inc.) fitted with a peltier block. Transmittance of polymer solutions was monitored at 550 nm as a function of temperature (cell path length 10 mm). Heating /cooling cycles were conducted at a rate of 0.1°C min⁻¹.

Hydrodynamic diameters (D_h) for polymers and conjugates in aqueous 0.1 M NaCl solutions were determined by dynamic light scattering (DLS). The DLS instrumentation consisted of a MALVERN Instruments HPPS-ET 5002 operating at 25 °C with a 633-nm laser module. Measurements were made at a detection angle of 173° (back scattering), and Malvern DTS 4.20 software was utilized to analyze the data. All determinations were made in duplicate.

pH Measurements were recorded with a Hanna HI 98103 instrument that was calibrated daily with commercial buffer solutions (Sigma-Aldrich). The adjustment of pH was carried out using concentrated NaOH and concentrated HCl (or NaOD and DCl if required) solutions to avoid any significant increase in sample volume.

Synthetic Procedures

p-(2-Methacryloxyethoxy)benzaldehyde (**MAEBA**) was prepared according to the procedure reported¹ by Antonucci.



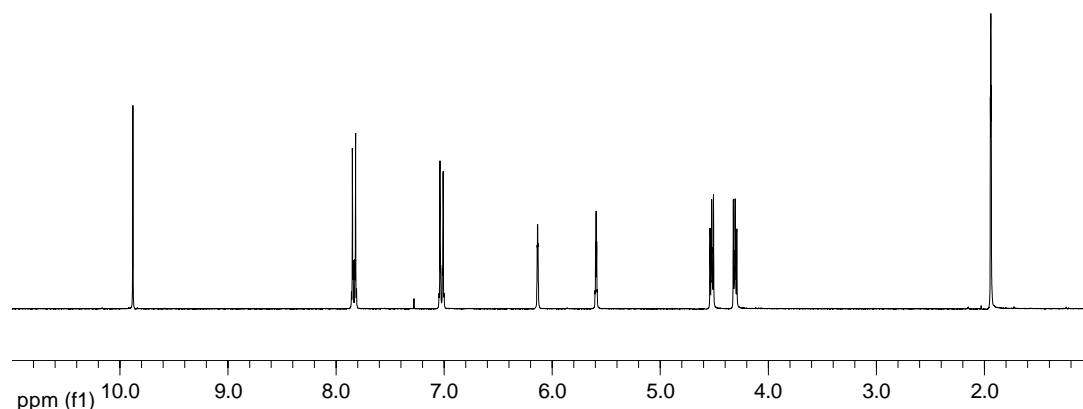
S1. Synthesis of *p*-(2-Methacryloxyethoxy)benzaldehyde (**MAEBA**): (i) K₂CO₃, DMF, Reflux, 24 h. (ii) Methacryloyl chloride, Et₃N, CH₂Cl₂, 0 °C, 2 h.

***p*-(2-Hydroxyethoxy)benzaldehyde (1):**

Potassium carbonate (17.0 g, 0.123 mol) was suspended in a solution of 4-hydroxybenzaldehyde (5.0 g, 0.041 mol), 2-bromoethanol (5.1 g, 0.041 mol) and dimethylformamide (100 mL) under reflux conditions for 24 h. After filtration and evaporation to dryness crude material was transferred into a separating funnel in CH₂Cl₂ (100 mL). The organic layer was washed with NaCl_(aq) (2 × 100 mL), dried over MgSO₄, filtered and evaporated to dryness to obtain a crude solid which was purified by column chromatography [SiO₂, Hexane-EtOAc (1:1)] to afford 4-(2-hydroxyethoxy)benzaldehyde as a clear oil (4.2 g, 61.6 %). ¹H NMR (CDCl₃): δ 3.10 (br s, 1H), 3.95 (t, 2H, J = 4.8 Hz), 4.11 (t, 2H, J = 4.8 Hz), 6.95 (d, 2H, J = 8.7 Hz), 7.75 (d, 2H, J = 8.7 Hz), 9.79 (s, 1H). ¹³C NMR (CDCl₃): δ 61.4, 71.0, 115.2, 130.4, 132.4, 164.2, 191.5.

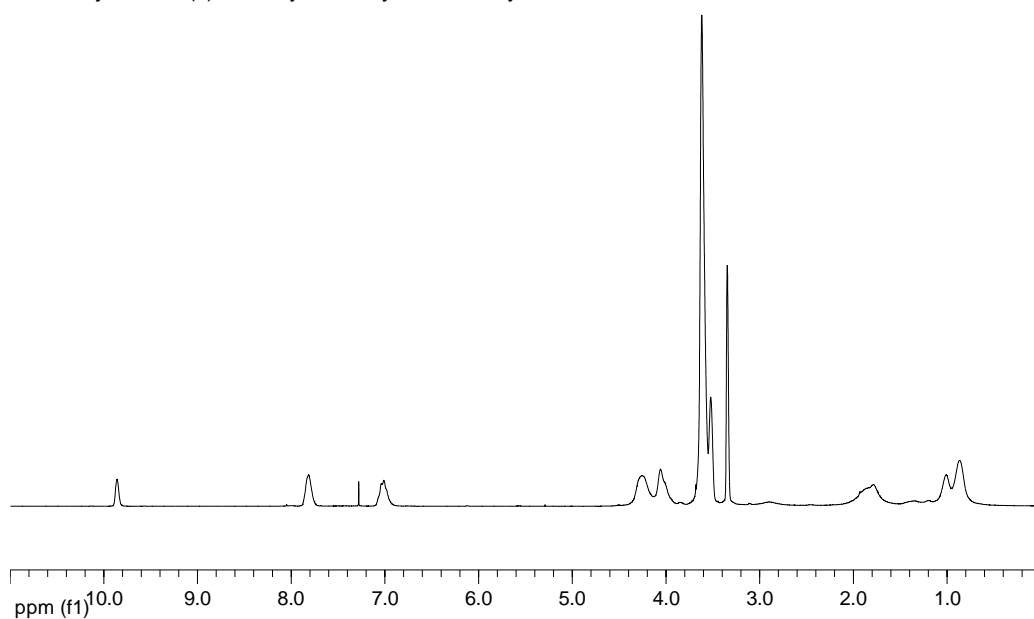
***p*-(2-Methacryloxyethoxy)benzaldehyde (MAEBA):**

p-(2-Hydroxyethoxy)benzaldehyde (3.8 g, 0.023 mol) and Et₃N (2.8 g, 0.027 mol) in CH₂Cl₂ (75 mL) were cooled to 0 °C. To this solution methacryloyl chloride (2.7 g, 0.026 mol) in CH₂Cl₂ (25 mL) was added dropwise over 30 min while stirring under N₂. Reaction mixture was allowed to reach room temperature and was left to stir for 2 h before transferring to a separating funnel with H₂O (100 mL). Organic layer was washed with NaCl_(aq) (2 × 100 mL), dried over MgSO₄, filtered and evaporated to dryness to obtain a crude oil which was purified by column chromatography [SiO₂, Hexane-EtOAc (4:1)] to afford 4-(2-Methacrylateethoxy)-benzaldehyde as a white solid (2.8 g, 52 %). ¹H NMR (CDCl₃): δ 1.92 (s, 3H), 4.29 (t, 2H, J = 5.1 Hz), 4.51 (t, 2H, J = 5.1 Hz), 5.57 (s, 1H), 6.12 (s, 1H), 7.00 (d, 2H, J = 8.7 Hz), 7.81 (d, 2H, J = 8.7 Hz), 9.86 (s, 1H). ¹³C NMR (CDCl₃): δ 18.7, 63.0, 66.6, 115.3, 126.7, 130.7, 132.3, 136.2, 163.9, 167.5, 191.1.

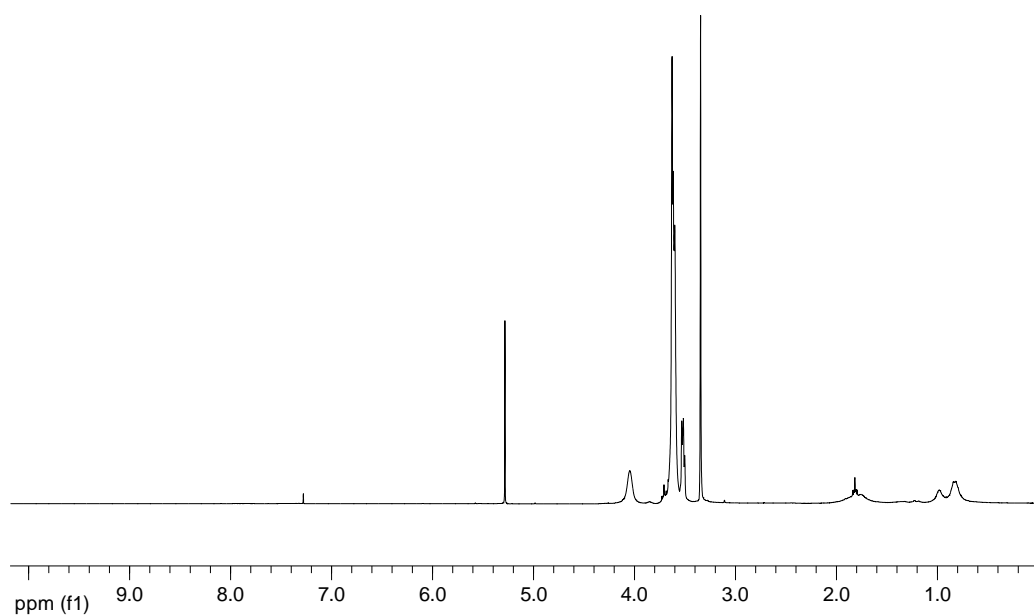


S2. ¹H NMR (CDCl₃) spectra of *p*-(2-Methacryloxyethoxy)benzaldehyde (MAEBA).

General RAFT copolymerization of OEGMA₃₀₀ and MAEBA: 4-(4-cyanopentanoic acid)dithiobenzoate (CPADB) (1 eq) and AIBN (0.2 eq) were added to a small schlenk tube. OEGMA₃₀₀ (66 eq) and MAEBA (33 eq) was then added followed by dioxane (100 eq). The reaction mixture was degassed five times via freeze-pump-thaw, and then the vessel was backfilled with N₂, purged with N₂, and allowed to warm to room temperature. The reaction mixture was then placed in an oil bath at 70 °C, and the polymerization was quenched after 23 h by rapid cooling and solvent was removed on the rotary evaporator. The resulting pink oil was dissolved in a minimal amount of THF and added dropwise to a large excess of ice-cold hexane, and the polymer isolated by filtration. Copolymers **P1** – **P6** were obtained as pink oils with yields typically between 50 and 70 %. A typical ¹H NMR spectrum of copolymer **P1** is shown in **S1**.



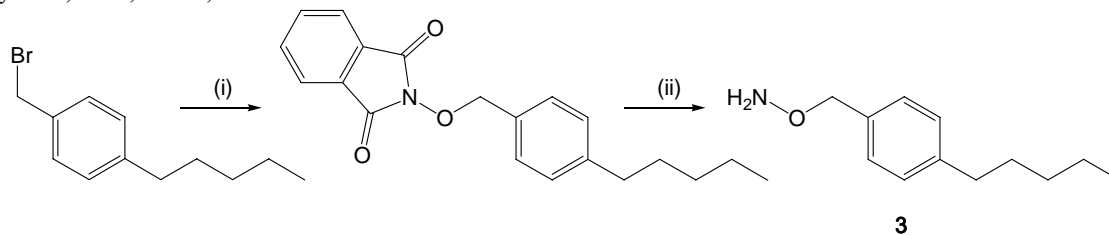
S1.(a) ¹H NMR spectrum (300 MHz, CDCl₃) of poly(OEGMA₃₀₀)-c-(MAEBA) (**P1**).



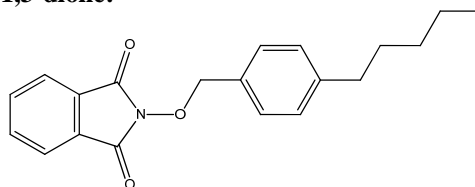
S1.(b) ¹H NMR spectrum (300 MHz, CDCl₃) of poly(OEGMA₃₀₀) (**P6**).

Compounds **1a**,² **1b**,³ **4**,⁴ **5**,⁵ **6**,⁶ and **8**⁷ were prepared according to literature procedures to afford the corresponding compounds possessing satisfactory spectral and physical properties to those already reported. *O*-(4-pentylbenzyl)hydroxylamine **3** was prepared in two steps from 1-(bromomethyl)-4-pentylbenzene⁸ as shown in **S2**. *O*-(pyren-1-ylmethyl)hydroxylamine **7** was prepared in two steps from 1-(bromomethyl)pyrene⁹ as shown in **S3**. ¹H and ¹³C NMR spectra for **3** and **7** and their precursors are shown in **S4-S7**.

S2. Synthesis of *O*-(4-pentylbenzyl)hydroxylamine: (i) *N*-hydroxyphthalimide, NEt₃, THF, reflux, 3 h. (ii) Hydrazine monohydrate, THF, reflux, 4 h.

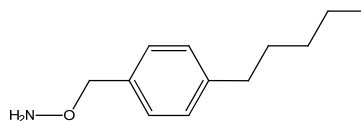


2-((4-Pentylbenzyl)oxy)isoindoline-1,3-dione:



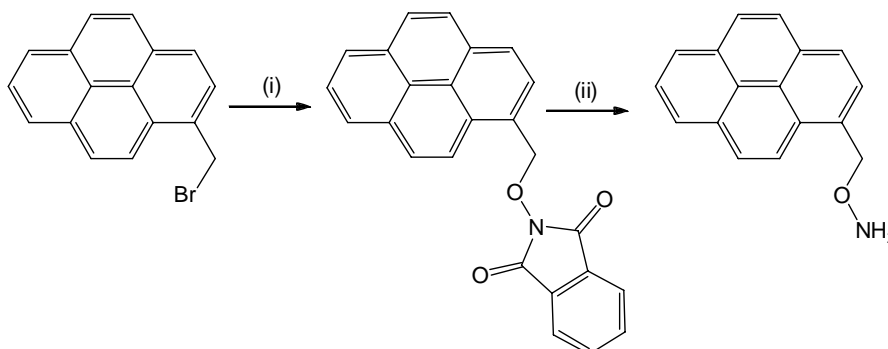
A solution of 1-(bromomethyl)-4-pentylbenzene (1.13 g, 4.69 mmol), *N*-hydroxyphthalimide (1.53 g, 9.38 mmol) and NEt₃ (1.31 mL, 9.4 mmol) in THF (50 mL) was heated at reflux for 3 h. The reaction mixture was left to cool, then H₂O (300 mL) was added to precipitate a solid that was collected by filtration then further washed with H₂O (30 mL). The solid was dried under reduced pressure then recrystallised from hot EtOH (50 mL) to yield the desired compound as a waxy white solid (0.75 g, 2.32 mmol, 49 %); ¹H NMR (CDCl₃, 300 MHz): δ 7.83 – 7.71 (m, 4H, phthalimide), 7.44 (d, 2H, J = 8.0 Hz, Ar), 7.19 (d, 2H, J = 8.0 Hz, Ar), 5.18 (s, 2H, CH₂O), 2.60 (t, 2H, J = 8.0 Hz, pentyl), 1.57 (m, 2H, pentyl), 1.31 (m, 4H, pentyl), 0.88 (t, 3H, J = 7.0 Hz, CH₃); ¹³C NMR (CDCl₃, 75 MHz): δ 163.4 (C=O), 144.3 (Ar_(q)), 134.2 (Ar), 131.2 (Ar_(q)), 129.9 (Ar), 129.4 (Ar_(q)), 128.6 (Ar), 123.4 (Ar), 79.9 (CH₂O), 35.8, 31.5, 30.9, 22.5, 13.8 (pentyl); MS (ES⁺) *m/z* 346.2 [M + Na]⁺ 100 %; m.p. 99-101 °C.

***O*-(4-Pentylbenzyl)hydroxylamine:**

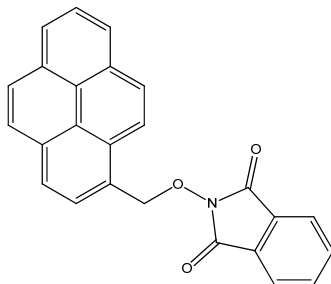


Hydrazine monohydrate (0.54 mL, 11.1 mmol) was added to a stirred solution of 2-((4-pentylbenzyl)oxy)isoindoline-1,3-dione (0.74 g, 2.29 mmol) in THF (230 mL) at reflux to immediately yield a precipitate. The suspension was left at reflux for 4 h. at which point TLC analysis confirmed all starting material had been consumed. The suspension was allowed to cool then filtered, and the filtrate evaporated to dryness. The resultant crude yellow oil was purified by precipitation from hot MeOH to yield the desired product as a light yellow oil (0.42 g, 2.17 mmol, 95 %); ¹H NMR (CDCl₃, 400 MHz): δ 7.27 (d, 2H, J = 8.0 Hz, Ar), 7.18 (d, 2H, J = 8.0 Hz, Ar), 5.36 (br, 2H, ONH₂), 4.67 (s, 2H, CH₂ONH₂), 2.60 (t, 2H, J = 8.0 Hz, pentyl), 1.61 (m, 2H, pentyl), 1.33 (m, 4H, pentyl), 0.89 (t, 3H, J = 8.0 Hz, CH₃); ¹³C NMR (CDCl₃, 100 MHz): δ 143.0 (Ar_(q)), 134.7 (Ar_(q)), 128.7, 128.6 (Ar), 78.1 (CH₂O), 35.8, 31.6, 31.3, 22.7, 14.2 (pentyl); MS (ES⁺) *m/z* 194.2 [M + H]⁺ 100 %.

S3. Synthesis of *O*-(pyren-1-ylmethyl)hydroxylamine: (i) *N*-hydroxyphthalimide, NEt₃, THF, reflux, 2 h (ii) Hydrazine monohydrate, THF, 2 h.

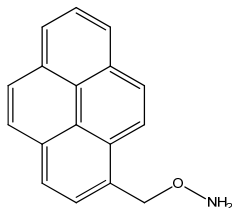


2-(Pyren-1-ylmethoxy)isoindoline-1,3-dione:



NEt₃ (1.9 mL, 13.6 mmol) was added to a refluxing solution of 1-(bromomethyl)pyrene (1.0 g, 3.4 mmol) and *N*-hydroxyphthalimide (1.1 g, 6.7 mmol) in THF to yield a deep red solution in which a precipitate rapidly formed (5 min). The suspension was left at reflux for 2 h., allowed to cool then filtered. The collected solid was washed with THF (5 mL), H₂O (10 mL) and ether (10 mL) to leave a straw coloured powder that was recrystallised from CH₂Cl₂-hexane to yield the desired compound as fibrous yellow crystals (0.90 g, 2.4 mmol, 71 %); ¹H NMR (CDCl₃, 400 MHz): δ 8.84 (d, 1H, J = 8.0 Hz, pyrene), 8.29 – 8.03 (m, 8H, pyrene), 7.82 (m, 2H, phthalimide), 7.73 (m, 2H phthalimide), 5.91 (s, 2H, CH₂-O); ¹³C NMR (CDCl₃, 100 MHz): δ 163.8 (C=O), 134.6, 134.2, 132.7, 131.3, 131.1, 130.9, 129.4, 129.1, 128.8, 128.4, 127.7, 127.5, 126.6, 126.2, 125.9, 125.3, 124.6, 123.8, 123.7 (pyrene + phthalimide), 78.3 (CH₂O); MS (ES⁺) *m/z* 378.2 [M + H]⁺ 100 %; mp = 190-194 °C (dec).

***O*-(pyren-1-ylmethyl)hydroxylamine:**



Hydrazine monohydrate (0.23 mL, 4.7 mmol) was added to a suspension of 2-(pyren-1-ylmethoxy)isoindoline-1,3-dione (0.90 g, 2.4 mmol) in THF (50 mL) at reflux to yield immediately a clear yellow solution followed by the precipitation of a white solid. The suspension was left at reflux for 2 h, allowed to cool to room temperature and then filtered. The filtrate was diluted by the addition of CH₂Cl₂ (50 mL) then washed with H₂O (2 x 50 mL). The organic fraction was dried over Na₂SO₄ then solvent was removed under reduced pressure to yield the desired compound as a yellow powder (0.4 g, 1.6 mmol, 67 %); ¹H NMR (CDCl₃, 400 MHz): δ 8.41 (d, 1H, J = 8.0 Hz, pyrene), 8.22 – 8.00 (m, 8H, pyrene), 5.48 (br, 2H, ONH₂), 5.42 (s, 2H, CH₂ONH₂); ¹³C NMR (CDCl₃, 100 MHz): δ 131.7, 131.3, 130.9, 130.5, 129.9, 2 x 128.0, 127.7, 127.5, 126.1, 125.4, 125.1, 124.8, 124.6, 123.5 (pyrene), 76.5 (CH₂ONH₂); MS (ES⁺) *m/z* 248.1 [M + H]⁺ 100 %; mp = 73 75 °C.

