Supporting information

pH-responsive diblock copolymers with two different fluorescent labels for simultaneous monitoring of micellar self-assembly and degree of protonation

Jeppe Madsen, George Madden, Efrosyni Themistou, Nicholas J. Warren, Steven P. Armes

Materials

4,4′-Azobis(4-cyanovaleric acid) (ACVA, ≥98 %), benzoic anhydride (≥95%), 2,2'-bipyridine (bpy, 99%), deuterated chloroform (CDCl$_3$, 99.8 %), Cresyl Violet Perchlorate (CV ClO$_4$, ≥96.5 %), Copper(I) bromide (CuBr, 99.999 %), Delta-gluconolactone (>99%), deuterated DMSO (DMSO-d6, ≥99.9 %), Methacrylic Anhydride (94 %) and Pyrene-1-ylmethanol (98 %) were purchased from Sigma Aldrich UK (Dorset, UK) and used as received.

Cresyl violet acetate (≥65 %) was obtained from Acros Organics (Geel, Belgium) and was used as received.

Ethanol (EtOH, Absolute) and triethylamine (≥99%) was purchased from VWR Lutterworth, UK

2-(Diisopropylamino)ethyl methacrylate (DPA, 97 %) was purchased from Scientific Polymer Products Inc. and was used as received.

Glycerol monomethacrylate (GMMA, 99.8%) was kindly donated by Cognis Performance Chemicals (Hythe, UK) and was used as received.

HPLC grade acetonitrile, dichloromethane, methanol, dialysis membranes (Spectra/Por 6, MWCO 1,000), pyridine (≥99%), were obtained from Fisher Scientific (Loughborough, UK) and were used as received. Magnesium sulfate (MgSO4), sodium chloride (NaCl) and sodium sulfate (Na$_2$SO4) were laboratory reagent grade from Fisher Scientific (Loughborough, UK) and were used as received.

Deuterated methanol (CD$_3$OD, 99.96 atom %) was purchased from Goss Scientific (Nantwich, U.K.).

2 (Methacryloyloxy)ethyl phosphorylcholine monomer (MPC, 99.9 % purity) was donated by Biocompatibles UK Ltd.

Experimental methods

Synthesis of Pyrene-1-ylmethyl-methacrylate (PyMA) monomer

The pyrene methacrylate monomer was synthesised similar to a previously published procedure:[1] Pyrene-1-ylmethanol (1.0 g, 4.3 mmol) was dissolved in dichloromethane (50 mL) in a 100 mL round-bottom flask. Triethylamine (2.5 mL, 18 mmol) was added to the flask, which was subsequently stoppered using a rubber septum. The head-space was then purged with nitrogen for 1 minute. Methacrylic anhydride (2.6 mL, 18 mmol) was added via a syringe. After 14 h, the solution was washed with water (3 portions of 50 mL), saturated sodium hydrogen carbonate (50 mL), water (50 mL) and brine (50 mL). The organic phase was dried with magnesium sulfate, filtered and evaporated. The crude product was recrystallized twice from ethanol. Yield: 0.60 g, 46 %. The proton NMR spectrum was consistent with the previously reported spectrum[1] and no further characterisation was carried out.

Synthesis of 4-Cyano-4-(2-phenylethane sulfanylthiocarbonyl) Sulfanylpentanoic Acid (PETTC) Chain Transfer Agent
The PETTC CTA was synthesised according to a previously published procedure.[2]

Preparation of macro-CTAs based on Glycerol Monomethacrylate (GMMA) and Pyrene-1-ylmethyl-methacrylate (PyMA) using RAFT polymerisation
The general polymerisation conditions followed a previously published procedure for non-labelled PGMMA macro-CTAs.[3] A stock solution consisting of GMMA (14.06 g, 87.78 mmol, 55 eq.), ACVA (0.0414 g, 0.148 mmol, 0.09 eq.) and PETTC (0.5377 g, 1.584 mmol, 1 eq.) was dissolved in absolute ethanol (14.08 g). The solution was purged with nitrogen for 30 minutes (which led to a mass loss of less than 0.2 % w/w). Accurately weighed aliquots of the degassed polymerisation mixture were then added to PyMA in molar ratios between PyMA and PETTC of 0 to 1.06 under nitrogen. After complete dissolution of the PyMA monomer, a sample was taken for analysis prior to placing the mixture at 70 °C using an aluminium heating block.

0.05 mL samples were removed at predetermined time points using a nitrogen-purged syringe and needle. These samples were diluted approximately 10 times with deuterated methanol, which quenched the reaction and enabled analysis by ^{1}H NMR spectroscopy to determine conversion and GPC to determine molecular weight. The samples were further analysed by Reverse-Phase HPLC to determine conversion of individual monomers as described in further detail below.

After 3.5 h, the reaction mixtures were removed from heat. All samples were dialysed against 500 mL water for 3 days with a change of dialysis water twice a day using a 1,000 MWCO dialysis bag. After dialysis, samples were freeze-dried to yield a yellow powder. All operations were conducted in containers that were protected from the light using aluminium foil.

NMR spectroscopy
^{1}H NMR of kinetics samples of the GMMA polymerisation were recorded in CD_3OD. The composition of the final macro-CTAs after purification was assessed using dry DMSO-d_6. The composition of PGMMA-PDPA diblock copolymers was determined using a 1:1 v/v mixture of DMSO-d_6 and $CDCl_3$.

All spectra were recorded using either a 400 MHz Bruker Avance 400 or a Bruker Avance III HD 400 spectrometer.

GPC of PGMMA
Molecular weight distributions were determined by DMF GPC. The GPC setup comprised two Polymer Laboratories PL gel 5 μm Mixed-C columns and a guard column maintained at 60 °C in series with a Varian 390 LC refractive index detector (also thermostated to 60 °). The flow rate was 1.0 mL min^{-1}, and the mobile phase contained 10 mM LiBr. Ten near-monodisperse PMMA standards (M_p = 625−618,000 g mol^{-1}) were used for calibration.

RP-HPLC for assessing conversion of individual monomers
The chromatographic system for assessing relative monomer conversion comprised two LC-20AD XR pumps, a DGU-20A3 degasser, a SIL-20A XR autosampler, a SPD-M20A diode array detector, a CBM-20A communications bus module, a CTO-10AS VP column oven. The system was fitted with a Genesis C18 4μ 3

mm x 150 mm column kept at 25 °C. The following gradient of deionised water and acetonitrile was used for the separation: 0-15 min: 5-100 % Acetonitrile. 15-20 min: 100 % Acetonitrile. 20-25 min: Reconstitution with 5 % Acetonitrile.

The evolution of the individual monomer conversion could be assessed by calculating their relative areas at 219 nm from the chromatograms and relating this to the known starting composition by assuming a linear relationship between absorption and concentration.

Kinetic NMR samples were diluted ten times with acetonitrile prior to injection. 5 µL sample was injected.

Assessment of reactivity ratios

Reactivity ratios for the two monomers were calculated assuming a non-terminal model according to reference 4.

According to this model, the overall conversion as a function of the conversion of each individual component is given by:

(1) $p_{AB}(p_A) = 1 - n_A(1-p_A) - (1-n_A)(1-p_A)^{r_B}$

(2) $p_{AB}(p_B) = 1 - n_A(1-p_B)^{r_A} - (1-n_A)(1-p_B)$

where p_{AB} is the overall conversion, p_A and p_B are conversions of each individual monomer, n_A is the initial mole fraction of monomer A and r_A and r_B are reactivity ratios for monomers A and B, respectively. A plot of the overall conversion as a function of the conversion of the individual monomers can then be fitted to give the reactivity ratios.

The overall conversion of methacrylic monomers was calculated from the ^{1}H NMR spectra by comparing the ratio between integrals assigned to methacrylic protons and integrals assigned to methylene protons alpha to the C-O bond in the ester (at 4-4.5 ppm) relative to the same ratio prior to initiation of polymerisation..

The individual conversion of monomers could be calculated by the relative integrals of each individual absorption peak in the HPLC chromatogram through knowledge of the initial composition (from ^{1}H NMR) and the overall conversion (from ^{1}H NMR):

The ratio between the monomer concentrations, M_A and M_B is given by the ratio between the absorption intensities as:

(3) $M_A/M_B = A_A/A_B * a/b$

Where the quantities *a* and *b* are the coefficients relating the absorption (*A*) intensity and the concentration of each component. This is assumed to be constant over the concentration range used:

(4) $M_Y = A_Y * y$, Y=A,B, y=a,b

The overall conversion can be written:

(5) $1 - p_{AB} = (M_A + M_B)/(M_A(0) + M_B(0)) = M_A/(M_A(0) + M_B(0)) + M_B/(M_A(0) + M_B(0))$

Where $M_A(0) + M_B(0)$ is the overall monomer concentration at t=0.

Combination of equations (3) and (5) allows calculation of the individual concentrations and thereby their conversion. These values were used in equations (1) and (2) to plot Figure S2.

Fits were obtained by initially calculating the right side of equations (1) and (2) using initial values of R_A and R_B of 1 for each monomer for each conversion. The values for each monomer then subtracted from the

measured overall conversion. The resulting numbers were summed for each monomer. The sum was fitted to give a value as close to zero as possible using the 'Solver' function in the Microsoft Excel software package. The optimised reactivity ratios and the fits are shown in Figure S2.

RAFT polymerisation of DPA using PGMMA-macro-CTAs
Polymerisation with addition of Cresyl Violet at high conversion:

A solution consisting of PGMMA macro-CTA (0.4007 g, 40 µmol) and ACVA (0.0030 g, 11 µmol) in ethanol (10.0240 g) was prepared. The solution was purged with nitrogen for 10 minutes. In a separate vial, the DPA(5 mL) was purged with nitrogen for 10 minutes. Then purged DPA (2.0383 g, 9.5551 mmol) was added to the PGMMA-ACVA solution using a nitrogen-purged syringe. The opaque mixture was purged for further 10 minutes with nitrogen. The solution was then placed at 70 °C and sampled at regular intervals. After 8 h, a nitrogen-purged solution of CV ClO$_4$ (0.0193 g, 5.36 µmol) and ACVA (0.0080 g, 2.85 µmol) in ethanol (1 mL) was added. The solution was left at 70 °C for a further 17 h. After cooling to room temperature, the solution was transferred to a dialysis bag (MWCO 1,000) and dialysed against ethanol for 1 day with two solvent shifts, methanol for 1 day with two solvent shifts and finally water for 3 days with two daily solvent shifts. The resulting opaque solution was frozen and freeze-dried to yield a reddish-brown powder.

Polymerisation with Cresyl Violet present throughout:

A solution consisting of PGMMA macro-CTA (0.4021 g, 40 µmol), ACVA (0.0055 g, 19 µmol) and CV ClO$_4$ (0.0018 g, 5.0 µmol) in ethanol (9.658 g) was prepared. The solution was purged with nitrogen for 10 minutes. In a separate vial, the DPA (5 mL) was purged with nitrogen for 10 minutes. Then purged DPA (2.0664 g, 9.6869 mmol) was added to the PGMMA-CV-ClO$_4$-ACVA solution using a nitrogen-purged syringe. The opaque mixture was purged for further 10 minutes with nitrogen. The solution was then placed at 70 °C and sampled at regular intervals. After a total of 25 h, the solution was cooled to room temperature. After transfer to a dialysis bag (MWCO 1,000), the solution was dialysed against ethanol for 1 day with two solvent shifts, methanol for 1 day with two solvent shifts and finally water for 3 days with two daily solvent shifts. The resulting opaque solution was frozen and freeze-dried to yield a reddish-brown powder.

GPC of PGMMA-PDPA
In order to analyse the diblock copolymer samples by GPC, it was necessary to benzoylate the hydroxy-groups according to a literature protocol:[5]

A stock solution of benzoic anhydride (0.0954 g, 0.421 mmol) in pyridine (5 mL) was prepared. For the benzoylation, 1 mL of this stock solution (0.08 mmol benzoic anhydride) was added to the solid PGMMA-PDPA polymer (10 mg, approximately 0.002 mmol OH). The resulting solution was kept overnight at room temperature (approximately 20 °C). Then the pyridine was allowed to evaporate at room temperature in the back of the fume cupboard and the polymers were analysed using a THF GPC.

The GPC set-up comprised two 5 µm Mixed-C columns maintained at 35 °C; an integrated PL-GPC 50 chromatographic system was used for measuring the samples. The THF eluent contained 2.0% v/v triethylamine and 0.05% w/v butylhydroxytoluene (BHT) and a flow rate of 1.0 mL min^{-1} was employed. Ten near-monodisperse PMMA standards ($M_p = 625−618,000$ g mol^{-1}) were used for calibration in conjunction with the integrated refractive index detector in the above system.

Preparation of 2-Phenoxyethanol-2-bromoisobutyrate initiator for ATRP polymerisation
The 2-phenoxyethanol-2-bromoisobutyrate ATRP initiator was synthesised according to a previously published procedure.[6]

ATRP polymerisation of PMPC polymers in the presence of Cresyl Violet
Preparation of stock solution of initiator and monomer

2-Phenoxyethanol-2-bromoisobutyrate initiator (194 mg, 0.68 mmol, 1 eq.) was dissolved in ethanol (35 ml) under nitrogen. MPC (10.0 g, 33.9 mmol, 50 eq.) was added and dissolved in the solution. The solution was stirred and purged with nitrogen for twenty minutes and stored at -20 °C. Before each use, this stock solution was allowed to equilibrate at room temperature and purged with nitrogen for 20 minutes.

ATRP polymerisation of MPC to give PhO-PMPC$_{50}$

A round-bottom flask was purged with nitrogen for ten minutes at 30 °C. Cu(I)Br (9.8 mg, 68.0 µmol, 1 eq.) and 2,2'-bipyridine (21.2 mg, 0.136 mmol, 2 eq.) were mixed and added to the round-bottom flask. Stock solution (3.50 ml) was transferred to the round-bottom flask under a nitrogen atmosphere to begin the reaction. The reaction was kept under nitrogen at 30 °C until ^{1}H NMR showed conversion no further change in the ratio between integrals of signals assigned to vinylic protons and signals assigned to the backbone. The reaction was then quenched with methanol (50 ml). The polymer was purified by extensive dialysis against water and freeze dried overnight to obtain a white powder. Yield: 0.3505 g, 35.05 %, ^{1}H NMR, (400 MHz, CD$_3$OD), δ 1 (3H), 1.9 (2H), 3.6 (2H), 4.1 (2H), 4.3 (2H), 4.4 (2H), 7 (2H), 7.3 (2H).

ATRP polymerisation of MPC in the presence of cresyl violet acetate

For the polymerisation in the presence of Cresyl Violet, Cresyl Violet Acetate was added to the round-bottom flask prior to the addition of the ATRP catalyst. ATRP of MPC in the presence of 0.5, 1.0, and 2.0 Eq. CV was investigated. A stock solution of the initiator and MPC monomer was generally used to ensure a constant monomer/initiator molar ratio for all reactions. Kinetic samples (0.10 ml) were taken from each reaction at ten and thirty minutes, and then at every hour until no further conversion was observed. Work-up was done as described for the polymer without added dye.

pH Measurements
The pH was measured using a calibrated pH probe (Hanna Instruments).

Absorption measurements
Absorption spectra were recorded using a Shimadzu UV 1800 instrument using disposable UV-grade fluorescence cuvettes.

Absorption measurements of PGMMA macro-CTAs with a variable PyMA content
The PGMMA macro-CTAs (5.0-30.0 mg depending on pyrene content) were dissolved in approximately 10 g water (determined to 6 digits). These stock solutions were further diluted with water to give a series of solutions with formal Pyrene content down to approximately 0.5 µM (and 0 µM for the macro-CTA without pyrene). Absorption spectra shown in Figure 2 have formal pyrene content of 5-10 µM and 0.05-0.5 mg polymer / g solution.

Absorption measurements of PGMMA-PDPA-CV diblock copolymers and of CV reference

Polymer (10-20 mg for the shorter PDPA block, 30-35 mg for the longer PDPA block, determined to 3 significant digits) was weighed into a 10 mL volumetric flask. For solutions in acid, 1 mL 1 M HCl was added and the flask was swirled until the polymer had dissolved, which gave a blue solution. The flask was then filled to the mark with deionised water. For solutions in ethanol, 1-2 mL ethanol was added and the flask was swirled until no visible precipitate was present. The resulting reddish-brown solution was diluted to the 10 mL mark.

The solutions were diluted using volumetric glassware to a total of 5 dilutions, with the lowest having a concentration of one tenth of the stock.

Cresyl Violet Perchlorate (4.5 mg, 12.4 µmol) was transferred to a 50 mL volumetric flask with 0.1 M HCl. The flask was filled to the mark with 0.1 M HCl to give a stock solution (c=0.25 mM). The resulting stock solution was diluted 10-fold and this diluted solution was used to prepare a series of dilutions down to a concentration of 1.5 µM.

The integrated absorption coefficients for cresyl violet 0.1 M HCl between 400 nm and 800 nm was calculated using Excel according to the procedure given in reference 7. Similarly, the integrated absorption for the cresyl violet-labelled block copolymers were evaluated. The ratio between this value and the absorption of the pyrene at 342 nm, combined with knowledge of the integrated absorption coefficient of cresyl violet perchlorate, the absorption of pyrene monomer at 342 nm and the literature value of the extinction coefficient of the pyrene monomer at 342 nm ($32,520$ $M^{-1}cm^{-1}$ [1]) allowed the evaluation of the amount of cresyl violet *relative* to the amount of pyrene in the polymer. Knowledge of the amount of pyrene in the macro-CTA then allowed the determination of the absolute amount of cresyl violet in the polymer. The advantage of this relative method is that it is not sensitive to residues of water or other non-absorbing impurities in the copolymer. In addition, some uncertainty on weighing out the polymer (which can be highly static when dry) is not a problem. Of course, the CTA will give a small contribution to the absorption signal at 342 nm (see Figure 2) if present and this will lead to an underestimation of the cresyl violet content. However, according to Scheme S1, the final content of trithiocarbonate is expected to be almost insignificant, at least where cresyl violet is added at high conversion. In addition, the absorption of trithiocarbonate is low relative to pyrene methacrylate, and therefore the error introduced by the presence of small amounts of CTA should be insignificant.

Fluorescence measurements

For fluorescence measurements of the PGMMA macro-CTAs, the stock solution was diluted up to 100-fold with water. The fluorescence spectra shown in Figure 2 are recorded using a 50-fold dilution of the stock solutions, corresponding to formal pyrene concentrations of 0.5-1.5 µM and 5-50 µg polymer / g solution.

For fluorescence measurements of the diblock copolymers, each of the absorption solutions were diluted 5-fold.

A Fluoromax-3 fluorimeter was used for obtaining fluorescence spectra. Spectrophotometer settings were adjusted to give spectra with high signal-to-noise ratio without overloading the detector. For spectra recorded using an excitation wavelength of 342 nm, values of the excitation and emission slit widths were 2.5 nm. For spectra recorded using excitation wavelengths of 405 nm, 488 nm and 543 nm, slit widths of 5 nm were used. In all cases a photomultiplier tube voltage of 950 was maintained.

UV-grade disposable fluorescence cuvettes were used.

Calculation of quantum yields of cresyl violet

Quantum yields were calculated using the method of Fery-Forgues.[8] For diblock copolymers, solutions with a maximum absorption of approximately 0.1, prepared by 10-fold dilution of solutions with maximum absorption of approximately 1 in 0.1 M HCl (determined by absorption spectroscopy) were used. As a standard was used a 1 μM cresyl violet solution in 0.1 M HCl. The quantum yield of cresyl violet at a concentration of 1 μM in water is reported to be 0.44.[9]

For labelled PMPC homopolymers, the quantum yield was determined in water, and cresyl violet perchlorate in water was used as a standard.

Dynamic Light Scattering

DLS measurements were conducted at 25 °C using a scattering angle of 173° with a Malvern Instruments Zetasizer Nanoseries instrument equipped with a 4 mW He−Ne laser operating at 633 nm, an avalanche photodiode detector with high quantum efficiency, and an ALV/LSE-5003 multiple t digital correlator electronics system. The intensity-average diameter and polydispersity of the diblock copolymer particles were calculated by cumulants analysis of the experimental correlation function using Dispersion Technology Software version 6.20.

A solution of $PGMMA_{61}$-$PyMA_{0.55}$-$PDPA_{64}$-$CV_{0.15}$ in 0.1 M HCl (0.13 g/L) was prepared. The pH of this solution was carefully increased to 7.2 by addition of 1 M NaOH while stirring.

Confocal Laser Scanning Microscopy with spectral imaging

An Olympus FV1000 confocal BX61 microscope having spectral imaging capability was used for the acquisition of fluorescence images and for assessing the effect of pH on the fluorescence.

A 20 x magnification air lens with numerical aperture of 0.75 was used for imaging.

Pictures were recorded using Olympus Fluoview FV-ASW software. Post-acquisition analysis was performed using the free image analysis software ImageJ and extracted spectral data were treated further using Microsoft Excel.

A drop of a solution of 0.13 g/L $PGMMA_{61}$-$PyMA_{0.55}$-$PDPA_{64}$-$CV_{0.15}$ in 0.1 M aqueous HCl (pH ~3) was placed on a glass microscopy slide and covered with a cover glass prior to imaging. After increasing the pH to 7.2 with 1 M NaOH, the procedure was repeated using the now opaque solution.

Measurements of fluorescence emission using CLSM in the presence of gluconolactone

The pH of a solution of $PGMMA_{61}$-$PyMA_{0.55}$-$PDPA_{64}$-$CV_{0.15}$ (1.11 g/L) in 0.1 M HCl was increased to 7.2 using 1 M NaOH. 1 mL of this solution (approximately 50 nmol polymer, corresponding to approximately 3 mmol base) was added to solid gluconolactone (0.0101 g, 56.1 mmol) at t=0 in a vial.

The vial was closed and briefly shaken to dissolve the gluconolactone. Then it was placed on a microscopy slide, covered with a cover slip. The solution was imaged using the Olympus FV1000 microscope by excitation using the 405 nm laser. Emission at 450-550 nm and emission at 550-650 nm was recorded separately as a function of time. The resulting intensities were extracted using ImageJ and their ratio plotted as a function of time.

The pH was measured in a solution of gluconolactone in the absence of diblock copolymer as a function of time. The resulting graph is shown in Figure S4.

1) $M_n^\bullet$ + ⟶ M_n−H + + H^+

2) $M_n^\bullet$ + ⟶
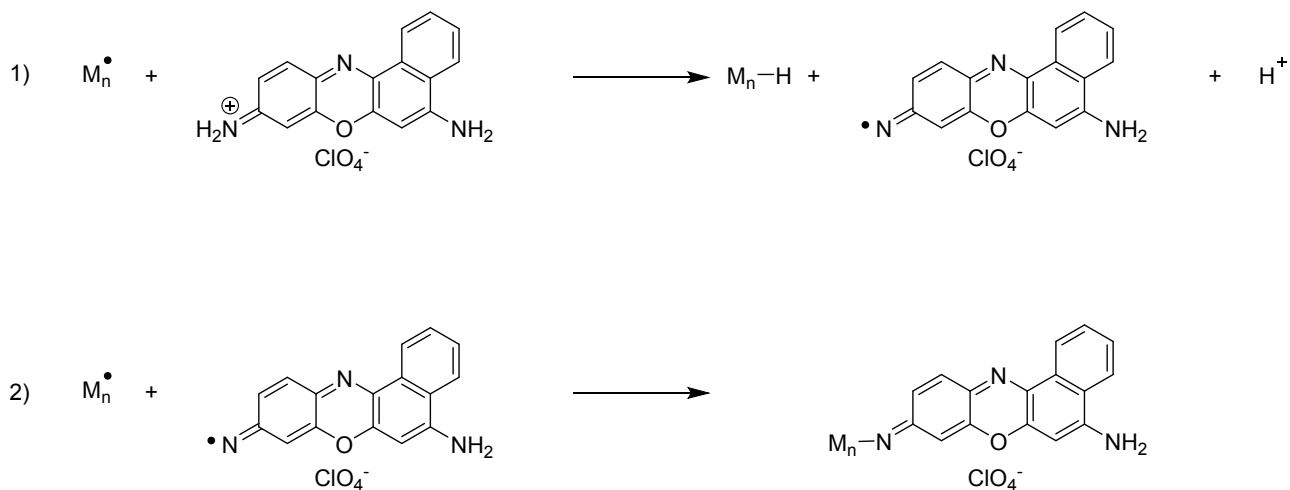
Scheme S1: Probable reaction of polymer radicals with cresyl violet following references 10–12

Table S1: PGMMA macro-CTA characterisation

	Target composition		Measured			
Entry	[GMMA]:[PETTC][a]	[PyMA]:[PETTC][b]	NMR DP[c]	NMR [PyMA]:[PETTC][d]	M_n[e]	M_w/M_n[e]
1	55	0.00	56	0.00	15,700	1.15
2	55	0.06	59	0.04	15,300	1.13
3	55	0.13	59	0.18	15,900	1.13
4	55	0.57	61	0.54	15,500	1.15
5	55	1.06	61	1.03	15,200	1.16

[a] Molar ratio between GMMA and PETTC as added to the polymerisation mixture.

[b] Molar ratio between PyMA and PETTC as added to the polymerisation mixture.

[c] Degree of polymerisation determined by integrating aromatic signals assigned to the PETTC end-group and signals assigned to PGMMA after purification by aqueous dialysis (MWCO 1,000).

[d] Ratio between PyMA and PETTC in the final polymer determined by integrating aromatic signals assigned to PyMA and to PETTC.

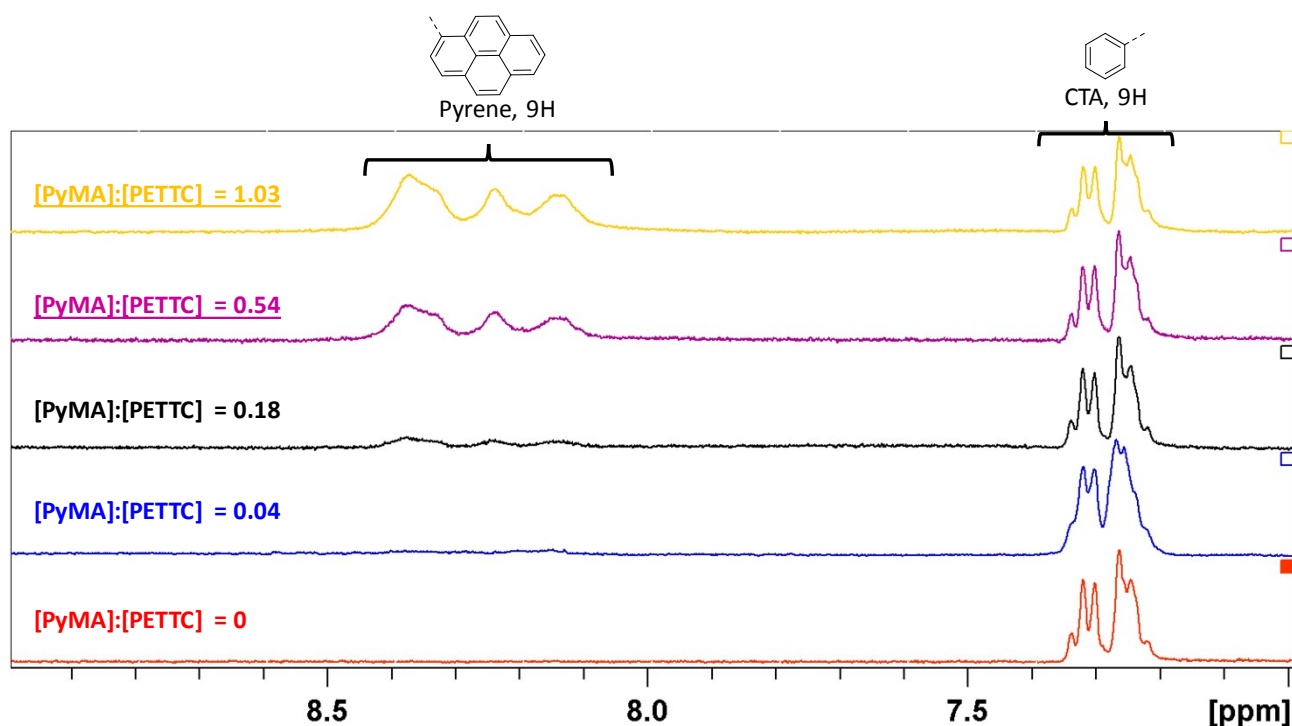[e] Determined by GPC using a DMF eluent.

Figure S1: Aromatic region of ^{1}H NMR spectra of PGMMA-MacroCTAs with signals assigned to the aromatic fragment of the CTA and to the pyrene fragment.
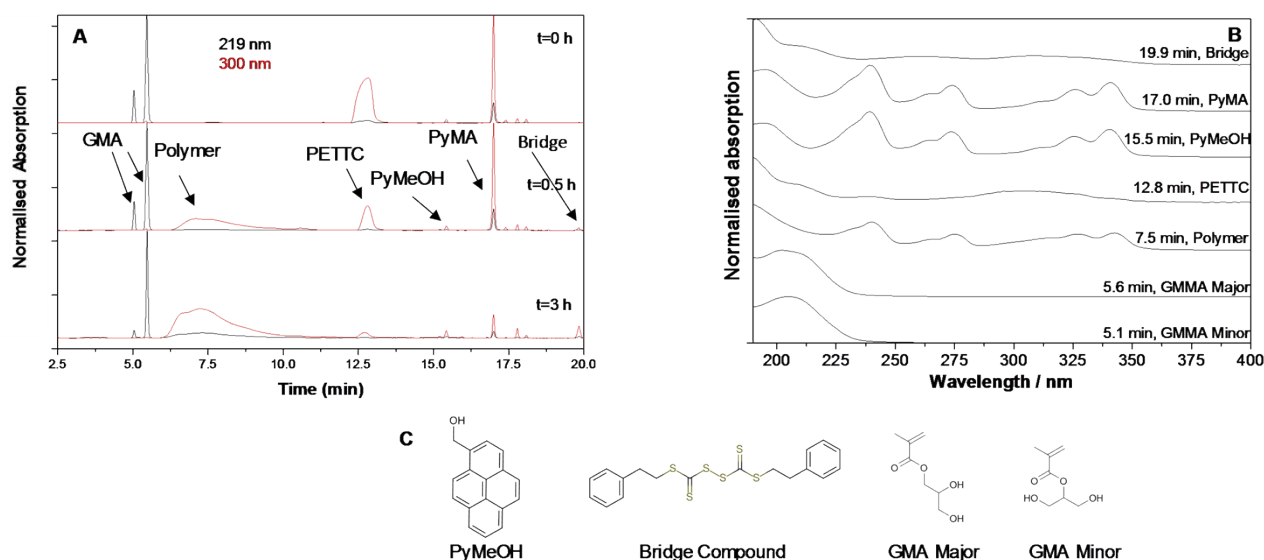

Figure S2: Analysis of the statistical copolymerisation of glyceryl monomethacrylate (GMA) and pyrene methacrylate (PyMA) using HPLC. (A) HPLC chromatograms for polymerisation mixtures recorded after 0 h, 0.5 h and 3 h. Black trace: 219 nm, Red trace: 300 nm (B) Normalised absorption spectra for the individual assigned peaks. Polymerisation conditions: [PGMA]: [PyMA]:[PETTC] = 55: 1.06: 1. See above for experimental details. HPLC protocol: A: Water. B: Acetonitrile. 0-15 min: 5-100 % B. 15-20 min: 100 % B Column: Genesis C18 4µ 3 mm x 150 mm (C) Chemical structures of pyrenemethanol, bridge compound, major and minor isomers of GMA. For the chemical structures of PETTC and PyMA, see Scheme 1.
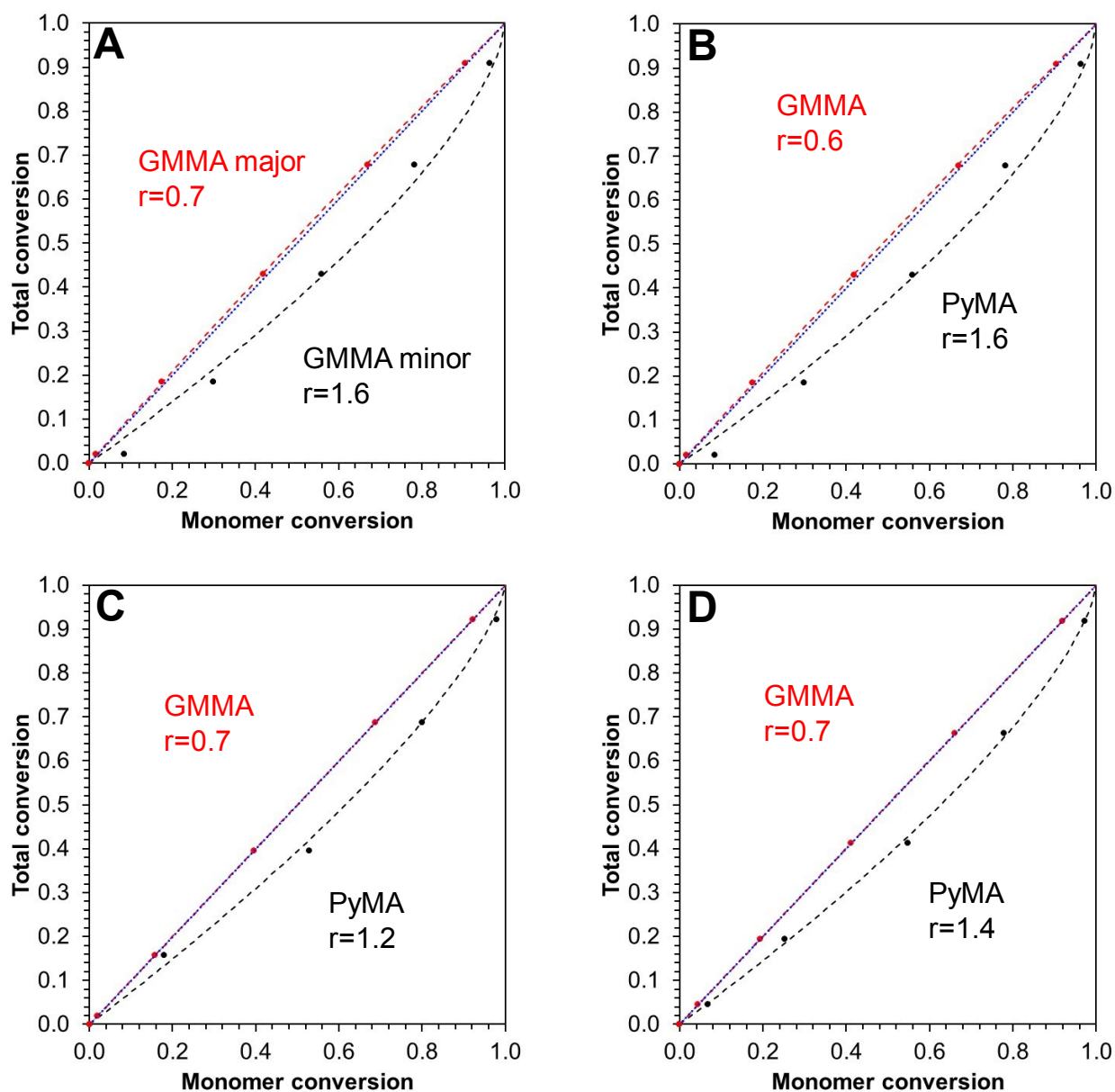
Figure S3: Overall conversion versus individual monomer conversion for A) The two Glycerol Monomethacrylate (GMMA) isomers in molar ratio 8:92 B) Pyrene methacrylate (PyMA) and the combined glycerol methacrylate monomers (GMMA) in molar ratio 1:1000 C) PyMA:GMMA 1:100 D) PyMA:GMMA 1:50. Reactivity ratios are obtained from the fits (dotted lines) by adopting a non-terminal copolymerisation model as described in reference [4]. The straight line corresponds to a strictly random copolymerisation.
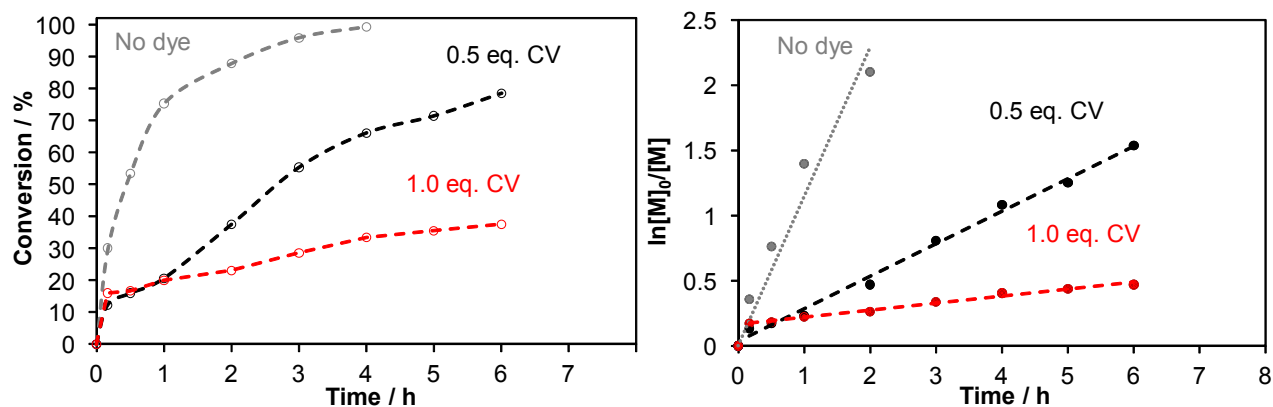
Figure S4: Kinetic data for the polymerization of MPC in the presence of Cresyl Violet. Conditions: [MPC]=1 M. [PhOBr]=0.02 M. [CuBr]=0.02 M, [bpy]=0.04 M, 30 °C. Solvent: Anhydrous ethanol. A) Conversion versus time B) Semilogarithmic plot of the evolution of $[M]_0/[M]$. Straight lines are linear fits.
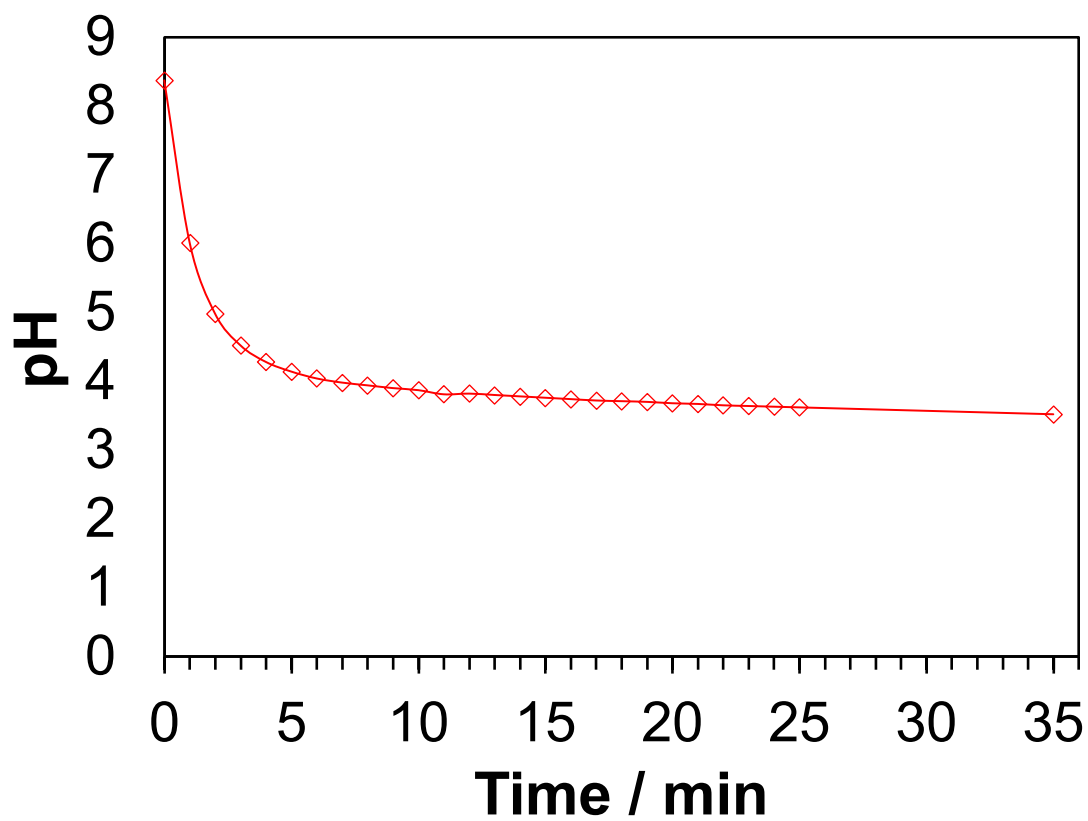

Figure S5: pH as a function of time in water with added gluconolactone (7 mM)
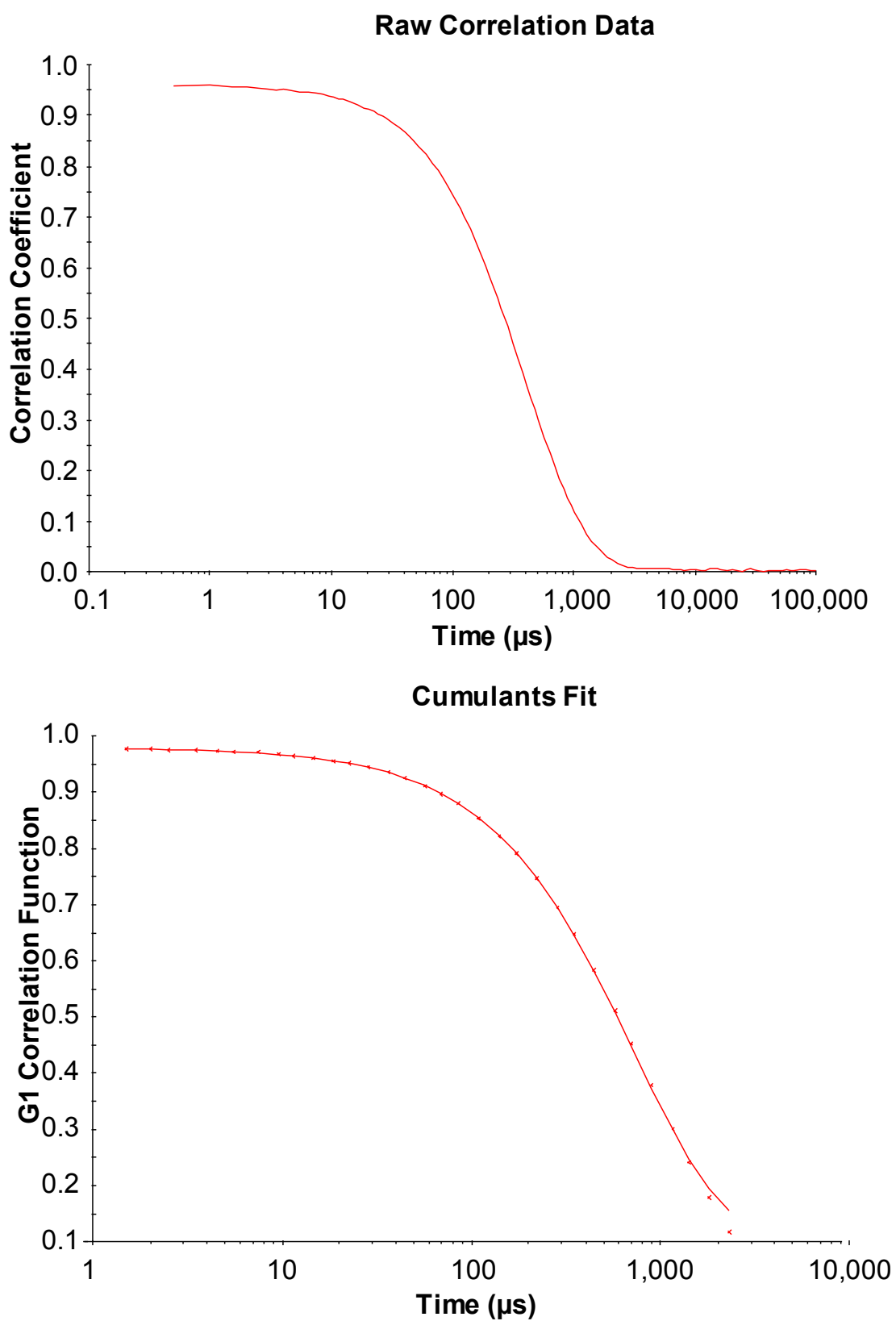
Figure S6: Raw correlation data and cumulants fit used for calculating the DLS intensity-average size distributions in Figure 7A

Table S2: Summary of data obtained for all PMPC polymers.

No.	Polymer target composition	Conversion / % [a]	Polymer composition [b]	Abs λ_{max} / nm [c]	Em. λ_{max} / nm [c]	Quantum Yield [d]
1	PhO-P(MPC$_{50}$-CV$_{0.5}$)	80	PhO-P(MPC$_{40}$-CV$_{0.22}$)	556	624	0.68 ± 0.03
2	PhO-P(MPC$_{50}$-CV$_1$)	61	PhO-P(MPC$_{31}$-CV$_{0.48}$)	534	622	0.62 ± 0.03
3	PhO-P(MPC$_{50}$-CV$_2$)	0	N/A	N/A	N/A	N/A

a) Conversion was determined using ¹H NMR spectroscopy. The value given is the value where no further change could be detected as measured over a period of approximately 24 h

b) Composition based on the integrated absorption

c) Measured in PBS, pH 7.3

d) Relative quantum yield determined in water using laser grade Cresyl Violet in methanol as fluorescence standard

(1)   Pietsch, C.; Vollrath, A.; Hoogenboom, R.; Schubert, U. S. *Sensors* **2010**, *10* (9), 7979–7990.

(2)   Semsarilar, M.; Ladmiral, V.; Blanazs, A.; Armes, S. P. *Langmuir* **2012**, *28* (1), 914–922.

(3)   Ladmiral, V.; Semsarilar, M.; Canton, I.; Armes, S. P. *J. Am. Chem. Soc.* **2013**, *135* (36), 13574–13581.

(4)   Beckingham, B. S.; Sanoja, G. E.; Lynd, N. A. *Macromolecules* **2015**, *48* (19), 6922–6930.

(5)   Zhang, H.; Ruckenstein, E. *Macromolecules* **2000**, *33* (13), 4738–4744.

(6)   Madsen, J.; Armes, S. P.; Bertal, K.; MacNeil, S.; Lewis, A. L. *Biomacromolecules* **2009**, *10* (7), 1875–1887.

(7)   Grabolle, M.; Brehm, R.; Pauli, J.; Dees, F. M.; Hilger, I.; Resch-Genger, U. *Bioconjug. Chem.* **2012**, *23* (2), 287–292.

(8)   Fery-forgues, S.; Lavabre, D. *J. Chem. Educ.* **1999**, *76* (9), 1260–1264.

(9)   Isak, S. J.; Eyring, E. M. *J. Phys. Chem.* **1992**, *58* (15), 1738–1742.

(10)   Das, N. K.; Mandal, B. M. *Polymer (Guildf).* **1982**, *23* (11), 1653–1658.

(11)   Lucarini, M.; Pedrielli, P.; Pedulli, G. F.; Valgimigli, L.; Gigmes, D.; Tordo, P. *J. Am. Chem. Soc.* **1999**, *121* (49), 11546–11553.

(12)   Wei, Y.; Hariharan, R.; Patel, S. a. *Macromolecules* **1990**, *23* (3), 758–764.