Electronic Supporting Information

Aqueous Photoinduced Living/Controlled Polymerization: Tailoring for Bioconjugation

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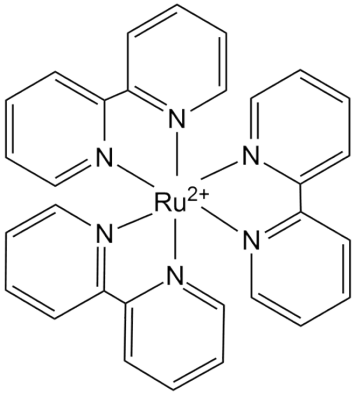
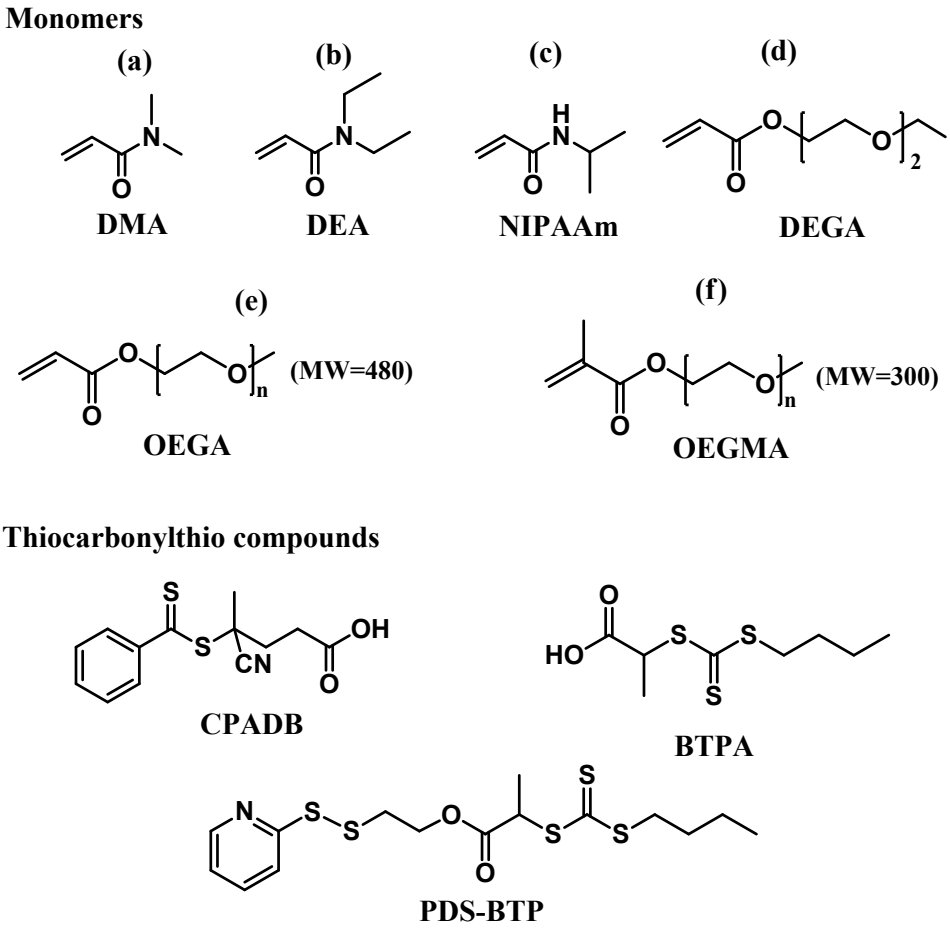
Experimental Section

Materials. *N,N*-dimethylacrylamide (99%, DMA), *N,N*-diethylacrylamide (99%, DEA), *N*-isopropylacrylamide (99%, NIPAAm), di(ethylene glycol) ethyl ether acrylate (>90%, DEGA), oligo(ethylene glycol) methyl ether methacrylate (M_n = 300) (OEGMA), and oligo(ethylene glycol) methyl ether acrylate (M_n = 480) (OEGA) were all purchased from Aldrich and were deinhibited *via* basic activated alumina oxide column chromatography before use. 2,2'-dithiodipyridine (99%), 4-dimethylaminopyridine (99%, DMAP), *N,N'*-dicyclohexylcarbodiimide (99%, DCC), fetal bovine serum, and bovine serum albumin lyophillized powder (>96%, BSA) were purchased from Aldrich and used as received. Tris(2,2′-bipyridyl)dichlororuthenium(II) hexahydrate ($Ru(bpy)_3Cl_2$, 99%) was freshly prepared into stock solutions at concentrations of 0.5 mg/mL and 0.05 mg/mL for each solvent used for the experiments. *N,N*-dimethylformamide (DMF, 99.8%, Ajax Chemical), dimethyl sulfoxide (DMSO, Ajax Chemical), acetonitrile (Ajax Chemical), toluene (Ajax Chemical), *n*-hexane (Ajax Chemical), methanol (Ajax Chemical), diethyl ether (Ajax Chemical), and petroleum spirit (Ajax Chemical) were used as received. Chain transfer agents (CTA) 4-cyanopentanoic acid dithiobenzoate (CPADB) and 2-(*n*-butyltrithiocarbonate)-propionic acid (BTPA) were synthesized according to literature procedures.[1]



Scheme S1. Chemical Structure of commercially available water soluble photoredox catalyst $Ru(bpy)_3Cl_2$ (bpy = 2,2'-bipyridyl) – Tris(2,2'-bipyridyl)ruthenium(II) chloride hexahydrate.



Scheme S2. Chemical structures of monomers and thiocarbonylthio compounds (chain transfer agents, CTAs): (a) *N,N'*-dimethylacrylamide (DMA), (b) *N,N'*-diethylacrylamide (DEA), (c) *N*-isopropylacrylamide (NIPAAm), (d) di(ethylene glycol) ethyl ether acrylate (DEGA), (e) oligoethylene glycol methyl ether acrylate (OEGA), (f) oligoethylene glycol methyl ether methacrylate (OEGMA); 4-cyanopentanoic acid dithiobenzoate (CPADB), 2-(*n*-butyltrithiocarbonate)-propionic acid (BTPA) and 2-(pyridin-2-yldisulfanyl)ethyl 2-(((butylthio)carbonothioyl)thio)propanoate (PDS-BTP).

Instrumentation.

Gel permeation chromatography (GPC) was performed using tetrahydrofuran (THF), dimethylacetamide (DMAc) or deionized water as the eluent. The GPC system was a Shimadzu modular system comprising an auto injector, a Phenomenex 5.0 μm beadsize guard column (50 × 7.5 mm) followed by four Phenomenex 5.0 μm bead-size columns (10^5, 10^4, 10^3 and 10^2 Å) for DMAc system, two Phenomenex 5.0 μm bead-size columns (MIX C provided by Polymer Lab) for THF system, and a differential refractive-index detector and a UV-vis. detector. The system was calibrated with narrow molecular weight distribution polystyrene standards with molecular weights of 200 to 10^6 g mol^{-1}. Aqueous GPC was conducted using a Shimadzu modular system comprising a DGU-12A solvent degasser, on LC-10AT pump, a CTO-10A column oven, and a RID-10A refractive index detector (flow rate: 0.8 ml/min). The column was equipped with a Polymer Laboratories 5.0 mm bead-size guard column (50 × 7.8 mm2) followed by three PL aquagel-OH columns (50, 40, 8μm). Calibration was performed with PEO standards ranging from 500 to 500,000 g/mol.

UV-vis Spectroscopy. UV-vis spectra were recorded using a CARY 300 spectrophotometer (Varian) equipped with a temperature controller.

Nuclear magnetic resonance (NMR) spectroscopy was carried out on a Bruker DPX 300 spectrometer operating at 400 MHz for ^1H and 100 MHz for ^{13}C using $CDCl_3$, DMSO-d_6, acetonitrile-*d3* and D_2O as solvents and tetramethylsilane (TMS) as a reference. Data was reported as follows: chemical shift (δ) measured in ppm downfield from TMS.

Fluorescence spectroscopy. Fluorescence spectra were recorded using Agilent fluorescent spectrometer.

Poly(acrylamide) Gel Electrophoresis (PAGE). Poly(acrylamide) gel electrophoresis (PAGE) was performed using a Bio-Rad Ready Gel® Precast Gel System. Samples were prepared at 1 mg/ml concentration in TRIS buffer containing bromophenol blue and run through a precast Tris-HCl gradient gel (4-20%) under non-denaturing conditions (voltage 150 V, 400 mA, 50-55 min).

Reaction setup. Photopolymerizations were carried out under visible light irradiation by a 1 m blue LED strip (λ_{max} = 435 nm, 4.8 Watts) surrounding the reaction vessels.

Figure S1. Experimental setup for photo-polymerization using 4.8 Watts blue LED light.

Experimental procedure for the kinetic study of DMA in DMSO. In a typical kinetic study experiment of DMA, a 6 mL glass vial equipped with a rubber septum was charged with DMA (1.68 g, 16.95 mmol), BTPA (20 mg, 0.084 mmol), $Ru(bpy)_3Cl_2$ (0.013 mg, 1.74×10^{-5} mmol, 260 µL of 0.05 mg/mL DMSO solution) and DMSO (1460 µL, total solvent = 1720 µL) at a molar ratio of [Monomer] : [CTA] : $[Ru(bpy)_3Cl_2]$ = 202 : 1 : 0.000202 (leading to a catalyst concentration of 1 ppm with respect to the monomer) and a molar concentration of 10 M of the monomer with respect to the solvent. The reaction mixture was covered with aluminum foil and degassed with N_2 in a water bath for 30 min. After purging, the reaction vessel was sealed and was irradiated with blue LED light (LED strip, 4.8 Watts) at room temperature. Aliquots were withdrawn using nitrogen-purged syringes and predetermined time points and subsequently analyzed via ^1H NMR ($CDCl_3$) and GPC (DMAc) to measure the conversion, number-average molecular weight (M_n) and polydispersity (PDI), respectively.

Experimental procedure for the kinetic study of DMA in H_2O. In a similar manner to the method prescribed for the kinetic study of DMA in DMSO, the experiments in the different solvents utilized the same molar ratios; [Monomer] : [CTA] : $[Ru(bpy)_3Cl_2]$ = 202 : 1 : 0.000202 (leading to a catalyst concentration of 1 ppm with respect to the monomer) at a molar concentration of 10 M of the monomer with respect to the solvent. A 6 mL glass vial equipped with a rubber septum was charged with DMA (1.68 g, 16.95 mmol), BTPA (20 mg, 0.084 mmol), $Ru(bpy)_3Cl_2$ (0.013 mg, 1.74×10^{-5}

mmol, 260 μL of 0.05 mg/mL H_2O solution) and milliQ H_2O (1460 μL, total solvent = 1720 μL). Following addition of the reactants to a 6 mL glass vial covered with aluminum foil, the reaction mixture was degassed with N_2 in an ice bath for 30 min. After purging, the reaction vessels were irradiated under blue LED light (4.8 Watts) at room temperature. Nitrogen-purged syringes were used to withdraw aliquots at predetermined time points. Again, ^{1}H NMR (D_2O) and GPC (DMAc) analyses were performed to measure the conversion, number-average molecular weight (M_n) and the polydispersity (PDI).

Experimental procedure for the kinetic study of DMA in acetonitrile. In a similar manner to the method prescribed for the kinetic study of DMA in DMSO, the experiments in the different solvents utilized the same molar ratios; [Monomer]:[CTA]:[$Ru(bpy)_3Cl_2$] = 202 : 1 : 0.000202 (leading to a catalyst concentration of 1 ppm with respect to the monomer) at a molar concentration of 10 M of the monomer with respect to the solvent. A 6 mL glass vial equipped with a rubber septum was charged with DMA (1.68 g, 16.95 mmol), BTPA (20 mg, 0.084 mmol), $Ru(bpy)_3Cl_2$ (0.013 mg, 1.74×10^{-5} mmol, 260 μL of 0.05 mg/mL acetonitrile solution) and acetonitrile (1460 μL, total solvent = 1720 μL). Following addition of the reactants to a 6 mL glass vial covered with aluminum foil, the reaction mixture was degassed with N_2 in an ice bath for 30 min. After purging, the reaction vessels were irradiated under blue LED light (4.8 Watts) at room temperature. Nitrogen-purged syringes were used to withdraw aliquots at predetermined time points. Again, ^{1}H NMR (acetonitrile-d_3) and GPC (DMAc) analyses were performed to measure the conversion, number-average molecular weight (M_n) and the polydispersity (PDI).

Experimental procedure for the kinetic study of DMA in methanol. In a similar manner to the method prescribed for the kinetic study of DMA in DMSO, the experiments in the different solvents utilized the same molar ratios; [Monomer]:[CTA]:[$Ru(bpy)_3Cl_2$] = 202: 1 : 0.000202 (leading to a catalyst concentration of 1 ppm with respect to the monomer) at a molar concentration of 10 M of the monomer with respect to the solvent. A 6 mL glass vial equipped with a rubber septum was charged with DMA (1.68 g, 16.95 mmol), BTPA (20 mg, 0.084 mmol), $Ru(bpy)_3Cl_2$ (0.013 mg, 1.734×10^{-5} mmol, 260 μL of 0.05 mg/mL methanol solution) and methanol (1460 μL, total solvent = 1720 μL). Following addition of the reactants to a 6 mL glass vial covered with aluminum foil, the reaction mixture was degassed with N_2 in an ice bath for 30 min. After purging, the reaction vessels were irradiated under blue LED light (4.8 Watts) at room temperature. Nitrogen-purged syringes were used to withdraw aliquots at predetermined time points. Again, ^{1}H NMR ($CDCl_3$) and GPC (DMAc) analyses were performed to measure the conversion, number-average molecular weight (M_n) and the polydispersity (PDI).

Experimental procedure for the kinetic study of DMA in toluene. In a similar manner to the method prescribed for the kinetic study of DMA in DMSO, the experiments in the different solvents utilized the same molar ratios; [Monomer] : [CTA] : [$Ru(bpy)_3Cl_2$] = 202 : 1 : 0.000202 (leading to a catalyst concentration of 1 ppm with respect to the monomer) at a molar concentration of 10 M of the monomer with respect to the solvent. A 6 mL glass vial equipped with a rubber septum was charged with DMA (1.68 g, 16.95 mmol), BTPA (20 mg, 0.084 mmol), $Ru(bpy)_3Cl_2$ (0.013 mg, 1.74×10^{-5} mmol, 260 µL of 0.05 mg/mL toluene solution) and toluene (1460 µL, total solvent = 1720 µL). Following addition of the reactants to a 6 mL glass vial covered with aluminum foil, the reaction mixture was degassed with N_2 in an ice bath for 30 min. After purging, the reaction vessels were irradiated under blue LED light (4.8 Watts) at room temperature. Nitrogen-purged syringes were used to withdraw aliquots at predetermined time points. Again, ^1H NMR ($CDCl_3$) and GPC (DMAc) analyses were performed to measure the conversion, number-average molecular weight (M_n) and the polydispersity (PDI).

Experimental procedure for the "ON"/"OFF" study of DMA in H_2O. In a similar manner to the method prescribed for the kinetic study of DMA in DMSO, the experiments in the different solvents utilized the same molar ratios; [Monomer] : [CTA] : [$Ru(bpy)_3Cl_2$] = 202 : 1 : 0.000202 (leading to a catalyst concentration of 1 ppm with respect to the monomer) at molar concentration of 10 M of monomer with respect to the solvent. A 6 mL glass vial equipped with a rubber septum was charged with DMA (1.68 g, 16.94 mmol), BTPA (20 mg, 0.084 mmol), $Ru(bpy)_3Cl_2$ (0.013 mg, 1.74×10^{-5} mmol, 260 µL of 0.05 mg/mL H_2O solution) and milliQ H_2O (1460 µL, total solvent = 1720 µL). Following addition of the reactants to a 6 mL glass vial covered with aluminum foil, the reaction mixture was degassed with N_2 in an ice bath for 30 min. After purging, the reaction vessels were irradiated under blue LED light (4.8 Watts) at room temperature. For the light "ON"/ "OFF" study, the reaction mixture was initially irradiated for 2h. Following this initial irradiation period, the light was turned off for an hour, then turned on again for ***x*** hours (*x* corresponds to 1h, 2h, 4h and 6h). Nitrogen-purged syringes were used to withdraw aliquots at 1 h (ON), 2 h (ON), 3 h (OFF), 4 h (ON), 5 h (OFF) and 6 h (ON). Again, ^1H NMR (D_2O) and GPC (DMAc) analyses were performed on the aliquots to measure the conversion, number-average molecular weight (M_n) and the polydispersity (PDI).

Experimental procedure for the chain extension of PDMA with DEGA, NIPAAm or OEGA in H_2O. In a similar manner to the method prescribed for the kinetic study of DMA in DMSO, PDMA was synthesized using DMA (847 mg, 8.540 mmol), BTPA (10 mg, 0.042 mmol), $Ru(bpy)_3Cl_2$ (0.0325 mg, 4.34×10^{-5} mmol, 65 µL of 0.5 mg/mL H_2O solution) and milliQ H_2O (795 µL, total solvent = 860 µL) in a 6 mL glass vial equipped with a rubber septum. The reaction mixture was covered with foil then

degassed with N_2 in an ice bath for 30 mins. Following degassing, the reaction vessel was placed under blue LED light and was irradiated for 3 h. The reaction mixture was then purified by dialysis against water for 24 h with water changed at 3 h and 16 h. The sample was then freeze dried overnight and was analyzed via ^{1}H NMR ($CDCl_3$) and GPC (DMAc). The purified sample was then chain extended with OEGA in H_2O. PDMA (50 mg, 0.00313 mmol, M_n = 17 150 g/mol (GPC)), OEGA (63 mg, 0.131 mmol), $Ru(bpy)_3Cl_2$ (0.0002 mg, 2.67×10^{-7} mmol, 10 μL of 0.05 mg/mL H_2O solution) and milliQ H_2O (1000 μL, total solvent = 1010 μL). The ratio of [Monomer]: [macroCTA]: [$Ru(bpy)_3Cl_2$] was 42 : 1 : 0.0002. The reaction mixture was covered with aluminum foil then degassed with N_2 in an ice bath for 30 mins. Following degassing, the reaction vessel was placed under blue LED light and was irradiated for 40 h. After 40 h, the reaction mixture was analyzed *via* ^{1}H NMR ($CDCl_3$) and GPC (DMAc) to measure the final conversion, number average molecular weight (M_n) and the polydispersity (PDI).

Experimental procedure for the chain extension of PNIPAAm with DMA in H_2O. In a similar manner to the method prescribed for the kinetic study of DMA in DMSO, PNIPAAm was synthesized using NIPAAm (957 mg, 8.540 mmol), BTPA (10 mg, 0.042 mmol), $Ru(bpy)_3Cl_2$ (0.0065 mg, 8.68×10^{-6} mmol, 130 μL of 0.05 mg/mL H_2O solution) and milliQ H_2O (730 μL, total solvent = 860 μL) in a 6 mL glass vial equipped with a rubber septum. The reaction mixture was covered with foil then degassed with N_2 in an ice bath for 30 mins. Following degassing, the reaction vessel was placed under blue LED light and was irradiated for 4 h. The reaction mixture was then purified by dialysis against water for 24 h with water changed at 3 h and 16 h. The sample was then freeze dried overnight and was analyzed via ^{1}H NMR ($CDCl_3$) and GPC (DMAc). The purified sample was then chain extended with DMA in H_2O. PNIPAAm (50 mg, 0.00256 mmol, M_n = 18,250 g/mol (GPC)), DMA (50 mg, 0.505 mmol), $Ru(bpy)_3Cl_2$ (0.0002 mg, 2.67×10^{-7} mmol, 10 μL of 0.05 mg/mL H_2O solution) and milliQ H_2O (1000 μL, total solvent = 1010 μL). The ratio of [Monomer]: [macroCTA]:[$Ru(bpy)_3Cl_2$] was 200 : 1 : 0.0002. The reaction mixture was covered with aluminum foil then degassed with N_2 in an ice bath for 30 mins. Following degassing, the reaction vessel was placed under blue LED light and was irradiated for 4 h. After 4 h, aliquots were removed for ^{1}H NMR ($CDCl_3$) and GPC (DMAc) analyses. The remainder of the reaction mixture was kept in darkness for 10 hr. Degased DMA (100 mg, 1010 mmol) in water (0.5 mL) was added to the solution and then irradiated under blue LED light for a further 10 h. Finally, the reaction mixture was analyzed GPC (DMAc) to measure the final conversion, number average molecular weight (M_n) and the polydispersity (PDI).

Experimental procedure for the kinetic study of DMA in biologic media. In a similar manner to the method prescribed for the kinetic study of DMA in water, using the molar ratio of

[Monomer]:[BTPA]: [$Ru(bpy)_3Cl_2$] = 202 : 1 : 0.00202 (leading to a catalyst concentration of 10 ppm with respect to the monomer) at a molar concentration of 10 M of the monomer with respect to the solvent. A 6 mL glass vial equipped with a rubber septum was charged with DMA (1.68 g, 16.95 mmol), BTPA (20 mg, 0.084 mmol), $Ru(bpy)_3Cl_2$ (0.13 mg, 1.74×10^{-4} mmol, 260 μL of 0.5 mg/mL H_2O solution) and H_2O/fetal bovine serum (90/10 v/v) (1460 μL, total solvent = 1720 μL). Following addition of the reactants to a 6 mL glass vial covered with aluminum foil, the reaction mixture was degassed with N_2 in an ice bath for 30 min. After purging, the reaction vessels were irradiated under blue LED light (4.8 Watts) at room temperature. Nitrogen-purged syringes were used to withdraw aliquots at predetermined time points. Again, ^{1}H NMR ($CDCl_3$) and GPC (DMAc) analyses were performed to measure the conversion, number-average molecular weight (M_n) and the polydispersity (PDI).

Synthesis of 2-(pyridin-2-yldisulfanyl)ethyl 2-(((butylthio)carbonothioyl)thio)propanoate (**PDS-BTP**). First, hydroxyethyl pyridyldisulfide was prepared according to the previously reported procedure.[2] The yield was 60%. The product was analyzed by ^{1}H NMR: ($CDCl_3$, 400 MHz), δ (ppm from TMS): 3.00 ppm (2H, p ,-C*H*$_2$-S-S-), 3.80 ppm (2H, t,-C*H*$_2$-OH), 5.30 (1H, s, -O*H*), 7.1 (1H, m, aromatic hydrogen meta to nitrogen, 7.70 (2H, m, para to nitrogen and ortho to thiol derivatized carbon), 8.45 (1H, q, aromatic hydrogen ortho to nitrogen); and by ^{13}C NMR, δ (ppm from TMS): 30.50 (*C*H$_2$-S-), 58.85 (HO-*C*H$_2$), 119.30 121.70, 138.02, 149.51, 159.23 (*C*H of Ar).

2-(*n*-Butyltrithiocarbonate)-propionic acid (BTPA) (1g, 4.20×10^{-3} mol) was introduced in round bottom flask (50 mL). 20 mL of dichloromethane, 4-dimethylaminopyridine (DMAP, 25 mg, 2.10×10^{-4} mol) and *N,N'*-dicyclohexylcarbodiimide (0.95 g, 4.62×10^{-3} mol) were introduced in the round bottom flask and the flask was placed in ice bath. Hydroxyethyl pyridyldisulfide (0.863 g, 4.62×10^{-3} mol) was added to the solution. The solution was stirred overnight. The solution was filtered, and then concentrated to yield a yellow product. The crude product was purified by column chromatography, using a mixture of ethyl acetate/hexane (30/70, v/v). The solvent was removed by vacuum to yield yellow oil (yield 65%). The product was analyzed by ^{1}H NMR spectroscopy (**SI, Figure S14**).

Synthesis of BSA-macroinitiator (BSA-MI). This method was adapted from a previous publication.[4] 81 mg (1×10^{-4} mol) of 2-(pyridin-2-yldisulfanyl)ethyl 2-(((butylthio)carbonothioyl)thio)propanoate (PDS-BTP) was dissolved in 1 ml of DMF and added dropwise to bovine serum albumin (BSA) solution (50 g/L, 7.5×10^{-6} mol diluted in phosphate buffer solution (pH = 6), total volume: 10 mL) to prepare BSA-macroinitiator. The mixture was gently shaken for 14 h at room temperature. An aliquot was taken and analyzed by UV-vis spectrometer to detect the presence of 2-pyridinethione, a by-product of the conjugation reaction, which appears at the maximum

of 350 nm. The excess of PDS-BTP was precipitated in water (40 mL), and the solution was centrifuged (5000 rcf for 5 min) to eliminate the excess of unreacted PDS-BTP. The solution was dialyzed against water to remove the trace of DMF and other impurities for 1 day. Then, the solution was freeze dried to yield a white/yellow powder (35 mg, yield 70%). BSA-MI (50 g/l) was re-dispersed in water.

Polymerization of DMA and OEGA using BSA-macroinitiator (BSA-MI). 200 mg (3.0 μmol) of BSA, (i.e. 55 mol% free BSA and 45 mol% BSA-MI) was dissolved in 5 ml of phosphate buffer (pH = 6). A DMA monomer solution (4 mL, 0.5 M, 2 mmol) in phosphate buffer was added slowly to the BSA-MI solution. The flask was covered by aluminum foil. A solution of $Ru(bpy)_3Cl_2$ was added to the mixture. The final concentration ratios were as follows: [DMA]:[BSA-MI]:[$Ru(bpy)_3Cl_2$] = 1200.0: 1.0: 12×10^{-3}. Following the sealing of the vials with rubber septa, the polymerization solutions were deoxygenated for 30 min in an ice bath. After purging, the reaction vessels were irradiated under blue LED light (4.8 Watts) at room temperature. Nitrogen-purged syringes were used to withdraw aliquots at predetermined time points. Aliquots were taken at predetermined time intervals and quenched via rapid cooling and exposure to oxygen. These samples were directly analyzed by ^{1}H NMR to determine the molecular weight and the monomer conversion, respectively and also by aqueous GPC analysis. Polymerization samples were treated with a solution containing tris(2-carboxyethyl)phosphine hydrochloride (TCEP) (0.5 mg/ml, 1.7 M) and incubated at 25 ^{o}C for 4 hrs. Samples were freeze dried and re-dissolved in DMAc (for 14 hrs at room temperature). The samples were then filtered through a 0.45 μm filter and analyzed by DMAc GPC.

Measurement of enzyme-like activity of BSA. The esterase-like activity of BSA towards *p*-nitrophenylacetate was performed according to published procedure.[4] 0.100 mL of BSA or BSA conjugate solution ([BSA] = 0.27 mM) in phosphate buffer (pH 8), 10 μL of nitrophenyl acetate dissolved in acetonitrile (10 mM) and 0.900 mL of phosphate buffer solution (pH 8) were rapidly mixed and incubated at room temperature for 20 min. At the end of exact incubation time, absorbance at 405 nm was measured for each sample to evaluate the activity, and normalized using native BSA. Activity measurements were performed with two different samples in triplicates. The results represent the average of 6 measurements ± standard deviation.

Part I: Investigation of photoinduced living polymerization mechanism using fluorescence spectroscopy.

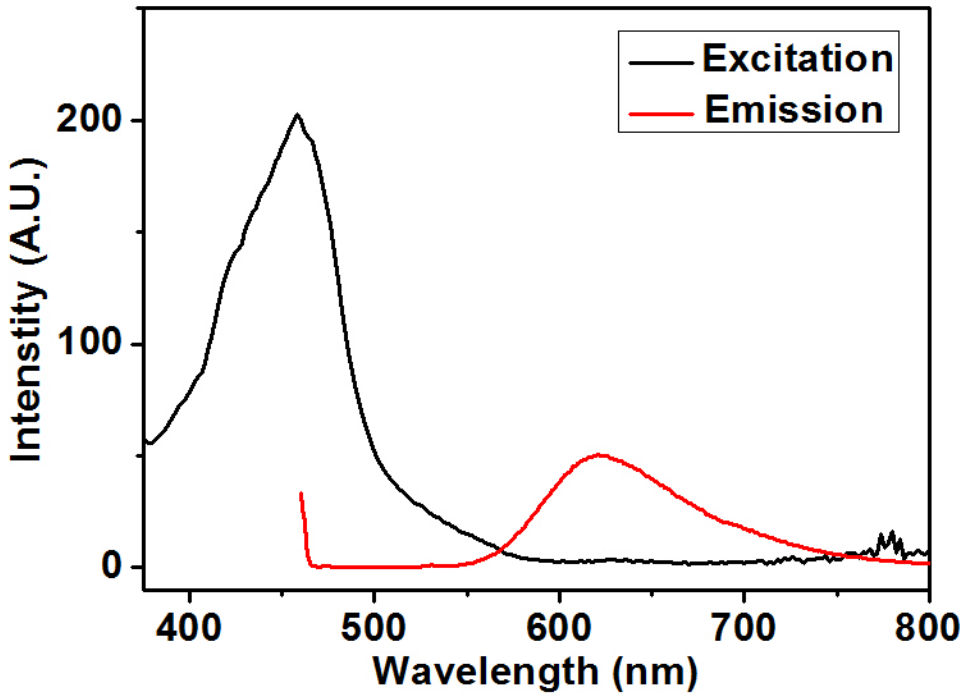
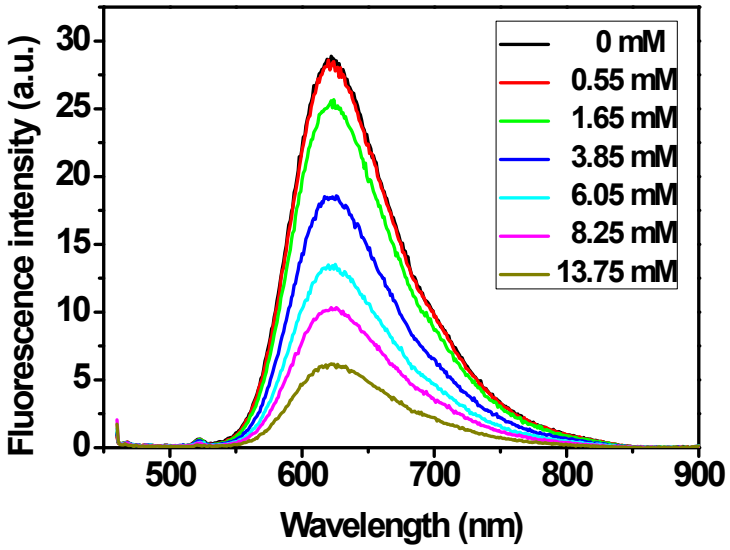
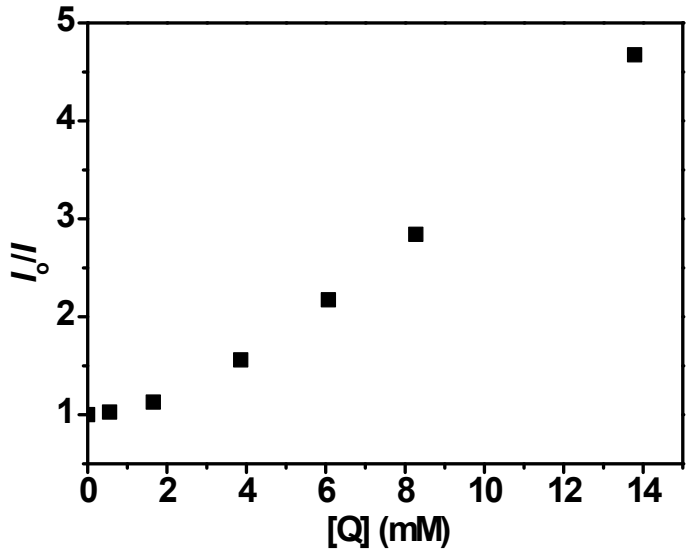


Figure S2. Excitation and emission spectra of photoredox catalyst $Ru(bpy)_3Cl_2$ in DMSO. $\lambda_{max,\ ex}$ = 458 nm, $\lambda_{max,\ em}$ = 620 nm.



(a)



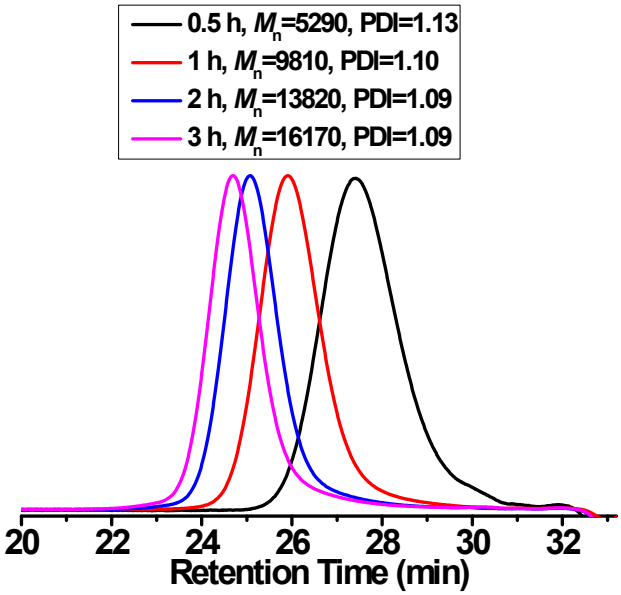
(b)

Figure S3. Fluorescence quenching (Stern-Volmer) studies of a 6.68 μM solution of $Ru(bpy)_3Cl_2$ in DMSO with varying concentrations of thiocarbonylthio compound CPADB. (a) Fluorescent emission intensity versus different concentrations of CPADB; (b) Plots of the ratio I_o/I versus quencher concentration. I_o and I correspond to the emission intensity in the absence and presence of quencher, respectively.

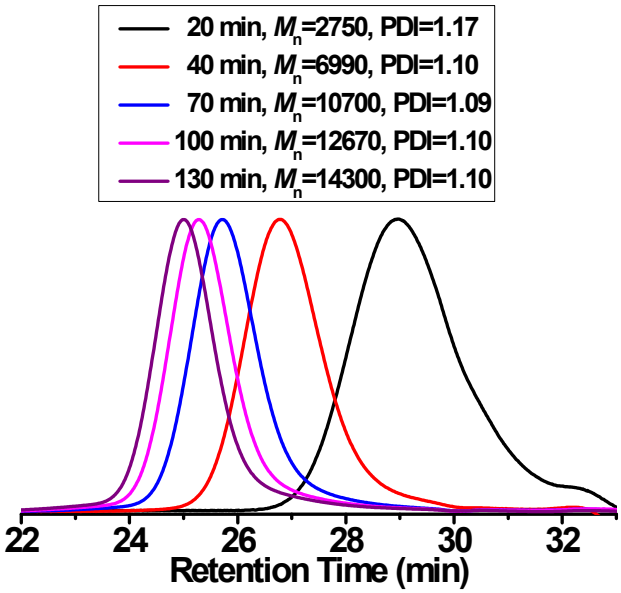
Plotting the ratio I_0/I versus the quencher concentration showed a non-linear relationship, indicative of both dynamic and static quenching behaviors. In the case of dynamic quenching (also called collisional quenching), the excited state of photoredox catalyst $Ru(bpy)_3Cl_2$ transfers the energy to the thiocarbonylthio compound, whereas static quenching results in the formation of a complex. This plot

demonstrates that a reductive or oxidative quenching is operative via photoinduced electron transfer (PET).[3]

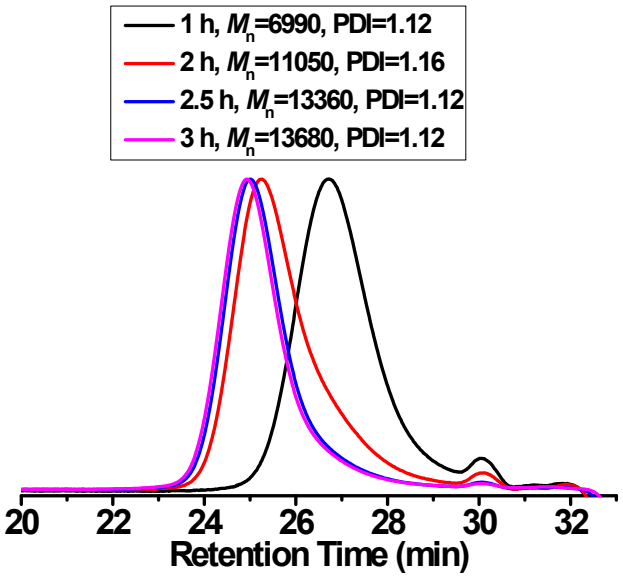
Part II: Kinetic study of aqueous photoinduced living polymerization of DMA in different solvents



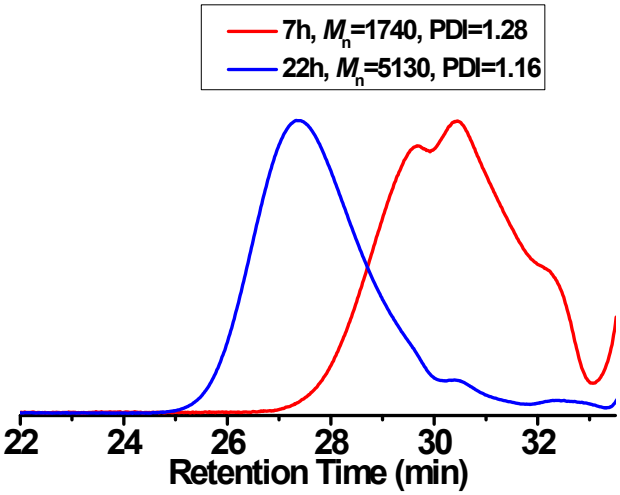
(a) DMSO



(b) Acetonitrile



(c) Methanol



(d) Toluene

Figure S4. GPC traces for the aqueous *photoinduced living polymerization* of DMA in different solvents: (a) DMSO; (b) acetonitrile; (c) methanol; (d) toluene. Experimental condition: [DMA]:[BTPA]:[$Ru(bpy)_3Cl_2$] = 202 : 1 : 2×10^{-4}, room temperature under 4.8 W blue LED light.

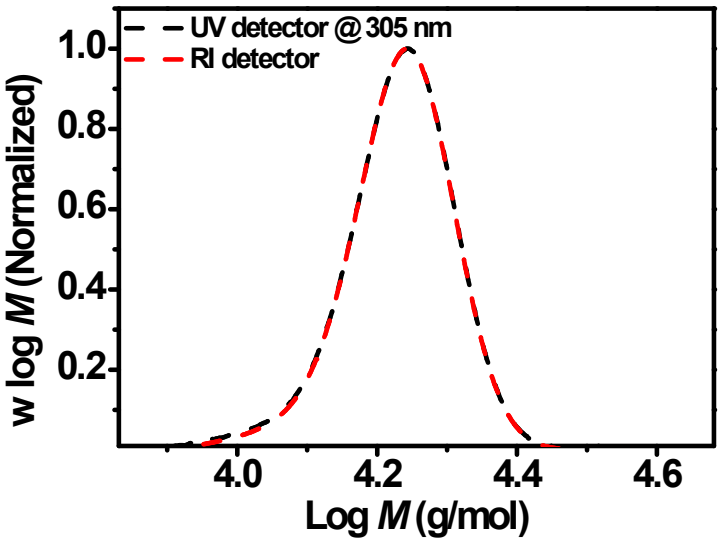


Figure S5. Molecular weight distribution (MWD) recorded by UV (black line) and RI (red line) detector for the aqueous photoinduced living polymerization of DMA at 3 h in DMSO. Experimental condition: [DMA]:[BTPA]:[$Ru(bpy)_3Cl_2$] = 202 : 1 : 2×10^{-4}, room temperature under 4.8 W blue LED light.

Part III: "ON"/"OFF" kinetic study of aqueous photoinduced living polymerization of DMA in water

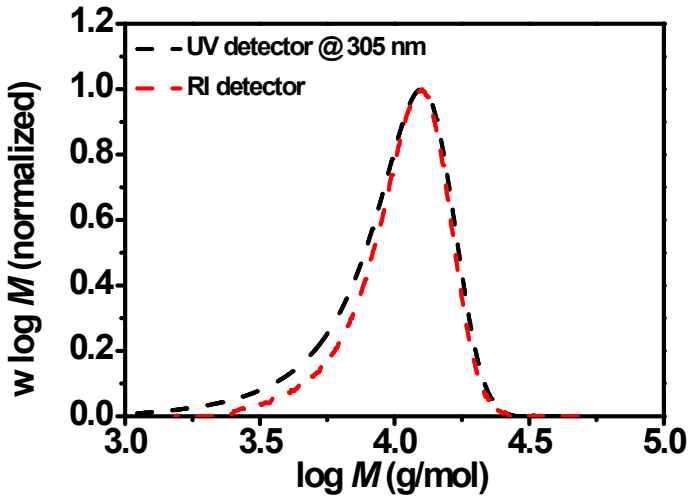
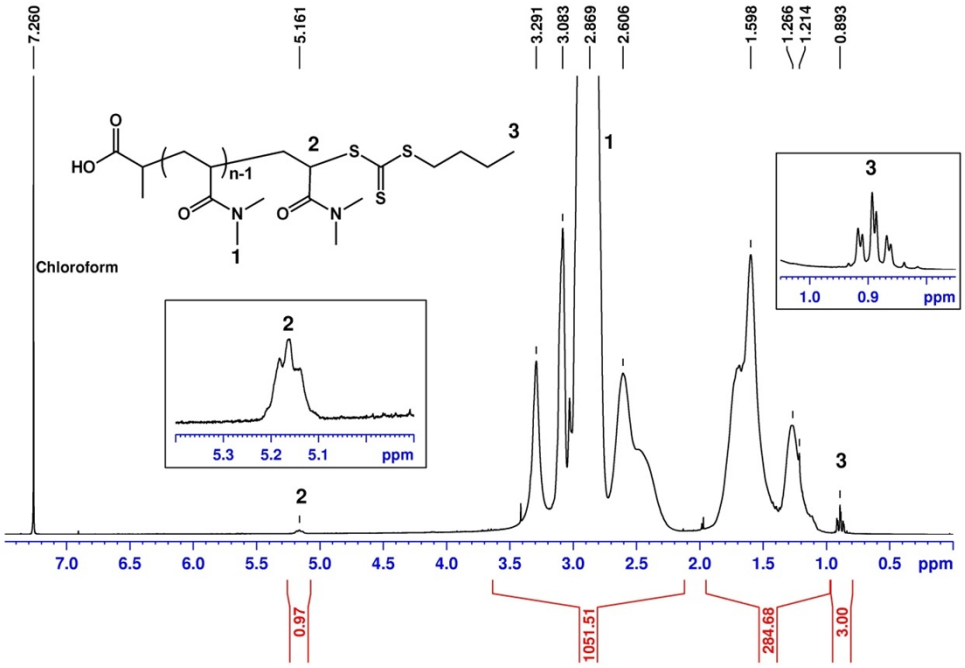
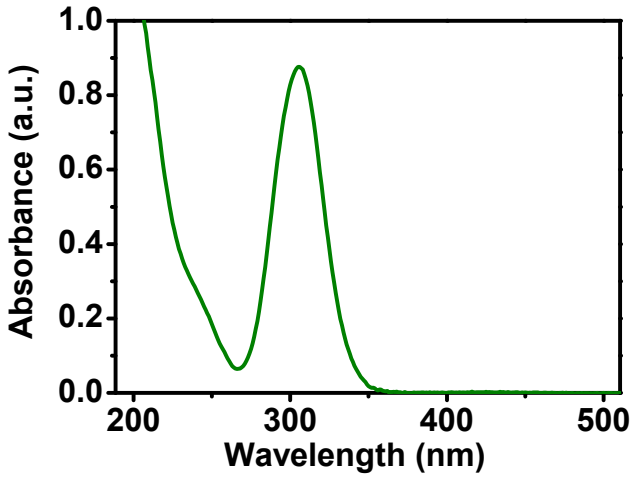


Figure S6. Molecular weight distribution (MWD) recorded by UV (black line) and RI (red line) detector for the aqueous photoinduced living polymerization of DMA at 4 h in water. Experimental condition: [DMA]:[BTPA]:[$Ru(bpy)_3Cl_2$] = 202 : 1 : 2×10^{-4}, room temperature under 4.8 W blue LED light.



(a)



(b)

Figure S7. Characterization of purified PDMA: (a) ^{1}H NMR spectrum for purified PDMA prepared by aqueous photoinduced living polymerization of DMA at 4 h in water; (b) UV-vis spectrum of purified PDMA in acetonitrile. Experimental condition: [DMA]:[BTPA]:[$Ru(bpy)_3Cl_2$] = 202 : 1 : 2×10^{-4}, room temperature under 4.8 W blue LED light. $M_{n,\,GPC}$ = 17 150 g/mol, PDI=1.10.

Note: the absorbance at 305 nm confirms the presence of trithiocarbonate (C=S). The trithiocarbonate end group functionality was determined to be ~100% using the following equation: $F^{end\ group}$ =

(Abs/ε^{BTPA})/[PDMA], where Abs, ε^{BTPA} and [PDMA] correspond to absorbance, extension coefficient of BTPA agent[4] and PDMA concentration, respectively. PDMA concentration was calculated using the molecular weight determined by NMR.

Part IV: Aqueous photoinduced living polymerization of other water soluble monomer in water

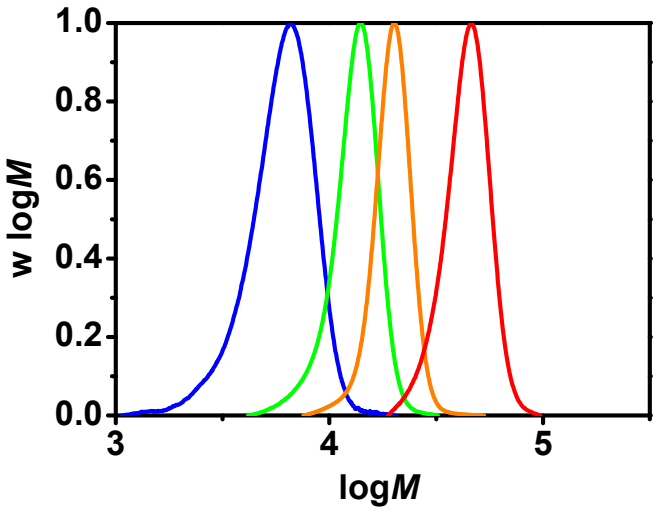


Figure S8. Example of different molecular weight distributions (MWD) recorded by RI detector for the aqueous photoinduced living polymerization of PDMA in water at room temperature under 4.8 W blue LED light.

Notes: Experimental condition:

Blue line: [DMA]:[BTPA]:[$Ru(bpy)_3Cl_2$] = 100:1:1 × 10^{-4}, Table 1, Entry 9;

Green line: [DMA]:[BTPA]:[$Ru(bpy)_3Cl_2$] = 200:1:2 × 10^{-4},Table 1, Entry 5;

Orange line: [DMA]:[BTPA]:[$Ru(bpy)_3Cl_2$] = 500:1:5 × 10^{-4},Table 1, Entry 8;

Red line: [DMA]:[BTPA]:[$Ru(bpy)_3Cl_2$] = 1000:1:10 × 10^{-4},Table 1, Entry 7.

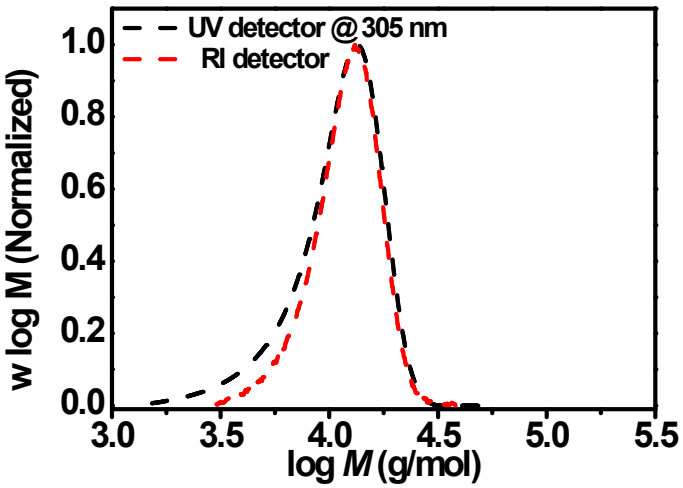
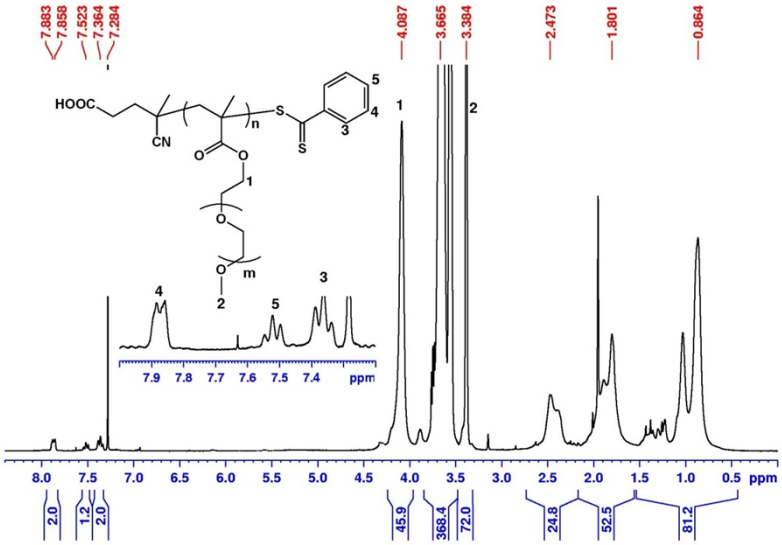
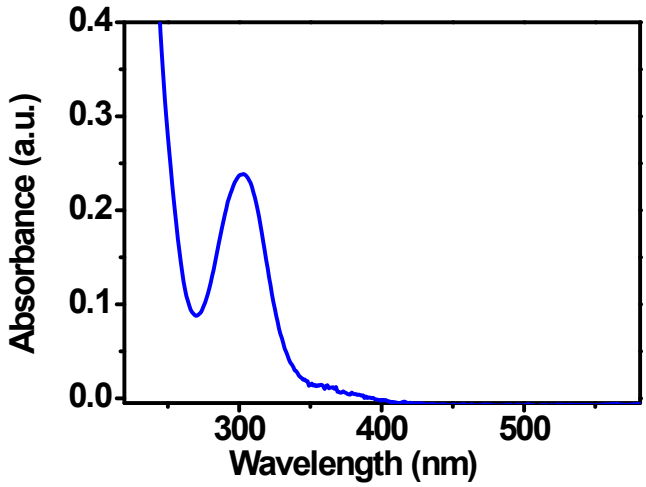


Figure S9. Molecular weight distribution (MWD) recorded by UV (black line) and RI (red line) detector for the aqueous photoinduced living polymerization of NIPAAm at 3 h in water. Experimental condition: [NIPAAm]:[BTPA]:[$Ru(bpy)_3Cl_2$] = 202 : 1 : 2×10^{-4}, room temperature under 4.8 W blue LED light.



(a)



(b)

Figure S10. Characterization of purified POEGMA: (a) ^{1}H NMR spectrum for purified POEGMA prepared by aqueous photoinduced living polymerization of OEGMA at 22 h in water. Experimental condition: [OEGMA]:[CPADB]:[$Ru(bpy)_3Cl_2$] = 70:1:3.5 × 10^{-4}, room temperature under 4.8 W blue LED light. $M_{n,\,GPC}$ = 9470 g/mol, PDI=1.18 (Entry 1 in **Table 1**).

Note: the absorbance at 305 nm confirms the presence of dithioester (C=S). The dithioester end group functionality was determined to be ~100% using the following equation: $F^{end\ group}$ = (Abs/ε^{CPADB})/[OEGMA], where Abs, ε^{CPADB} and [OEGMA] correspond to absorbance, extension

coefficient of CPADB agent[4] and OEGMA concentration, respectively. OEGMA concentration was calculated using the molecular weight determined by NMR.

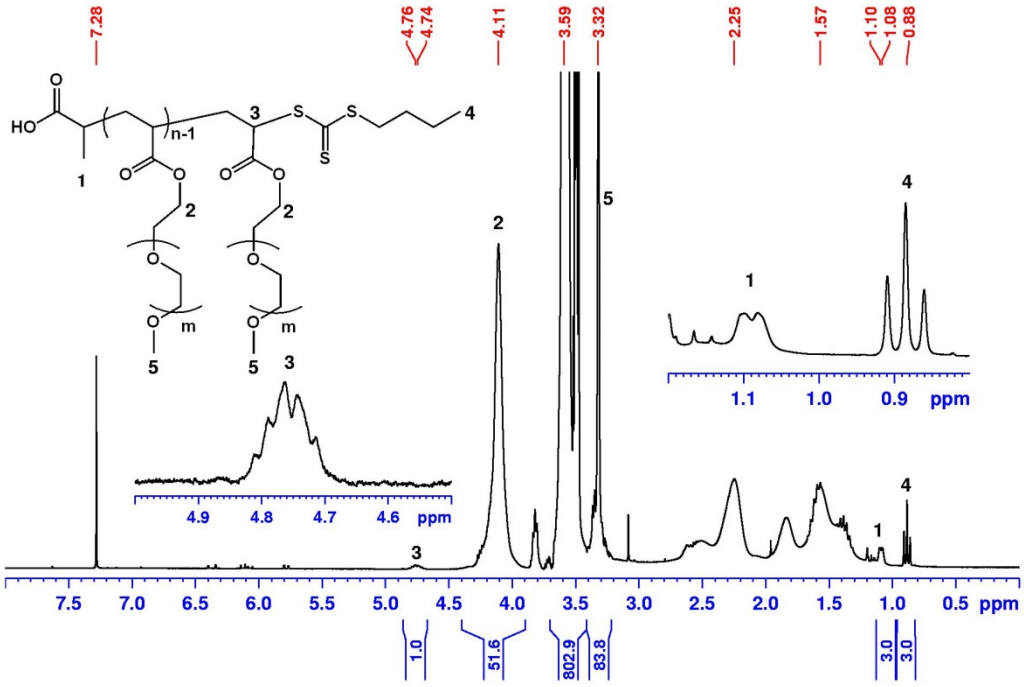
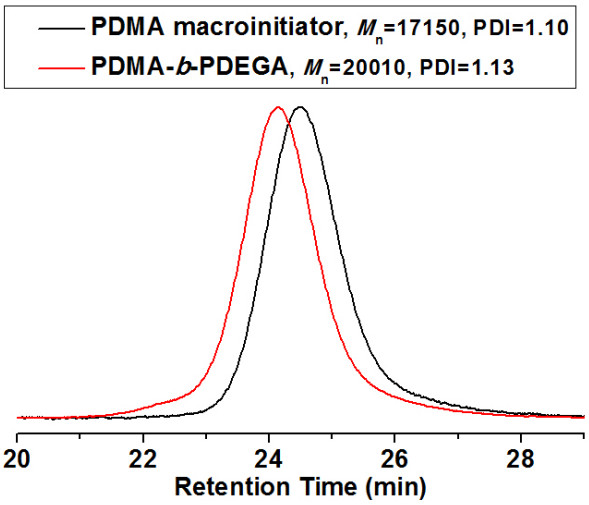
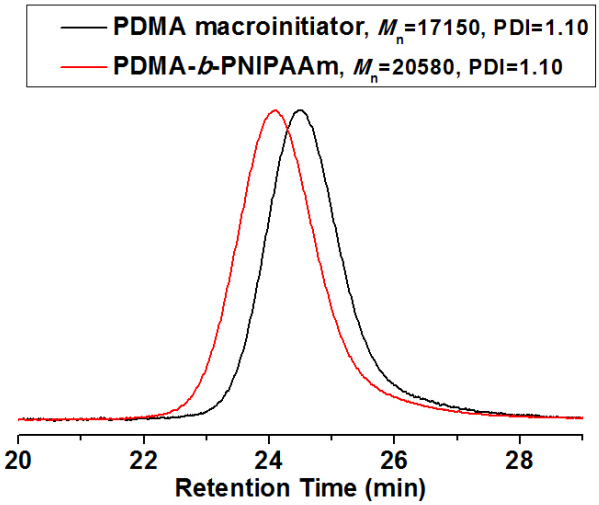


Figure S11. ^{1}H NMR spectrum for purified POEGA prepared by aqueous photoinduced living polymerization of OEGA at 22 h in water. Experimental condition: [OEGA]:[BTPA]:[$Ru(bpy)_3Cl_2$] = 50:1:2.5 × 10^{-4}, room temperature under 4.8 W blue LED light. $M_{n,\,GPC}$=15400 g/mol, PDI=1.29 (Entry 2 in Table 1).

Part V: Diblock copolymers preparation by aqueous photoinduced living polymerization in water



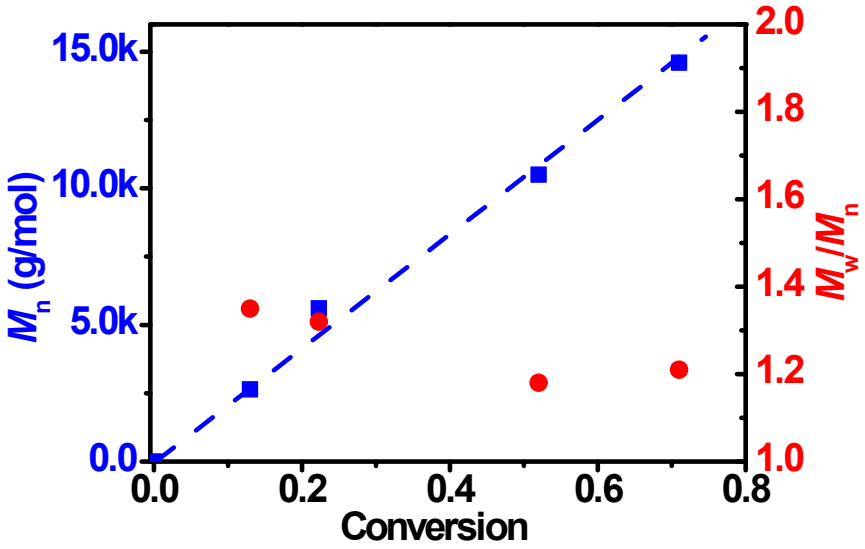
(a)



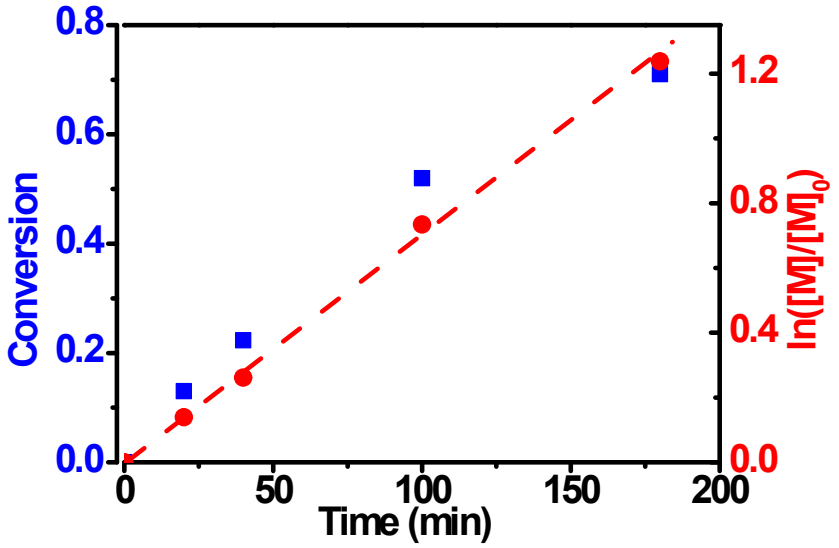
(b)

Figure S12. GPC traces for PDMA macroinitiator (black line), PDMA-*b*-PDEGA (red line, a), and PDMA-*b*-PNIPAAm (red line, b) . Experimental condition: [monomer]:[macroinitiator]:[$Ru(bpy)_3Cl_2$] = 202 : 1 : 2×10^{-4} for PDMA-*b*-PDEGA and PDMA-*b*-PNIPAAm, , room temperature under 4.8 W blue LED light in water.

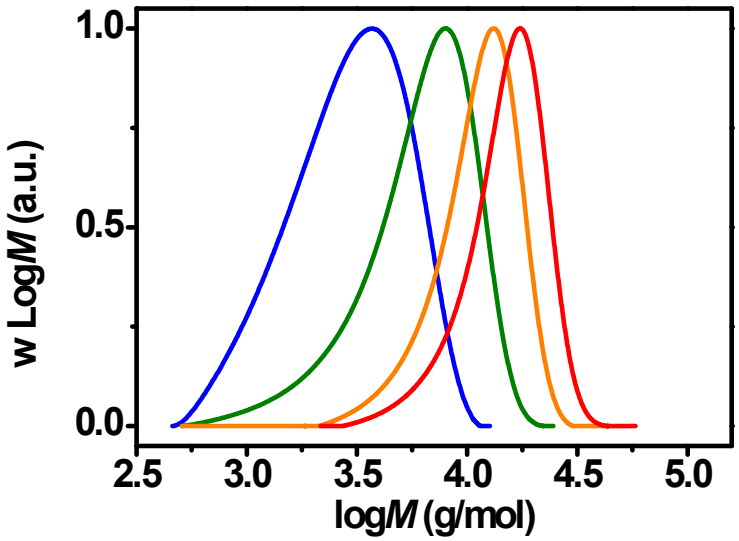
Part VI: Aqueous photoinduced living polymerization in biological media.



(a)



(b)



(c)

Figure S13. Aqueous photoinduced living polymerization of *N,N'*-dimethylacrylamide (DMA) in fetal bovine serum using BTPA as chain transfer agent and $Ru(bpy)_3Cl_2$ as photoredox catalyst under 4.8 W blue LED light: (a) M_n (■) and M_w/M_n (●) *vs.* conversion; (b) $\ln([M]_0/[M]_t)$ (●) and conversion (■) *vs.* time of exposure; (c) molecular weight distribution (MWD) at different times of exposure. Experimental condition: $[DMA]:[BTPA]:[Ru(bpy)_3Cl_2] = 202:1:2\times10^{-3}$, room temperature.

Part VII: BSA-polymer bioconjugates

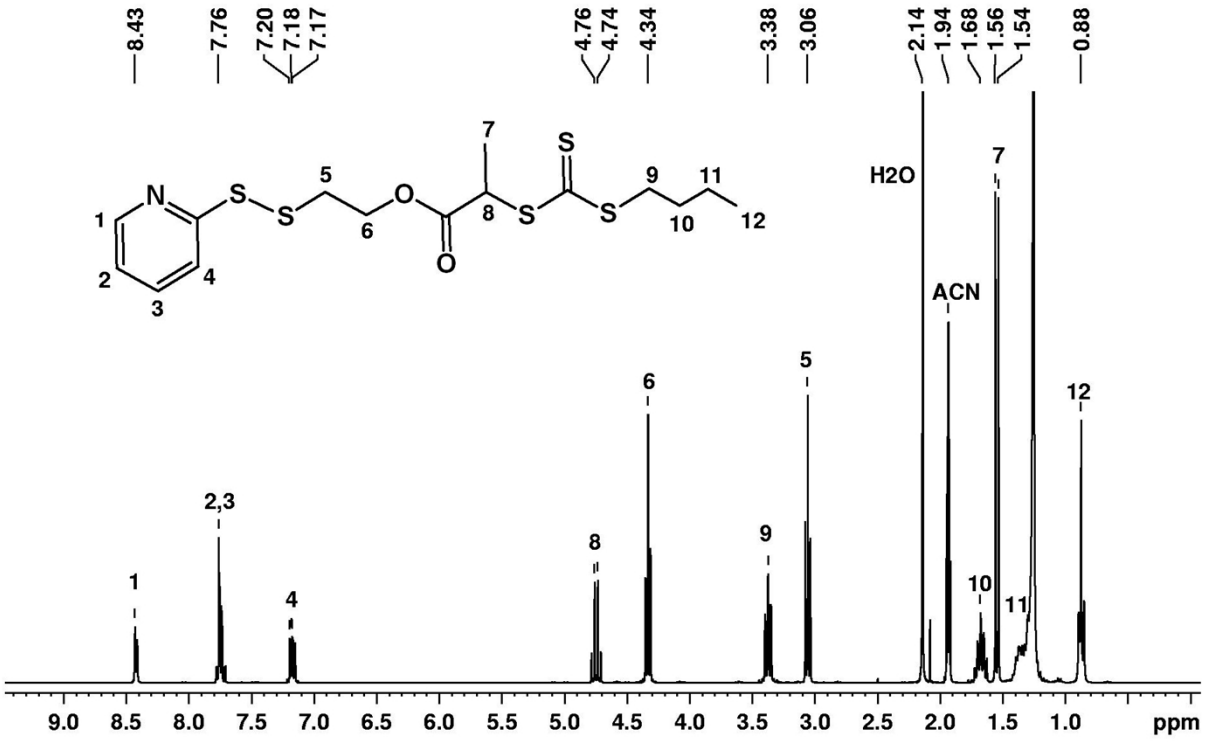


Figure S14. ^{1}H NMR spectrum of thiocarbonylthio compound (PDS-BTP) (Solvent: CD_3CN). Note: the signal at 1.3ppm was attributed to the trace of cyclohexane.

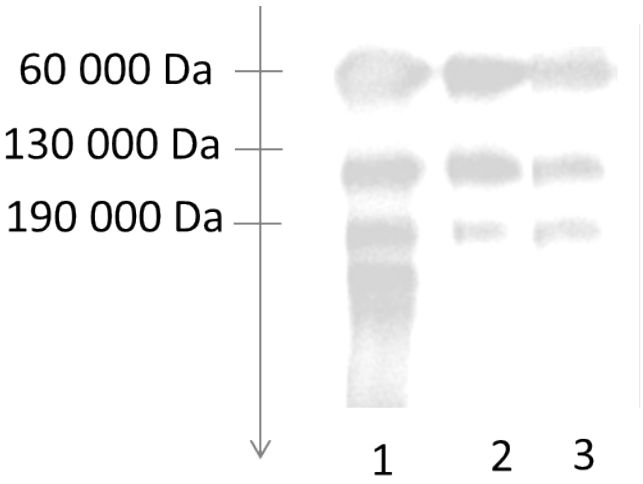


Figure S15. SDS-PAGE analysis: 1- BSA-poly(DMA) M_n = 42 000 g/mol; B- BSA-MI; C- native BSA.

Note: The conjugates appeared on the gel as higher molecular weight smears while control BSA sample appeared as two distinct bands corresponding to molecular weights of ca. 60,000 and 130,000 Da. With the polymer conjugate samples, the bands for BSA not modified with a RAFT agent (55mol% of total BSA due to the absence of free thiol) was also observed.

Additional References

[1] a) J. Xu, L. Tao, C. Boyer, A. B. Lowe, T. P. Davis, *Macromolecules* **2009**, *43*, 20-24; b) M. H. Stenzel, L. Cummins, G. E. Roberts, T. P. Davis, P. Vana, C. Barner-Kowollik, *Macro. Chem. Phys.* **2003**, *204*, 1160-1168; c) C. Boyer, A. Granville, T. P. Davis, V. Bulmus, *J. Polym. Sci. Part A: Polym. Chem.* **2009**, *47*, 3773-3794.

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