Electronic Supporting Information

for

Fluorogenic Monomer Activation for Protein-Initiated Atom Transfer Radical Polymerization

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General Methods

All air- and moisture-sensitive reactions were carried out in glassware that was oven-dried (>130°C) and cooled under nitrogen (N_2) gas. Reaction vessels were sealed with rubber septa and maintained in an inert environment under a positive pressure of anhydrous N_2. Stirring was accomplished via magnetic, Teflon-coated stir bars. Solid reagents were measured on a Mettler Toledo MS204TS balance. Air- and moisture-sensitive liquids were transferred via syringe under an atmosphere of N_2. Reaction temperatures refer to the bath temperature in which the reaction vessel was partially immersed. Room temperature indicates an external temperature of 20-25°C. Elevated temperatures were achieved by the use of a mineral oil bath heated by a VWR 620-HPS hot plate/stirrer. Buffer solutions were freshly prepared and standardized for pH with an Orion Star A211 pH meter from Thermo Scientific.

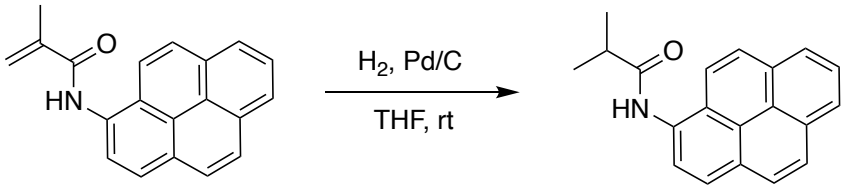
Materials

Unless otherwise noted, all commercial solvents and reagents were purchased from Millipore-Sigma USA and used as received. ATRP initiator *N*-hydroxysuccinimidyl ester **3** was synthesized as previously described.[1] Protein-initiator conjugates were purified by centrifugal filtration with Centricon Plus-20 centrifugal dialysis-filtration tubes with a 30 kDa cutoff membrane, purchased from Millipore-Sigma USA. The pyrene and anthracene methacrylamide fluorogenic monomers **1** and **5**, respectively, were synthesized as described previously.[2] Oligo(ethylene oxide) methyl ether methacrylate (**4**, 99%, average molecular weight 500) was passed over a column of basic alumina (Millipore-Sigma USA) prior to use. Water was obtained from a Barnstead Nanopure Infinity water system (18 MΩ cm). Fetal bovine serum (FBS) was obtained from Omega Scientific (USDA Certified). Gases (H_2, N_2) were 5.0 grade supplied by Praxair and used without further purification.

Physical and Spectroscopic Measurements

Protein concentrations were determined by a Nanodrop 2000 Spectrophotometer (Thermo Scientific) by absorbance analysis at 280 nm. Circular Dichroism data were obtained with a Jasco J-815 spectrophotometer. Spectra were collected at 25 ºC from 250 to 200 nm with a scan speed of 20 nm/min, a bandwidth of 1 nm, a resolution of 1 nm, a response time of 1 s, and a cycle time of 2 min. Fluorescence spectra and intensities were determined with a Photon Technology International Quanta-Master model QM-1 fluorimeter with 2 mm slit width. Samples were run in quartz cuvettes (4 clear walls, 10 mm with screw cap and septum) purchased from Science Outlet. Gel permeation chromatography (GPC) was performed in THF at a flow rate of 0.5 mL/min on a Tosoh Biosciences EcoSEC Elite GPC system with refractive index detector. Samples were run on a TSKgel GMHHR-M mixed bed polymer column and calibrated with polystyrene. High resolution mass spectrometry (HRMS) was performed at the University of Texas San Antonio mass spectrometry core with a Bruker microTOFMS or at Trinity University with an Angilent 6230 TOF LC/MS mass spectrometer with an electrospray ion source in the positive ion mode. Separations were performed on C18 columns with acetonitrile:water solvent systems spiked with 0.1% formic acid. Photos of polymerization reactions were taken with iPhone cameras and illuminated by long-wave (365 nm) ultraviolet (UV) light using a hand-held UV lamp (UVP compact).

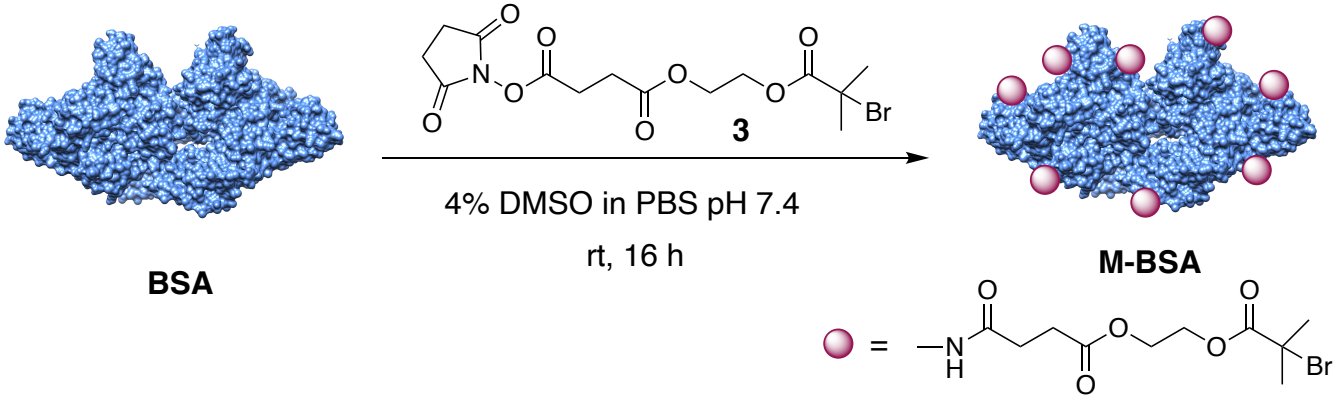
Hydrogenation of Pyrene Methacrylamide



1 **2**

Pyrene methacrylamide 1[19] (40 mg, 0.14 mmol) was dissolved in 10 mL of THF. An aliquot (3 mL) of this solution was transferred to a cuvette and observed for fluorescence by fluorimeter emission scans and photographs after irradiation by long-wave UV light. Following transfer of the aliquot back into the round bottom flask, 10% Pd/C (15 mg, 0.14 mmol) was added to the solution. Hydrogen gas (H_2) was bubbled through the solution for 1 h at rt. A 3 mL aliquot was again removed for fluorescence measurements. Full conversion of starting material to the reduced product was observed by TLC (9:1 CH_2Cl_2:hexanes).

Synthesis of BSA-Initiator Conjugate, M-BSA

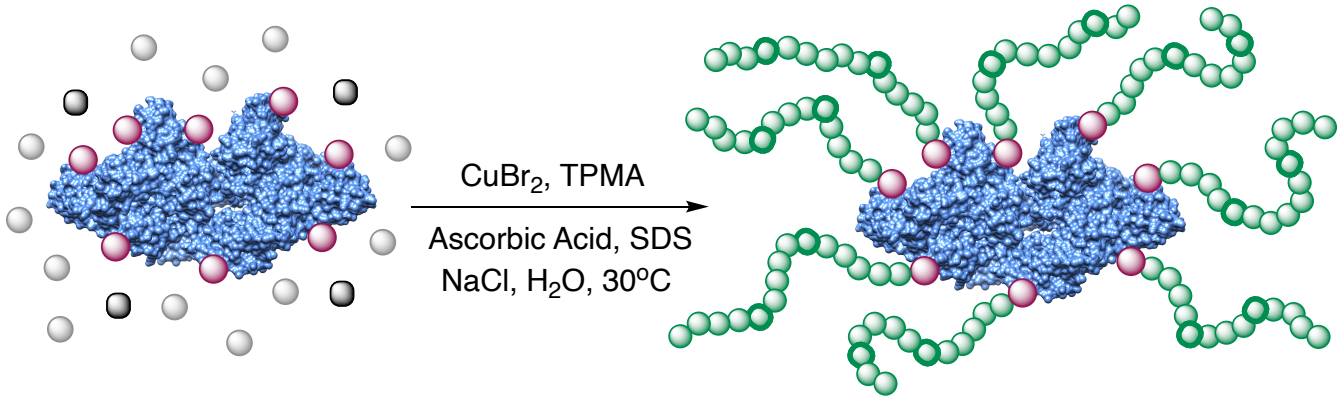


N-hydroxysuccinimidyl ester **3** (1.0 g, 5.5 mmol) was dissolved in 2 mL of DMSO and sonicated. Unmodified BSA (**BSA**, 1.0 g, 0.5 mmol Lysine residues) was dissolved in 500 mL of 0.1 M PBS (pH 7.4) and the DMSO solution containing **3** was added dropwise. The reaction was stirred for 18 h at rt, then purified by centrifugal filtration in Centricon Plus-20 centrifugal dialysis-filtration tubes with a 30 kDa cutoff membrane. Each aliquot was washed 4x with deionized water, then eluted with 0.5 mL H_2O. Aliquots were combined, protein concentrations determined by Nanodrop, and diluted with deionized water to provide 100 mM (4 mg/mL) protein stock solutions of **M-BSA** (11 initiator equivalents per lysine). The reaction was performed similarly with a reduction in amount of *N*-hydroxysuccinimidyl ester **3** (0.5 g, 2.75 mmol) to afford **M-BSA** with 5.5 initiator equivalents per lysine. In all cases, total protein recovery was > 90%.

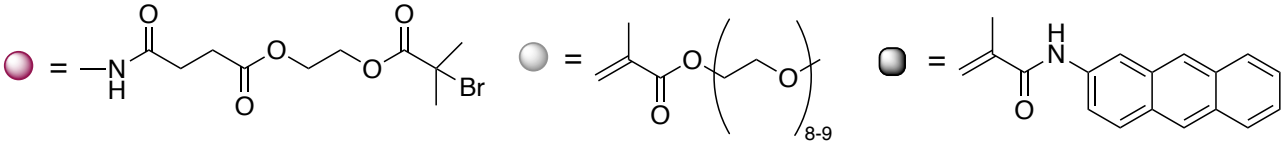
Circular Dichroism Denaturation Experiments

Protein solutions (**BSA**, **M-BSA** with 5.5 or 11 initiator equivalents per lysine) in water were diluted with PBS buffer (pH 7.4) to prepare samples with final protein concentrations of 0.05 mg/mL (total volume 0.350 mL). A guanidinium hydrochloride (Gdn-HCl) solution was also prepared (8 M in PBS pH 7.4). Denatured protein samples were accessed by mixing **BSA** (0.05 mg/mL) and Gdn-HCl (7 M), adding PBS as necessary for a total volume per sample of 0.350 mL. The samples were equilibrated for 1 h at rt before CD spectral analysis (250 to 200 nm spectra at 25 ºC).

Fluorogenic ATRP from Protein Surface



M-BSA **M-BSA Fluorescent Polymer**



4 **5**

Fluorogenic ATRP reactions from the surface of the BSA protein were conducted with slight modifications from previously published fluorogenic ATRP small-molecule methods.[2] **BSA** or **M-BSA** protein (0.006 mmol, 60 μL of 100 mM stock solution) were combined with PEG methacrylate monomer **4** (0.7043 g, 1.5 mmol), sodium chloride (NaCl, 17.4 mg, 0.3 mmol), stock solutions of 25 mM copper bromide ($CuBr_2$) and 200 mM tris(2-pyridylmethyl)amine (TPMA) in water (6 μL, final concentrations 0.15 μmol of $CuBr_2$ and 1.2 μmol of TPMA) and a 5 mM stock solution of fluorogenic methacrylamide anthracene monomer **5** in water with 366 mM sodium dodecyl sulfate (SDS, 0.300 mL, final concentrations 0.150 mmol monomer **5**, 0.102 mmol SDS). Deionized water was added to give a final volume of 2.30 mL in a round-bottom flask. DMF was added (0.03 mL) as an internal standard for monitoring the reaction by ^{1}H NMR. The flask was sealed and purged with nitrogen bubbling through the solution for 30-60 min, and then the solution was transferred to a sealed quartz cuvette containing a stir bar and placed in a 30°C oil bath under nitrogen. A 16 mM ascorbic acid solution in water was separately purged with nitrogen for 30-60 min, then 0.090 mL (1.4 μmol) was slowly added to the cuvette to start the reaction. At various times, the cuvettes were removed from the oil bath and examined for fluorescence by fluorimeter emission scans and/or photographs after irradiation by long-

wave UV light. After 1 h, an additional 0.090 mL (1.4 μmol) of degassed ascorbic acid solution was added and the reaction continued to be monitored for up to 24 h. In select cases, the synthesized polymers were cleaved from the protein by adding 200 μL of the reaction mixture to 200 μL of 5% KOH solution for 2 h at room temperature, then diluted in THF and analyzed by GPC.

For experiments probing the bioorthogonality of the assay, fluorogenic ATRP reactions were performed as described above with the addition of 80 μL fetal bovine serum (FBS, Omega Scientific) into the polymerization mixture.

Supplemental Figures

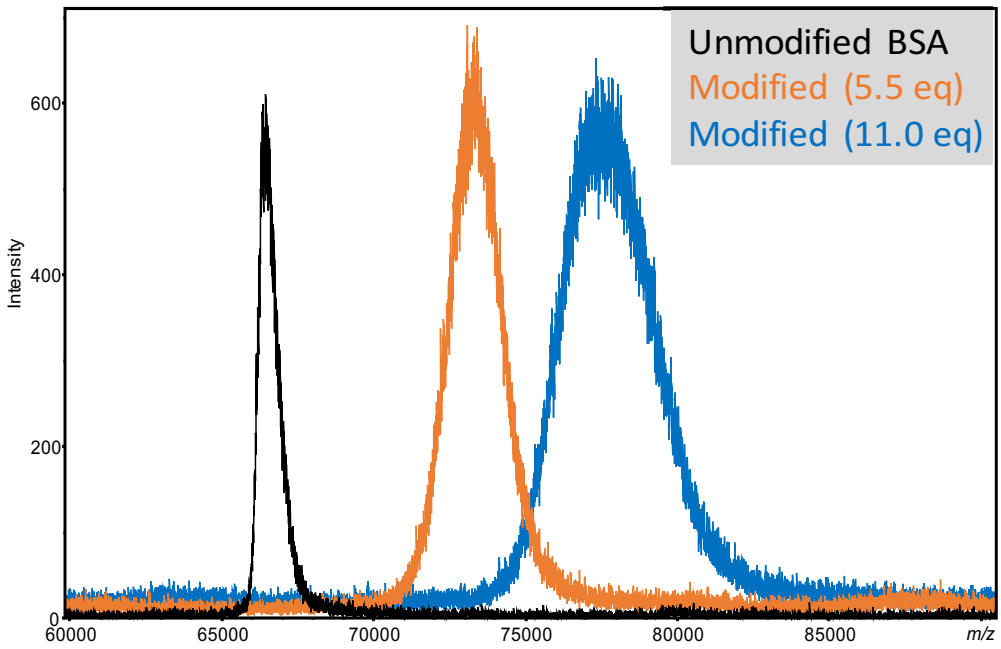


Figure S1. Mass Spectrometry Analysis Demonstrates an Increase in Molecular Weight upon Initiator Conjugation. Relative to Unmodified BSA (**BSA**), ATRP initiator conjugates (**M-BSA**) give higher m/z ratios with increasing initiator reaction concentration (5.5 vs 11 equivalents of initiator per BSA lysine). **M-BSA** with 5.5 equivalents demonstrated a mass increase consistent with 23-24 modifications per protein, with 11 equivalents giving a mass increase consistent with 37-38 modifications.

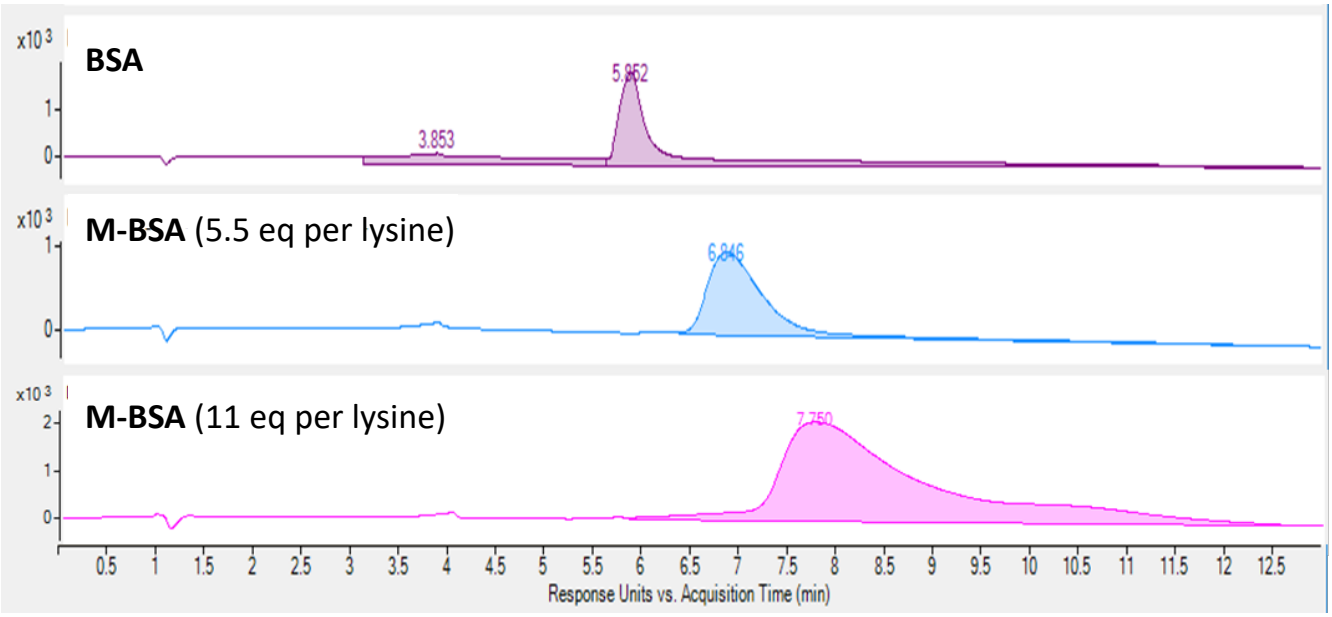


Figure S2. Initiator-Modified BSA Protein Conjugates Observe a Reduction in Surface Polarity and an Increase in Heterogeneity as a Function of Initiator Modification by Reverse-Phase HPLC. Higher retention times indicate higher acetonitrile concentration for elution (run on a 40:60 to 100:0 CH_3CN:H_2O gradient for 10 minutes with C18 column), which represents a change in physical properties from a reduction in surface polarity moving from unmodified BSA (**BSA**) to modified BSA samples with increasing number of surface modifications (**M-BSA**, 5.5 versus 11 equivalents initiator / lysine). Peak broadening indicates an increase in sample heterogeneity from variable surface modification with higher initiator load.

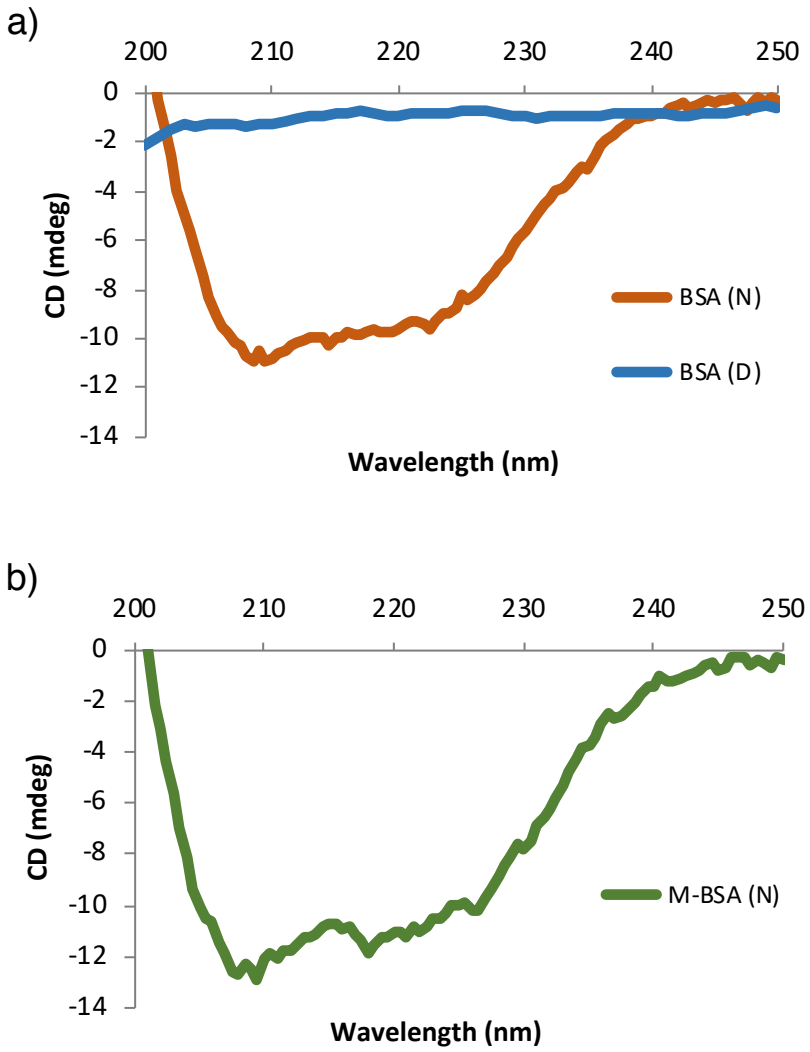


Figure S3. M-BSA with 5.5 equivalents initiator / BSA Lysine Retains Protein Fold. a) Circular dichroism (CD) spectra of unmodified **BSA** (native (N) and denatured (D) by 7 M Gdn-HCl). b) CD spectrum of native **M-BSA**, 5.5 equivalents of initiator per BSA lysine upon conjugation.

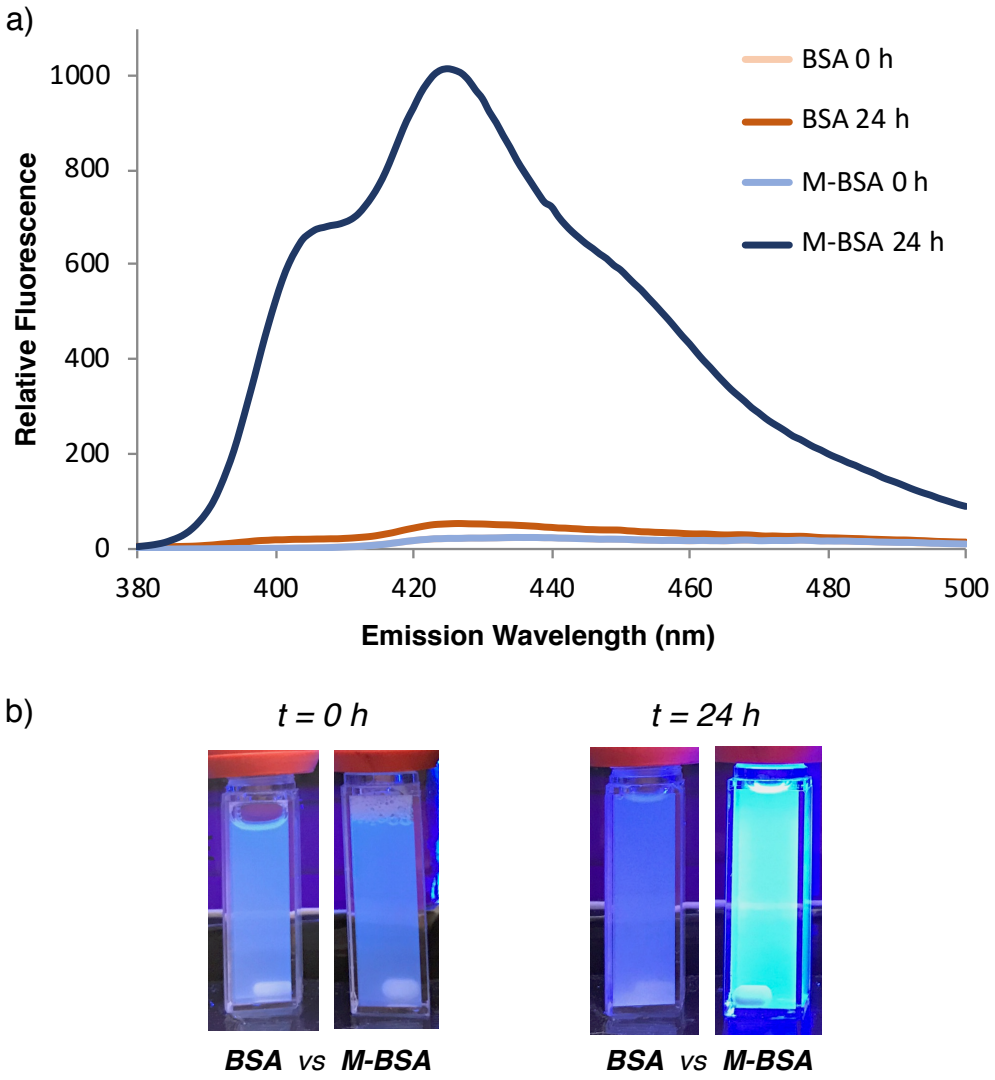


Figure S4. M-BSA with 11 equivalents initiator / BSA Lysine is Similarly Capable of Fluorogenic ATRP. Plot of fluorescence emission (a) and photographs (b) at indicated times of fluorogenic ATRP reaction shown in **Scheme 2** utilizing **M-BSA** initiator with increased initiator modification (11 equivalents initiator / BSA lysine) compared to unmodified **BSA** following 371 nm excitation.

References

1 S. Averick, A. Simakova, S. Park, D. Konkolewicz, A. J. D. Magenau, R. A. Mehl and K. Matyjaszewski, *ACS Macro Lett.*, 2012, **1**, 6–10.

2 Z. T. Allen, J. R. Sackey-Addo, M. P. Hopps, D. Tahseen, J. T. Anderson, T. A. Graf and C. B. Cooley, *Chem. Sci.*, 2019, **10**, 1017–1022.