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Supporting Information

for

Oxygen tolerant, photoinduced Controlled Radical Polymerization approach for the synthesis of *Giant Amphiphiles*

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1. Materials

Chemicals were purchased from Fluka Chemica, Sigma-Aldrich or Acros and used as received, unless otherwise specified. Bovine serum albumin (BSA) was purchased from Sigma (>99%). Tris-(2-(dimethylamino)ethyl)amine (Me_6Tren)[1, 2] and the biomacroinitiator BSA-Br (I_o)[3] were synthesized according to the literature and stored at 4 ºC. Dialysis bags (Spectra/Por® Biotech Regenerated Cellulose Dialysis Membranes (MWCO 10 and 25 kDa) were purchased from Spectrum Labs.

2. Analytical Techniques

Size Exclusion Chromatography (SEC).

Aqueous size exclusion chromatography (SEC) was conducted using a Shimadzu modular system comprising a DGU-14A solvent degasser, a LC-10AD pump, a CTO-10A column oven, a SIL-10AD auto-injector, a RID-10A refractive index detector and an SPD-10A Shimadzu U.V. Vis. Spectrometer. The system was equipped with a Polymer Laboratories 30 × 7.8mm 5µm BioBasic SEC 60 guard column followed by a 300x7.8mm 5µm BioBasic SEC 300 Polymer Laboratories column, using a mixture of 5 mM phosphate buffer, and 10% MeCN as the eluent at room temperature (flow rate: 0.5 or 1.0 mL/min). Chromatograms were acquired at 254 nm and 280 nm wavelength and were processed with the EZStart 7.3 chromatography software.

Gel Permeation Chromatography (GPC) was performed on a Shimadzu modular system comprising a CBM-20A system controller, an LC-20AD pump (flow rate at 1 mL min^{-1}), an SIL-20A automatic injector, a 10.0 µm bead-size guard column (50 × 7.5 mm) followed by PLgel Mixed-D column (300 × 7.5 mm, bead size: 5 µm, pore size maximum: 5000 Å), an SPD-20A ultraviolet detector, and an RID-20A differential refractive index detector. The columns' temperature was maintained at 40 °C using a CTO20AC oven. HPLC grade THF was used as eluent. Calibration was performed using commercial narrow molecular weight distribution polystyrene standards with molecular weights ranging from 580 to 299400 $g \cdot mol^{-1}$ (Polymer Laboratories). Before injection, all samples were passed through 0.45 µm filters.

Native Polyacrylamide Gel Electrophoresis (PAGE).

Discontinuous Native PAGE (Ornstein-Davis) electrophoresis was run using a 4% stacking gel and a 10% resolving under standard nondenaturing conditions. Samples were dissolved in TRIS buffer containing bromophenol blue and were visualized using Coomasie Brilliant Blue or Silver Staining.

NMR Spectroscopy

^{1}H and ^{13}C NMR spectra were recorded on Bruker 500 MHz spectrometer system. All chemical shifts are reported in ppm (δ) relative to tetramethylsilane, referenced to the chemical shifts of residual solvent resonances (^{1}H and ^{13}C). The following abbreviations were used to explain the multiplicities: s = singlet, bs = broad singlet, d = doublet, t = triplet, m = multiplet.

IR Spectroscopy

Infrared spectroscopy was performed with a Nicolet 6700 Attenuated Total Reflection Fourier Transform Infrared (ATR FT-IR) spectrometer using Omnic (Thermo Electron Corporation) software.

UV Spectroscopy

Activity tests were performed on a Shimadzu UV-1900 UV-VIS spectrophotometer.

Scanning Electron Microscopy.

Scanning electron microscopy was performed with a JEOL JSM 6390LV Scanning Electron Microscope operated at 10-20 kV. SEM microscopy was also performed using a ZEISS Gemini SEM - Field Emission Scanning Electron Microscope. Before sample imaging all samples were dried and sputter coated with ca. 10 nm of gold (Au).

Transmission Electron Microscopy.

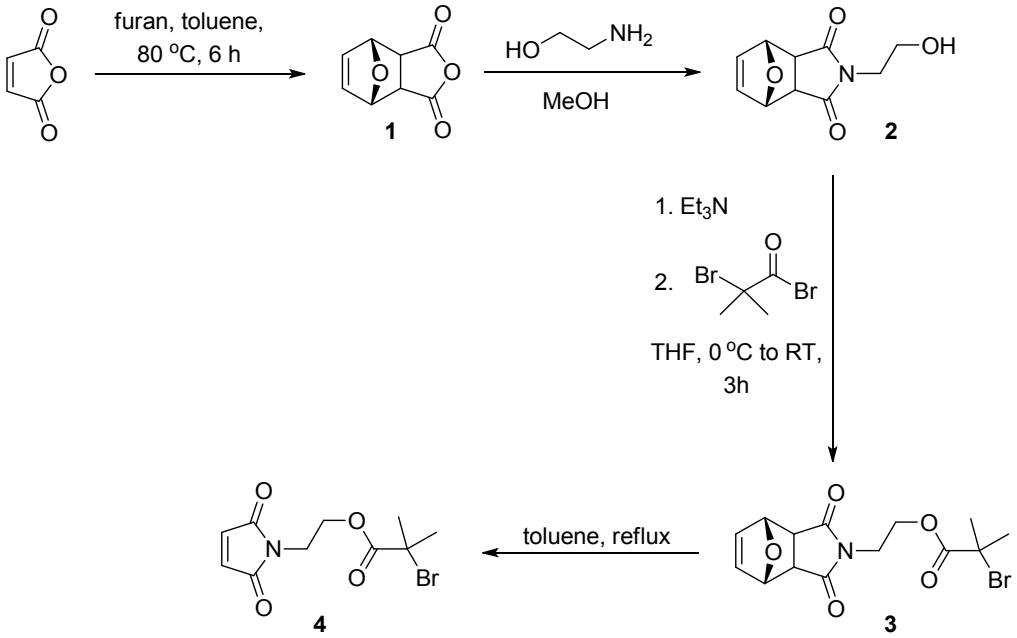
TEM micrographs were obtained using a JEOL JEM-2100 transmission electron microscope at an accelerating voltage of 200 kV. The samples for TEM were prepared by dropcasting 7 µL of the diluted solution (1 µL of the sample dissolved in 1 mL of DI H_2O) onto lacey carbon-copper grids supplied by Agar Scientific and were left to dry at room temperature for ~2 h. TEM experiments were also performed using a JEOL JEM-100C microscope operating at 80 kV.

UV Source.

A UV nail gel curing lamp (-365 nm) equipped with four 9 Watt lamps (36 Watt) was used.

3. Experimental Procedures and Results

3.1. Synthesis of the Maleimido-ATRP Initiator[2, 3]



4,10-Dioxatricyclo[5.2.1.02,6]dec-8-ene-3,5-dione (1) Maleic anhydride (30.0 g, 306 mmol) was suspended in 150 mL of toluene and the mixture warmed to 80 °C. Furan (33.4 mL, 459 mmol) was added via syringe and the turbid solution stirred for 6 h. The mixture was then cooled to ambient temperature and the stirring stopped. After 1 h, the resulting white crystals were collected by filtration and washed with 2 × 30 mL of petroleum ether to obtain 44.4 g (267 mmol, 87% yield) of the product as small white needles.

^{1}H NMR (500 MHz, $CDCl_3$, 298 K): δ = 3.18 (s, 2H, CH), 5.47 (t, *J* =0.9 Hz, 2H, OCH), 6.58 (t, *J* = 0.9 Hz, 2H, CH_{vinyl}); ^{13}C NMR (125 MHz, $CDCl_3$, 298 K): δ = 48.69 (2C, CH), 82.20 (2C, OCH), 136.97 (2C, CH_{vinyl}), 169.86 (2C, CO).

4-(2-Hydroxyethyl)-10-oxa-4-aza-tricyclo[5.2.1.02,6]dec-8-ene-3,5-dione (2) The anhydride **1** (2.00 g, 12.0 mmol) was suspended in MeOH (50 mL) and the mixture cooled to 0 °C. A solution of ethanolamine (0.72 mL, 12.0 mmol) in 20 mL of MeOH was added dropwise (over ~ 10 min) and the resulting solution was stirred for 5 min at 0 °C, then 30 min at ambient temperature, and finally refluxed for 4 h. After cooling the mixture to ambient temperature, the solvent was removed under reduced pressure, and the white residue was dissolved in 150 mL of CH_2Cl_2 and washed with 3 x 100 mL of water. The organic layer was dried over $MgSO_4$ and filtered. Removal of the solvent under reduced pressure furnished an off-white residue that was purified by flash chromatography to give the product (1.04 g, 5.00 mmol, 42% yield) as a white solid.

^{1}H NMR (500 MHz, $CDCl_3$, 298 K): δ = 2.14 (bs, 1H, OH), 2.90 (s, 2H, CH), 3.70-3.73 (m, 2H, NCH_2), 3.76-3.79 (m, 2H, OCH_2), 5.29 (t, *J* = 0.9 Hz, 2H, CH), 6.53 (t, *J* = 0.9 Hz, 2H, CH_{vinyl}); ^{13}C NMR (125 MHz, $CDCl_3$, 298 K): δ = 41.81

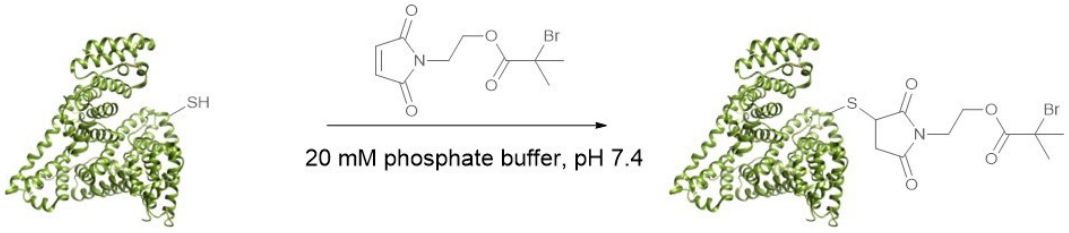
(1C, NCH_2), 47.48 (2C, CH), 60.46 (OCH_2), 80.99 (2C, OCH), 136.52 (2C, CH_{vinyl}), 176.78 (2C, CO).

2-Bromo-2-methyl-propionic acid 2-(3,5-dioxo-10-oxa-4-aza-tricyclo[5.2.1.02,6]dec-8- en-4-yl)-ethyl ester (3) A solution of the alcohol **2** (2.22 g, 10.6 mmol) and Et_3N (1.60 mL, 11.7 mmol) in 120 mL of THF (the solution remained slightly turbid) was cooled to 0 °C, and a solution of 2-bromo isobutyryl bromide (1.40 mL, 11.1 mmol) in 40 mL of THF was added dropwise (30 min). The white suspension was stirred for 3 hours at 0 °C and subsequently at ambient temperature overnight. TLC revealed the complete disappearance of the starting material. The ammonium salt was filtered off and the solvent removed under reduced pressure to give a pale-yellow residue that was purified by flash chromatography (CC, SiO_2, petroleum ether/ethyl acetate 1:1). We obtained 3.54 g (9.88 mmol, 93% yield) of **3** as a white solid.

^{1}H NMR (500 MHz, CDCl3, 298 K): δ = 1.89 (s, 6H, CH_3), 2.87 (s, 2H, CH), 3.81 (t, *J* = 5.3 Hz, 2H, NCH_2), 4.33 (t, *J* = 5.3 Hz, 2H, OCH_2), 5.27 (t, *J* = 0.9 Hz, 2H, OCH), 6.51 (t, *J* = 0.9 Hz, 2H, CH_{vinyl}); ^{13}C NMR (125 MHz, $CDCl_3$, 298 K): δ = 30.55 (1C, CH_2), 37.57 (1C, NCH_2), 47.46 (2C, CH), 55.65 (1C, $C(CH_3)_2Br$), 62.17 (1C, OCH_2), 80.82 (2C, OCH), 136.52 (2C, CH_{vinyl}), 171.39 (1C, CO_{ester}), 175.87 (2C, CO_{imide}).

2-Bromo-2-methyl-propionic acid 2-(2,5-dioxo-2,5-dihydro-pyrrol-1-yl)-ethyl ester (4) A solution of the maleimido-protected initiator **3** (0.15 gr, 0.419 mmol) was suspended in dry toluene (5 ml) and heated to reflux under nitrogen atmosphere for 16 hours. The solvent was removed under reduced pressure to give a pale-yellow residue which was subsequently purified by flash chromatography (SiO_2, petroleum ether/ethyl acetate 4:1) to yield **4** as a slightly yellow solid (90% yield). ^{1}H NMR (500 MHz, $CDCl_3$, 298 K): δ = 1.89 (s, 6H, CH_3), 3.83-3.88 (m, 2H, NCH_2), 4.31-4.35 (t, *J* = 5.3 Hz, 2H, OCH_2), 6.73 (s, 2H, CH_{vinyl}); ^{13}C NMR (125 MHz, $CDCl_3$, 298 K): δ = 30.58 (2C, CH_3), 36.57 (2C, NCH_2), 55.42 (1C, $C(CH_3)_2Br$), 62.87 (OCH_2), 134.25 (2C, CH_{vinyl}), 170.30 (1C, CO_{ester}), 171.45 (2C, CO_{imide}).

3.2 Preparation of Bovine Serum Albumin Macroinitiator BSA-Br (I_o)



A solution of 2-bromo-2-methyl-propionic acid 2-(2,5-dioxo-2,5-dihydro-pyrrol-1-yl)-ethyl ester (**4**) (157.5 mM, 0.8 mL) in DMSO was slowly added to 9.0 mL of a 0.35 mM solution of BSA in 20 mM sodium phosphate buffer (pH 7.4). The reaction mixture was gently shaken for 48 hours at 7°C. To eliminate the excess

of the maleimido-ester **4**, the mixture was then extensively dialyzed initially against 2 % DMSO in 5 mM phosphate buffer, then against 5 mM phosphate buffer and finally against 20 mM phosphate buffer using regenerated cellulose dialysis membranes (MWCO 10 kDa). The macroinitiator BSA-Br (I_o) was characterized by Polyacrylamide Gel Electrophoresis (PAGE), Size Exclusion Chromatography (SEC) and Infrared spectroscopy (IR, Figure S1). The biomacroinitiator BSA-Br (I_o) was stored at 4 °C until further use.

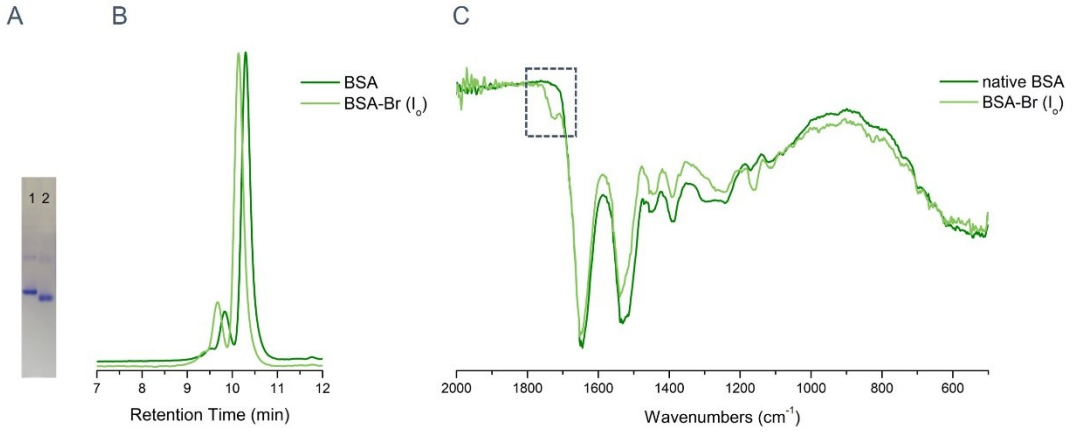


Figure S1 **A.** Native PAGE, lane 1: native BSA, lane 2: BSA-Br (I_o). **B.** SEC, olive trace: native BSA, green trace: BSA-Br (I_o). **C.** IR spectra of the native BSA (olive trace) and BSA-Br (I_o) macroinitiator (green trace).

The biomacroinitiator (BSA-Br, **I_o**) displayed slightly shorter elution time than native BSA under the chromatographic conditions (eluent: 10% MeCN in 5 mM phosphate buffer, flow rate: 1.0 mL/min). As previously observed,[2, 3] the biomacroinitiator displayed a higher electrophoretic migration rate than the native BSA, a fact that can be attributed to change of either shape or total charge in the bioconjugate.

The biomacroinitiator was also characterized via IR spectroscopy. The IR spectrum presented in Figure S1, clearly shows a new peak corresponding to the stretching vibration of the ester carbonyl group of the maleimide initiator moiety at 1728 cm^{-1} accompanied by the stretching of the corresponding ester oxygen at 1161 cm^{-1}.

3.3 Oxygen Tolerant Photoinduced Grafting of Acrylates *from* BSA-Br (I_o)

3.3.1 General procedure of oxygen tolerant, photoinduced grafting of methyl acrylate (MA) from BSA-Br (I_o) in the presence of 22 ppm Cu (Table S1, Entries 1-4)

A solution of methyl acrylate (500-2000 equiv.) in nanopure water (0.48-0.42 mL) was sonicated for 30 sec to disperse the monomer (2000 eq. of MA form an emulsion). Me_6TREN (1.4 µL, 5.244 × 10^{-3} mmol, 12 equiv.) was added to 0.1 mL of a 1.5 mg/mL aqueous solution of $CuBr_2$ (0.655 × 10^{-3} mmol, 1.5 equiv.) to form a light blue colored solution due to the immediate copper-ligand complex formation. The $CuBr_2$/Me_6TREN solution was added to the monomer emulsion and immediately transferred to a 5 mL plastic polypropylene syringe equipped with a stirring bar and containing a solution of the macroinitiator BSA-Br (I_o) (1.25 mL, 0.437 × 10^{-3} mmol, 1 equiv.). Headspace was eliminated to avoid the presence of undissolved oxygen, the reaction syringe was hermitically capped and exposed to the UV-light source under gentle stirring for 2 to 5 hours. The resulting bioconjugate solution was subsequently enriched through extensive dialysis initially against 10 % DMSO, 2% EDTA in 5 mM phosphate buffer, then against 5 mM phosphate buffer and finally against 20 mM phosphate buffer using regenerated cellulose dialysis membranes (MWCO 10 kDa).

3.3.2 General procedure of oxygen tolerant, photoinduced grafting of acrylates from BSA-Br (I_o) in the presence of 22 ppm Cu and 5 mM NaBr (Table S1, Entries 7 and 11)

A solution consisting of the acrylate monomer (n-butyl acrylate (*n*-BuA) or t-butyl acrylate (*t*-BuA) 2000 equiv.) in nanopure water (0.421 mL) was sonicated for 30 sec to form an emulsion. NaBr (1.03 mg, 0.01 mmol, 23 equiv.) was dissolved in 0.1 mL of a 1.5 mg/mL aqueous solution of $CuBr_2$ (0.655 × 10^{-3} mmol, 1.5 equiv.) and Me_6TREN (1.4 µL, 5.244 × 10^{-3} mmol, 12 equiv.). The resulting $CuBr_2$/Me_6TREN/NaBr solution was added to the monomer emulsion and immediately transferred to a 5 mL plastic syringe equipped with a stirring bar and containing a solution of the macroinitiator BSA-Br (I_o) (1.25 mL, 0.437 × 10^{-3} mmol, 1 equiv.). Headspace was eliminated to avoid the presence of undissolved oxygen, the syringe was hermitically capped and exposed to the UV-light source under gentle stirring. The resulting bioconjugate solution was subsequently enriched through extensive dialysis, initially against 10 % DMSO, 2% EDTA in 5 mM phosphate buffer, then against 5 mM phosphate buffer and finally against 20 mM phosphate buffer using regenerated cellulose dialysis membranes (MWCO 10 kDa).

3.3.3 General procedure of oxygen tolerant, photoinduced grafting of acrylates from BSA-Br (I_o) in the presence of 45 ppm Cu (Table S1, Entries, 8, 12 and 13)

A solution consisting of the acrylate monomer (n-BuA or t-BuA , 2000 equiv.) in nanopure water (0.321 mL) was sonicated for 30 sec to form an emulsion. Me_6TREN (2.8 μL, 10.488 × 10^{-3} mmol, 24 equiv.) was added to 0.2 mL of a 1.5 mg/mL aqueous solution of $CuBr_2$ (1.310 × 10^{-3} mmol, 3 equiv.) to form a light blue colored solution due to the immediate copper-ligand complex formation. The resulting $CuBr_2$/Me_6TREN solution was then added to the monomer emulsion and immediately transferred to a 5 mL plastic syringe equipped with a stirring bar and containing a solution of the macroinitiator BSA-Br (I_o) (1.25 mL, 0.437 × 10^{-3} mmol, 1 equiv.). Headspace was eliminated to avoid the presence of undissolved oxygen, the syringe was hermitically capped and exposed to the UV-light source under gentle stirring for fixed periods of time. The resulting bioconjugate solution was subsequently enriched through extensive dialysis, initially against 10 % DMSO, 2% EDTA in 5 mM phosphate buffer, then against 5 mM phosphate buffer and finally against 20 mM phosphate buffer using regenerated cellulose dialysis membranes (MWCO 10 kDa).

3.3.4 Optimization Studies

To optimize the oxygen tolerant, photoinduced RDRP grafting of acrylates *from* BSA-Br (I_o) in order to achieve quantitative yields under biologically relevant conditions**,** the effect of monomer, $Cu^{(II)}$ concentration and/or of the presence of non-impeding additives (DMSO, SDS, NaBr, EtOAc) was studied under the conditions described in Table S1. In all reactions the total volume of the reaction mixture was kept constant at 1.85 mL via the addition of the appropriate amount of nanopure water when necessary. The only exception was during grafting of *n*-BuMA (Table S1, Entry 14), where the concentration of all reagents was lowered by increasing the total volume of the reaction.

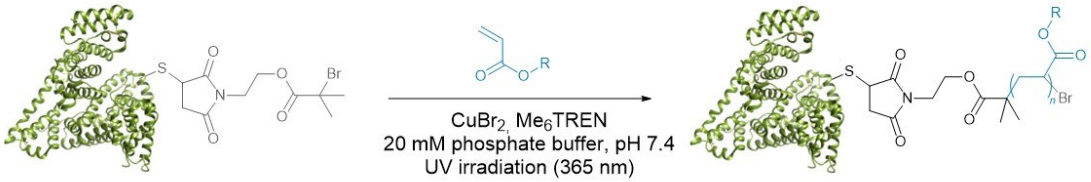


Table S1 Optimization of the oxygen tolerant, photoinduced grafting of acrylates *from* BSA-Br (I_o)

Entry	$M_n/I_o/Cu^{II}/L$	M_n	M_n (mmole)	Cu^{II} (μmole)	Cu^{II} (ppm)	Me_6TREN (μmole)	Additive	Time (min)	BSA-Br (I_o) Consumption
1	100/1/1.5/12	MA	0.044	0.655	22	5.244	-	300	No reaction or partial reaction
2	500/1/1.5/12	MA	0.218	0.655	22	5.244	-	300	Partial reaction
3	1000/1/1.5/12	MA	0.437	0.655	22	5.244	-	300	Partial reaction
4	2000/1/1.5/12	MA	0.874	0.655	22	5.244	-	300	Partial reaction
5	2000/1/1.5/12	MA	0.874	0.655	22	5.244	5 % v/v DMSO	300	Traces of I_o
6	2000/1/1.5/12	MA	0.874	0.655	22	5.244	0.095 % w/v SDS	300	Traces of I_o
7	2000/1/1.5/12	MA	0.874	0.655	22	5.244	5 mM NaBr	300	Quantitative
8	2000/1/3/24	MA	0.874	1.310	45	10.488	-	180	Quantitative
9	5000/1/1.5/12	MA	2.185	0.655	22	5.244	10 % v/v DMSO	480	Quantitative
10	2000/1/3/24	MA	0.874	1.310	45	10.488	pH 5.5	180	Quantitative
11	2000/1/1.5/12	*t*-BuA	0.874	0.655	22	5.244	5 mM NaBr	300	Quantitative
12	2000/1/3/24	*t*-BuA	0.874	1.310	45	10.488	-	300	Quantitative
13	2000/1/3/24	*n*-BuA	0.874	1.310	45	10.488	-	300	Quantitative
14[a]	2000/1/4.5/18	*n*-BuA	0.874	1.965	45	15.732	-	300	Quantitative

I_o: Initiator BSA-Br (I_o), M_n: Monomer, L: Ligand (Me_6TREN).
[a] Total volume of the reaction mixture: 2.78 mL.

3.3.5 BSA-poly(MA) *Giant Amphiphiles*

A Effect of Monomer Molar Feed Ratio (Table S1, Entries 1-4)

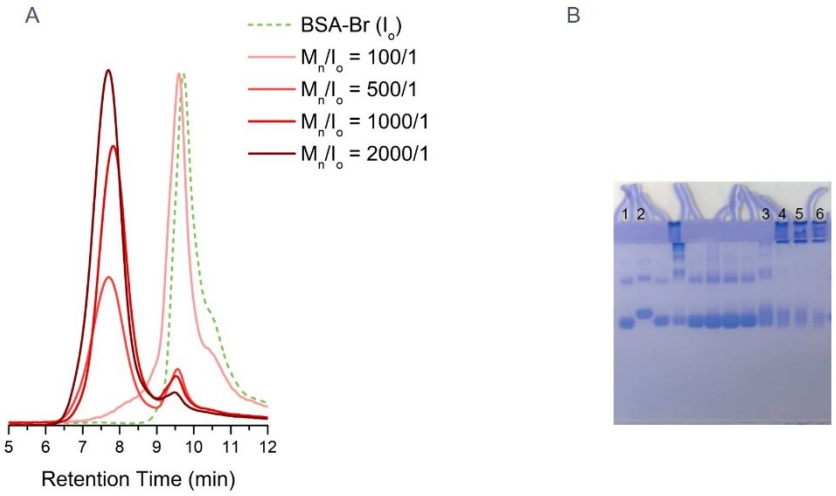


Figure S2 BSA-poly(MA) synthesized via the oxygen tolerant photoinduced grafting of MA *from* BSA-Br (I_o) using increasing monomer feed ratios (100 to 2000 equivalents over BSA-Br (I_o)). **A.** SEC (Table S1, Entries 1-4); **B.** Native PAGE, lane 1: BSA-Br (I_o), lane 2: native BSA, lane 3: M_n/I_o = 100/1 (Table S1, Entry 1), lane 4: M_n/I_o = 500/1 (Table S1, Entry 2), lane 5: M_n/I_o = 1000/1 (Table S1, Entry 3), lane 6: M_n/I_o = 2000/1 (Table S1, Entry 4).

B Effect of Additives or Catalyst Loading (Table S1, Entries 5-8)

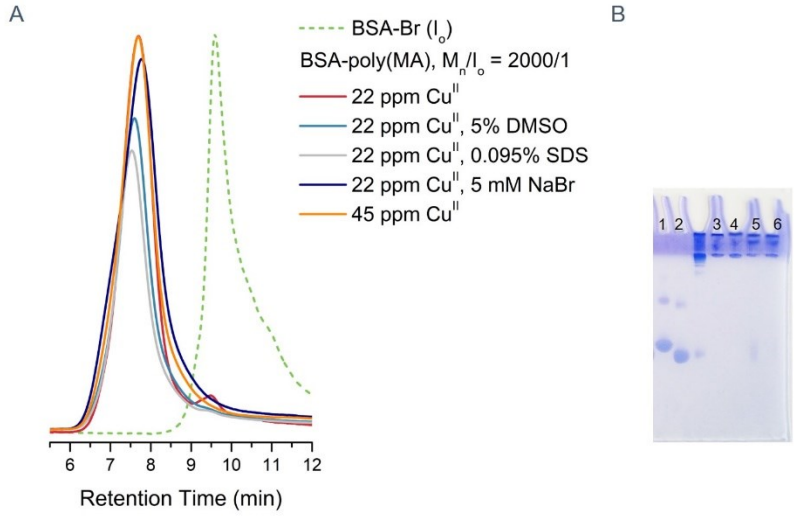


Figure S3 BSA-poly(MA) produced via the oxygen tolerant photoinduced grafting of MA from BSA-Br (I_o) using additives or catalyst loadings. **A.** SEC (Table S1, Entries 4-8 respectively); **B.** Native PAGE, lane 1: Native BSA, lane 2: BSA-Br (I_o), lane 3: 45 ppm Cu^{II} (Table S1, Entry 8), lane 4: 22 ppm Cu^{II}, 5 mM NaBr (Table S1, Entry 7), lane 5: 22 ppm Cu^{II}, 0.095% w/v SDS (Table S1, Entry 6), lane 6: 22 ppm Cu^{II}, 5% v/v DMSO (Table S1, Entry 5).

C Time course of MA grafting under the optimum polymerization conditions (Table S1, Entries 7 and 8)

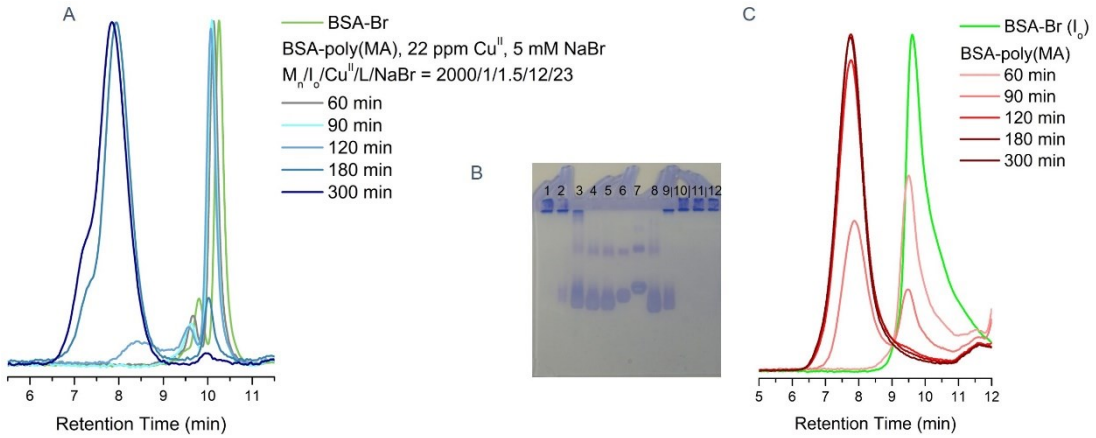


Figure S4 Kinetic studies for the synthesis of BSA-poly(MA) produced via the oxygen tolerant photoinduced grafting of MA *from* BSA-Br**. A.** SEC, M_n/I_o = 2000/1, 22 ppm Cu^{II}, 5 mM NaBr (Table S1, Entry 7). **B.** Native PAGE, lanes 1-5: M_n/I_o = 2000/1, 22 ppm Cu^{II}, 5mM NaBr, 300, 180, 120, 90 and 60 min respectively; lane 6: BSA-Br (I_o), lane 7: native BSA, lanes 8-12: M_n/I_o = 2000/1, 45 ppm Cu^{II}, 60, 90, 120, 180 and 300 min respectively. **C.** M_n/I_o = 2000/1, 45 pm Cu^{II} (Table S1, Entry 8).

Grafting of MA was performed under the conditions judged to be optimal for the grafting of MA from BSA-Br (I_o) as described in Table S1 (Entries 7 and 8). Minute samples (~50 µL) of the reaction mixture were withdrawn from the reaction syringe at fixed time points and analyzed by SEC and native gel electrophoresis (Figure S4).

D Control experiments (Table S2)

Table S2 Blank experiments

Entry	$M_n/I_o/Cu^{II}/L$	protein	Monomer	Cu^{II} (ppm)	Time (min)	Energy Source
1	2000/1/3/24	BSA	MA	45	180	UV
2	2000/1/3/24	BSA-Br (I_o)	MA	45	180	Heat 40 °C
3	2000/1/0/0	BSA-Br (I_o)	MA	-	180	UV
4	2000/1/15/120	BSA	MMA	220	180	UV
5	2000/1/15/120	BSA-Br (I_o)	MMA	220	180	Heat 40 °C
6	2000/1/0/0	BSA-Br (I_o)	MMA	-	180	UV
7[a]	2000/1/3/24	BSA-Br (I_o)	MA	45	180	Heat 40 °C
8[a]	2000/1/0/0	BSA-Br (I_o)	MA	-	180	UV

I_o: Initiator, BSA-Br (I_o), M_n: Monomer, L: Ligand (Me_6TREN).
[a] Reaction performed at pH 5.5

Control experiments aimed to elucidate the effect of MA upon incubation with BSA-Br (I_o) or the native BSA with MA were performed under irradiation or heat (40 °C) in the presence or absence of the catalytic components ($Cu^{(II)}$ or Me_6TREN) under standard polymerization conditions.

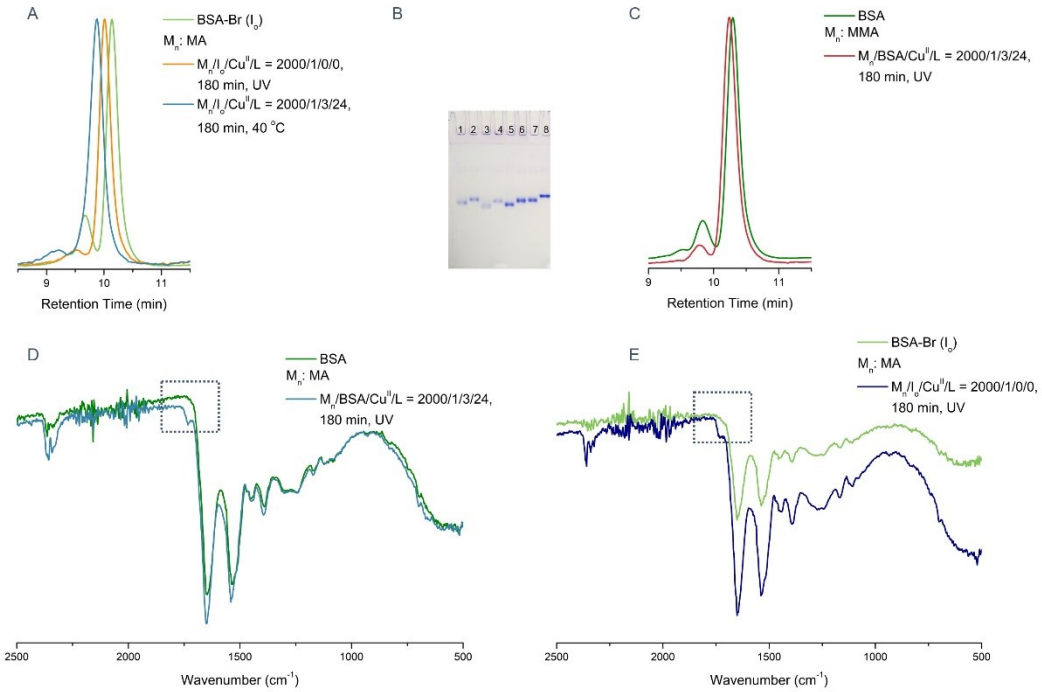


Figure S5 Control experiments performed to study the interaction between MA or MMA and BSA or BSA-Br (I_o). **A**: SEC, olive trace: macroinitiator BSA-Br (I_o), orange trace: MA/ I_o/Cu^{II}/L = 2000/1/0/0, 180 min UV irradiation (Table S2, Entry 3), blue trace: MA/ I_o/Cu^{II}/L = 2000/1/3/24, 180 min, 40 °C (Table S2, Entry 2). **B.** Native PAGE, lane 1: MA/ I_o/Cu^{II}/L = 2000/1/0/0, 180 min UV irradiation (Table S2, Entry 3), lane 2: MMA/I_o/Cu^{II}/L = 2000/1/0/0, 180 min UV irradiation (Table S2, Entry 6), lane 3: MA/ I_o/Cu^{II}/L = 2000/1/3/24, 180 min, 40 °C (Table S2, Entry 2), lane 4: MMA/I_o/Cu^{II}/L = 2000/1/15/120, 180 min, 40 °C (Table S2, Entry 5), lane 5: MA/BSA/Cu^{II}/L = 2000/1/3/24, 180 min, 40 °C (Table S2, Entry 1), lane 6: MMA/BSA/Cu^{II}/L = 2000/1/15/120 (Table S2, Entry 4), lane 7: BSA-Br (I_o), lane 8: native BSA. **C**. SEC, olive trace: native BSA, blue trace: MMA/BSA/Cu^{II}/L = 2000/1/15/120, 180 min UV irradiation (Table S2, Entry 4). **D.** IR spectra, olive trace: native BSA, blue trace: MA/BSA/Cu^{II}/L = 2000/1/3/24, 180 min UV irradiation (Table S2, Entry 1). **E.** IR spectra, green trace: BSA-Br (I_o), blue trace: MA/I_o/Cu^{II}/L = 2000/1/0/0, 180 min UV irradiation (Table S2, Entry 3).

IR spectroscopy of the biomolecules obtained from the control experiments supported the assumption of non-specific bioconjugation via the detection a new band that can be attributed to the stretching of ester carbonyls (1734 cm^{-1}, Figure S5). Formation of BSA-poly(MA) was not observed in any of the blank experiments through either SEC or PAGE.

E Effect of pH (Table S1, Entry 10 and Table S2, Entry 8)

Control experiments aimed to elucidate the nature of the interaction between MA and BSA-Br (I_o) or native BSA were performed at lower pH under standard polymerization conditions and in dark.

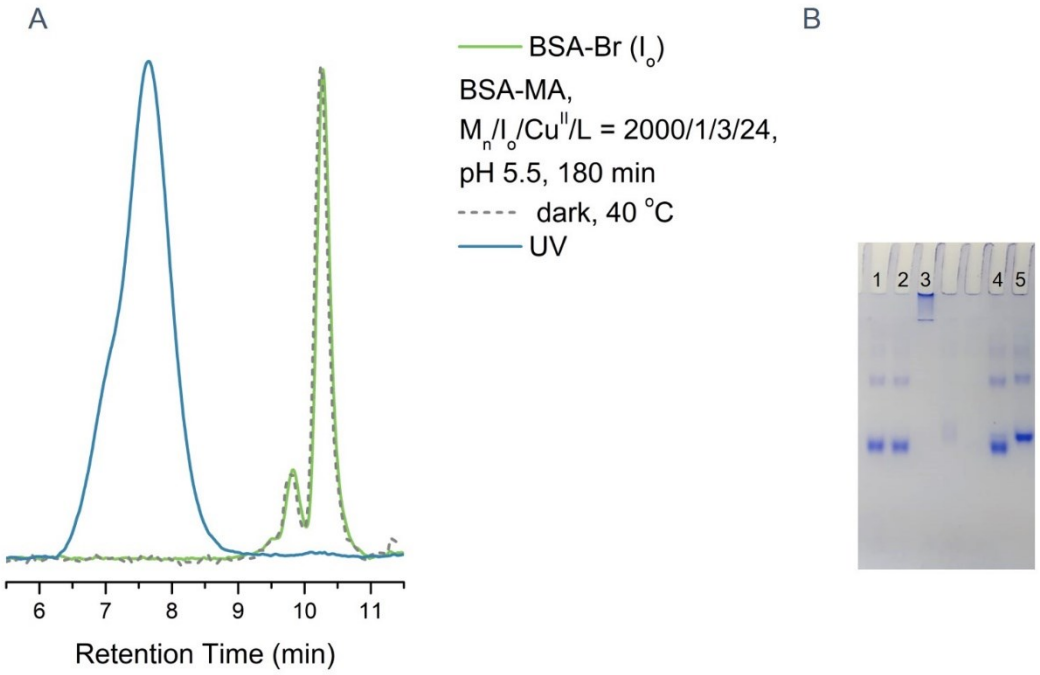


Figure S6 Control experiment and grafting of MA *from* BSA-Br (I_o) performed at pH 5.5**. A**: SEC, olive trace: BSA-Br (I_o), grey trace: MA/I_o/Cu^{II}/L = 2000/1/3/24, 180 min at 40 °C (Table S2, Entry 7), blue trace: MA/I_o/Cu^{II}/L = 2000/1/3/24, 180 min UV irradiation (Table S1, Entry 10). **B**: PAGE, lane 1: MA/I_o/Cu^{II}/L = 2000/1/0/0, 180 min UV irradiation (Table S2, Entry 8), lane 2: MA/I_o/Cu^{II}/L = 2000/1/3/24, 180 min at 40 °C (Table S2, Entry 7), lane 3: MA/I_o/Cu^{II}/L = 2000/1/3/24, 180 min UV irradiation (Table S1, Entry 10).

3.3.6 BSA-poly(*n*-BuA) and BSA-poly(*t*-BuA) *Giant Amphiphiles*

A Synthesis of BSA-poly(n-BuA) and BSA-poly(t-BuA) through the oxygen tolerant photoinduced RDRP (Table S1, Entries 11-14)

BSA-poly(*n*-BuA) and BSA-poly(*t*-BuA) were synthesized using the general synthetic procedure described for the grafting of acrylates from BSA-Br (I_o).

B Time course of t-BuA grafting under the optimum polymerization conditions (Table S1, Entries 11 and 12)

Grafting of *t*-BuA was performed under the conditions judged to be optimal for the grafting of MA from BSA-Br (I_o) as described in Table S1 (Entries 7 and 8). Minute samples (~50 μL) of the reaction mixture were withdrawn from the

reaction syringe at fixed time points and analyzed by SEC and native gel electrophoresis (Figure S7).

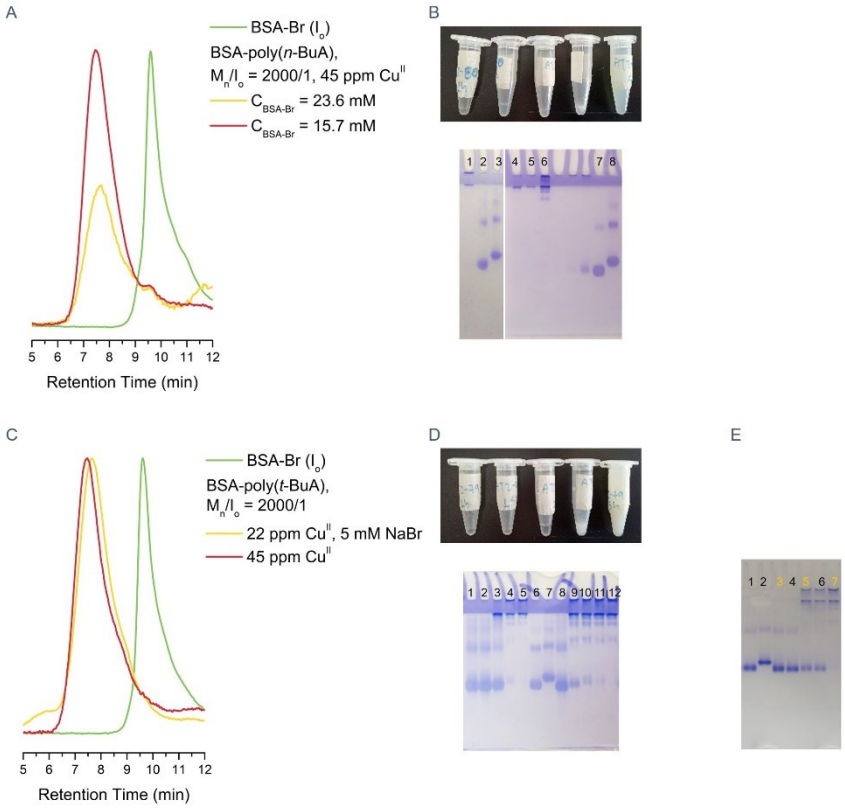


Figure S7 BSA-poly(*n*-BuA) and BSA-poly(*t*-BuA) produced via oxygen tolerant photoinduced grafting from BSA-Br. **A.** SEC, green trace: BSA-Br (I_o), yellow trace: BSA-poly(*n*-BuA), M_n/I_o = 2000/1, 45 ppm Cu^{II}, (Table S1, Entry 13), red trace: BSA-poly(*n*-BuA), M_n/I_o = 2000/1, 45 ppm Cu^{II}, lower BSA-Br (I_o) concentration reaction (Table S1, Entry 14). **B.** *top*: samples withdrawn during the course *n*-BuA grafting; *bottom*: native PAGE: lane 1: BSA-poly(*n*-BuA), M_n/I_o = 2000/1, 45 ppm Cu^{II}, lower concentration reaction (Table S1, Entry 14), lanes 2 & 7: macroinitiator BSA-Br (I_o), lanes 3 & 8: native BSA, lane 4: BSA-poly(*t*-BuA), M_n/I_o = 2000/1, 45 ppm Cu^{II} (Table S1, Entry 12), lane 5: BSA-poly(*n*-BuA), M_n/I_o = 2000/1, 45 ppm Cu^{II} (Table S1, Entry 13), lane 6: BSA-poly(*t*-BuA), 22 ppm Cu^{II}, 5 mM NaBr (Table S1, Entry 11). **C.** SEC, green trace: BSA-Br (I_o), yellow trace: BSA-poly(*t*-BuA), 22 ppm Cu^{II}, 5 mM NaBr (Table S1, Entry 11), red trace: BSA-poly(*t*-BuA), M_n/I_o = 2000/1, 45 ppm Cu^{II} (Table S1, Entry 12). **D.** *top*: samples withdrawn during the course *t*-BuA grafting. **D.** PAGE from BSA-Br (I_o) consumption study during *t*-BuA grafting: lanes 1-5: M_n/I_o = 2000/1, 22 ppm Cu^{II}, 5 mM NaBr, withrdawn at 60, 90, 120, 180 and 300 min respectively, lane 6: BSA-Br (I_o), lane 7: native BSA, lanes 8-12: M_n/I_o = 2000/1, 45 ppm Cu^{II}, 60, 90, 120, 180 and 300 min respectively. **E**. ON/OFF time course during *t*-BuA grafting, [*t*-BuA]/[BSA-Br]/[Cu^{II}]/[Me_6TREN] = 2000/1/3/24 (Table 1, Entry 12). Native PAGE, lane 1: BSA-Br (I_o), lane 2: native BSA, lane 3: 45 min ON, lane 4: 90 min OFF, lane 5: 45 min ON, lane 6: 90 min OFF, lane 7: 210 min ON.

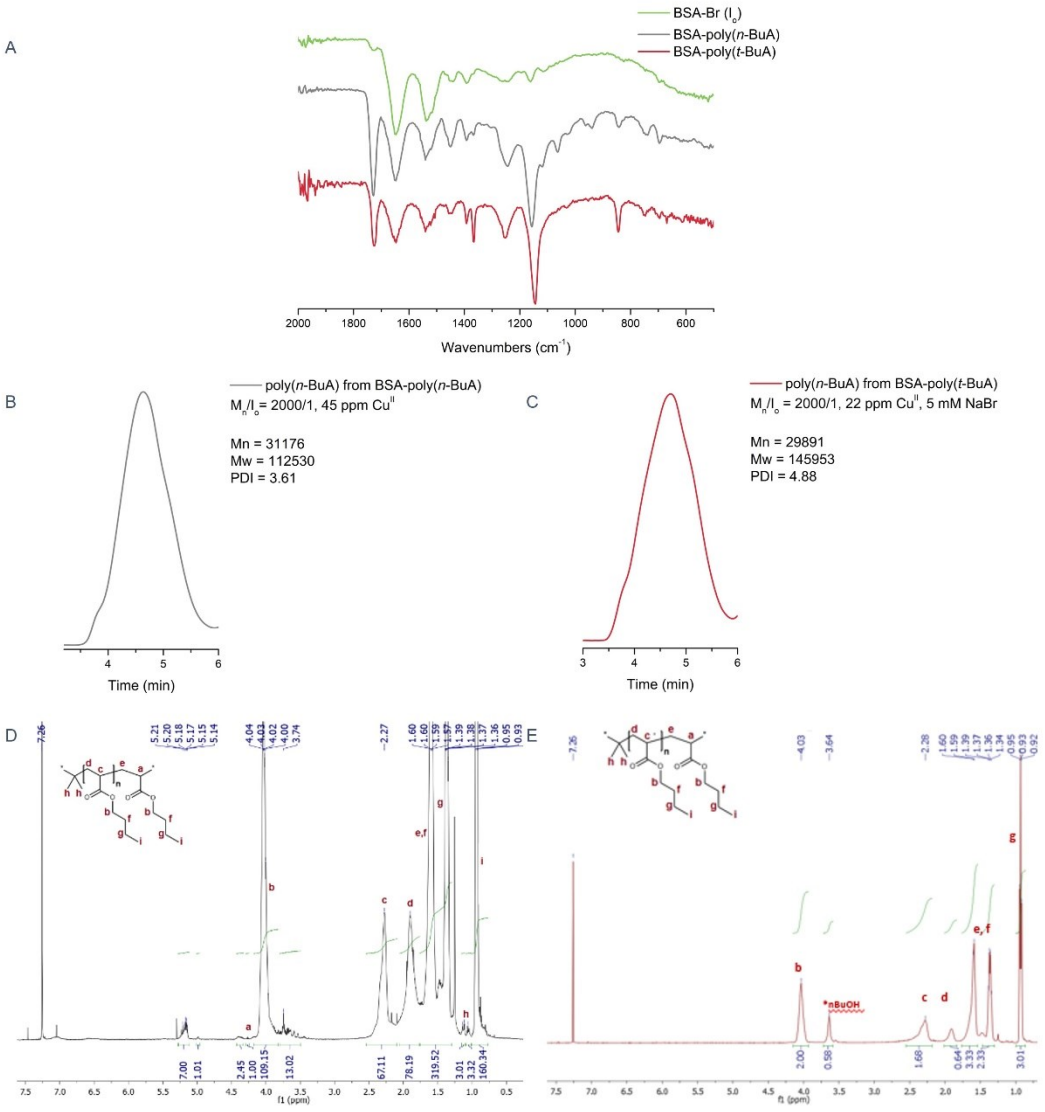


Figure S8 BSA-poly(*n*-BuA) and BSA-poly(*t*-BuA) *Giant Amphiphiles*. **A**. IR spectra, green trace: BSA-Br (I_o), grey trace: BSA-poly(*n*-BuA), red trace: BSA-poly(*t*-BuA). GPC chromatograms of polymers derived from chemical digestion of **B.** BSA-poly(*n*-BuA) and **C.** BSA-poly(*t*-BuA). ^{1}H-NMR spectra of **D.** poly(*n*-BuA) isolated from BSA-poly(*n*-BuA) and **E.** poly(*n*-BuA) isolated from BSA-poly(*t*-BuA) chemical digestion.

3.4 Oxygen Tolerant Photoinduced Grafting of Methacrylates *from* BSA-Br (I_o)

Table S3 Optimization of the oxygen tolerant, photoinduced grafting of methacrylates and acrylamides from BSA-Br (I_o)

Entry	$M_n/I_o/Cu^{II}/L$	Monomer	Monomer quantity. (mmole)	Cu^{II} (µmole)	Cu^{II} (ppm)	Me_6TREN (µmole)	Additive	Time (min)	BSA-Br (I_o) Consumption
1	2000/1/1.5/12	MMA	0.874	0.655	22	5.244	5 mM NaBr	300	No reaction
2	2000/1/3/24	MMA	0.874	1.310	45	10.488	-	180	partial reaction
3	2000/1/3/24	MMA	0.874	1.310	45	10.488	5 mM NaBr	180	partial reaction
4	2000/1/3/24	MMA	0.874	1.310	45	10.488	10 mM NaBr	300	Traces of I_o
5	2000/1/6/48	MMA	0.874	2.620	90	20.98	-	300	Traces of I_o
6	2000/1/6/48	MMA	0.874	2.620	90	20.98	-	360	Traces of I_o
7[a]	2000/1/9/72	MMA	0.874	3.930	90	31.47	-	360	Traces of I_o
8	2000/1/6/48	MMA	0.874	2.620	90	20.98	5 mM NaBr	300	Traces of I_o
9	2000/1/6/48	MMA	0.874	2.620	90	20.98	20 mM NaBr	300	Traces of I_o
10	2000/1/15/120	MMA	0.874	6.55	220	52.44	-	360	quantitative
11	5000/1/15/120	MMA	2.185	6.55	220	52.44	-	360	quantitative
12	2000/1/1.5/12	*t*-BuMA	0.874	0.655	22	5.244	5 mM NaBr	300	No reaction
13	1000/1/3/24	*t*-BuMA	0.437	1.310	45	10.488	-	360	quantitative
14	1000/1/15/120	*t*-BuMA	0.437	6.55	220	52.44	-	135	quantitative
15	2000/1/15/120	*t*-BuMA	0.874	6.55	220	52.44	-	135	quantitative
16	4000/1/15/120	*t*-BuMA	1.748	6.55	220	52.44	-	135	quantitative
17	80/1/3/24	NIPAM	0.035	1.310	45	10.488	-	180	quantitative
18	400/1/3/24	NIPAM	0. 175	1.310	45	10.488	-	180	quantitative

I_o: Initiator, BSA-Br (I_o), M_n: Monomer, L: Ligand (Me_6TREN).
[a] Total volume of the reaction mixture: 2.78 mL.

3.4.1 General procedure for the polymerization of methacrylates in the presence of 220 ppm Cu (Table S3, Entry 10)

A solution consisting of the methacrylate monomer (0.874 mmol, 2000 equiv.), in nanopure water (0.3 mL) was sonicated for 30 sec to form an emulsion. Me_6TREN (14µL, 0.05244 mmol, 120 equiv.) was added to 100 µL of a 15 mg/mL aqueous solution of $CuBr_2$ (6.55×10^{-3} mmol, 15 equiv.) to form a light blue colored solution due to the immediate copper-ligand complex formation. The $CuBr_2/Me_6TREN$ solution was transferred to the monomer emulsion and

immediately transferred to a 5 mL syringe equipped with a stirring bar and containing a solution of the macroinitiator BSA-Br (I_o) (1.25 mL, 0.437 × 10^{-3} mmol, 1 equiv.). Headspace was eliminated to avoid the presence of undissolved oxygen and the reaction syringe was hermitically capped and placed under the UV-light source. After completion of the reaction the resulting bioconjugate was enriched through extensive dialysis, initially against 10 % DMSO in 5 mM phosphate buffer, 2% EDTA, then against 5 mM phosphate buffer and finally against 20 mM phosphate buffer using 10 kDa MWCO regenerated cellulose dialysis membranes.

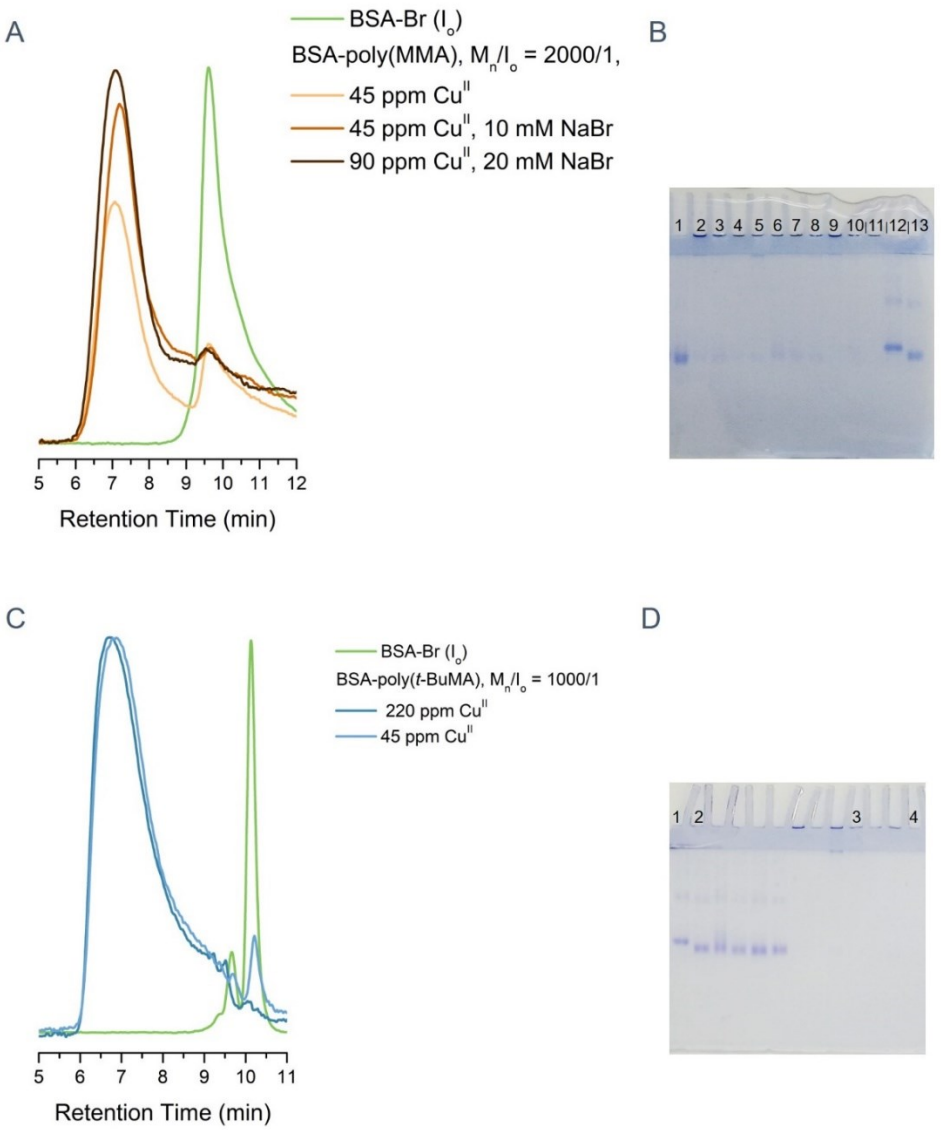


Figure S9 Oxygen tolerant, photoinduced grafting of methacrylates from BSA-Br (I_o). **A**. SEC, green trace: BSA-Br (I_o), orange trace: M_n/I_o = 2000/1, 45 ppm Cu^{II}, (Table S3, Entry 2), brown trace: M_n/I_o = 2000/1, 45 ppm Cu^{II}, 10 mM NaBr, (Table S3, Entry 4), dark brown trace: M_n/I_o = 2000/1, 90 ppm Cu^{II}, 20 mM NaBr, (Table S3, Entry 9). **B**. Native PAGE, lanes 1-11: Table S3 Entries 1-11, respectively, lane 12: native BSA, lane 13: BSA-Br (I_o). **C**. SEC, green trace: BSA-Br (I_o), light blue trace: M_n/I_o = 1000/1, 220 ppm Cu^{II}, (Table S3, Entry 14), blue trace: M_n/I_o = 1000/1, 45 ppm Cu^{II} (Table S3, Entry 13). **D**. Native PAGE, lane 1: native BSA, lane 2: BSA-Br (I_o), lane 3: M_n/I_o = 2000/1, 220 ppm Cu^{II} (Table S3, Entry 15), lane 4: M_n/I_o = 4000/1, 220 ppm Cu^{II} (Table S3, Entry 16).

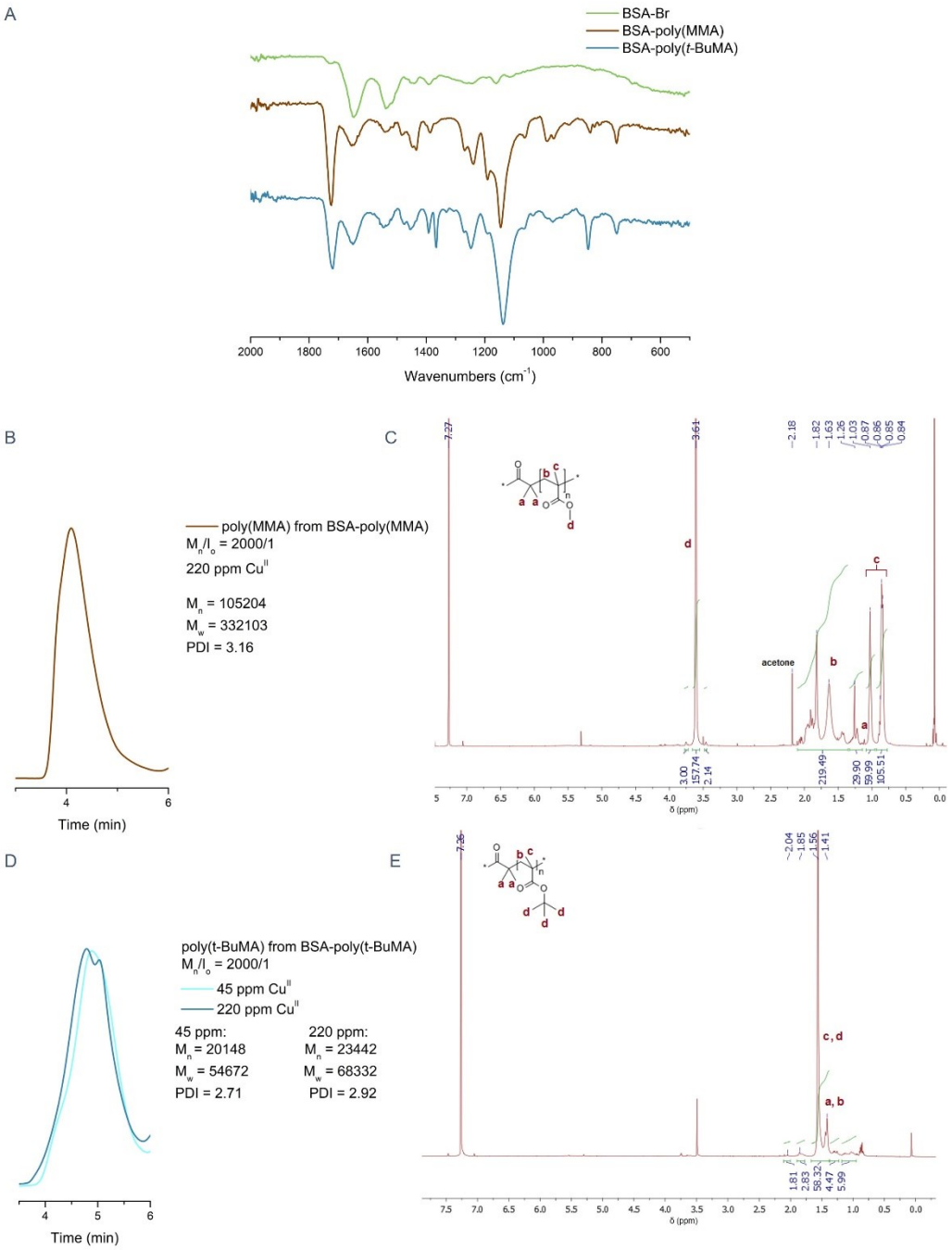


Figure S10 BSA-poly(MMA) and BSA-poly(*t*-BuMA) produced by the oxygen tolerant photo-RDRP. **A.** IR spectra, green trace: BSA-Br (I_o), brown trace: BSA-poly(MMA), blue trace: BSA-poly(*t*-BuMA). **B**. GPC chromatogram and **C.** ^{1}H-NMR spectrum of poly(MMA) isolated from BSA-poly(MMA). **D**. GPC chromatogram and **E.** ^{1}H-NMR spectrum of poly(*t*-BuMA) from BSA-poly**(***t*-BuMA).

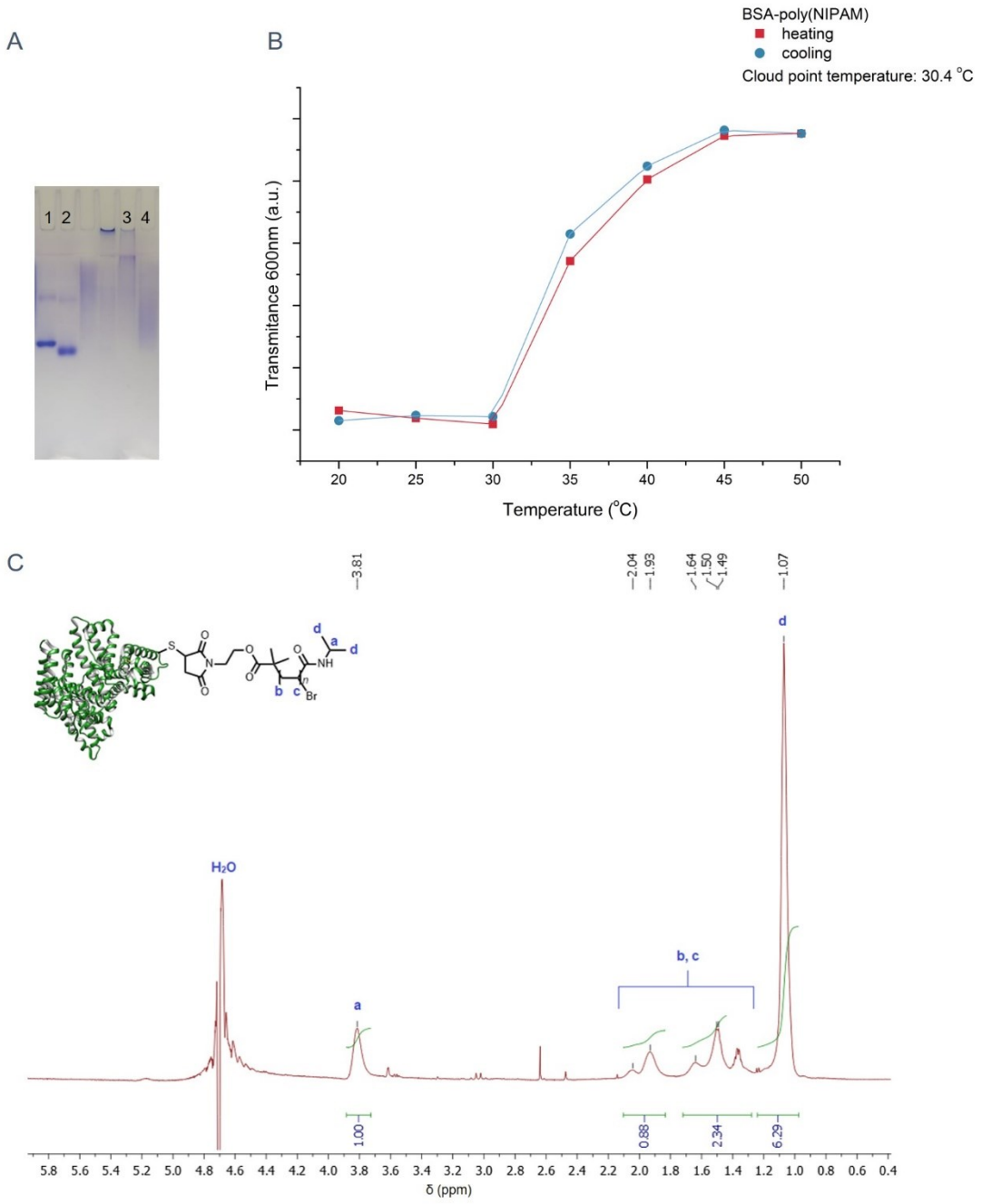


Figure S11 BSA-poly(NIPAM) produced via the RDRP, oxygen tolerant photoinduced grafting from BSA-Br (I_o). **A**. Native PAGE, lane 1: native BSA, lane 2: BSA-Br (I_o), lane 3: M_n/I_o = 400/1, 45 ppm Cu^{II}, Table S3, Entry 18, lane 4: M_n/I_o = 80/1, 45 ppm Cu^{II}. **B**. ^{1}H-NMR spectrum of BSA-poly(NIPAM) in D_2O. **C**. Cloud point temperature of BSA-poly(NIPAM) (Table S3, Entry 18).

3.5 Chemical Digestion of the Protein

All protein-polymer bioconjugates were extensively dialyzed against nanopure water to remove salts and subsequently freeze dried prior to chemical digestion of the biomolecule moiety. 37 mg of a BSA-polymer conjugate were placed in

a round bottom flask. 3.7 mL of *n*-butanol (MeOH in case of BSA-poly(MA)) and 102.3 mg of K_2CO_3 (0.2M in butanol) were added and the reaction mixture was stirred at 75 °C for 48 hours. After cooling to room temperature, the solvent was removed in vacuo. 10 mL THF were subsequently added and the mixture stirred at room temperature for 30 min. After removing the precipitate through filtration, the solvent was removed under reduced pressure. The recovered polymer was analyzed with ^{1}H-NMR and ^{13}C-NMR spectroscopy without further purification.

3.6 Self-Assembly

All protein-polymer bioconjugates were extensively dialyzed against nanopure water prior to imaging with SEM or TEM.

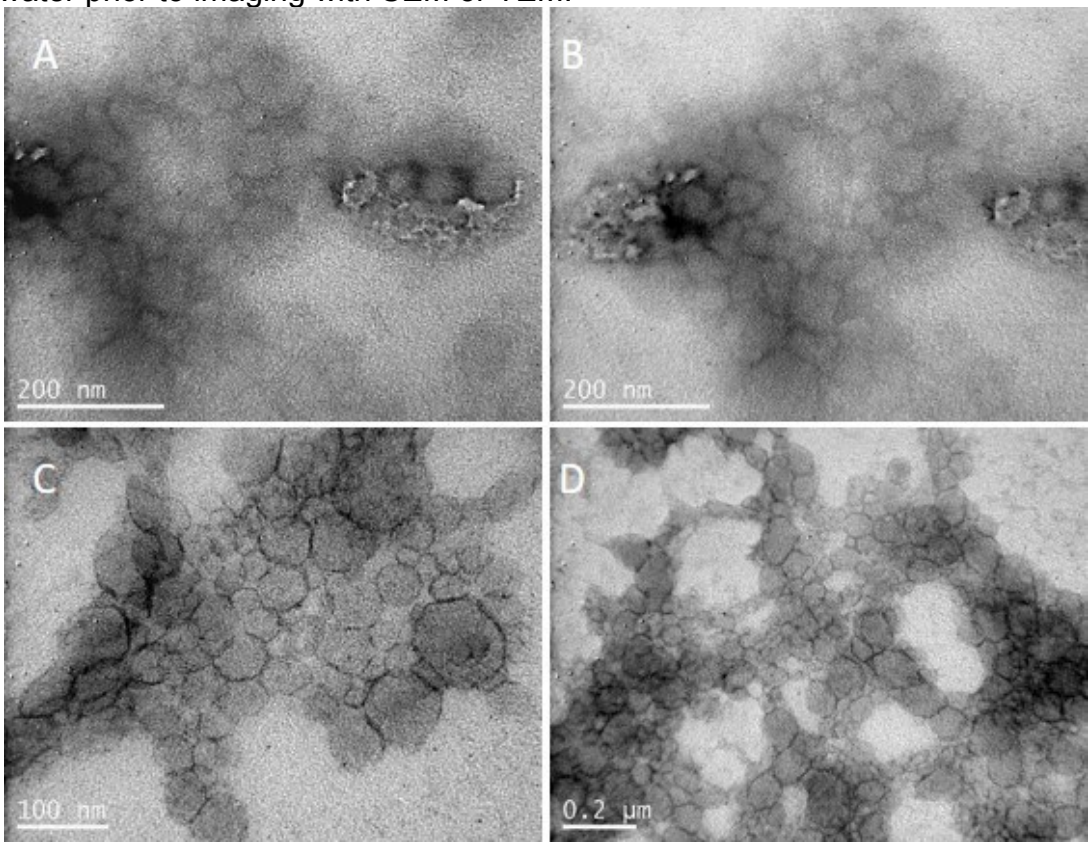


Figure S12 SEM TEM micrographs of BSA-poly(MA) with (**A, B**) and without uranyl acetate staining (**C**, **D**) synthesized using monomer molar feed ratio M_n/I_o = 5000/1 (Table S1, Entry 9).

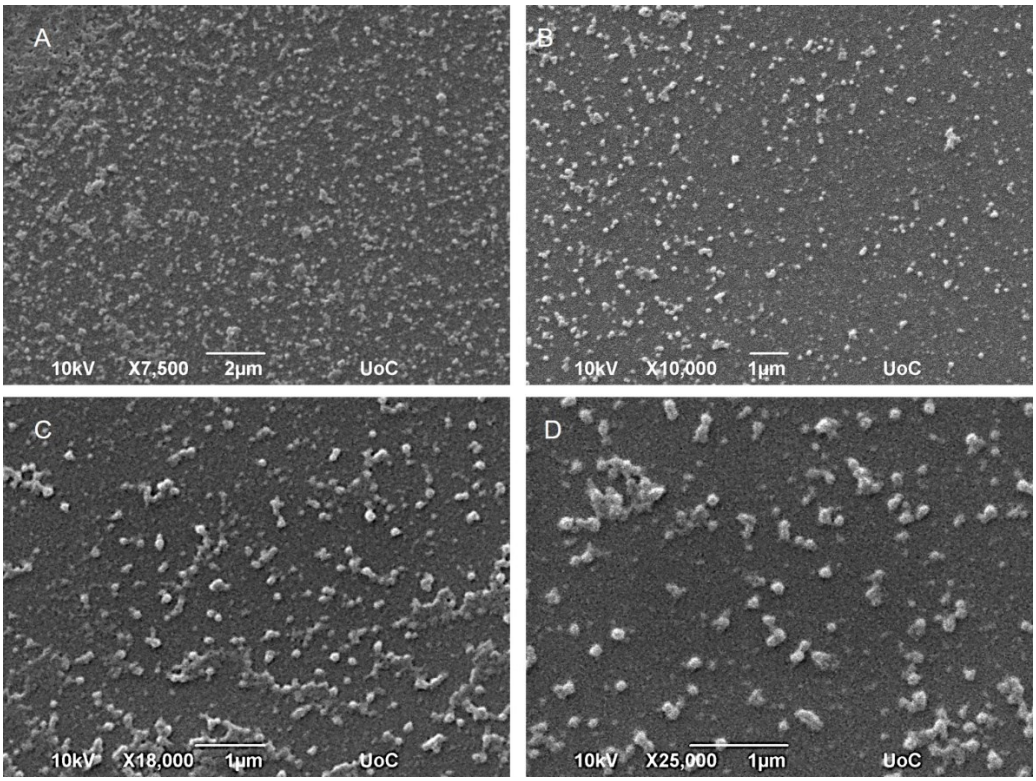


Figure S13 SEM micrographs of BSA-poly(*t*-BuA) synthesized using molar feed ratio M_n/I_o = 2000/1 (Table S1, Entry 12).

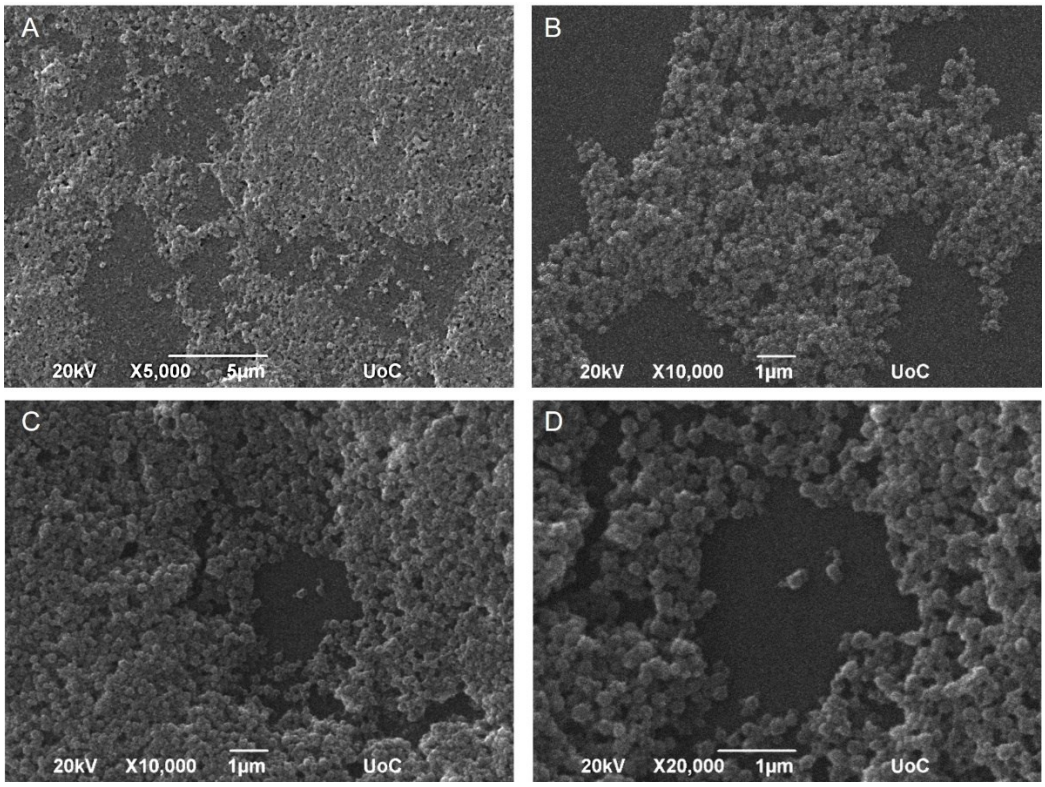


Figure S14 SEM micrographs of BSA-poly(MMA) synthesized using molar feed ratio M_n/I_o = 5000/1 (Table S3, Entry 11).

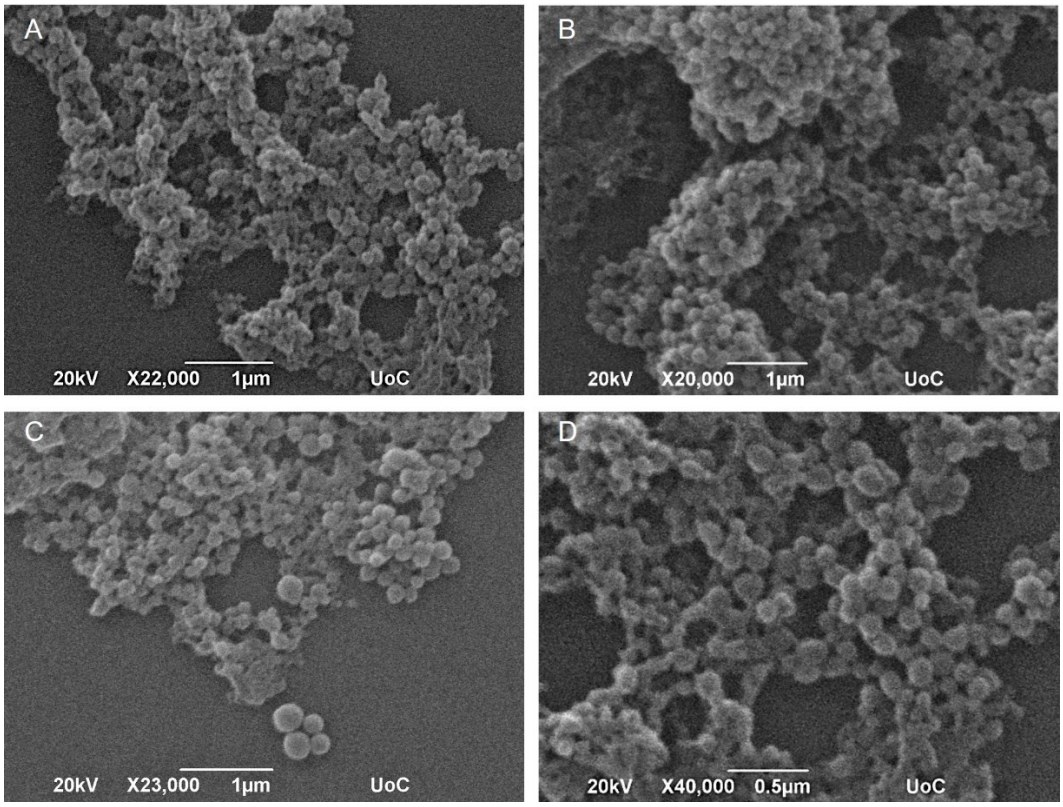


Figure S15 SEM micrographs of BSA-poly(*t*-BuMA) synthesized using molar feed ratio M_n/I_o = 4000/1 (Table S3, Entry 16).

3.6 BSA-Polymer Conjugates: Esterase-Like Activity[3, 4]

32 mg of p-nitrophenyl acetate (pNPA) were dissolved in 600 μL MeOH to form a 300 mM stock solution which was fractionated and stored at -20 °C. 21 μL of a 0.35 mM BSA solution were diluted with 20 mM tris buffer saline pH 7.4 to form a 3 μM protein solution (2.4 mL). Immediately prior to measurement, 6 μL of the 300 mM pNPA solution in methanol were diluted with nanopure water to form 0.6 mL of a 3 mM pNPA solution. The reaction was initiated by the addition of the 3 mM pNPA solution to the BSA solution and the esterase-like activity of BSA was monitored by UV at 400 nm at 20 °C. The ability of BSA-Br (I_o) and BSA-polymer conjugates to hydrolyze *p*NPA was also tested following the same protocol. A blank experiment was performed using the same protocol in the absence of the protein. The effect of UV light on BSA was studied by studying the activity of native BSA after 3 hours of UV irradiation.

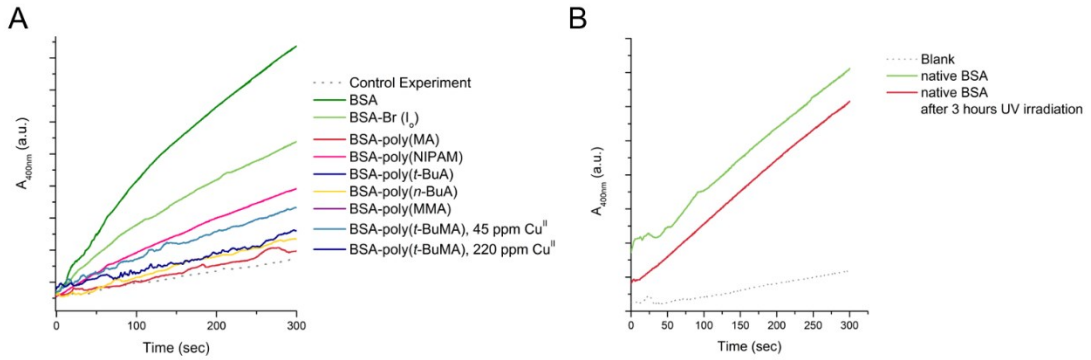


Figure S16 (A) Esterase-like activity of BSA, BSA-Br (I_o) and BSA-bioconjugates; (B) Effect of UV irradiation on the esterase-like activity of native BSA.

Supporting Information References

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