Electronic Supplementary Material (ESI) for Polymer Chemistry.

Supplementary Information

Oxygen-tolerant, Eosin Y mediated synthesis of protein-polymer biohybrids and protein-coated polymer nanoparticles

Errika Voutyritsa, [a, b] Thomai Lazou, [a] Jonida Bushi, [a] Stavroula Margaritaki, [a] Myrto Charitaki, [a] Sune M. Christensen, [c] Nikos Hatzakis, [b] and Kelly Velonia *[a]

Contents

Materials

Chemicals were purchased from commercial sources and were used as received, unless otherwise stated. Bovine serum albumin (BSA) was purchased from Sigma (> 99%). Dialysis bags (Spectra/Por® Biotech Regenerated Cellulose Dialysis Membranes, MWCO 10, 25, and 50 kDa) were purchased from Spectrum Labs

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 LC10AD pump, a CTO-10A column oven, a SIL-10AD auto-injector, a RID-10A refractive index detector, and an SPD-10A Shimadzu UV Vis spectrometer. The system was equipped with a Polymer Laboratories 30 × 7.8 mm 5 µm BioBasic SEC 60 guard column followed by a 300 × 7.8 mm 5 µm BioBasic SEC 300 Polymer Laboratories column, using a mixture of 5 mM phosphate buffer, pH 7.4, 10% acetonitrile (MeCN) as the eluent at room temperature (flow rate: 1.0 mL/min). Chromatograms were acquired at 254 nm and 280 nm wavelength and were processed with the EZStart 7.3 chromatography software.

Native Polyacrylamide Gel Electrophoresis (native PAGE)

Discontinuous Native PAGE (Ornstein-Davis) electrophoresis was run using a 4 % stacking gel and a 10 % resolving gel under standard non-denaturing conditions. Samples were dissolved in Tris buffer containing bromophenol blue and were visualized using Coomassie Brilliant Blue. The freely available ImageJ software (https://imagej.nih.gov) was used for semi-quantitative digital analysis of the electrophoresis gel in ON/OFF experiments.[1,2]

Sodium dodecyl-sulfate polyacrylamide gel electrophoresis (SDS-PAGE)

PAGE electrophoresis was run using a 4 % stacking gel and a 12 % resolving gel. Samples mixed with an equal volume of electrophoresis sample buffer (125 mM Tris-HCl, pH 6.8, 5 % SDS, 20 % v/v glycerol, 0.004 % bromophenol blue, 10 % *β*-mercaptoethanol) and denaturated at 95 °C for 5-10 min prior to loading.

UV-Vis Spectroscopy

Activity and response studies were performed on a Shimadzu UV-1900 UV Vis spectrophotometer.

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.

Scanning Electron Microscopy

Samples were diluted 20 times three consecutive times with nanopure water and subsequently dialyzed against nanopure water using a regenerated cellulose dialysis membrane with MWCO 10 kDa. After dialysis, three samples of each reaction mixture were prepared by sequentially diluting 20 times. 20 µL of each sample were placed on a glass slide and water was allowed to evaporate (ca. 8 hours room temperature). Before sample imaging, all samples were dried, and sputter coated with ca. 10 nm of gold (Au) under nitrogen atmosphere. 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.

Transmission Electron Microscopy

Transmission electron microscopy (TEM) imaging was performed with a JEOL JEM-2100 microscope (JEOL Ltd., Tokyo, Japan) equipped with a LaB6 filament, using an acceleration voltage of 80 kV. Dilute protein-polymer conjugate solutions (1000 dilution with nanopure water) were dialyzed against nanopure water using a regenerated cellulose dialysis membrane with MWCO 10 kDa. TEM samples were prepared after further diluting (100 times), by placing ca. 5 µL of the sample onto a perforated carbon-coated Cu grid (Agar Scientific). The samples were allowed to dry at room temperature for ca. 8 hours. TEM experiments were also performed using a JEOL JEM- 100C microscope operating at 80 kV.

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.

Irradiation Source

Blue LED flexible light strip, 60 LEDs/m, 10.8 w/m, 1000 lm/m.

Total inverted TIRF fluorescence microscopy

Fluorescence microscopy experiments were accomplished using an inverted TIRF microscope (model IX83) from Olympus. The microscope was equipped with an EMCCD camera (model imagEM X2) from Hamamatsu, a ×100 oil immersion objective (model UAPON 100XOTIRF) from Olympus. All the data were acquired using a 100 nm penetration depth. A solid-state laser line from Olympus 532 nm was used to excite the Eosin Y (EY).

Experimental Procedures

Candida Antarctica Lipase B (CALB) purification

The lipase was expressed in *A. Oryzae*. The culture broth was filtered through a 0.2 mm filtration unit (Nalgene) to remove the host cells. A solution of 2 M NaCl was added to the supernatant to reach 1M NaCl final concentration. The adjusted lipase solution was applied to a decylamin-agarose column and equilibrated in 50 mm HEPES/NaOH, NaCl (1.0M), pH 7.0. After washing the column with the equilibration buffer, the column was eluted with 50 mm HEPES/NaOH, 30 % EtOH, pH 7.0. The eluted peak, containing the lipase, was desalted using a G25-sephadex column (Cytiva), into 20 mm AcOH/ NaOH, pH 4.5 and applied to an SP-sepharose FF column (Cytiva) that had been equilibrated in 20 mm AcOH/NaOH, pH 4.5. The column was washed with the equilibration buffer and eluted with a linear NaCl gradient (0 to 0.5m) over three column volumes. The lipase which eluted as a sharp peak was collected, and the purity was analyzed by SDS-PAGE; The major band observed on the Coomassie-stained gel, belonged to the lipase. The purified enzyme was characterized by intact mass electrospray ionization mass spectrometry (ESI-MS) and activity confirmed by fluorometry (Figure S0).

Intact mass electrospray ionization mass spectrometry (ESI-MS)

The intact molecular weight analyses (Figure S0) were performed using a MAXIS II electrospray mass spectrometer (Bruker Daltonik GmbH, Bremen, DE). The samples were first diluted to 0.1mg/ml in 50mM NH_4Ac pH5.5. The diluted samples were applied to an AdvanceBio Desalting-RP column (Agilent Technologies) followed by washing and elution from the column running an acetonitrile linear gradient and introduced to the electrospray source with a flow of 400 ml/min by an Ultimate 3000 LC system (Dionex). Data analysis was performed with DataAnalysis version 5.1 (Bruker Daltonik GmbH, Bremen, DE). The molecular weight of the samples was calculated by deconvolution of the raw data in the range 20.000 to 60.000 Da.

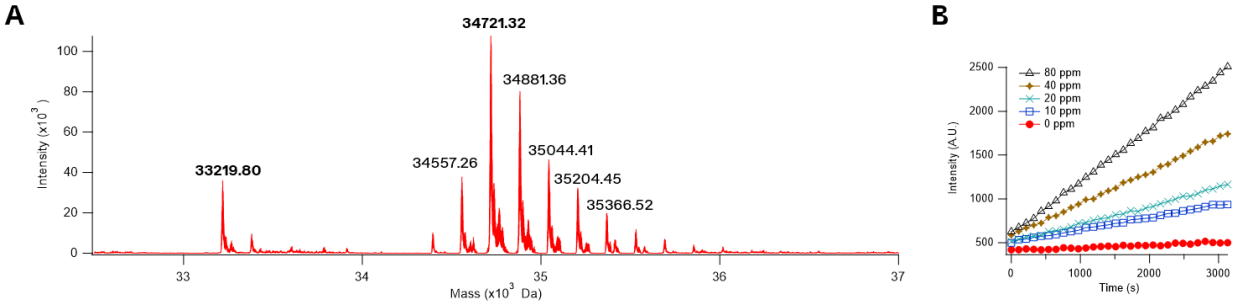


Figure S 0. **A.** Purified CalB was characterized by ESI-MS. The peak at 33219.80 Da matches the expected sequence with calculated mass 33219.21 Da.[3] The spectrum shows a high mannose N-glycosylation (Hex6-16HexNAc2), with the prevalent species corresponding to Hex8HexNAc2 (calculated mass 34719.54, observed mass 34721.32 Da). **B.** Lipase activity was measured in 96 well-plate format on a fluorometer using the substrate 4-methylumbelliferyl oleate (Sigma 75164)

in 0.01% TRITON X-100, 50 mM Tris, 100 mM NaCl pH 7.0. Upon hydrolysis, the lipase generates a fluorescent product causing a rise in fluorescence intensity.

UV-Vis spectra of Eosin Y

The UV Vis absorbance spectrum of Eosin Y (EY) (0.03 mg/mL) in 20 mM phosphate buffer, pH=7.4 showed an absorption band in the blue light region (516.5 nm). UV Vis spectra were also acquired in the presence of ascorbic acid (AscA) and tetramethylethylenediamine (TEMED) before and after incubation under blue LED for 2 hours.

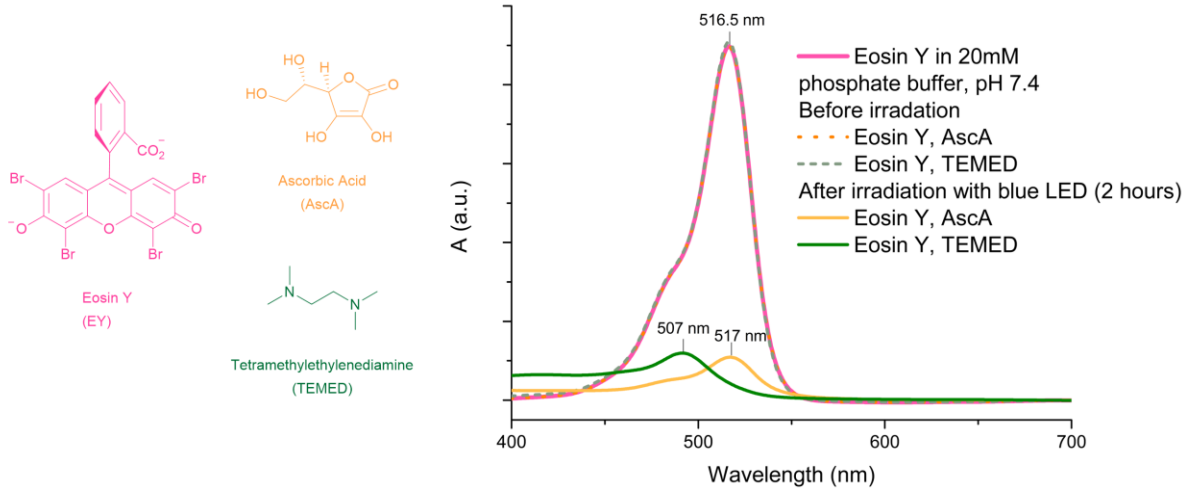


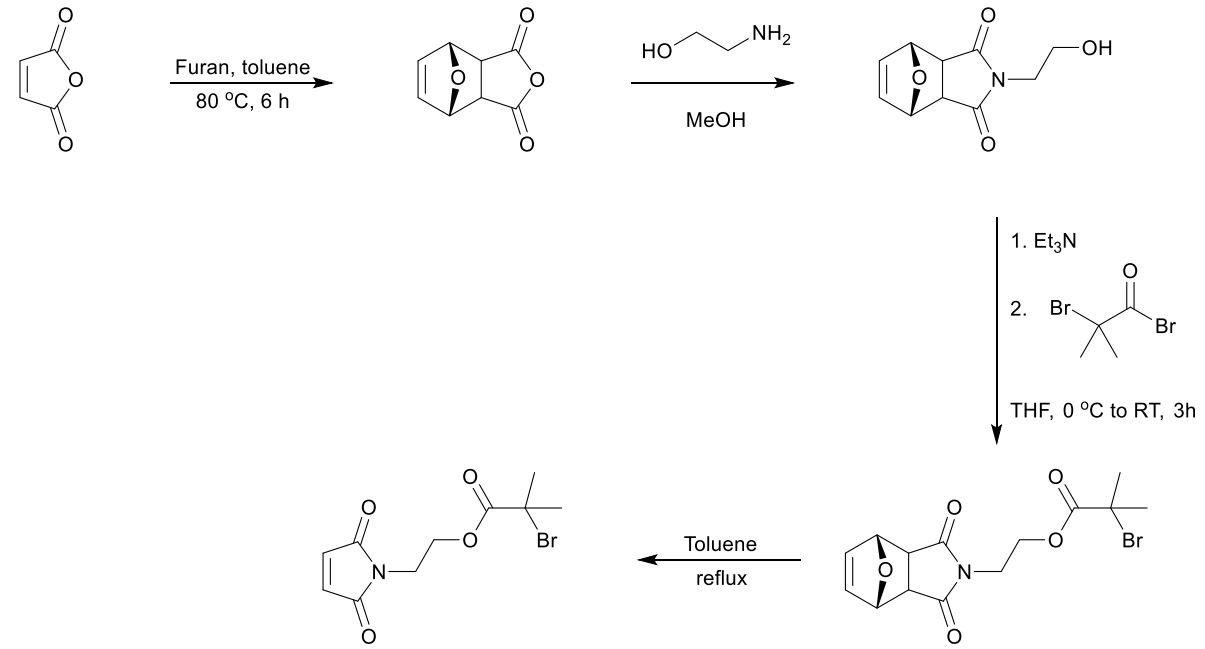
Figure S 1. UV Vis absorption spectra of EY and EY in the presence of AscA or TEMED, before and after irradiation under blue LED for 2 hours.

Synthesis of the bovine serum albumin macroinitiator (BSA-Br, I_o)

A. Synthesis of 2-bromo-2-methyl-propionic acid 2-(2,5-dioxo-2,5-dihydro-pyrrol-1-yl)-ethyl ester

The synthesis was performed according to published procedures as described in Scheme S 1.[4]

Scheme S 1 Synthesis of 2-bromo-2-methyl-propionic acid 2-(2,5-dioxo-2,5-dihydro-pyrrol-1-yl)-ethyl ester



4,10-dioxatricyclo[5.2.1.02,6]dec-8-ene-3,5-dione

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 subsequently added 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 (**1**) as white needles.

^{1}H NMR (400 MHz, $CDCl_3$) δ = 3.17 (s, 2H, CH), 5.45 (t, 2H, *J* = 1.0 Hz, CHO), 6.57 (t, 2H, *J* = 1.0 Hz, CH_{vinyl}).

4-(2-Hydroxyethyl)-10-oxa-4-aza-tricyclo[5.2.1.02,6]dec-8-ene-3,5-dione

4,10-Dioxatricyclo[5.2.1.02,6]dec-8-ene-3,5-dione (3.24 g, 19.5 mmol) (**1**) were suspended in MeOH (80 mL) and the mixture cooled to 0 °C. A solution of ethanolamine (1.18 mL, 19.5 mmol) in MeOH (320 mL) was added dropwise and the resulting solution was stirred for 5 min at 0 °C, then 30 min at ambient temperature, and finally refluxed for 3 h. After cooling the mixture to ambient temperature, the solvent was removed under reduced pressure and the white residue was dissolved in CH_2Cl_2 (130 mL) and washed with brine (2x65 mL). The organic layer was dried

over $MgSO_4$ and filtered. Removal of the solvent under reduced pressure furnished the product (**2**) (0.81 g, 19% yield) as a white solid.

^{1}H NMR (500 MHz, $CDCl_3$) δ = 6.53 (t, 2H, *J* = 0.9 Hz, 2x *H*C=C*H*), 5.29 (t, 2H, *J* = 0.9 Hz, 2 x CH), 3.82-3.76 (m, 2H, OCH_2), 3.72-3.64 (m, 2H, NCH_2), 2.90 (s, 2H, 2×CH), 2.14 (bs, 1H, OH).

2-Bromo-2-methyl-propionic acid 2-(3,5-dioxo-10-oxa-4-azatricyclo[5.2.1.02,6]dec-8-en-4-yl)-ethyl ester

A solution of the alcohol 4-(2-hydroxyethyl)-10-oxa-4-aza- tricyclo[5.2.1.0]dec-8-ene-3,5-dione (**2**) (0.81 g, 3.9 mmol) and Et_3N (0.70 mL, 5.1 mmol, 1.3 equiv.) in dry THF (60 mL) was cooled to 0 °C, and a solution of 2-bromo isobutyryl bromide (0.57 mL, 4.7 mmol, 1.2 equiv.) in dry THF (13 mL) was added dropwise over 30 min. The white suspension was stirred for 3 h at 0 °C and subsequently at ambient temperature overnight. TLC revealed the complete disappearance of the starting material. The ammonium salts were filtered off and the solvent was removed under reduced pressure to give a pale-yellow residue that was purified by flash column chromatography (petroleum ether/ethyl acetate 1:1). The pure product (**3**) was obtained as a white solid (1.37 g, 98% yield).

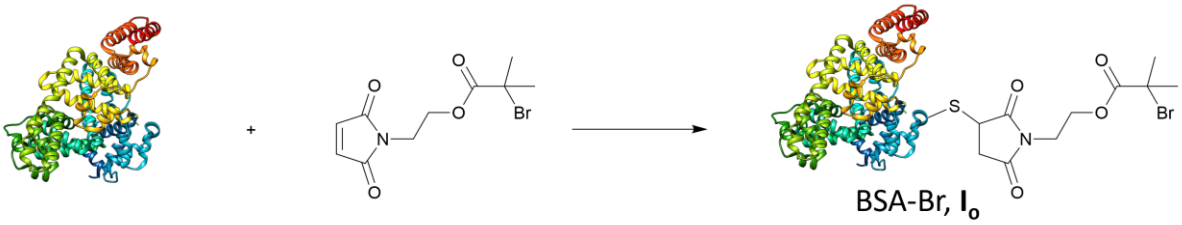
^{1}H NMR (500 MHz, $CDCl_3$) δ = 6.50 (t, 2H, *J* =1.0 Hz, 2×CH_{vinyl}), 5.25 (t, 2H, *J* = 1.0 Hz, 2×CHO), 4.31 (t, 2H, *J* = 5.3 Hz, OCH_2), 3.80 (t, 2H, *J* = 5.3 Hz, NCH_2), 2.85 (s, 2H, 2×CH), 1.83 (s, 6H, 2×CH_3).

2-Bromo-2-methyl-propionic acid 2-(2,5-dioxo-2,5-dihydro-pyrrol-1-yl)-ethyl ester

The maleimido-protected initiator (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**) (0.35 g, 0.90 mmol) was suspended in toluene (14 mL) and heated to reflux under Ar atmosphere for 20 hours. The solvent was removed under reduced pressure to give the pure product (**4**) as an off-white solid (0.24 g, 92% yield).

^{1}H NMR (400 MHz, $CDCl_3$) δ = 6.73 (t, 2H, *J* = 1.0 Hz, 2×CH_{vinyl}), 4.33 (t, 2H, *J* = 5.3 Hz, OCH_2), 3.85 (t, 2H, *J* = 5.3 Hz, NCH_2), 1.89 (s, 6H, 2×CH_3).

B. Synthesis of the bovine serum albumin macroinitiator (BSA-Br, I_o)



0.291 mL of a 120 mM solution of 2-bromo-2-methyl-propionic acid 2-(2,5-dioxo-2,5-dihydro-pyrrol-1-yl)-ethyl ester in DMSO (0.035 mmol, 20 equiv.) was slowly added to 4.7 mL of a 0.35 mM solution of BSA in 20 mM phosphate buffer, pH 7.4 (1.75×10^{-3} mmol, 1 equiv.). The reaction mixture was gently shaken for 2 hours at 7 °C and was then dialyzed using a 25 kDa MWCO regenerated cellulose membrane initially against 5 mM phosphate buffer, pH 7.4, 1 % DMSO, then against 5 mM phosphate buffer, pH 7.4, and finally against 20 mM phosphate buffer, pH 7.4. The

solution of the BSA-macroinitiator (BSA-Br, I_o) was subsequently characterized with FT-IR, SEC, and native PAGE electrophoresis and stored at 7 °C until further use.

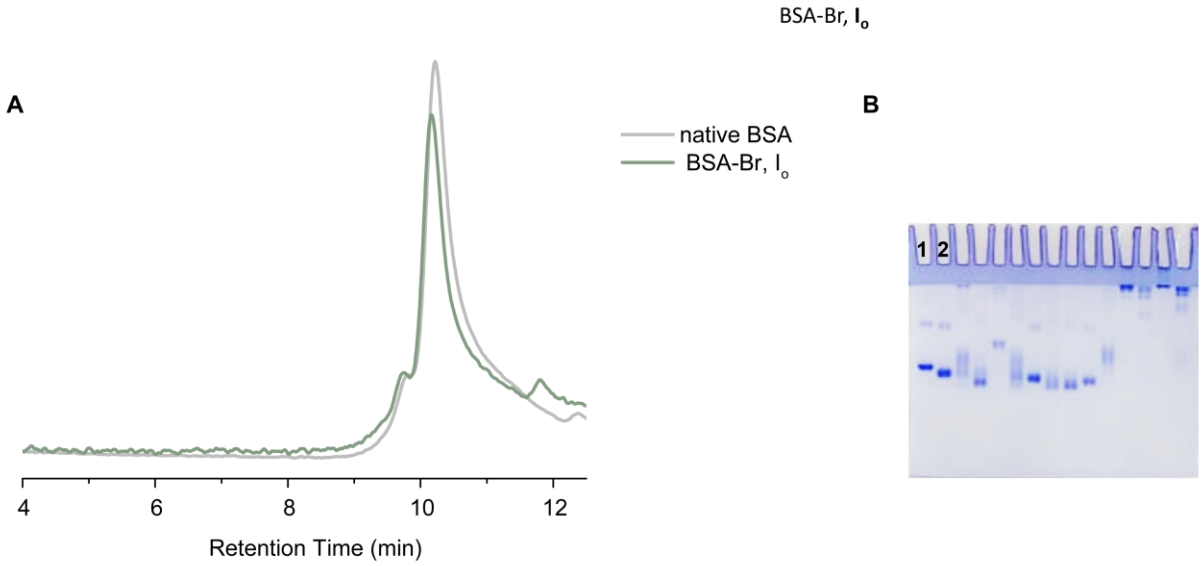


Figure S 2. ***A****. SEC chromatograms of native BSA (grey trace) and BSA-Br (I_o) (green traces).* ***B****. Native PAGE, lane 1: native BSA, lane 2: BSA-Br (I_o).*

In SEC, the biomacroinitiator (BSA-Br, I_o) was eluted with slightly shorter retention time than the native BSA (Figure S2 A). In accordance with previous reports, the macroinitiator BSA-Br (I_o) was found to migrate faster than native BSA, a fact that can be attributed to change of either shape or total charge of the bioconjugate.[5]

Oxygen tolerant, EY mediated grafting of styrene from BSA-Br (I_o)

A solution of EY was prepared by dissolving 1 mg EY (1.54 µmol) in 1 mL, 20 mM phosphate buffer pH 7.4 with the aid of sonication.

An emulsion of styrene was formed by adding styrene (872-1744 µmol, 4000-8000 equiv.) to a solution containing the selected quantity of EY (2.8-141.5 µL, 0.044-0.218 µmol, 0.02-1.0 equiv.) in nanopure water (385-425 µL) and sonicating for ca. 3 minutes. The volume of the water was adjusted for each feed molar ratio to retain a stable volume.

The styrene/ EY emulsion was immediately transferred to a 6 mL polypropylene syringe equipped with a stirring bar, containing 0.625 mL of a 0.35 mM solution of the protein macroinitiator (I_o) in 20 mM phosphate buffer, pH 7.4 (0.218 µmol, 1 equiv.). Headspace was eliminated to avoid the presence of undissolved oxygen and the reaction syringe was capped and placed under blue LED irradiation for specified amounts of time (20 min to 9 hours) with moderate stirring. A ventilator was used to avoid temperature increase maintaining the temperature between 25 and 32 °C. The reaction mixture was then dialyzed using a 10 kDa MWCO regenerated cellulose dialysis membrane initially against a mixture of 5 mM phosphate buffer, pH 7.4, 1 % DMSO, then against 5 mM phosphate buffer, pH 7.4, and finally against 20 mM phosphate buffer, pH 7.4. The product solutions were analyzed by means of native or SDS PAGE electrophoresis, SEC and FT-IR spectroscopy. ^{1}H-NMR spectra were acquired for hydrophilic products. Dilute suspensions of the products in nanopure water were imaged with SEM or FE-SEM. All products were stored at 7 °C until further use.

Oxygen tolerant, EY/AscA mediated grafting of styrene from BSA-Br (I_o)

An emulsion of the styrene was formed by adding the hydrophobic monomer (872 µmol, 4000 equiv.) to 298.5 µL nanopure water and sonicating for ca. 5 minutes.

A solution of EY was prepared by dissolving 1 mg EY (1.54 µmol) in 1 mL, 20 mM phosphate buffer pH 7.4 with the aid of sonication.

Ascorbic acid (1 µg, 5 µmol) was dissolved in 1 mL 20 mM phosphate buffer, pH 7.4.

The EY solution (141.5 µL, 0.218 µmol, 1.0 equiv.) and the AscA solution (21.8 µL, 0.109 µmol, 0.5 equiv.) were added to the monomer emulsion and the resulting mixture was immediately transferred to a 6 mL polypropylene syringe equipped with a stirring bar, containing 0.625 mL of a 0.35 mM solution of the protein macroinitiator (I_o) in 20 mM phosphate buffer, pH 7.4 (0.218 µmol, 1 equiv.). Headspace was eliminated to avoid the presence of undissolved oxygen and the reaction syringe was capped and placed under blue LED irradiation for two hours with moderate stirring. A ventilator was used to avoid temperature increase maintaining the temperature between 25 and 32 °C. The reaction mixture was then dialyzed using a 25 kDa MWCO regenerated cellulose dialysis membrane initially against a mixture of 5 mM phosphate buffer, pH 7.4, 1 % DMSO, then against 5 mM phosphate buffer, pH 7.4, and finally against 20 mM phosphate buffer,

pH 7.4. The product solutions were analyzed by means of native or SDS PAGE electrophoresis, SEC and FT-IR spectroscopy. The product was stored at 7 °C until further use.

Oxygen tolerant, EY/TEMED mediated grafting of monomers from protein macroinitiators

A. General Polymerization protocol for hydrophobic monomers

A solution of EY was prepared by dissolving 1 mg EY (1.54 µmol) in 1 mL, 20 mM phosphate buffer pH 7.4 with the aid of sonication.

0.21 M and 0.1 M TEMED stock solutions were prepared by dissolving 3.3 µL (22 µmol) in 100 µL nanopure water or 1.5 µL (10 µmol) in 98.5 µL nanopure water.

2.8 µL - 141.5 µL (0.044-0.218 µmol) of the stock solution of EY and the corresponding volume of the appropriate TEMED stock solution were dissolved in nanopure water to afford a solution with fixed total volume (460 µL). The emulsion of the monomer was formed by adding the hydrophobic monomer (872 µmol, 4000 equiv.) and sonicating for ca. 5 minutes. The volume of the water was adjusted for each feed molar ratio to retain a stable volume. The resulting emulsion was immediately transferred to a 6 mL polypropylene syringe equipped with a stirring bar, containing 0.625 mL of a 0.35 mM solution of the protein macroinitiator (I_o) in 20 mM phosphate buffer, pH 7.4 (0.218 µmol, 1 equiv.). Headspace was eliminated to avoid the presence of undissolved oxygen and the reaction syringe was capped and placed under blue LED irradiation for specified amounts of time (varying from 5 minutes to 9 hours) with moderate stirring. A ventilator was used to avoid temperature increase maintaining the temperature between 25 and 32 °C. The reaction mixture was then dialyzed using a 10 kDa MWCO regenerated cellulose dialysis membrane initially against a mixture of 5 mM phosphate buffer, pH 7.4, 1 % DMSO, then against 5 mM phosphate buffer, pH 7.4, and finally against 20 mM phosphate buffer, pH 7.4. The product solutions were analyzed by means of native or SDS PAGE electrophoresis, SEC, and FT-IR spectroscopy. ^{1}H-NMR spectra were acquired for hydrophilic products. Dilute suspensions of the products in nanopure water were imaged with SEM or FE-SEM. All products were stored at 7 °C until further use.

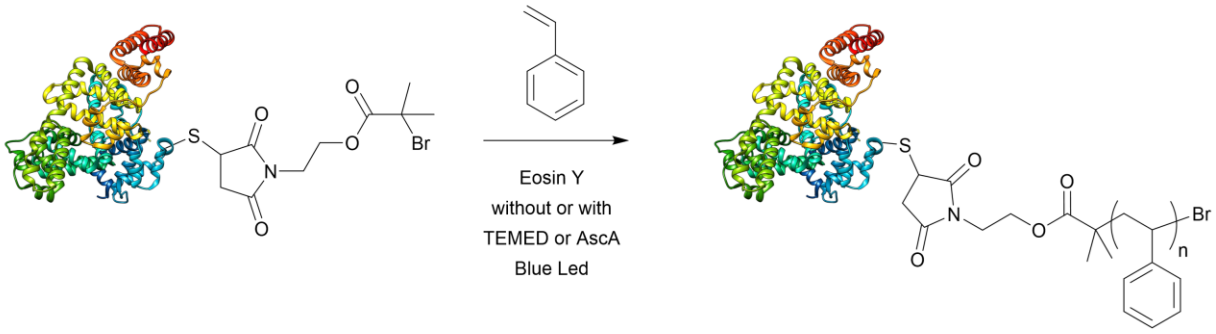
In the case of MMA, the monomer (94 µL, 872 µmol, 4000 equiv.) was mixed with 60 µL of toluene and added to 365 µL of nanopure water before sonicating to form an emulsion.

B. General Polymerization protocol for hydrophilic monomers

The protocol described for hydrophobic monomers (*vide supra*) was used without variations for hydrophilic monomers the grafting of which, was performed in solution.

BSA-polymer Conjugates

i. BSA-poly(styrene)



Styrene was used as model monomer and experiments to achieve and optimize grafting are included in Table 1 (manuscript). Experiments with higher and lower monomer (from 50 to 5000 equivalents) feed were also performed but not included in this study as the results were in agreement with previous reports.[5, 6]

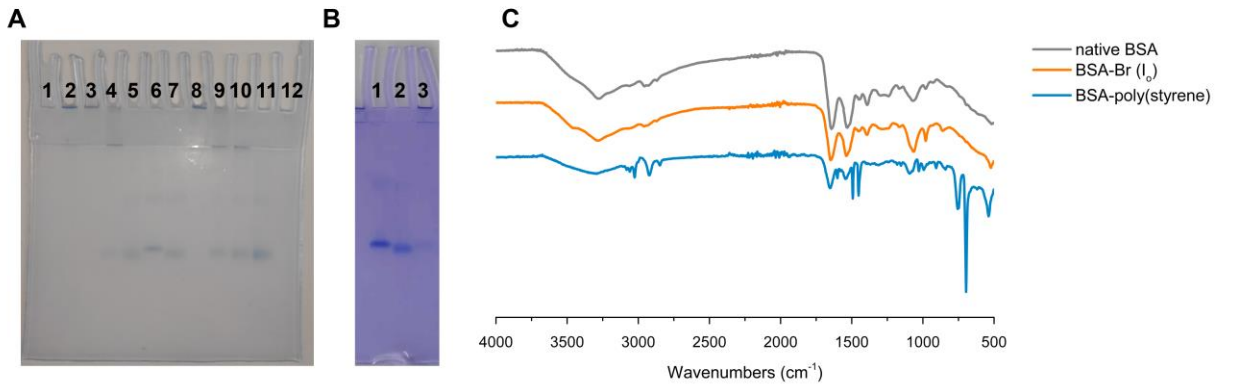


Figure S 3. Characterization of BSA-poly(styrene) formed during optimization experiments. ***A.*** *Native PAGE of the optimization experiments using different molar feed ratios. Lane 1: Styrene/BSA-Br, I_o/EY =4000/0/1 (control experiment), lane 2: Styrene/BSA-Br, I_o/EY/TEMED=4000/1/1/10, lane 3: Styrene/BSA-Br, I_o/EY/TEMED=4000/1/0.5/5, lane 4: Styrene/BSA-Br, I_o/EY/TEMED=4000/1/0.2/2, lane 5: Styrene/BSA-Br, I_o/EY/TEMED=4000/1/0.02/0.2, lane 6: native BSA, lane 7: BSA-Br (I_o), lane 8: Styrene/BSA-Br, I_o/EY=4000/1/1, lane 9: Styrene/BSA-Br, I_o/EY=4000/1/0.5, lane 10: Styrene/BSA-Br, I_o/EY=4000/1/0.2, lane 11: Styrene/BSA-Br, I_o/EY=4000/1/0.02.* ***B.*** *Native PAGE of BSA-poly(styrene) formed using AscA to deoxygenate. Lane 1: native BSA, lane 2: BSA-Br (I_o), lane 3: Styrene/BSA-Br, I_o/EY/AscA=4000/1/1/0.* ***C.*** *FT-IR spectra of native BSA (grey), BSA-Br (I_o, light grey) and BSA-poly(styrene) (magenta) produced during optimization experiments in which the characteristic C-H bending of the aromatic ring of poly(styrene) at 690.7 cm^{-1} together with the characteristic vibrations of amide I and I at 1652 and 1542 cm^{-1}, can be observed.*

Table 1, Entries 1-4: In the absence of sacrificial electron donor or oxygen scavenger

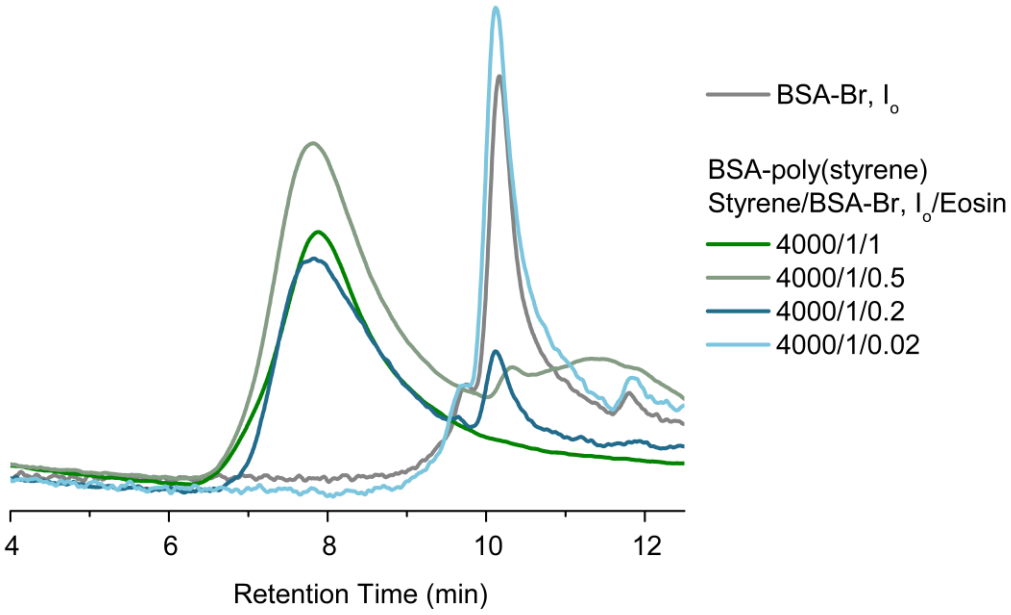


Figure S 4. SEC chromatograms of BSA-poly(styrene) produced by EY photocatalyzed polymerization (Table S1, Entries 1-4).

Table 1 Entries 5-7: Control Experiments

Control experiments were performed in the absence of a selected reaction component such as the catalyst, the monomer, irradiation or the macroinitiator.

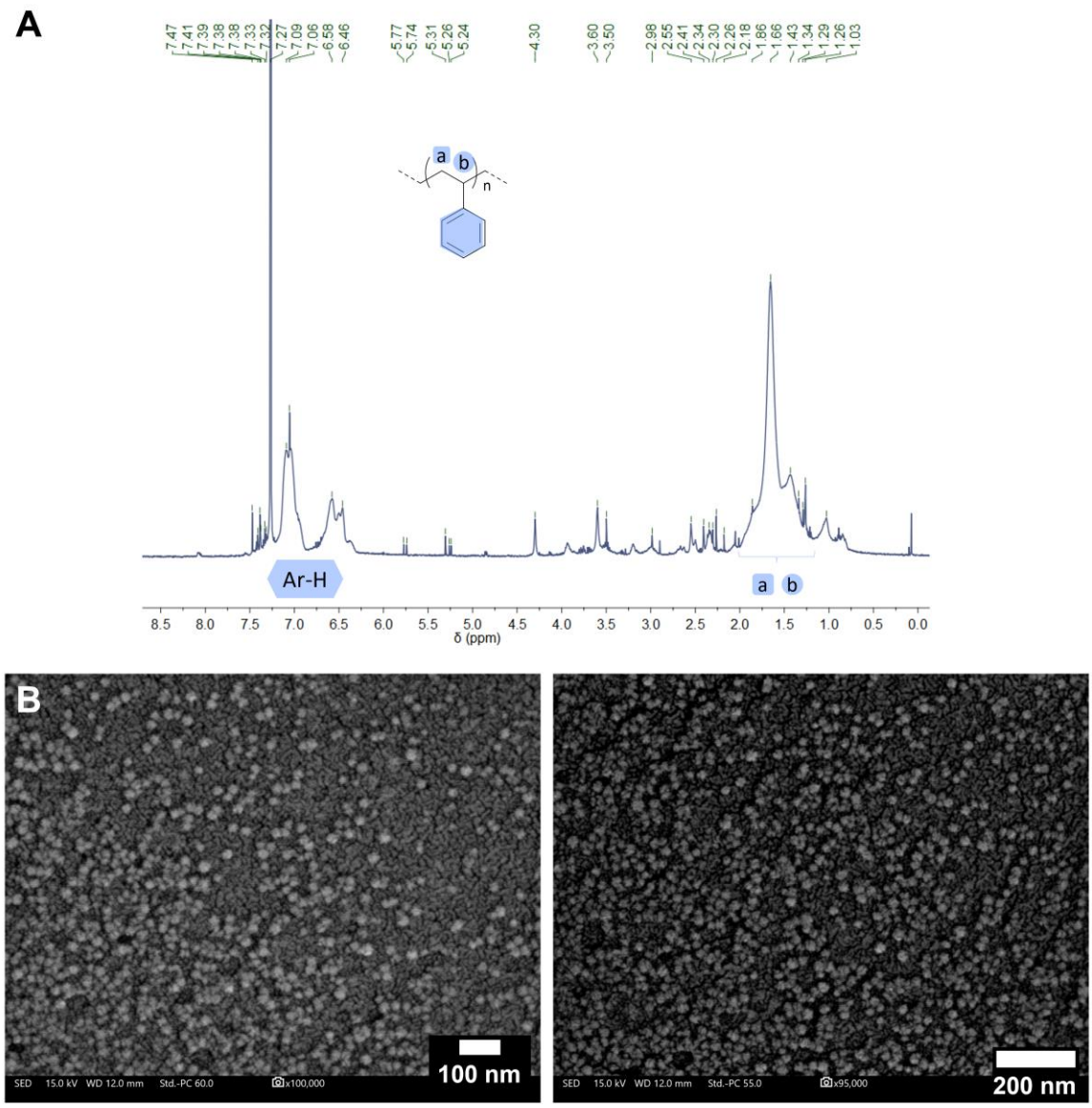


Figure S 5. Styrene oligomers and polystyrene isolated from the control experiment performed in the absence of macroinitiator. ***A****.* ^{1}*H-NMR spectrum of poly(styrene) isolated from the control experiment (Table S 1, Entry 7).* ***B****. FE-SEM micrographs of the produced nanoparticles with diameters varying from 10 to 40 nm.*

No product could be detected in the absence of EY or irradiation (Table 1, Entries 5 and 6). Poly(styrene) was formed when styrene was subjected to EY photoinduced emulsion polymerization (Table 1, Entry 7) in the absence of the macroinitiator BSA-Br (I_o). The polymer was isolated by freeze-drying the reaction products and then dissolving organic components in chloroform and sonicating. The chloroform was evaporated under reduced pressure, the organic component dissolved in deuterated chloroform and characterized with ^{1}H-NMR spectroscopy (Figure S 5A). When samples of the reaction mixture were imaged with FE-SEM, spherical

nanostructures with diameters between 10 and 40 nm were observed (Figure S 5B, C). Similarly, poly(styrene) was also formed in the presence of TEMED (Table 1, Entries 13 and 14).

Taking these results into account, control experiments were also performed with MMA, DPA, and NIPAM (Table S 1, Entries 1-2). Poly(MMA) was isolated after freeze-drying the reaction mixture while, for poly(DPA) and poly(NIPAM), ^{1}H-NMR spectra were acquired without isolating the produced polymers. In the case of poly(NIPAM), DMF was used as internal standard.

Table S 1. List of control experiments

Entry	Monomer/BSA-Br, I_o/EY/TEMED	Monomer	Reaction Time (min)
1	4000/0/1/10	MMA	120 [a, b]
2	4000/0/1/10	DPA	120 [a]
3	1000/0/1/10	NIPAM	30 [a]

[a] Formation of the corresponding polymer. [b] in the presence of toluene

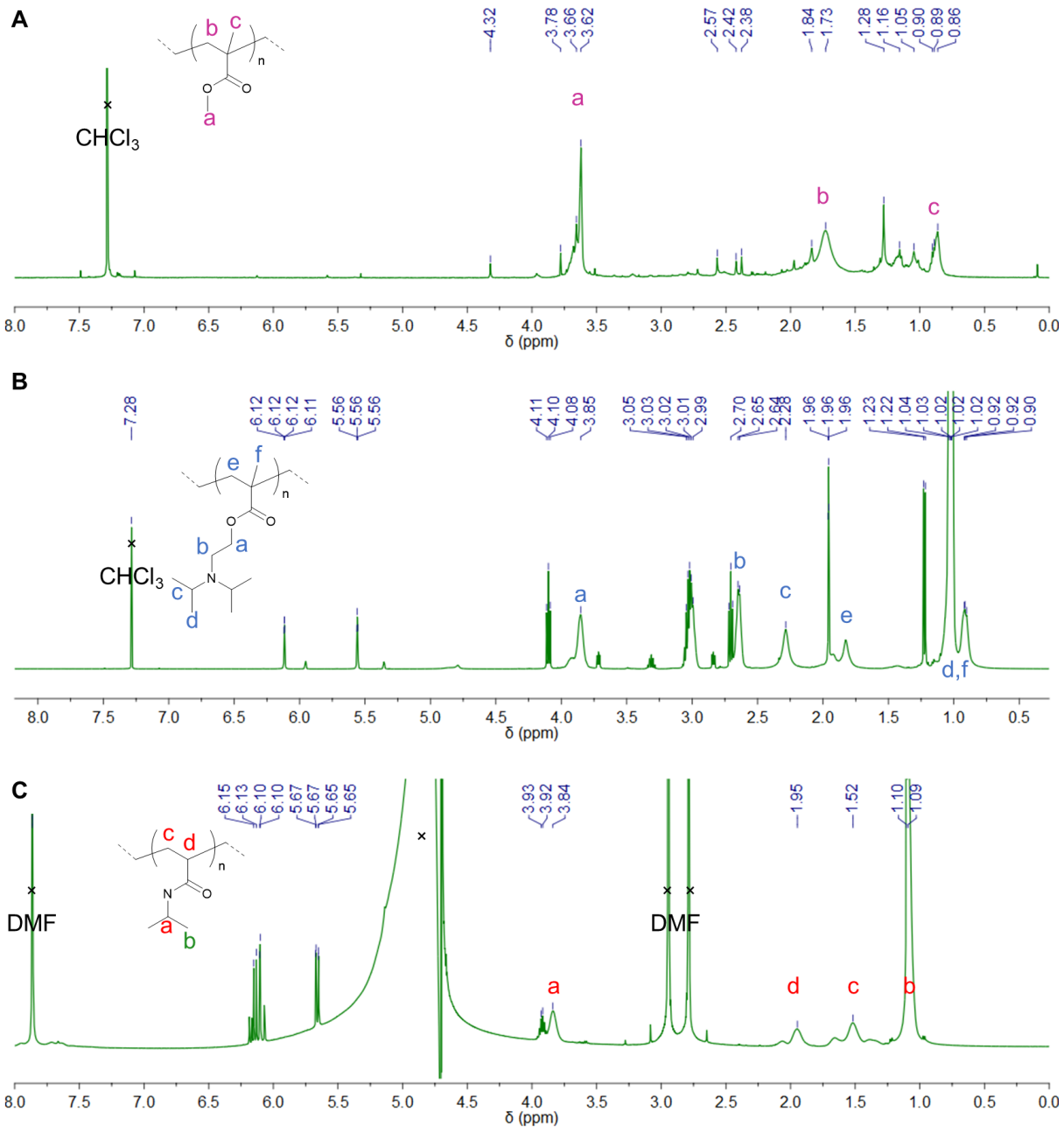


Figure S 6. [1]H-NMR spectra of polymers produced during control experiments performed in the absence of macroinitiator. ***A.*** *poly(MMA) (Table S 1, Entry 1),* ***B.*** *poly(DPA) (Table S 1, Entry 2),* ***C.*** *poly(NIPAM) (Table S 1, Entry 3).*

Table 1, Entry 8: with AscA

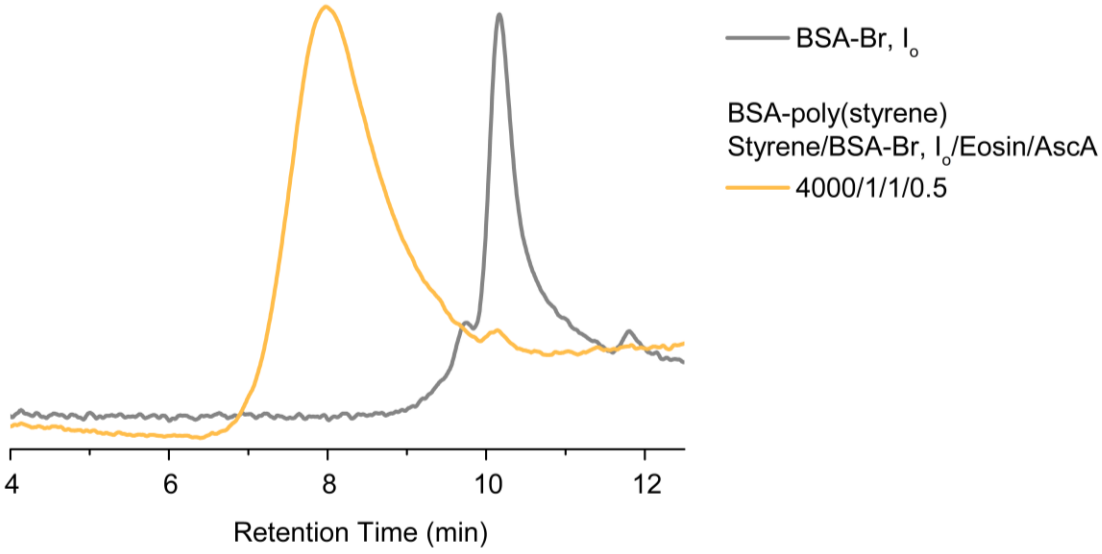


Figure S 7. SEC chromatograms of BSA-poly(styrene) produced by EY/AscA photocatalyzed polymerization (Table 1, Entry 8).

Table 1, Entries 9-12: With TEMED

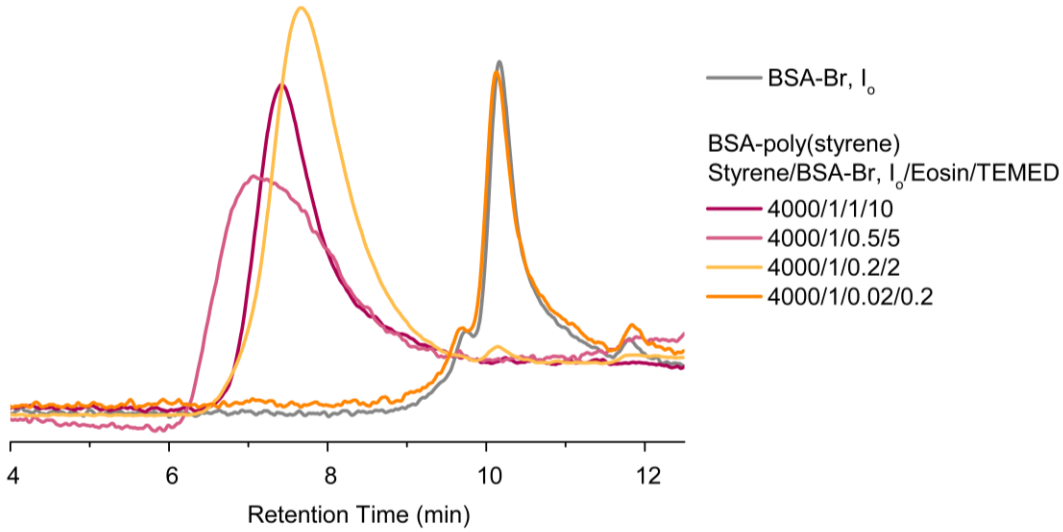


Figure S 8. SEC chromatograms of BSA-poly(styrene) produced by EY/TEMED photocatalyzed polymerization (Table S2, Entries 9-12).

Large Scale

The reaction was performed using the same experimental conditions in larger scale, i.e. using 8.75 µmol BSA-Br (0.58 gr dissolved in 17 mL 20 mM phosphate buffer pH 7.4), 35 mmol styrene,

8.75 µmol EY, 87.5 µmol TEMED and 12.4 mL nanopure water. Special attention was paid to retaining the temperature of the reaction mixture below 37 °C by using several ventilators.

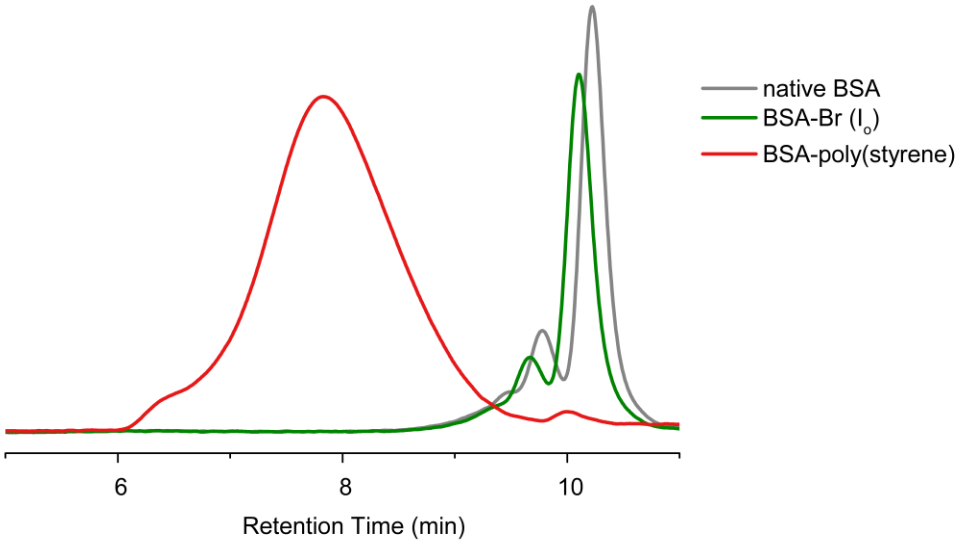


Figure S 9. SEC chromatograms of BSA-poly(styrene) produced by EY/TEMED photocatalyzed polymerization in large scale.

ii. BSA-poly(MA)

The general procedure of the oxygen tolerant EY/TEMED mediated polymerization protocol was applied to optimize the synthesis of BSA-poly(MA) and feed molar ratios were modified to achieve complete macroinitiator consumption.

Table S 2. Optimization of the EY/TEMED mediated synthesis of BSA-poly(MA).

Entry	MA/BSA-Br (I_o)/EY/TEMED	EY (µmol)	BSA-Br (I_o) Consumption
1	4000/1/0.2/10	0.044	partial reaction
2	4000/1/1/10	0.22	near quantitative
3	4000/1/1/10[a]	0.22	near quantitative

[a] In the presence of organic cosolvent (5 % v/v EtOAc, toluene or DMSO)

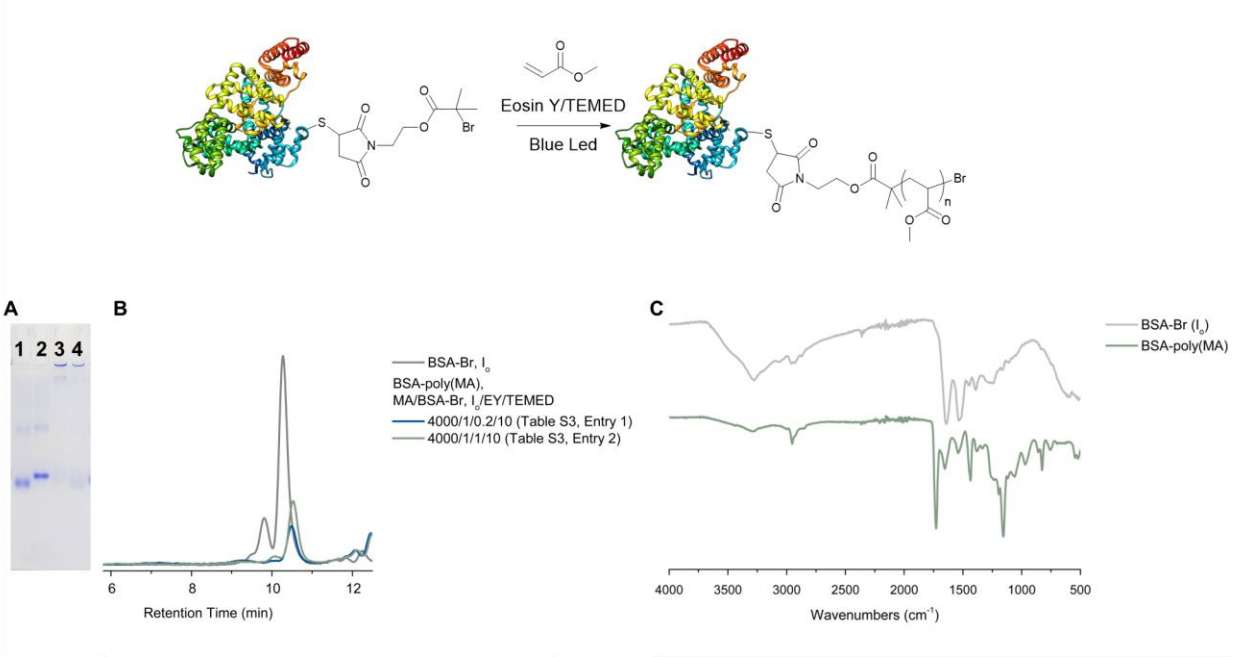


Figure S 10. ***A.*** *Native PAGE electrophoresis: Lane 1: BSA-Br (I_o), lane 2: native BSA, lane 3: BSA-poly(MA), Table S2, Entry 1, lane 4: BSA-poly(MA), Table S2, Entry 2.* ***B.*** *SEC chromatograms of BSA-Br (I_o) and BSA-poly(MA).* ***C.*** *FT-IR spectra of BSA-poly(MA) and BSA-Br (I_o). In the biohybrid spectrum the peak at 1658.5 cm^{-1} that can be attributed to the C=O ester bond, a peak at 1440.2 cm^{-1} that can be attributed to the bending vibrations of CH_2 polymer backbone bonds and at 1125.4 cm^{-1} that can be attributed to the stretching of C-C=O bond of the poly(MA) moiety. The peaks at 1653. 8 and 1541.6 cm^{-1} are attributed to amides I and II of the protein.*

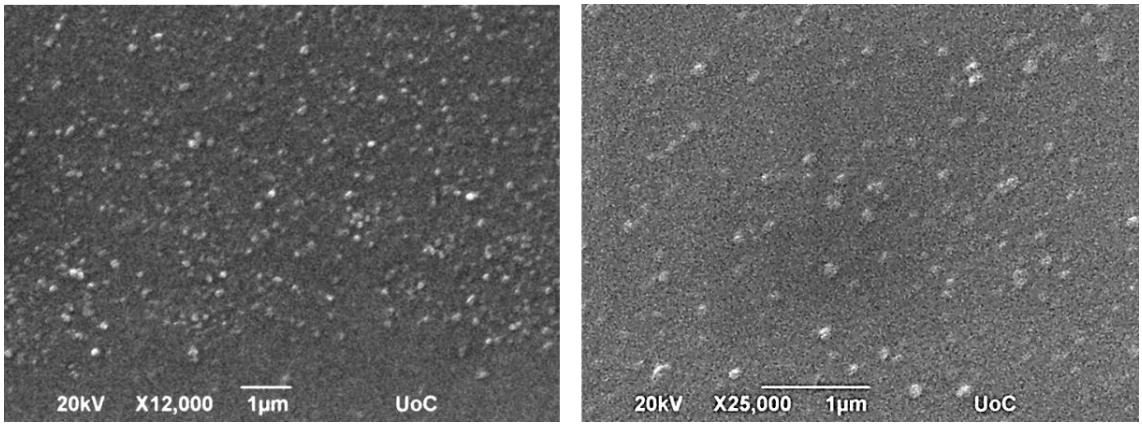


Figure S 11. SEM micrographs of BSA-poly(MA) synthesized using molar ratio MA/BSA-Br, (I_o)/EY/TEMED = 4000/1/1/10 (Table S2, Entry 2).

iii. BSA-poly(MMA)

The general procedure of the oxygen tolerant EY/TEMED mediated polymerization protocol was applied to optimize the synthesis of BSA-poly(MMA) and feed molar ratios were modified to achieve complete macroinitiator consumption. Quantitative macroinitiator consumption at feed molar ratio MMA/BSA-Br, I_o/EY/TEMED = 4000/1/1/10 could only be achieved in the presence of 5 % v/v toluene.

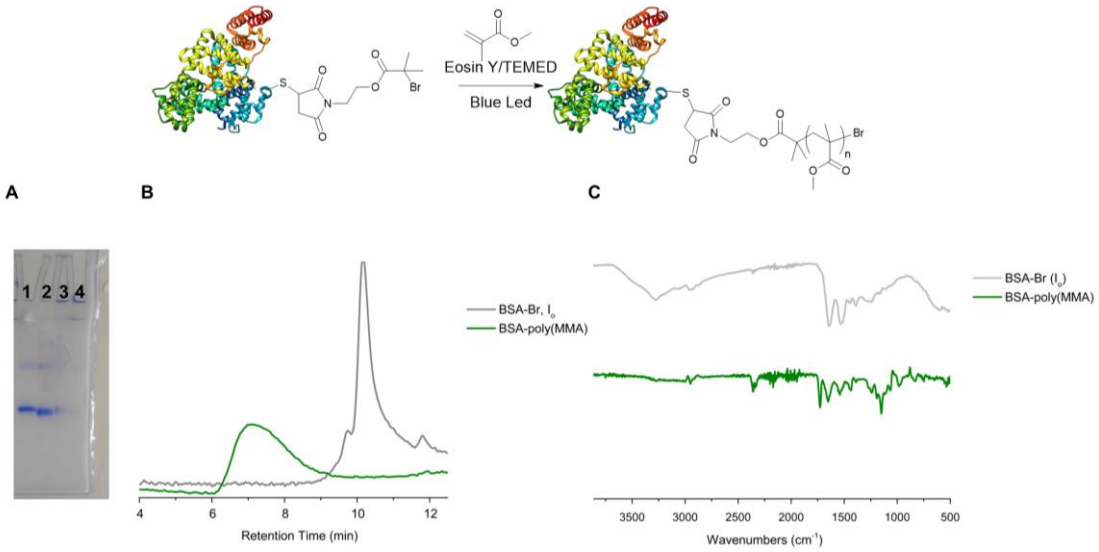


Figure S 12. ***A.*** *Native PAGE electrophoresis: Lane 1: native BSA, lane 2: BSA-Br (I_o), lane 3: BSA-poly(MMA), MMA/BSA-Br/EY/TEMED = 4000/1/1/10, lane 4: BSA-poly(MMA), MMA/BSA-Br/EY/TEMED = 4000/1/1/10, in the presence of toluene.* ***B.*** *SEC chromatograms of BSA-Br (I_o) and BSA-poly(MMA).* ***C.*** *FT-IR spectra of BSA-poly(MMA) and BSA-Br (I_o). In the biohybrid spectrum the peak at 1710.5 cm^{-1} can be attributed to the C=O ester bond, and at 1128.7 cm^{-1} can be attributed to the stretching of the C-C=O bond of poly(MMA). The amide I and II vibrations of the protein moiety are at 1652,3 and 1539.6 cm^{-1}.*

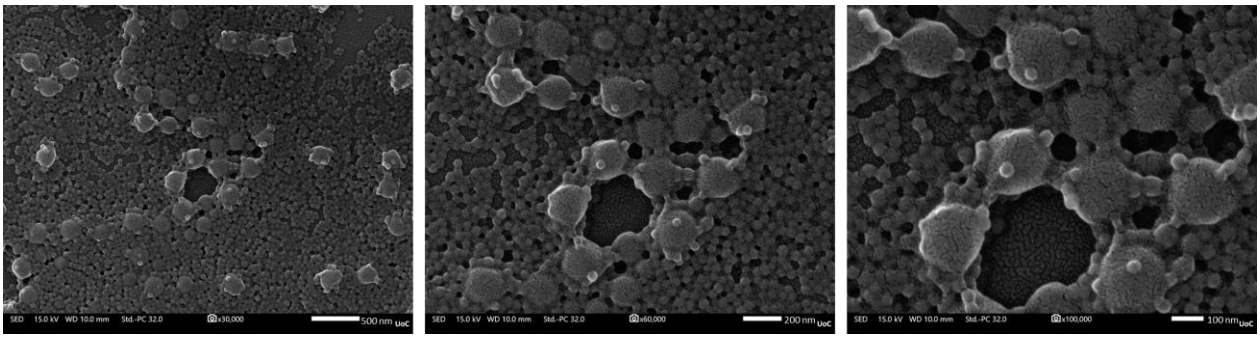


Figure S 13. SEM micrographs of BSA-poly(MMA) synthesized using molar ratio MMA/BSA-Br, (I_o)/EY/TEMED = 4000/1/1/10 in the presence of 5 % v/v toluene. BSA-poly(MMA) was found to assemble into hybrid polymer/biopolymer spherical nanostructures with two distinct populations with diameters between 120-150 nm and 10-30 nm.

iv. BSA-poly(VAc)

The general procedure of the oxygen tolerant EY/TEMED mediated polymerization protocol was applied to optimize the synthesis of BSA-poly(VAc) and feed molar ratios were modified in an effort to achieve complete macroinitiator consumption (experiments not presented).

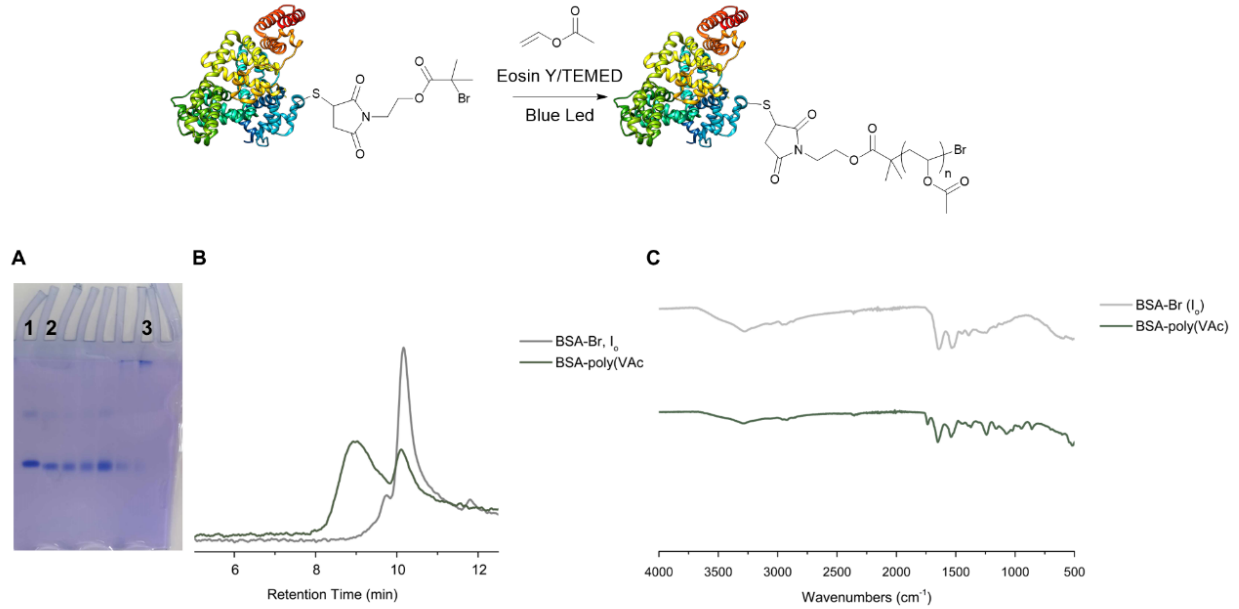


Figure S 14. ***A.*** *Native PAGE electrophoresis: Lane 1: native BSA, lane 2: BSA-Br (I_o), lane 3: BSA-poly(VAc).* ***B.*** *SEC chromatograms of BSA-Br (I_o) and BSA-poly(VAc).* ***C.*** *FT-IR spectra of BSA-poly(VAC) and BSA-Br (I_o). In the biohybrid spectrum the peak at 1755.9 cm-1 can be attributed to the stretching of C=O ester bond, and at 1206.7 cm^{-1} can be attributed to the vibration of O-C=O bond of the poly(VAc) moiety. The amide I and II vibrations of the protein moiety are at 1642.97 cm^{-1} and 1538.9 cm^{-1}.*

v. BSA-poly(VP)

The protocol described for hydrophobic monomers (*vide supra*) was used without variations for hydrophilic monomers the grafting of which was performed in solution. BSA-poly(vinyl pyrrolidone) (BSA-poly(VP)) was synthesized using feed molar ratio VP/BSA-Br, (I_o)/EY/TEMED = 4000/1/0.5/10. Full macroinitiator consumption could be observed after irradiating for 1 hour.

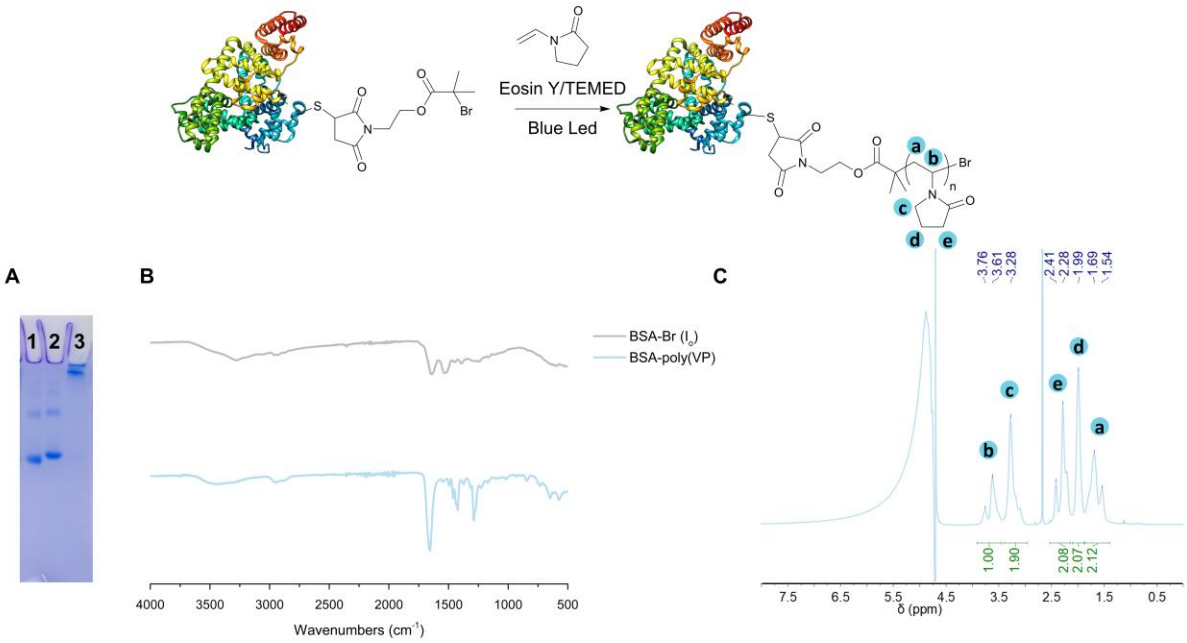


Figure S 15. Synthesis of BSA-poly(VP) ***A.*** *native PAGE: lane 1: BSA-Br (I_o), lane 2: native BSA, lane 3: BSA-poly(VP).* ***B.*** *FT-IR spectra of BSA-Br (I_o) and BSA-poly(VP). In the biohybrid spectrum the peak at 1620.1 cm^{-1} can be attributed to the stretching of the C=O ester bond, the peak at 1432.4 cm^{-1} can be attributed to the stretching vibrations of C-N bonds and the peak at 1250.6 cm^{-1} can be attributed to the C-N-C vibration of poly(VP). In the same spectrum, the amide I and II vibrations of the protein moiety are at 1657.9 cm^{-1} and 1543.5 cm^{-1}.* ***C.*** *^{1}H-NMR spectrum of BSA-poly(VP) in D_2O acquired with water suppression.*

vi. BSA-poly (NAM)

The catalyst and monomer loading were varied in an effort to achieve full macroinitiator consumption. A *feed molar ratio* NAM/BSA-Br, (I_o)/EY/TEMED = 5000/1/1/10 led to almost quantitative macroinitiator consumption after irradiating for 1 hour.

Table S 3. Optimization of the EY/TEMED mediated synthesis of BSA-poly(NAM).

Entry	NAM/BSA-Br, (I_o)/EY/TEMED	EY (µmol)	Reaction Time (min)	BSA-Br (I_o) Consumption
1	4000/1/0.2/10	0.044	60	no reaction
2	4000/1/0.5/10	0.11	60	partial reaction
3	4000/1/1/10	0.22	60	partial reaction
4	4000/1/1/10	0.22	120	near quantitative
5	5000/1/1/10	0.22	60	near quantitative

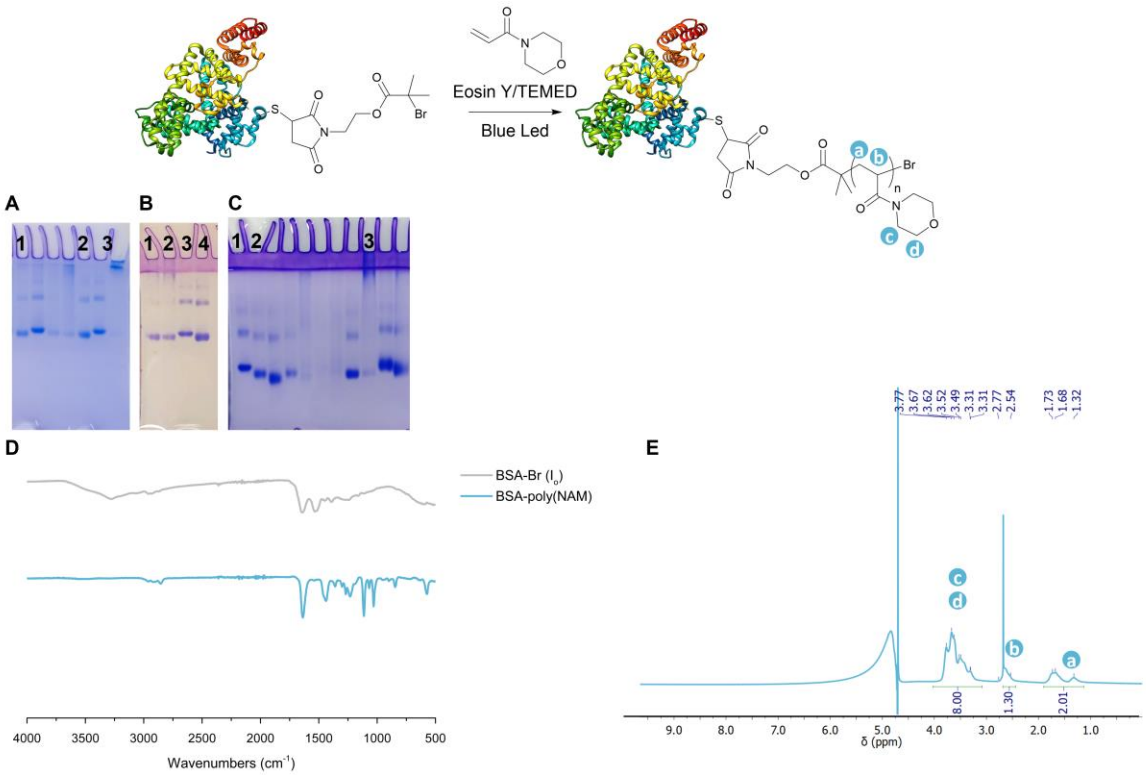


Figure S 16. Synthesis of BSA-poly(NAM) -optimization study. ***A.*** *Native PAGE, lane 1: NAM/BSA-Br, (I_o)/EY/TEMED = 4000/1/0.2/10 (Table S3, Entry 1), lane 2: BSA-Br (I_o), lane 3: native BSA.* ***B.*** *Native PAGE, lane 1: NAM/BSA-Br, (I_o)/EY/TEMED = 4000/1/0.5/10 (Table S3, Entry 2), lane 2: NAM/BSA-Br, (I_o)/EY/TEMED = 4000/1/1/10 (Table S3, Entry 3), lane 3: native BSA, lane 4: BSA-Br (I_o).* ***C.*** *Native PAGE, lane 1: native BSA, lane 2: BSA-Br (I_o), lane 3: NAM/BSA-Br, (I_o)/EY/TEMED = 4000/1/1/10, 120 minutes (Table S3, Entry 4).* ***D.*** *FT-IR spectra of BSA-Br (I_o) and BSA-poly(NAM). In the biohybrid spectrum the peak at 1750.2 cm^{-1} can be attributed to the stretching of the C=O ester bond and the peaks at 1500-500 cm^{-1} can be attributed to the stretching vibrations of C-H bonds of poly(NAM). In the same spectrum the amide I vibrations of the protein moiety are at 1637.3 cm^{-1}.* ***E.*** *^{1}H-NMR spectrum of BSA-poly(NAM) in D_2O acquired with water suppression.*

vii. BSA-poly (HEA)

The optimal conditions identified for the grafting of styrene were used to synthesize BSA-poly(HEA).

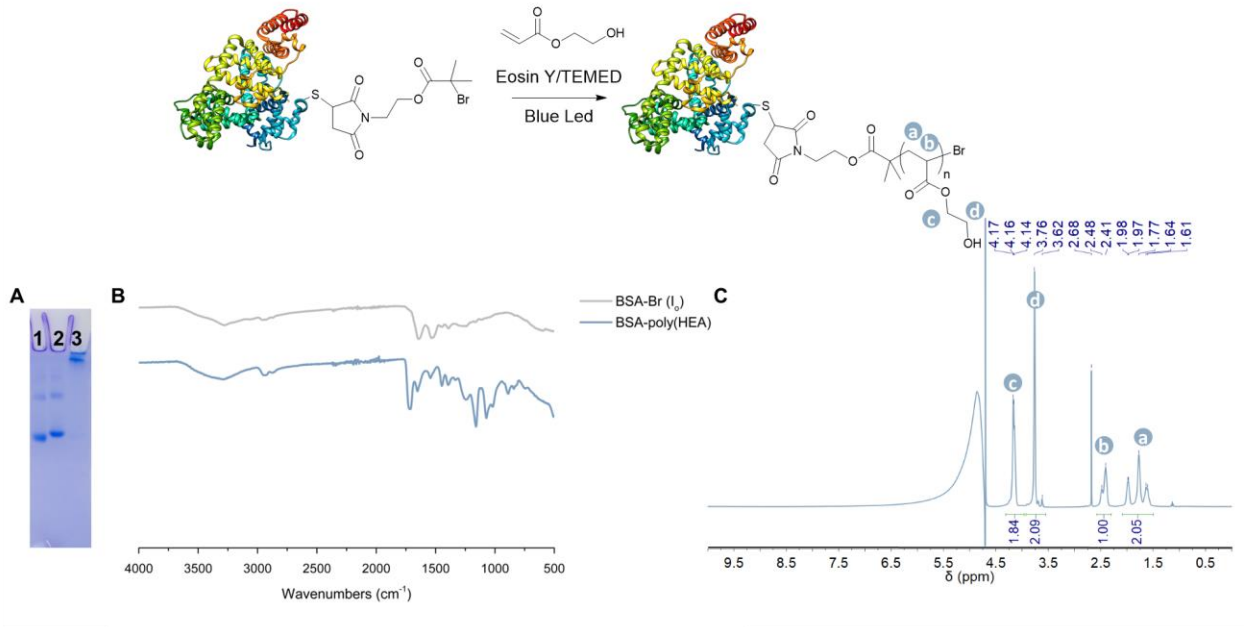


Figure S 17. Synthesis of BSA-poly(HEA). ***A.*** *Native PAGE, lane 1: BSA-poly(HEA) synthesized using feed molar ratio HEA/BSA-Br, (I_o)/EY/TEMED = 4000/1/1/10, lane 2: BSA-Br (I_o), lane 3: native BSA.* ***B.*** *FT-IR spectra of BSA-Br (Io) and BSA-poly(HEA). In the biohybrid spectrum the peak at 3302 cm^{-1} that be attributed to the stretching bond of OH, the peak at 1695.2 cm^{-1} can be attributed to the stretching of the C=O ester bond, the peak at 1176.8 cm^{-1} can be attributed to the stretching vibrations of the C-O of the ester bonds and the peak at 1050 cm^{-1} can be attributed to C-O stretching of the primary -OH of poly(HEA). In the same spectrum, the amide I and II vibrations of the protein moiety are at 1652.7 and 1543.8 cm^{-1}.* ***E.*** *^{1}H-NMR spectrum of BSA-poly(HEA) in D_2O acquired with water suppression.*

viii. BSA-poly (HEMA)

BSA-poly(2-hydroxyethyl methacrylate) (BSA-poly(HEMA)) was synthesized using the general EY/TEMED mediated photopolymerization protocol with molar loading HEMA/BSA-Br, (I_o)/EY/TEMED = 4000/1/1/10. Grafting was allowed to proceed for 1 hour after which time near quantitative macroinitiator consumption was attained.

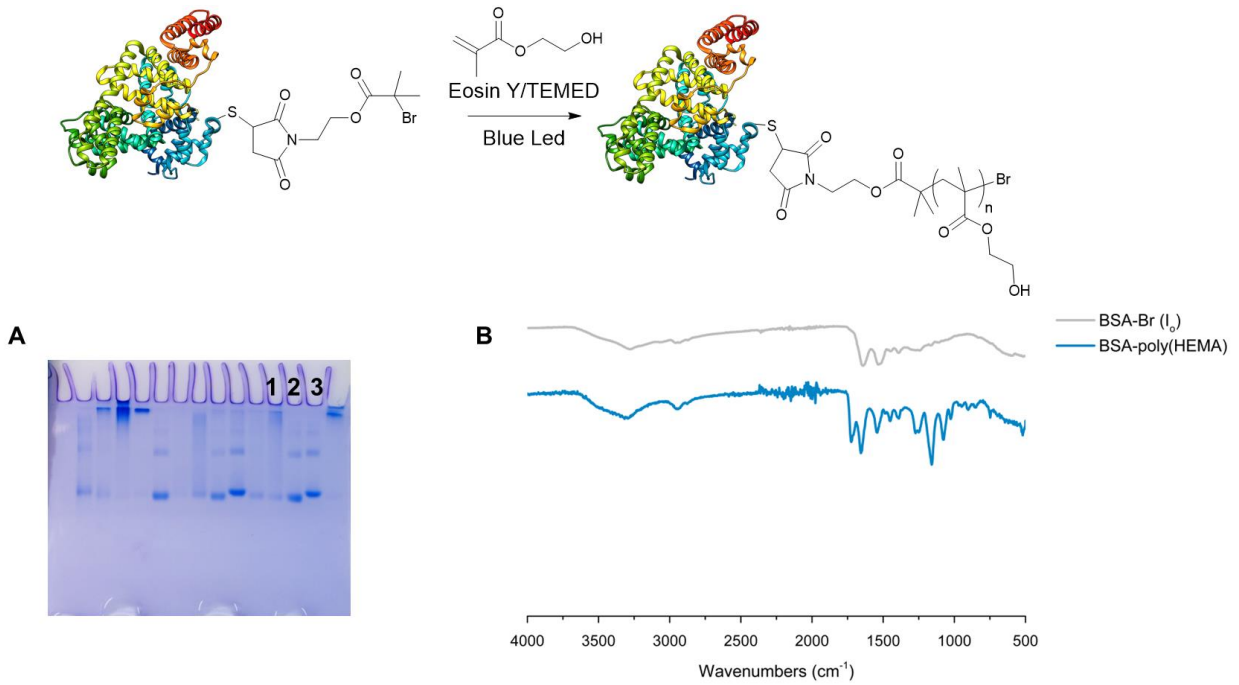


Figure S 18. Synthesis of BSA-poly(HEMA). ***A.*** *Native PAGE, lane 1: BSA-poly(HEMA), lane 2: BSA-Br (I_o), lane 3: native BSA.* ***B.*** *FT-IR spectra of BSA-Br (I_o) and BSA-poly(HEA). In the biohybrid spectrum the peak at 1725.3 cm^{-1} can be attributed to the stretching of the C=O ester bond, a peak at 1115.8 cm^{-1} can be attributed to the stretching vibrations of the C-O of the ester bonds and a peak at 1072 cm^{-1} can be attributed to C-O stretching of the primary -OH of poly(HEMA). In the same spectrum the amide I and II vibrations of the protein moiety are at 1652.2 and 1544.3 cm^{-1}.*

ix. BSA-poly (NIPAM)

BSA-poly(NIPAM) was synthesized using the general EY/TEMED mediated photopolymerization protocol. Low volume samples (ca. 50 µL) of the reaction mixture were withdrawn from the reaction at fixed time points and analyzed by native PAGE and ^{1}H-NMR spectroscopy. Following the same procedure (withdrawing low volume samples), ON/OFF experiments were also performed.

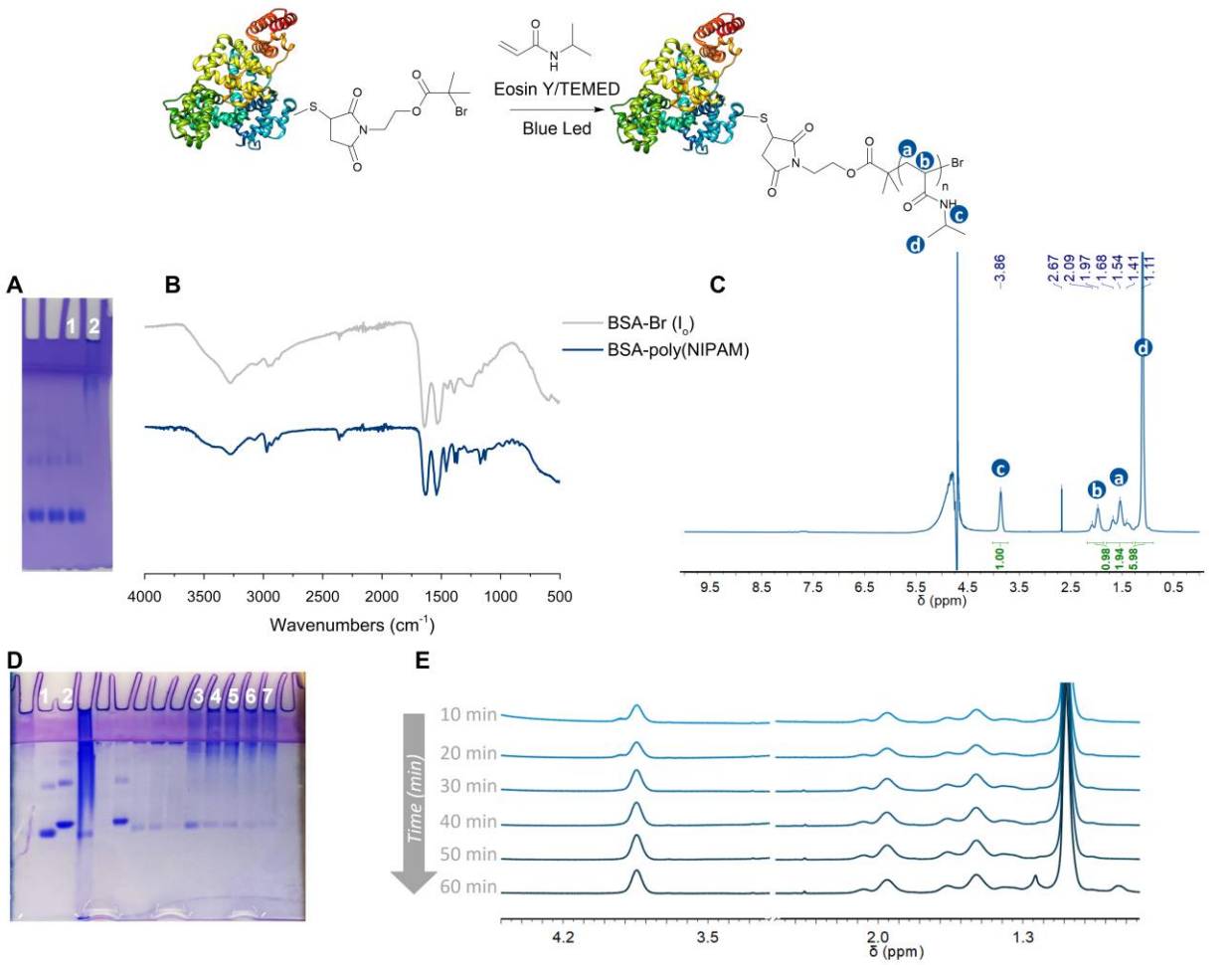


Figure S 19. Synthesis of BSA-poly(NIPAM). ***A.*** *Native PAGE, lane 1: BSA-Br (I_o), lane 2: BSA-poly(NIPAM).* ***B.*** *FT-IR spectra of BSA-Br (I_o) and BSA-poly(NIPAM). In the biohybrid spectrum, the peak at 1645.5 cm^{-1} can be attributed to the stretching of the C=O ester bond of the amides, the peak at 1547.1 cm^{-1} can be attributed to the bending of N-H bond of amides, and at 1308.7 cm^{-1} can be attributed to the stretching of C-N bond of poly(NIPAM). In the same spectrum the amide I and II vibrations of the protein moiety are at 1653.7 and 1540.8 cm^{-1}.* ***C.*** *^{1}H-NMR spectrum of BSA-poly(NIPAM) in D_2O acquired with water suppression.* ***D.*** *Native PAGE of the time course study of the synthesis of BSA-poly(NIPAM), lane 1: BSA-Br (I_o), lane 2: native BSA, lanes 3-7: samples withdrawn from the reaction performed using feed molar ratio NIPAM/BSA-Br, (I_o)/EY/TEMED = 2000/1/0.2/10 after 5, 10, 15, 30 and 60 min of irradiation time respectively.* ***E.*** *^{1}H-NMR spectra from samples withdrawn from the reaction performed using feed molar ratio NIPAM/BSA-Br, (I_o)/EY/TEMED = 1000/1/0.2/10 after 10, 20, 30, 40, 50 and 60 minutes of irradiation time respectively.*

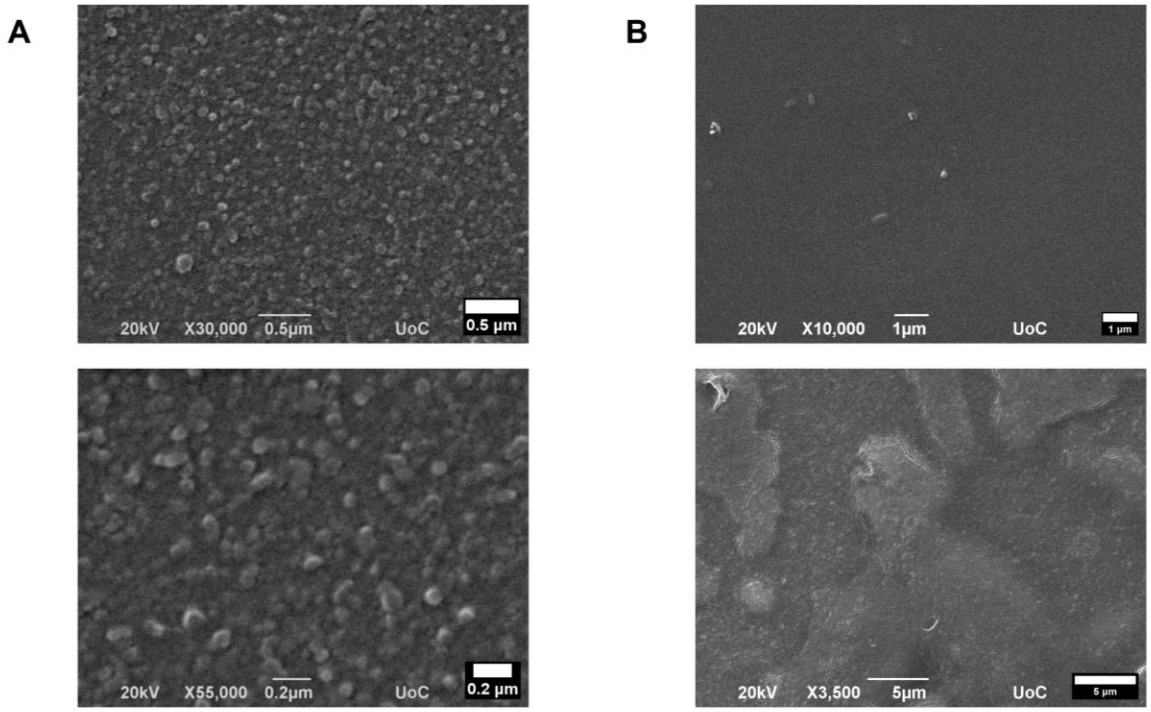


Figure S 20. ***A.*** *SEM micrographs of BSA-poly(NIPAM) -sample prepared and dried at 35 °C.* ***B.*** *SEM micrographs of BSA-poly(NIPAM) -sample prepared and dried at 8 °C. The sample was prepared in the same (top) and 100 times higher (bottom) concentration as compared to the concentration used at 35 °C.*

x. BSA-poly(DPA)

BSA-poly(2-(diisopropylamino)ethyl methacrylate) (BSA-poly(DPA)) was synthesized using the general EY/TEMED mediated photopolymerization protocol.

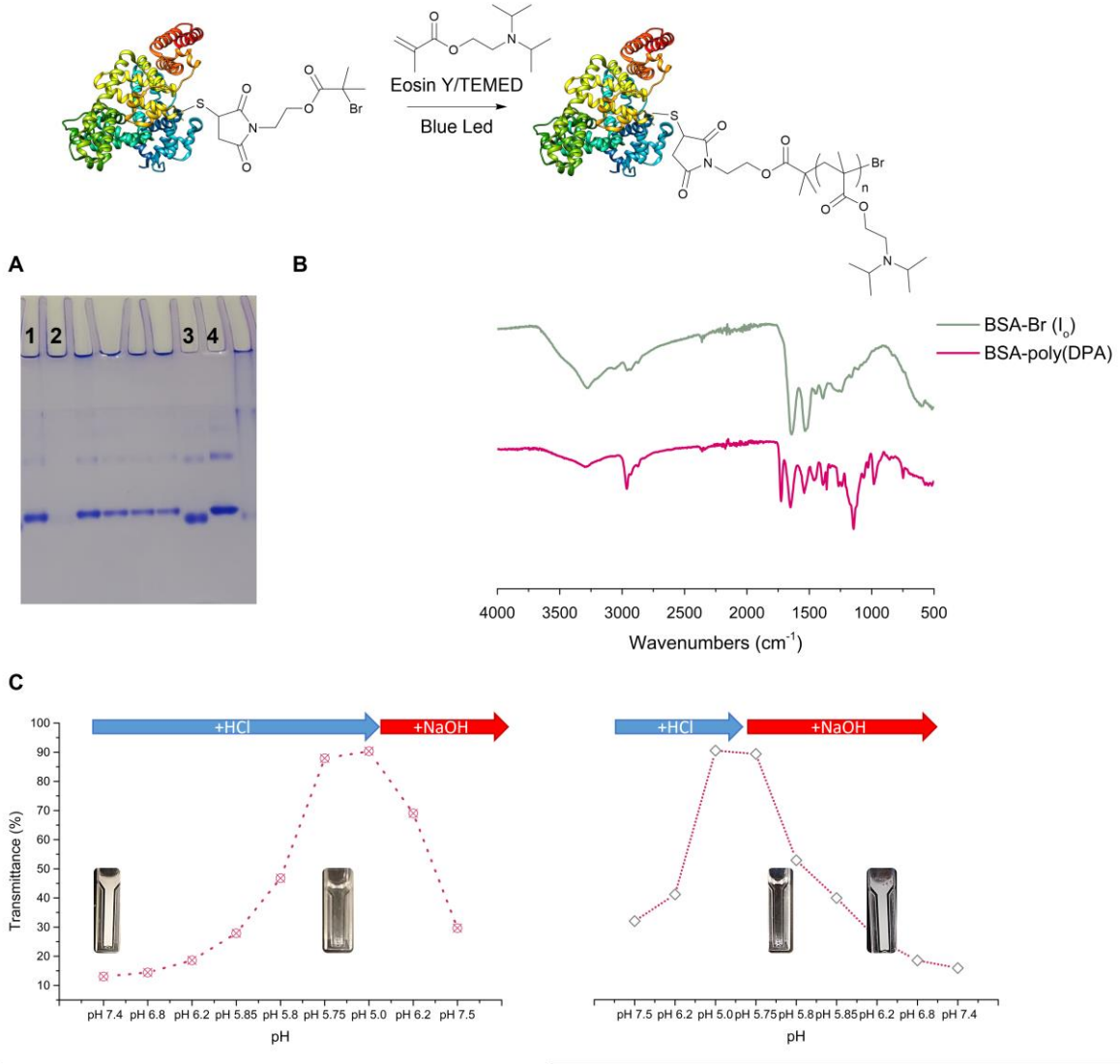


Figure S 21. Synthesis and characterization of BSA-poly(DPA). ***A.*** *Native PAGE, lane 2: BSA-poly(DPA) synthesized with feed molar ratio DPA/BSA-Br, I_o/EY/TEMED = 4000/1/1/10 (1 hour irradiation), lane 3: BSA-Br (I_o), lane 4: native BSA.* ***B.*** *FT-IR spectra of BSA-Br (I_o) and BSA-poly(DPA). In the biohybrid spectrum, the peak at 1645.5 cm^{-1} can be attributed to the stretching of the C=O ester bond of the amides, the peak at 1729.1 cm^{-1} that can be attributed to the stretching of the C=O ester bond, the peak at 1168.2 cm^{-1} can be attributed to the bending of N-H bond of tertiary amines of poly(DPA). In the same spectrum the amide I and II vibrations of the protein moiety are at 1653.7 and 1543.1 cm^{-1}.* ***C.*** *Transmittance vs. pH curve showing two cycles of the reversible, rapid response of BSA-poly(DPA).*

Taking advantage of the responsiveness of BSA-poly(DPA) to pH changes, the grafting of DPA was studied in more detail by characterizing the products of the EY/TEMED catalyzed reaction in the presence of biomacroinitiator and in the presence of native BSA. After isolation the products were dialyzed against acidic buffer (20 mM phosphate, pH 4.8) and the parent solution was characterized with native PAGE while the dialysate was characterized with ^{1}H-NMR spectroscopy.

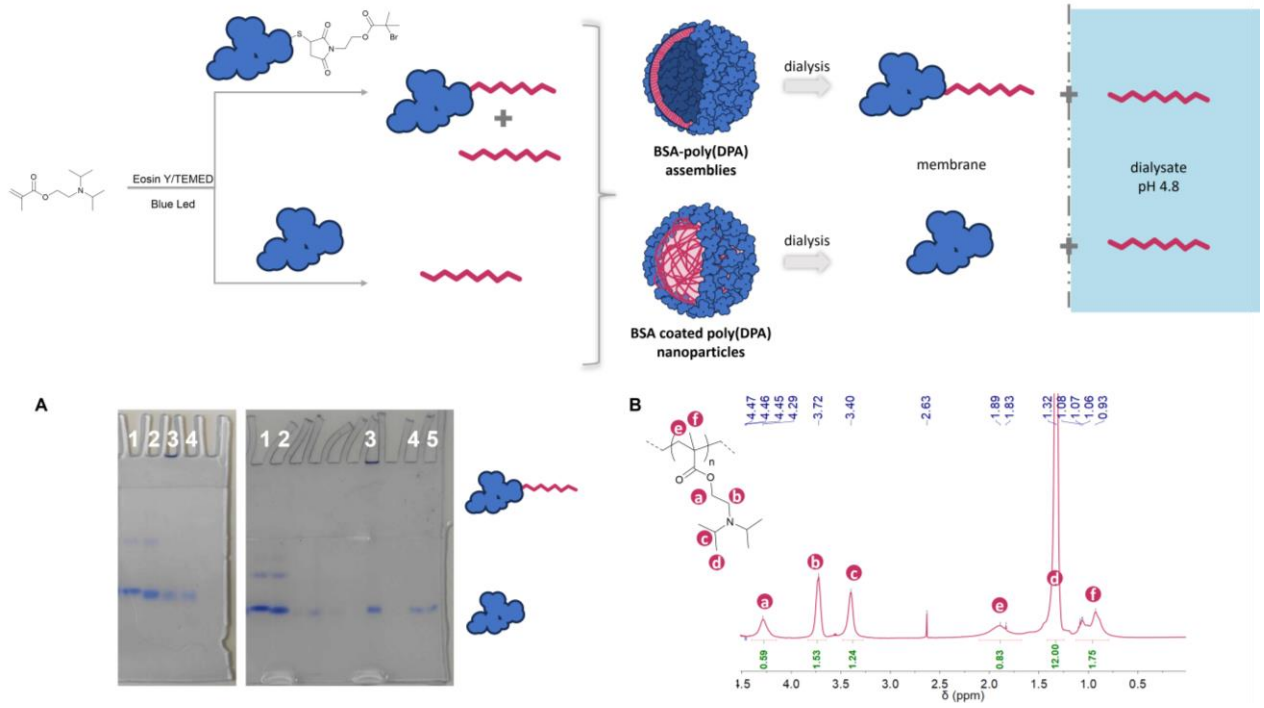


Figure S 22. Proposed pathways for the production of BSA-poly(DPA) and BSA-coated poly(DPA) nanoparticles. A. Native PAGE. Left, lane 1: native BSA, lane 2: BSA-Br (I_o), lane 3: BSA-poly(DPA) formed at DPA/BSA-Br, I_o/EY/TEMED = 4000/1/1/10, lane 4: BSA-poly(DPA) formed at DPA/BSA-Br, (I_o)/EY/TEMED = 4000/1/1/10 (dialysis at pH 7.4). Right, lane 1: native BSA, lane 2: BSA-Br (I_o), lane 3: BSA-coated poly(DPA) nanoparticles formed at DPA/EY/TEMED = 4000/1/10 in the presence of 1 equivalent of native BSA (dialysis at pH 7.4), lanes 4 and 5: BSA-coated poly(DPA) nanoparticles formed at DPA/EY/TEMED = 4000/1/1/10 in the presence of 1 equivalent of native BSA (dialysis at pH 4.8). B. ^{1}H-NMR spectrum of poly(DPA) isolated from the acidic (pH 4.8) dialysate of the reaction performed at DPA/EY/TEMED = 4000/1/10 in the presence of 1 equivalent of native BSA.

xi. BSA- poly(DMAEMA)

BSA-poly(2-(dimethylamino)ethyl methacrylate) (BSA-poly(DMAEMA)) was synthesized using molar loading DMAEMA/BSA-Br, I_o/EY/TEMED = 4000/1/0.2/10 upon blue LED irradiation for 1 hour. When the same reaction was performed without initiator, but in the presence of native BSA, coated nanoparticles and unbound protein were visualized in native PAGE.

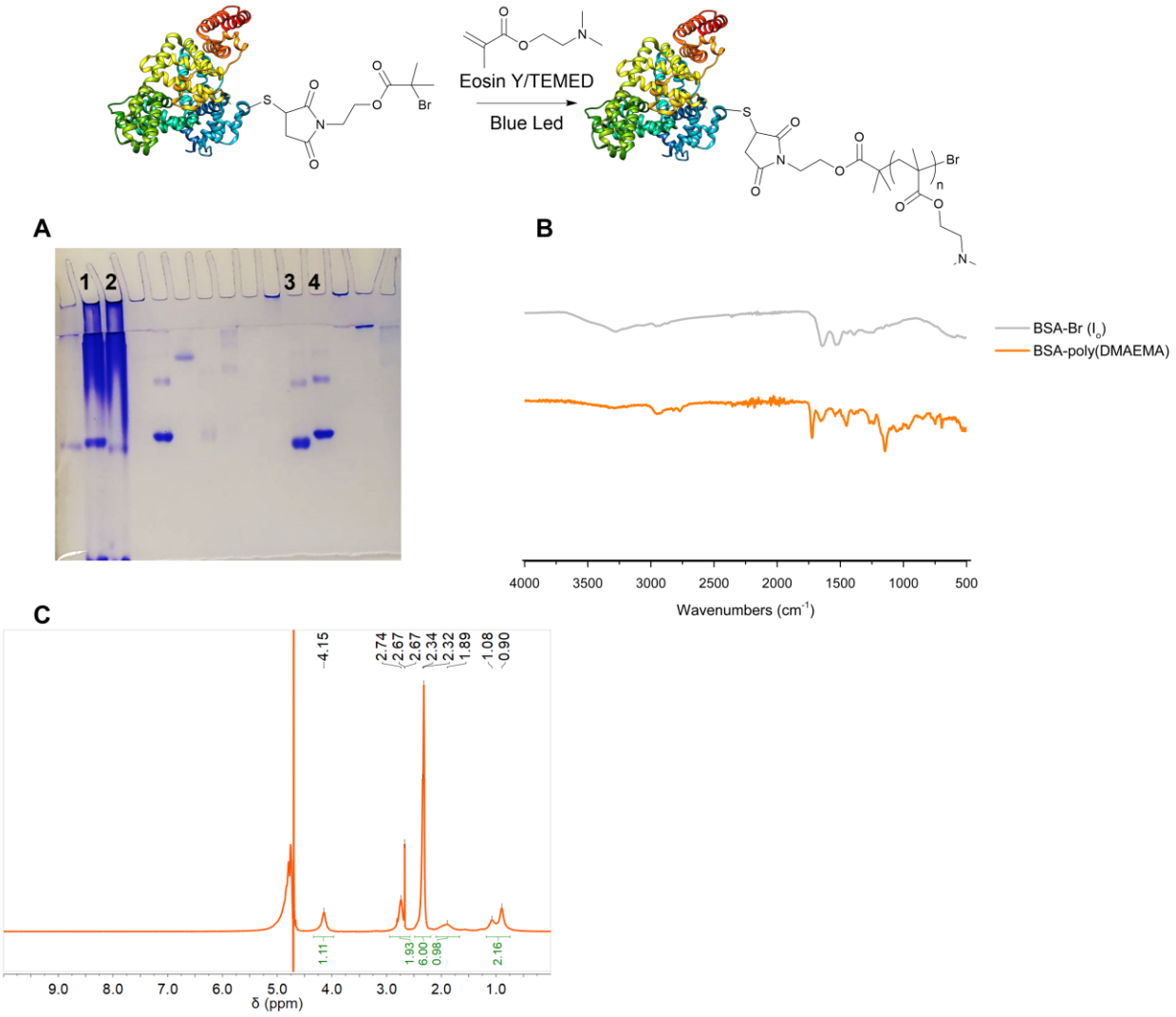


Figure S 23. Synthesis of BSA-poly(DMAEMA) and BSA-coated poly(DMAEMA) nanoparticles. ***A.*** *Native PAGE, lane 1: BSA-coated poly(DMAEMA) nanoparticles formed using DMAEMA/EY/TEMED = 4000/0.2/10 in the presence of 1 equiv. native BSA, lane 2: BSA-poly(DMAEMA) formed using feed molar ratio DMAEMA/BSA-Br, I_o/EY/TEMED = 4000/1/0.2/10, lane 3: BSA-Br (I_o), lane 4: native BSA.* ***B.*** *FT-IR spectra of BSA-Br (I_o) and BSA-poly(DMAEMA). In the biohybrid spectrum, the peak at 1720.1 cm^{-1} can be attributed to the stretching of the C=O bond of esters, and at 1156.1 cm^{-1} to the bending of N-H bond of tertiary amines of poly(DMAEMA). In the same spectrum the amide I and II vibrations of the protein moiety are at 1655.5 and 1540.7 cm^{-1}.* ***C.*** *^{1}H-NMR spectrum of BSA-poly(DMAEMA) in D_2O acquired with water suppression.*

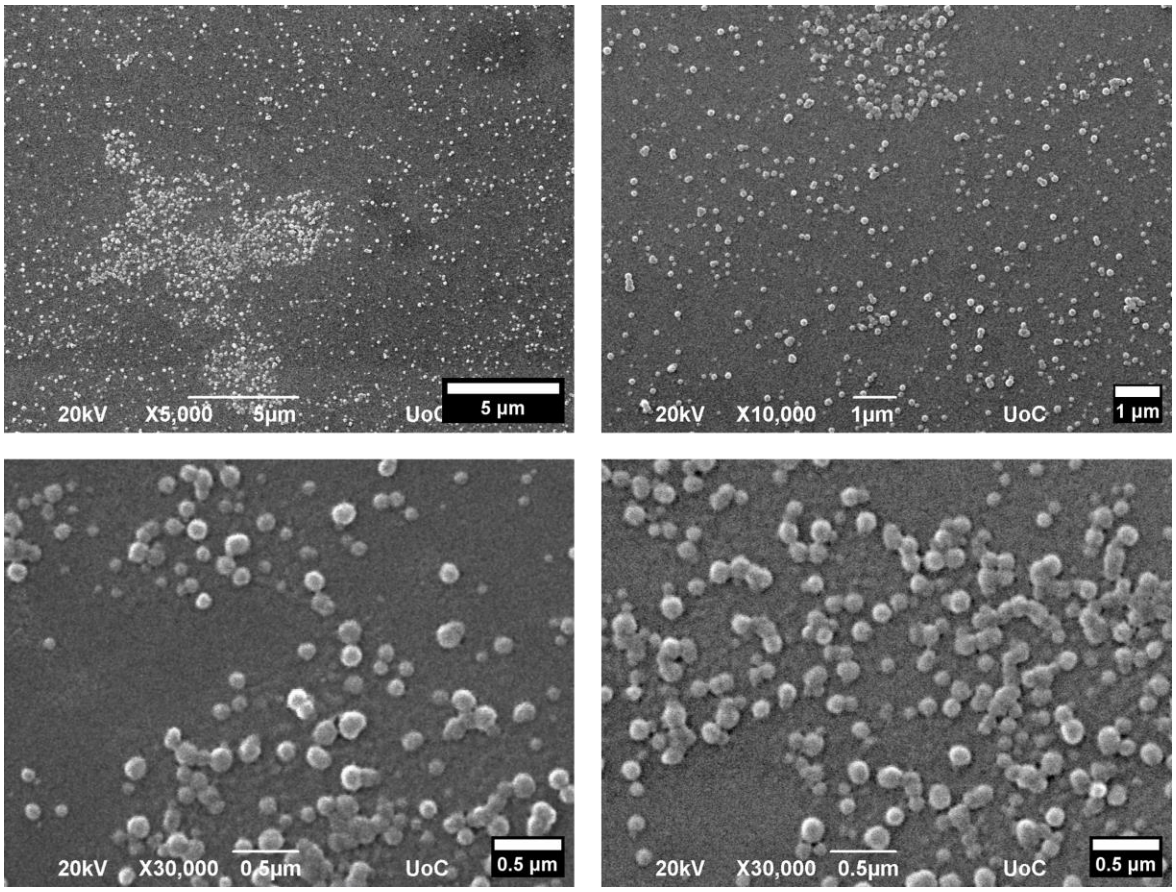


Figure S 24. SEM micrographs of BSA-poly(DMAEMA). Samples were prepared and dried in R.T.

Grafting multiple monomers

i. BSA-poly(NIPAM-co-DPA)

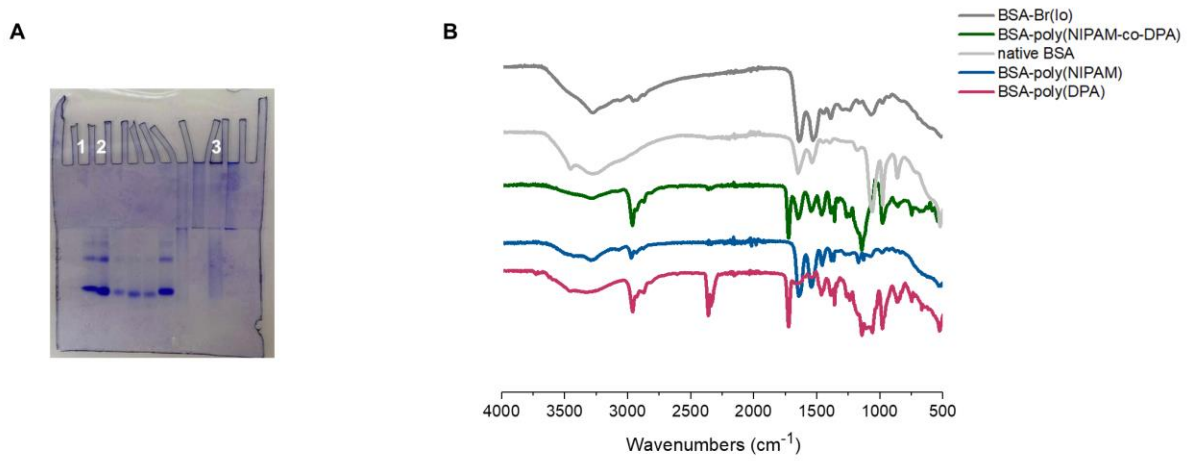


Figure S 25. Characterization of BSA-poly(NIPAM-co-DPA). ***A.*** *Native PAGE, lane 1: native BSA, lane 2: BSA-Br (I_o), lane 3: BSA-poly(NIPAM-co-DPA) formed using feed molar ratio NIPAM/DPA/BSA-Br, I_o/EY/TEMED = 4000/500/1/1/10.* ***B.*** *In the biohybrid spectrum, the peak at 1721.3 cm^{-1} can be attributed to the stretching of the C=O bond of esters, the peak at 1141.5 cm^{-1} can be attributed to the bending of N-H bond of tertiary amines of poly(DPA). At C-N group bending vibration at ca. 1170 cm^{-1} of poly(NIPAM). In the same spectrum the amide I and II vibrations of the protein moiety are at 1648.7 and 1546.1 cm^{-1}.*

ii. BSA-poly(NIPAM)-b-poly(styrene)

First, the conditions leading to quantitative grafting of 100 equiv. of NIPAM from BSA-Br (I_o) were determined (NIPAM/BSA-Br/EY/TEMED=100/1/0.2/10, 1 hour, Figure S 26). Upon determining that NIPAM was fully consumed within an hour (see also NMRs establishing fast NIPAM consumption in Figure S 19), an emulsion of the second monomer -styrene- was added (4000 equiv.) and the reaction was allowed to proceed for another 2 hours.

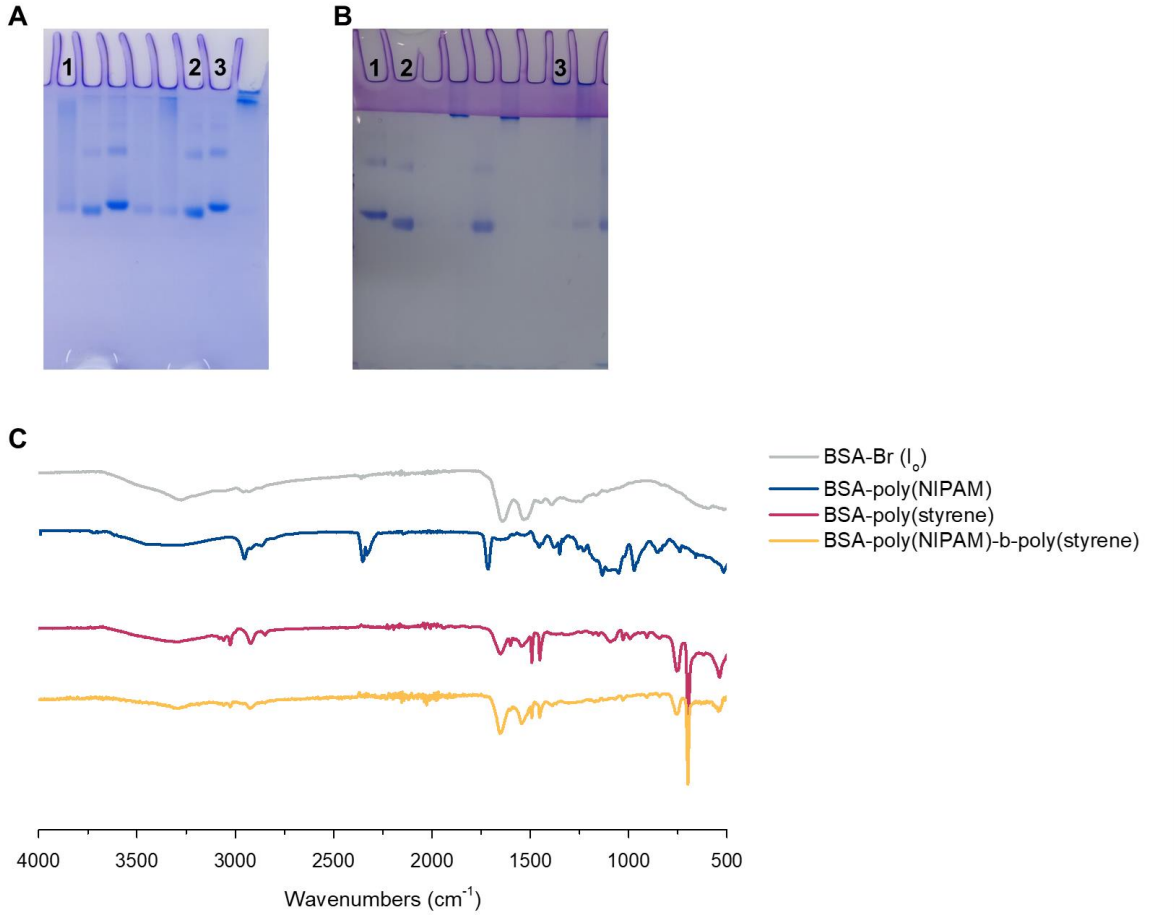


Figure S 26. Synthesis of BSA-poly(NIPAM)-b-poly(styrene). ***A.*** *Native PAGE, lane 1: BSA-poly(NIPAM), lane 2: BSA-Br (I_o), lane 3: native BSA.* ***B.*** *Native PAGE, lane 1: native BSA, lane 2: BSA-Br (I_o), lane 3: BSA-poly(NIPAM)-b-poly(styrene).* ***C****. FT-IR spectra of BSA-Br (I_o) and BSA-poly(NIPAM)-b-poly(styrene). In the biohybrid spectrum, the peak at 696.1 cm^{-1} is attributed to the C-H bending of the aromatic ring of poly(styrene). In the same spectrum the amide I and II vibrations of the protein moiety are at 1651.3 and 1541.7 cm^{-1}.*

iii. BSA-poly(NIPAM)-b-poly(VP)

Following the same protocol as for BSA-poly(NIPAM)-b-poly(styrene), 5000 equiv. of VP were used.

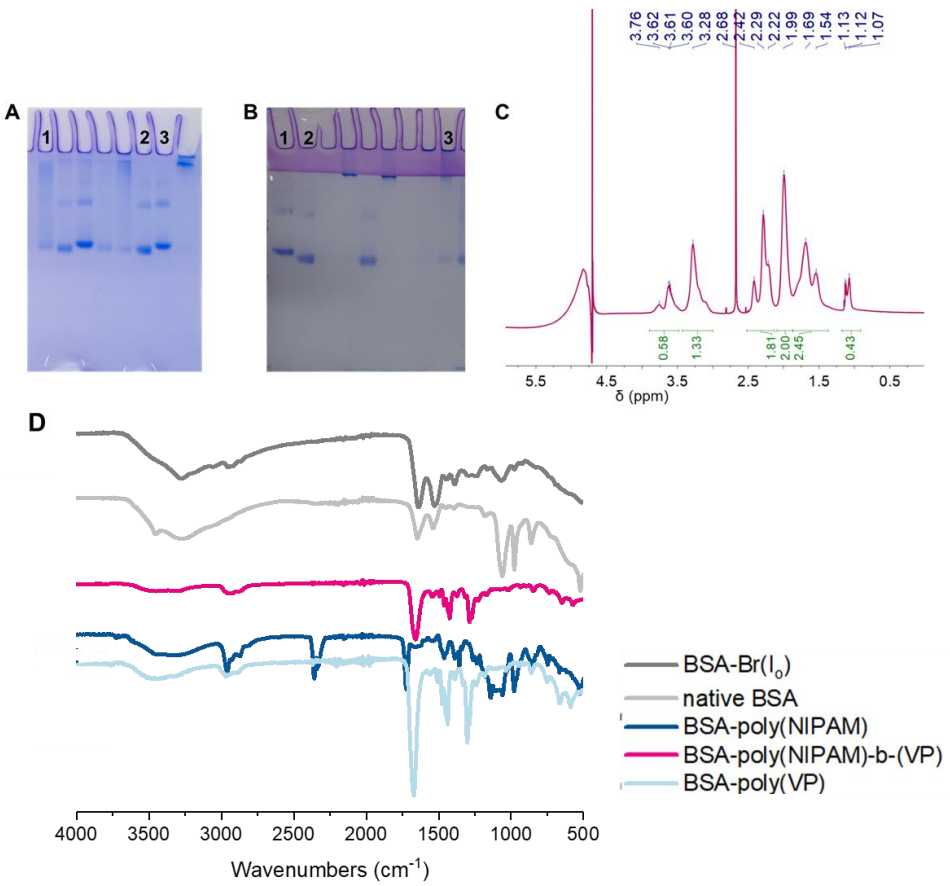


Figure S 27. Synthesis of BSA-poly(NIPAM)-b-poly(VP). **A.** Native PAGE, lane 1: BSA-poly(NIPAM), lane 2: BSA-Br (I_o), lane 3: native BSA. **B.** Native PAGE, lane 1: native BSA, lane 2: BSA-Br (I_o), lane 3: BSA-poly(NIPAM)-b-poly(VP). **C**. ^{1}H-NMR spectrum of BSA-poly(NIPAM)-b-poly(VP) in D_2O acquired with water suppression. **D.** FT-IR spectra of BSA-Br (I_o) and BSA-poly(NIPAM)-b-poly(VP). In the biohybrid spectrum the peak at 1432.4 cm^{-1} can be attributed to stretching vibrations of the C-N bonds and the peak at 1288.1 cm^{-1} can be attributed to the C-N-C vibration of poly(VP). Also, the characteristic stretching vibration of C-N at ca. 1371.24 cm^{-1} and the C-N group bending vibration at ca. 1170.2 cm^{-1} of poly(NIPAM) coexist in the spectrum with the amide I at 1661.3 cm^{-1} and, the amide II at 1544.8 cm^{-1} vibrations of BSA.

BSA-coated poly(styrene) nanoparticles

Synthesis

The reaction was performed in the absence of the ATRP initiator (BSA-Br). Native BSA was added following an otherwise identical experimental procedure. More specifically:

A solution of EY was prepared by dissolving 1 mg EY (1.54 μmol) in 1 mL, 20 mM phosphate buffer pH 7.4 with the aid of sonication.

A 0.21 M TEMED stock solution was prepared by dissolving 3.3 µL (22 µmol) of TEMED in 100 µL nanopure water.

141.5 µL (0.218 µmol) of the stock solution of EY and the appropriate volume of the TEMED stock solution were dissolved in nanopure water to afford a solution with fixed total volume (460 µL). The emulsion of the monomer was formed by adding the hydrophobic monomer styrene (872 µmol, 4000 equiv.) and sonicating for ca. 5 minutes. For different feed molar ratios (not included here), the volume of the water was adjusted to retain a stable volume. The resulting emulsion was immediately transferred to a 6 mL polypropylene syringe equipped with a stirring bar, containing 0.625 mL of a 0.35 mM solution of native BSA in 20 mM phosphate buffer, pH 7.4 (0.218 µmol, 1 equiv.). Headspace was eliminated to avoid the presence of undissolved oxygen and the reaction syringe was capped and placed under blue LED irradiation for 2 hours with moderate stirring. A ventilator was used to avoid temperature increase maintaining the temperature between 25 and 32 °C. The reaction mixture was then dialyzed using a 10 kDa MWCO regenerated cellulose dialysis membrane initially against 5 mM phosphate buffer, pH 7.4, 1 % DMSO, then against 5 mM phosphate buffer, pH 7.4, and finally against 20 mM phosphate buffer, pH 7.4. The product solutions were analyzed by means of native or SDS PAGE electrophoresis, SEC, and FT-IR spectroscopy. ^{1}H-NMR spectra were acquired for hydrophilic products. Dilute suspensions of the products in nanopure water were imaged with SEM or FE-SEM. All products were stored at 7 °C until further use.

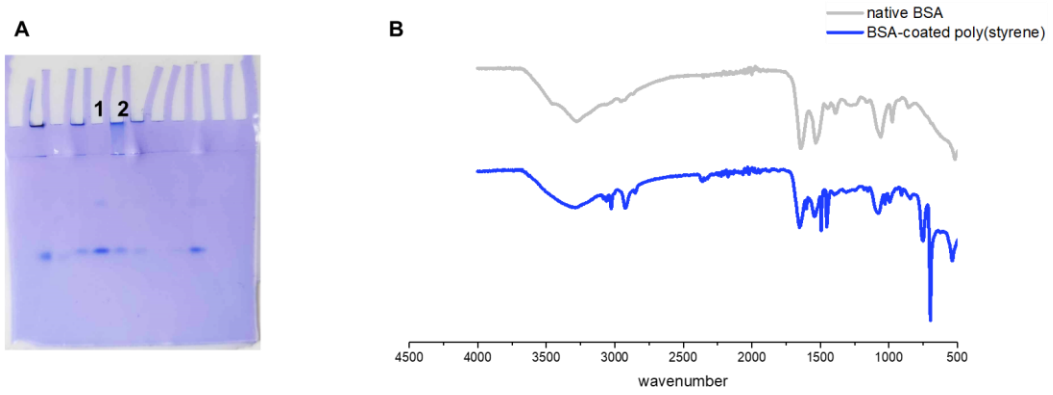


Figure S 28. Preparation of BSA-coated poly(styrene) nanoparticles. ***A.*** *Native PAGE, lane 1: native BSA lane 2: BSA-coated poly(styrene) nanoparticles.* ***B.*** *FT-IR spectra of native BSA-Br and BSA-coated poly(styrene) nanoparticles. In the coated nanoparticles spectrum, the peak at 698.1 cm^{-1} can be attributed to the C-H bending of the aromatic ring of poly(styrene) together with the characteristic vibrations of amide I and II at 1652.7 and 1546.3 cm^{-1} of BSA.*

TIRF microscopy of BSA-coated poly(styrene) nanoparticles.

Since EY has a strong binding affinity with proteins, the BSA-coated polymer nanoparticles could directly be visualized with total internal reflection fluorescence (TIRF) microscopy and detected in the green channel.

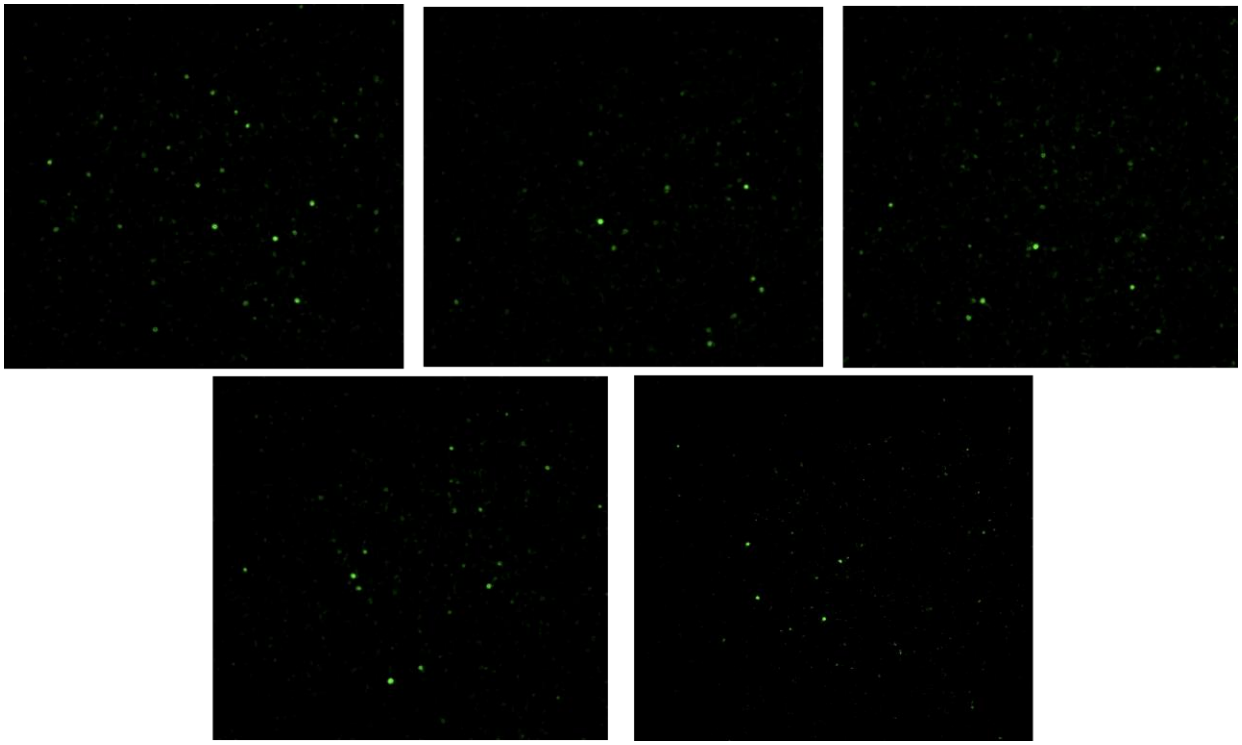


Figure S 29. Imaging of BSA-coated polymer nanoparticles with internal reflection fluorescence (TIRF) microscopy.

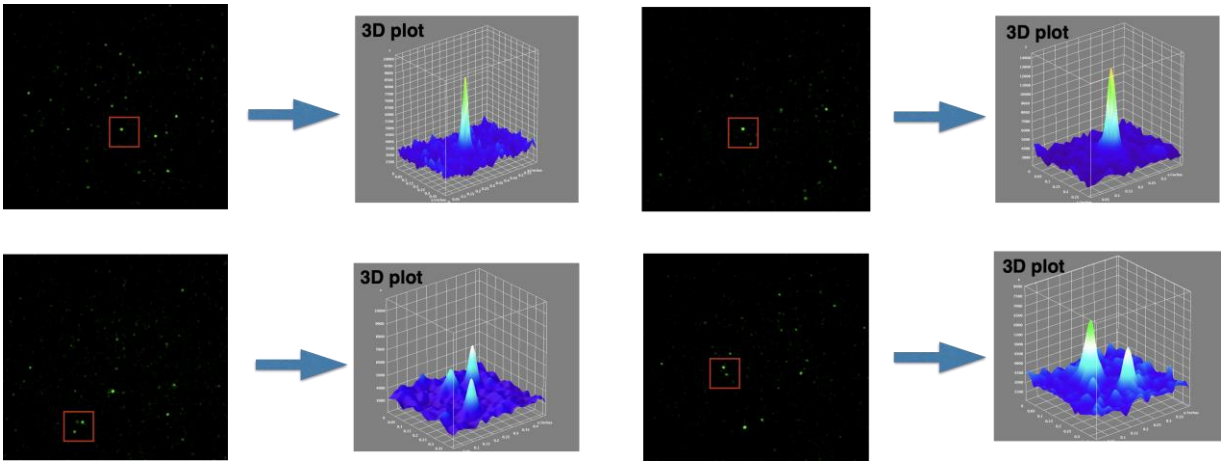
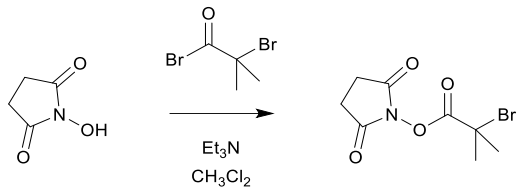


Figure S 30. 3D intensity plot of specific BSA-coated polymer nanoparticles.

Synthesis of lipase B from *Candida antarctica* macroinitiator (CALB-Br, I_o)

A. Synthesis of N-hydroxysuccinimide-2-bromo-2-methylpropionate[7]

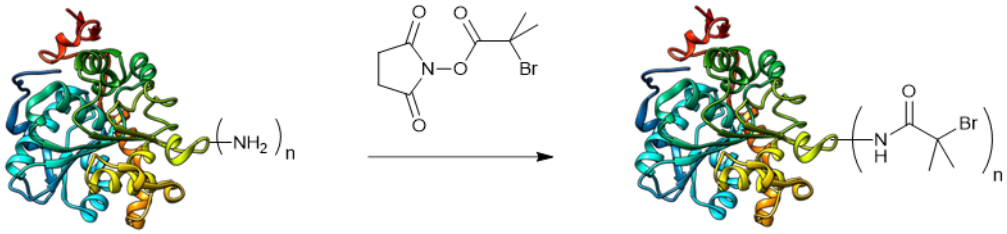


N-Hydroxysuccinimide (292 mg, 2.53 mmol) and triethylamine (0.7 mL, 5.02 mmol) were dissolved in 50 mL dichloromethane in a round-bottomed flask equipped with a magnetic stirrer under nitrogen atmosphere. The flask was cooled to 0 °C and 2-bromo-2-methylpropionyl bromide (0.34 mL, 2.75 mmol) was added dropwise. The mixture was stirred for 1 hour at 0 °C and then allowed to reach room temperature and stirred at room temperature for about 2 hours. The reaction mixture was then poured into an excess of cold water (74 mL) and extracted with diethyl ether (3×5 mL). The organic layer was washed with a saturated aqueous solution of sodium carbonate (3×5 mL), diluted HCl aqueous solution (pH 4.5, 3×5 mL), and again with saturated aqueous solution of sodium carbonate (3×5 mL). The combined organic layer was dried over anhydrous magnesium sulfate, filtered and the solvent was removed under reduced pressure to afford a white solid (237 mg, 0.89 mmol, 35.5 %).

^{1}H NMR ($CDCl_3$, 500 MHz), δ (ppm): 2.11 (s, 6H, $C(CH_3)_2Br$), 2.88 (s, 4H, *succ*), ^{13}C NMR ($CDCl_3$, 125 MHz), δ (ppm): 25.60 (2C, C_{succ}), 30.71 (2C, $C(CH_3)_2Br$), 51.11 (1C, $C(CH_3)_2Br$), 167.47 (1C, C=O), 168.51 (2C, C_{succ}=O).

B. Synthesis of lipase B from *Candida antarctica* macroinitiator (CALB-Br, I_o)

The ATRP initiator was non-specifically conjugated to the exposed primary amines of CALB via NHS-ester coupling.



More specifically, 1.68 mg of *N*-hydroxysuccinimide-2-bromo-2-methylpropionate (6.39×10^{-3} mmol, 20 equiv.) were dissolved in 45 µL DMSO and this solution was slowly added to a 900 µL of a 0.35 mM solution of CALB (0.139×10^{-3} mmol, 1 equiv.) in 100 mM sodium phosphate buffer, pH

7.4. The reaction mixture was gently shaken for 2 hours at 7 °C. The CALB-Br (I_o) initiator was enriched by dialysis using a 6-8 kDa MWCO regenerated cellulose membrane initially against 5 mM phosphate buffer, pH 7.4, 1 % DMSO, then against 5 mM phosphate buffer, pH 7.4, and finally against 20 mM phosphate buffer, pH 7.4. The solution of the enriched CALB-Br was subsequently characterized with FT-IR and native PAGE electrophoresis and stored at 7 °C until further use.

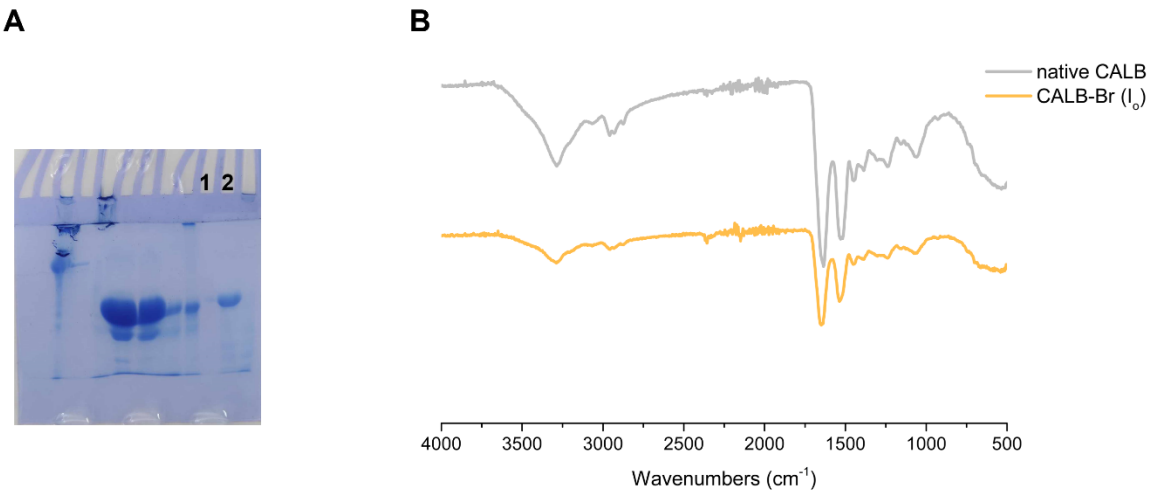
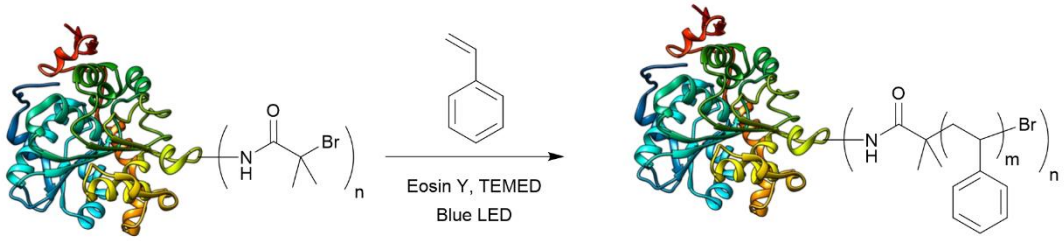


*Figure S 31. **A**. SDS-PAGE, lane 1: native CALB, lane 2: CALB-Br (I_o). **B.** FT-IR spectra of native CALB and CALB-Br (I_o). The characteristic vibrations of amide I and II of CALB at 1652.5 cm^{-1} and 1544.7 cm^{-1} can be observed in the FT-IR spectrum of CALB-Br (I_o).*

EY/TEMED mediated photoinduced synthesis of CALB-poly(styrene)



The synthesis was performed under the optimal conditions established for BSA.

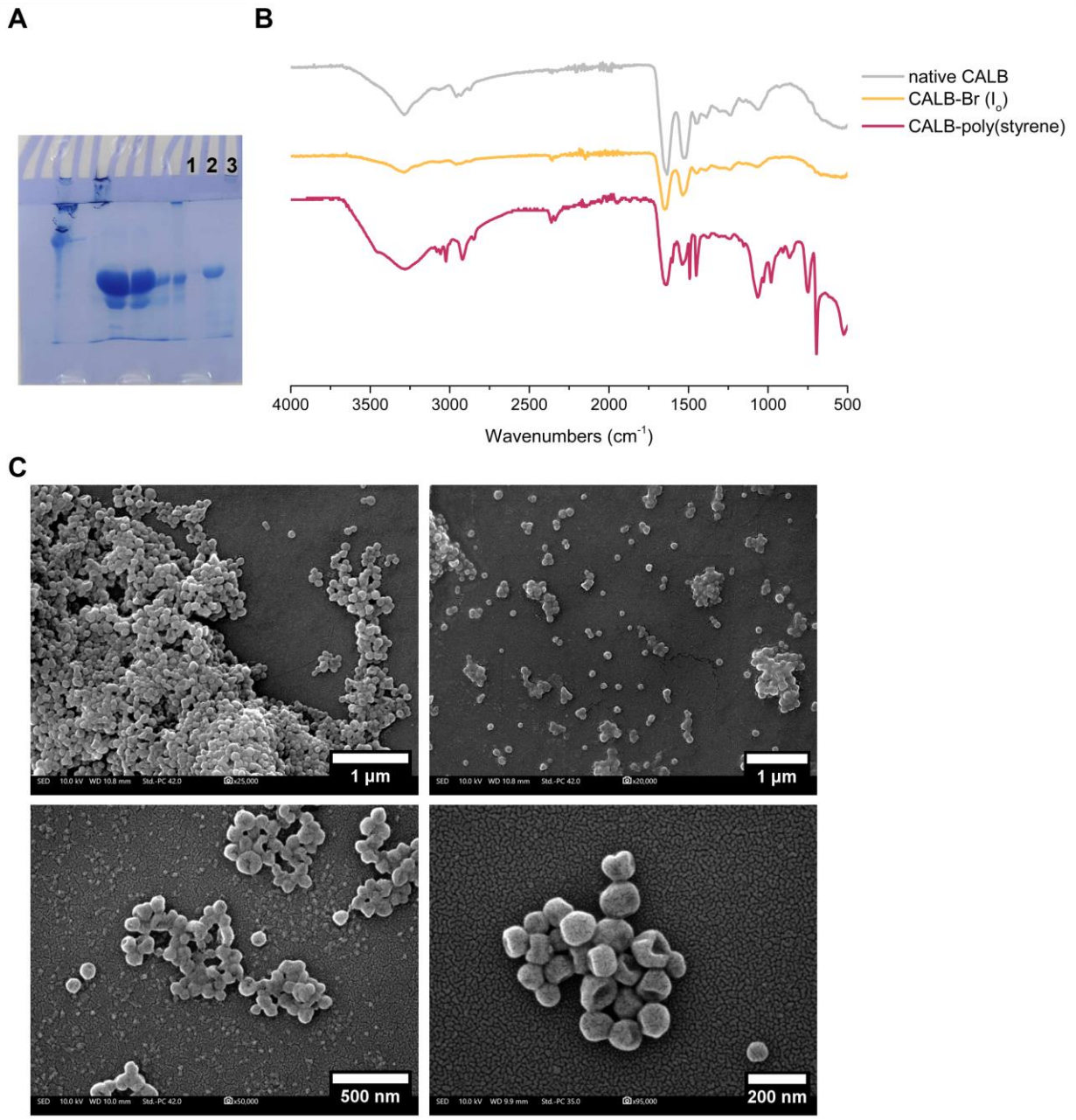


Figure S 32. ***A****. SDS-PAGE, lane 1: native CALB, lane 2: CALB-Br (I_o), lane 2: CALB-poly(styrene).* ***B.*** *FT-IR spectra of native CALB and CALB-Br (I_o), CALB-poly(styrene). In the FT-IR spectrum, the C-H bending of the aromatic ring of poly(styrene) is at 696.7 cm^{-1}. In the same spectrum, the amide I and II vibrations of the protein moiety at 1652.5 and 1544.7 cm^{-1} can be observed* ***C.*** *FE-SEM micrographs of CALB-poly(styrene). Samples were prepared and dried at R.T.*

CALB-coated poly(styrene) nanoparticles

The preparation of CALB-coated poly(styrene) nanoparticles was performed following the protocol established for BSA.

The activity and hydrodynamic diameter of the nanoparticles were measured in fresh samples and samples stored at 4 °C for a period longer than a year.

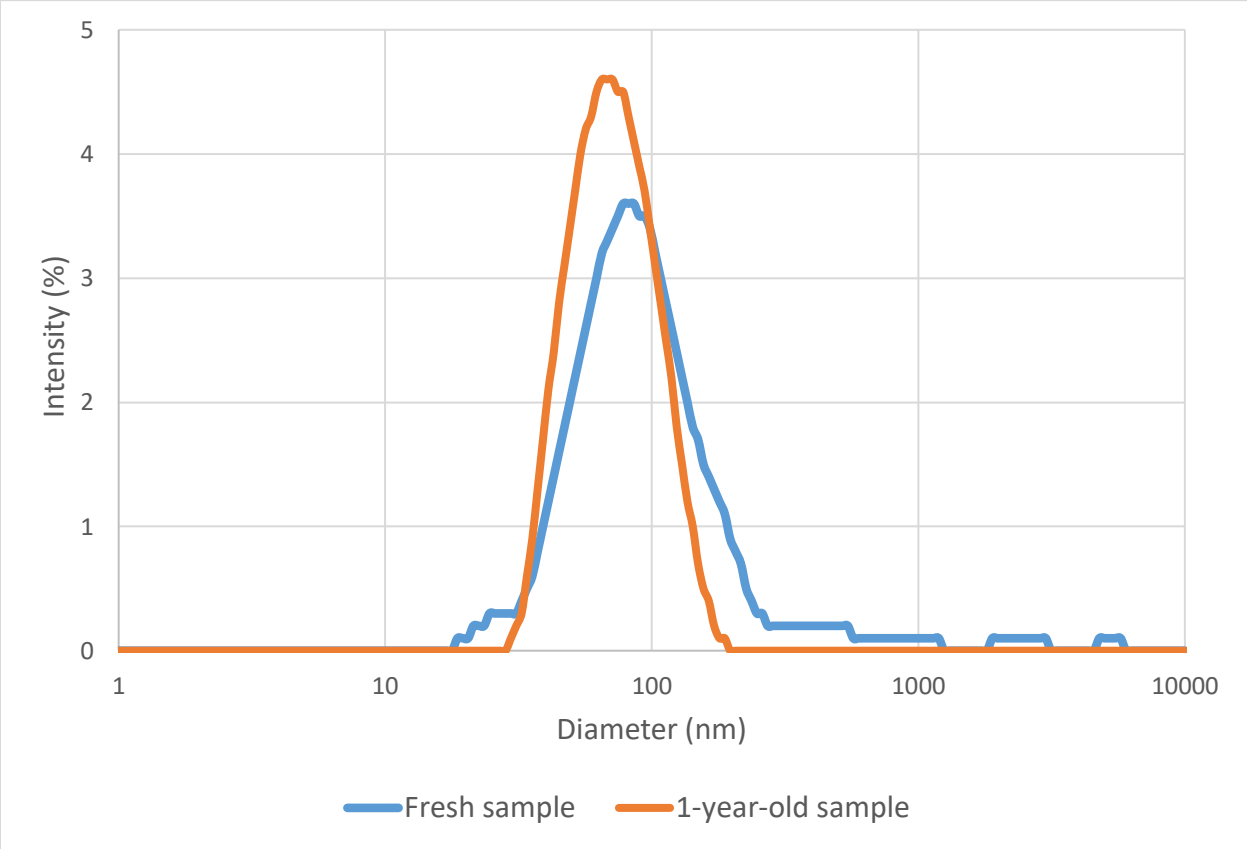


Figure S 33. Hydrodynamic diameter of CALB coated poly(styrene) nanoparticles. Fresh nanoparticles (blue trace) displayed average diameter: 83.55 +/- 47.98 nm (PDI: 0.330) while nanparticles stored at 4 °C for a period longer than a year displayed average diameter: 68.88 +/- 32.14 nm (PDI: 0.160).

Catalytic activity

Native CALB

7.89 µL of a 0.325 mg/mL solution of native CALB were diluted with 1005.6 µL 50 mM phosphate buffer, pH 7.4 to form a 2.5 µM CALB solution. The reaction was initiated by the addition of 12.5 µL of a 5(6)-carboxyfluorescein diacetate (CFDA) solution (4 mg/mL CFDA in DMSO) and the activity of CALB-poly(styrene) was monitored by UV-Vis through the absorption of the product

5(6)-carboxyfluorescein at 453 nm at 20 °C, 25 °C and 37 °C. All reactions were performed in triplicates.

CALB-poly(styrene)

16.65 µL of a 0.154 mM solution of CALB-poly(styrene) were suspended in 996.85 µL 50 mM phosphate buffer, pH 7.4 to form a 2.5 µM solution in terms of total lipase concentration. The reaction was initiated by the addition of 12.5 µL of a 5(6)-carboxyfluorescein diacetate (CFDA) solution (4 mg/mL CFDA in DMSO) and the activity of CALB-poly(styrene) was monitored by UV-Vis through the absorption of the product 5(6)-carboxyfluorescein at 453 nm at 20 °C, 25 °C and 37 °C. All reactions were performed in triplicates.

CALB-coated poly(styrene) nanoparticles

13.86 µL of 0.192 mM solution of CALB coated poly(styrene) nanoparticles were suspended in 999.6 µL 50 mM phosphate buffer, pH 7.4 to form a 2.5 µM solution in terms of total lipase concentration. The reaction was initiated by the addition of 12.5 µL of a 5(6)-carboxyfluorescein diacetate (CFDA) solution (4 mg/mL CFDA in DMSO) and the activity of CALB-poly(styrene) was monitored by UV-Vis through the absorption of the product 5(6)-carboxyfluorescein at 453 nm at 20 °C, 25 °C and 37 °C. All reactions were performed in triplicates.

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