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Supporting Information

for *Macromol. Rapid Commun.*, DOI: 10.1002/marc. 201300460

Sortase-Catalyzed Initiator Attachment Enables High Yield Growth of a Stealth Polymer from the C Terminus of a Protein

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Supporting Information

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Sortase-Catalyzed Initiator Attachment Enables High Yield in situ Growth of a Polyethylene Glycol Comb Polymer from the C Terminus of Green Fluorescent Protein by Atom Transfer Radical Polymerization

Yizhi Qi, Miriam Amiram, Weiping Gao, Dewey G. McCafferty, Ashutosh Chilkoti*

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1. Materials

All molecular biology reagents were purchased from New England Biolabs, unless otherwise specified. All chemical reagents were purchased from Sigma Aldrich and used as received, unless otherwise specified.

2. Molecular Biology Details

All nucleotides are denoted by their single letter code and all amino acids are denoted by their three letter abbreviations.

2.1 GFP-srt-ELP Cloning, Expression and Purification

The gene for GFP was PCR-amplified from an available GFP-containing pET32b(+) vector from a previous study[1] using the forward and reverse primers:

GFP-F: 5' TTCCCCTCTAGAAATAATTTTGT 3'

GFP-R: 3'

CTACTTGACATGTTGCAGCTGCCGCCACCCCCGTCGAACGGCCTTTGGCCGCC

ATTCGAAACGAAC 5'

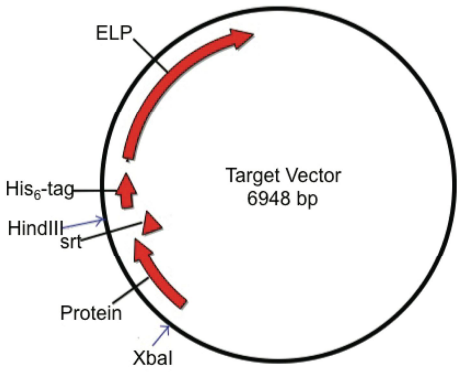


Figure S1. Vector map of target vector. Figure not drawn to scale.

The GFP-F primer was designed to anneal at the RBS site immediately upstream of the target GFP sequence and includes an XbaI site for cloning into the target vector

(**Figure S1**). The GFP-R primer was designed to anneal to the *C* terminus of GFP and includes an overhang that codes for a Gly_4Ser linker and the 'LPETG' SrtA recognition sequence as well as a HindIII site for cloning into the target vector.

The GFP-srt fragment was amplified in two 50 µl PCR reactions, each containing 25 µl GoTaq green master mix, 10 pmol each of forward and reverse primers, 0.25 µl template and nuclease-free water in a total volume of 50 µl. The PCR reaction conditions were: 95 °C for 2 min for initial denaturation, followed by 40 cycles at 95 °C for 30 s, 52 °C for 30 s, and 72 °C for 1 min. The resulting 'GFP-srt' PCR product was purified using a PCR purification kit and visualized on a 1% agarose gel stained with SYBR® Safe DNA stain. 1.5 µg of the GFP product was then digested with 2 µl each of XbaI and HindIII in 1X NEB buffer 2 and 1X bovine serum albumin (BSA) for 1.5 h at 37 °C and then purified using a PCR purification kit (QIAquick, QIAGEN).

A previously constructed pET25b(+) vector encoding a protein-srt-His_6-ELP fusion gene was used as the target vector. In this vector, the protein-srt insert was flanked by XbaI and HindIII restriction sites followed by codons that encode a His_6-tag, a thrombin cleavage site and an ELP with a sequence of $(VPGXG)_{90}$, where X represents alanine (A), glycine (G) and valine (V) at 2:3:5 molar ratio. 1.5 µg of this target vector was digested with 2 µl each of XbaI and HindIII in 1X NEB buffer 2 with 1X BSA for 1.5 h at 37 °C, enzymatically dephosphorylated with 1 µl CIP for 15 min to 1 h at 37°C (to prevent self-circularization of the vector), and then purified using a PCR purification kit (QIAquick, QIAGEN).

The 'GFP-srt' PCR insert (5 µl) was ligated into the target vector (3 µl) using 4 µl of T4 ligase in 1X T4 ligase buffer and nuclease-free water in a total volume of 20 µl. The ligation mixture was incubated at room temperature for 1 h, and BL21(DE) cells were then transformed with 7 µl of the ligation mixture for 15 min in an ice-water bath, heat-shocked at 42 °C for 30 s, and returned to the ice-water mixture for another 2 min. The cells were recovered in SOC media while horizontally shaking at 200 rpm at 37 °C for 40-60 min, and were then plated on TB agarose plates containing 100 µg ml^{-1} ampicillin (Calbiochem). Several clones were grown overnight in 3 ml TB media supplemented with 100 µg ml^{-1} ampicillin, and the plasmids were isolated by a miniprep plasmid purification kit (Qiagen) for DNA sequence verification.

Expression and purification of the fusion protein were done as previously described with minor changes. [1] Briefly, cells were cultured in Terrific Broth (TB, Mo Bio Laboratories, Inc.) supplemented with 100 μg ml^{-1} of ampicillin at 37°C. Once the optical density at 600 nm (OD600) of the culture reached 0.6, Isopropyl β-D-1-thiogalactopyranoside (IPTG, AMRESCO) was added to a final concentration of 0.5 mM to induce overnight expression. Cells were harvested 15 h post induction by centrifugation at 700×g for 10 min and were lysed by sonication on a Misonex Ultrasonic Liquid Processer (Qsonica, LLC.) at amplitude 85 for 3 min. Nucleic acids non-chromatographic purification were removed from the crude extract by addition of 1 vol % polyethyleneimine (PEI, Acros) followed by centrifugation at 4 °C at 21,000×g for 10 min. The ELP tag enable of the fusion by Inverse Transition Cycling (ITC), a non-chromatographic method we have developed for the purification of ELP fusion proteins that takes advantage of their inverse phase transtion behavior. After triggering the inverse phase transition of the fusion by addition of 1M NaCl, the aggregated proteins were collected by centrifugation at 21,000×g for 10 min at ~ 35 °C. The pellet was then resolubilized in cold PBS and the resulting solution was centrifuged at 4°C at 21,000×g for 10 min to remove any remaining insoluble material. The last two steps were repeated, typically three or four times, until satisfactory purity was achieved as verified by SDS-PAGE. In the final step, the protein was resolubilized in sortase buffer (50 mM Tris·Cl, 150 mM NaCl, 10 mM $CaCl_2$, pH 7.5) in preparation for sortase catalyzed initiator attachment (SCIA). Protein concentration and yield were assessed on an ND-1000 Nanodrop Spectrophotometer (Thermo Scientific) by UV-vis absorption spectroscopy.

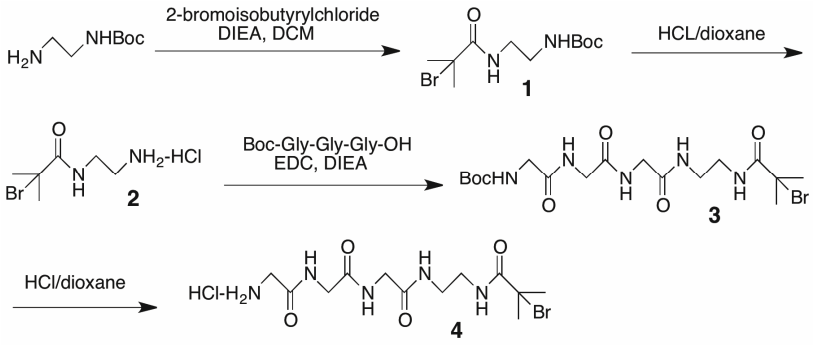
2.2 Sortase Cloning, Expression and Purification

The gene for SrtA with a 59 N-terminal amino acid truncation (previously shown to not affect its transpeptidase activity[2]) and an N-terminal His_6-tag in a pET15b vector was transformed into BL21 *E. coli* cells. Expression of protein and cell lysis was carried out identically as for the GFP-srt-ELP fusion protein. The SrtA fusion protein was purified by immobilized metal affinity chromatography (IMAC) on HisPur™ cobalt spin columns (Thermo Scientific) and following the manufacturer protocol. Briefly, the cell lysate was mixed with equal volume of equilibration buffer (50 mM sodium phosphate, 300 mM

sodium chloride, 10 mM imidazole; pH 7.4) and was loaded onto a pre-equilibrated HisPur™ column. After rotating the loaded columns at 4°C for 30 min to maximize binding, unbound proteins were eluted by centrifugation at 700×g for 2 min. Additional equilibration washes were performed until absorbance measurement at 280 nm of the eluent reached baseline as monitored on a ND-1000 Nanodrop Spectrophotometer. Concentration and yield at each step were calculated from the absorbance measurements. The bound $(His)_6$-SrtA fusion protein was eluted by centrifugation at 700×g for 2 min in elution buffer (50 mM sodium phosphate, 300 mM sodium chloride, 150 mM imidazole; pH 7.4). Typically the first two elution washes were collected and were solvent exchanged by overnight dialysis against sortase buffer in preparation for further use.

3. Synthetic Details

3.1 ATRP Initiator Synthesis



Schematic S1. Synthesis of ATRP initiator N-(2-(2-(2-(2-aminoacetamido)acetamido)acetamido)ethyl)-2-bromo-2-methylpropanamide (AEBMP) (**4**).

tert-butyl (2-(2-bromo-2-methylpropanamido)ethyl)carbamate (1): Over a period of 15 min, 2-bromoisobutyryl bromide (3.9 ml, 31.2 mmol) was added to a NaCl/ice cooled bath solution of N-Boc-ethylenediamine (5.01 g, 31.2 mmol) and diisopropylethylamine (6 ml, 34 mmol, 1.1 eq.) in anhydrous dichloromethane (35 ml). After 1 h, the ice bath was removed and the reaction was allowed to warm to room temperature and stirring was continued for 18 h. Silica gel (~10 g) was added and the mixture was concentrated to dryness under reduced pressure on a rotary evaporator. Flash column chromatography (RediSepR_f SiO_2 (80 g), 100% CH_2Cl_2→ 50% ethyl acetate (EtOAc) in CH_2Cl_2) gave **1**

as an off-white solid (7.36 g, 75%). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.2 (bs, 1H), 4.91 (bs, 1H), 3.33 (m, 4H), 1.93 (s, 6H), 1.43 (s, 9H). ^{13}C NMR ($CDCl_3$, 300 MHz): δ 172.9, 157.1, 80.1, 61.9, 42.0, 40.0, 32.5, 28.6. EIMS *m/z*: 331 ($[M+Na]^+$), 333 ($[M+Na]^+$).

N-(2-aminoethyl)-2-bromo-2-methylpropanamide hydrochloride (2): A solution of **1** (7.36 g, 23.8 mmol) in 4 M HCl in 1,4-dioxane (64 ml, 256 mmol) was stirred at room temperature for 1 h. The reaction mixture was concentrated to dryness on a rotary evaporator and further dried under high vacuum using a vacuum manifold connected to a vacuum pump, giving an off-white solid. The solid was triturated under diethyl ether (Et_2O, 3 x 100 ml) and the supernatant was removed by careful decantation. The insoluble material was dried under reduced pressure on a rotary evaporator giving **2** as a pale solid (5.8 g, 99%). ^{1}H NMR (CD_3OD, 300 MHz): δ 8.36 (bs, 1H), 3.65 (bs, 1H), 3.51 (s, 2H), 3.09 (s, 2H), 1.94 (s, 6H). ^{13}C NMR (CD_3OD, 300 MHz): δ 174.3, 58.9, 39.3, 37.8, 30.7. EIMS *m/z*: 209 ($[M-Cl]^+$), 211 ($[M-Cl]^+$).

tert-butyl (14-bromo-14-methyl-2,5,8,13-tetraoxo-3,6,9,12-tetraazapentadecyl)carbamate (3): Diisopropylethylamine (10.4 ml, 60 mmol, 2.5 eq.) was added in one portion to an ice-bath cooled suspension of **2** (5.8 g, 23.8 mmol), Boc-Gly-Gly-Gly-OH (6.9 g, 23.8 mmol), and EDC (6.84 g, 36 mmol, 1.5 eq.) in anhydrous CH_2Cl_2 (80 ml). The mixture was stirred overnight (16 h) then diluted with CH_2Cl_2 (80 ml). Insoluble material was isolated by vacuum filtration and the filter cake was washed sequentially with H_2O (100 ml), cold MeOH (3 x 20 ml), Et_2O (2 x 100 ml) and dried in vacuo giving **3** as a white powder (9.14 g, 80 %) ^{1}H NMR ($CDCl_3$, 300 MHz): δ 4.15-4.10 (m, 4H), 3.89 (s, 2H), 3.72 (m, 4H), 1.91 (s, 6H), 1.43 (s, 9H). ^{13}C NMR ($CDCl_3$, 300 MHz): δ 172.4, 168.9, 164.5, 156.8, 79.5, 52.8, 44.9, 44.4, 44.1, 38.2, 29.3, 25.2. EIMS *m/z*: 503 ($[M+Na]^+$), 505 ($[M+Na]^+$).

N-(2-(2-(2-(2-aminoacetamido)acetamido)acetamido)ethyl)-2-bromo-2-methylpropanamide hydrochloride (4): A solution of **3** (9.0 g, 18.8 mmol) in 4 M HCl in 1,4-dioxane (80 ml, 320 mmol) was stirred at room temperature for 1 h. The reaction mixture was diluted with Et_2O (300 ml). Insoluble material was collected and dried by vacuum filtration, giving the product as a white powder (7.7 g, 98%). ^{1}H NMR (CD_3OD, 500 MHz): δ 4.20 (m, 4H), 3.85 (s, 2H), 3.70 (m, 4H), 1.92 (s, 6H). ($CDCl_3$, 300 MHz): δ

171.0, 169.8, 166.5, 163.8, 52.6, 43.3, 42.7, 38.8, 30.3. EIMS *m/z*: 380 ($[MH\text{-}Cl]^+$), 382 ($[MH\text{-}Cl]^+$).

3.2 In situ ATRP from GFP-C-Br and Conjugate Purification

Three sets of ATRP reaction conditions were attempted and the parameters are summarized in **Table S1**.

Table S1. ATRP reaction conditions for reactions (Rxn) 1, 2, and 3.

	GFP-C-Br [µmol./eqv.]	**CuCl [µmol./eqv.]**	**$CuCl_2$ [µmol./eqv.]**	**HMTETA [µmol./eqv.]**	**OEGMA [µmol./eqv.]**	**time**
Rxn 1	0.2/1	5.1/25	15.0/75	25.0/125	110/550	30 min
Rxn 2	0.2/1	5.1/25	11.1/55	20.0/100	220/1100	30 min
Rxn 3	0.2/1	5.1/25	11.1/55	20.0/100	440/2200	2 h

4. Characterization Details

4.1 Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis (SDS-PAGE) and Initiator Attachment Efficiency

Samples were prepared in Laemmli loading dye containing 5 vol% β-mercaptoethanol. After brief heating at 98°C, the samples were loaded onto precast 4-20% Tris-HCl gels (Bio-Rad). Gels were run at 130 V and 400 mA for 55 min in 1x running buffer (25 mM Tris , 192 mM Glycine, and 0.1% SDS) on a Bio-Rad Mini-PROTEAN gel apparatus. Gels were stained with copper chloride.

To determine efficiency of SCIA, quantification of gel band intensity was performed using ImageJ. This method for quantification of the yield of initiator attachment to GFP is valid because we do not expect that attachment of the initiator to GFP should alter its staining by the dye. For each SDS-PAGE gel, bands in each lane were defined in ImageJ and converted into intensity profile plots using a built-in function, where each band was assigned a corresponding peak. After defining the baseline for each peak, band intensities were computed by calculating the area under each peak. Values were then imported into Excel (Microsoft) for analysis.

Because the errors involved in sample loading can be significant when the product of a sortase cleavage reaction is normalized to a standard amount of GFP-srt-ELP loaded in a separate lane, the yield of each SCIA reaction was calculated by internal normalization, wherein we assume that the intensity of the products of a sortase cleavage reaction sums to that of the parent fusion construct it was derived from. Hence, the band intensity of the initial amount of GFP-srt-ELP used in each reaction was determined by summing up all of its products after reaction, namely residual unreacted GFP-srt-ELP, cleaved ELP, and transpeptidized GFP-C-Br. The % unreacted product is thus the band intensity of unreacted GFP-srt-ELP divided by the sum of all products and multiplied by 100, and % transpeptidation is thus 100% - % unreacted. A very faint band slightly above 50 kDa could also be observed upon close inspection, which corresponds to an intermediate species of the reaction, where SrtA is linked to the *C* terminus of GFP via a thioacyl bond. The presence of SrtA in this species makes its staining not directly comparable to that of the other species, so that the intensity of this band was not incorporated into the overall calculation of reaction yield. However, including it in the sum of intensities and taking its percentage showed that this intermediate only comprised < 1% of the overall intensity at most (Table S2), so that omitting it in the calculation of % transpeptidation yield does not significantly change the results.

4.2 Liquid Chromatography Electrospray-Ionization Mass Spectrometry (LC/ESI-MS)

Samples at a concentration of 5 μM were first desalted by dialyzing against MilliQ water overnight. LC/ESI-MS was performed on an Agilent 1100 LC/MSD Quadrupole Mass Spectrometer. The instrument was calibrated with Cytochrome C and BSA. The ESI source was set to operate at 300°C with a nebulizer gas pressure of 20 psi and a dry gas flow rate of 7 L min^{-1}. 1 μl of sample was separated by reverse phase chromatography on a Zorbax SB-C_{18} column (Agilent) at 20%-80% acetonitrile/water gradient and a flow rate of 60 μl min^{-1}. Spectra were acquired in positive ion mode over the mass to charge range (m/z) of 400-1,600. Theoretical MW of GFP-C-Br was calculated using Molecular Weight Calculator (v. 6.49, Pacific Northwest National Laboratory, ncrr.pnl.gov/software).

4.3 Nano-Flow Liquid Chromatography Electrospray Ionization Tandem Mass Spectrometry (LC/MS-MS)

100 µl of ~8uM sample was loaded on to a 0.5 ml ZebaSpin desalting column (Thermo Scientific) for solvent exchange into 50 mM ammonium bicarbonate, pH 8.0, supplemented with 0.1% Rapigest SF surfactant (Waters Corp), by washing the loaded column with 300 µl of the solvent solution four times. The sample was then reduced with 5 mM dithiolthreitol for 30 min at 70°C and free sulfhydryls were alkylated with 10 mM iodoacetamide for 45 min at room temperature. Proteolytic digestion was accomplished by the addition of 500 ng sequencing grade trypsin (Promega) directly to the resin with incubation at 37°C for 18 h. Supernatant was collected following a 2 min centrifugation at 1,000 rpm, acidified to pH 2.5 with TFA and incubated at 60 °C for 1 h to hydrolyze remaining Rapigest surfactant. Insoluble hydrolyzed surfactant was cleared by centrifugation at 15,000 rpm for 5 min and the sample was then dried by vacuum centrifugation.

The dried sample was resuspended in 20 µl 2% acetonitrile, 0.1% formic acid, and subjected to chromatographic separation on a Waters NanoAquity UPLC equipped with a 1.7 µm BEH130 C18 75 µm I.D. X 250 mm reversed-phase column. The mobile phase consisted of (A) 0.1% formic acid in water and (B) 0.1% formic acid in acetonitrile. Following a 1 µl injection, peptides were trapped for 5 min on a 5 µm Symmetry C18 180 µm I.D. X 20 mm column at 20 µl min^{-1} in 99.9% A. The analytical column (BEH130) was then switched in-line and a linear elution gradient of 5% B to 40% B was performed over 60 min at 400 nl min^{-1}. The analytical column was connected to a fused silica PicoTip emitter (New Objective, Cambridge, MA) with a 10 µm tip orifice and coupled to a Waters Synapt G2 HDMS QToF mass spectrometer through an electrospray interface. The instrument was operated in a data-dependent mode of acquisition in resolution mode with the top three most abundant ions selected for MS/MS using a charge state dependent CID energy setting with a 60 s dynamic exclusion list employed.

Mass spectra were processed with Mascot Distiller (Matrix Science) and were then submitted to Mascot searches (Matrix Science) against a SwissProt_Ecoli database appended with the custom Aequorea victoria GFP sequence with 10 ppm precursor and 0.04 Da product ion mass tolerances. Static mass modifications corresponding to

carbamidomethylation on Cys residues, dynamic mass modifications corresponding to the ATRP initiator N-(2-(2-(2-(2-aminoacetamido)acetamido)acetamido)ethyl)-2-bromo-2-methylpropanamide (AEBMP), and oxidation of Met residues were included. Searched spectra were imported into Scaffold v4.0 (Proteome Software) and scoring thresholds were set to yield a minimum of 99% protein confidence (implemented by the PeptideProphet algorithm) based on decoy database searches.[3] A minimum of two unique peptides from each protein were required for identification. Extracted ion chromatograms of the expected C-terminal tryptic peptide modified by AEBMP were performed in MassLynx (v4.1) at a 20 ppm mass accuracy window and experimental isotope distributions of the triply charged precursor ion was compared to a theoretical isotope distribution modeled in Molecular Weight Calculator.

4.4 Size Exclusion Chromatography (SEC) and Conjugation Efficiency

Analytical SEC was performed on a Shimadzu HPLC system equipped with a UV-vis detector (SPD-10A VP) operating at 280 nm and a fluorescence detector (RF-10Axl) set at 460 nm excitation and 507 nm emission. 30 µl of samples at ~25 µM concentration were separated on a Protein KW-803 column (with a guard column) using Tris-HCl buffer (0.1M Tris-HCl, pH 7.4) as mobile phase at 25 °C and a flow rate of 0.5 ml min^{-1}. Preparative SEC to purify the conjugates was performed on an AKTA system (GE Healthcare) equipped with a photodiode detector set at 280 nm and a HiLoad 26/600 Superdex 200 PG column using PBS as mobile phase at 4 °C and a flow rate of 3 ml min^{-1}.

To determine conjugation efficiency of in situ ATRP from the *C* terminus of GFP, area under the curve (AUC) of the GFP-C-poly(OEGMA) conjugate peak and the residual unreacted GFP-C-Br macroinitiator peak in the chromatogram of each polymerization reaction mixture were computed by EZStart software (v. 7.4, Shimadzu). Sum of the areas of the two peaks corresponding to the macroinitiator and the conjugate in each chromatogram was regarded as 100% and % fraction of the conjugate peak was recorded as the conjugation efficiency of that particular polymerization reaction. Values from the three reactions were then used to calculate the mean and standard deviation of conjugation efficiency. The calculation was done for chromatograms detected by both

UV-vis absorbance (Table 3S) and fluorescence (Table 4S).

4.5 Size Exclusion Chromatography Multi-Angle Light Scattering (SEC-MALS)

The fluid line of the analytical HPLC system was connected downstream in series to a DAWN HELEOS II MALS detector followed by an Optilab T-rEX refractometer (both from Wyatt Technology). The system was calibrated with toluene and normalized with 2.0 mg ml^{-1} Bovine Serum Albumin (BSA). Samples were filtered with 0.1 μm filters before injection. The One-detector method involving only the refractometer was used due to low degree of UV absorbance detected when running poly(OEGMA) polymer. Online determination of dn/dc was performed using built-in method "dn/dc from peak" under the assumption of 100% mass recovery. The assumption was verified by confirming that mass recovered as measured by online UV detection at 280 nm and mass injected as measured by offline UV absorbance at 280 nm using Nanodrop Spectrophotometer were in close agreement. The full recovery of sample through the column was likely due to presence of the stealth poly(OEGMA) polymer on the conjugates that minimized binding to the column. A dn/dc value of 0.185 mL g^{-1} was used for GFP-C-Br. dn/dc values of conjugates from Rxn 1, Rxn 2, and Rxn 3 were determined to be 0.160 mL g^{-1}, 0.155 mL g^{-1}, and 0.149 mL g^{-1}, respectively. The actual mass injected was determined by lyophilization followed by weighing, and the number was entered into ASTRA (v. 6.0, Wyatt Technology) to compute dn/dc values of the conjugates. All results were analyzed using ASTRA 6.0.

4.6 Dynamic Light Scattering (DLS)

DLS was performed on a DynaPro Plate Reader (Wyatt Technology). Samples were prepared at 25 μM and filtered with 0.1 μm filters before analysis. The instrument was operating at a laser wavelength of 831.95 nm, a scattering angle of 90° and at 25 °C. Data were analyzed in Dynals mode using Dynamics 6.12.0.3.

4.7 Fluorescence Spectroscopy

Fluorescence spectra were recorded on a CARY Eclipse fluorescence spectrophotometer (Varian) in scan mode at 25 °C. The fluorescence of samples at a concentration of 20 μM

were measured with an excitation wavelength of 460 nm and the emission intensity was recorded from 485-530 nm.

5. Supplementary Figures

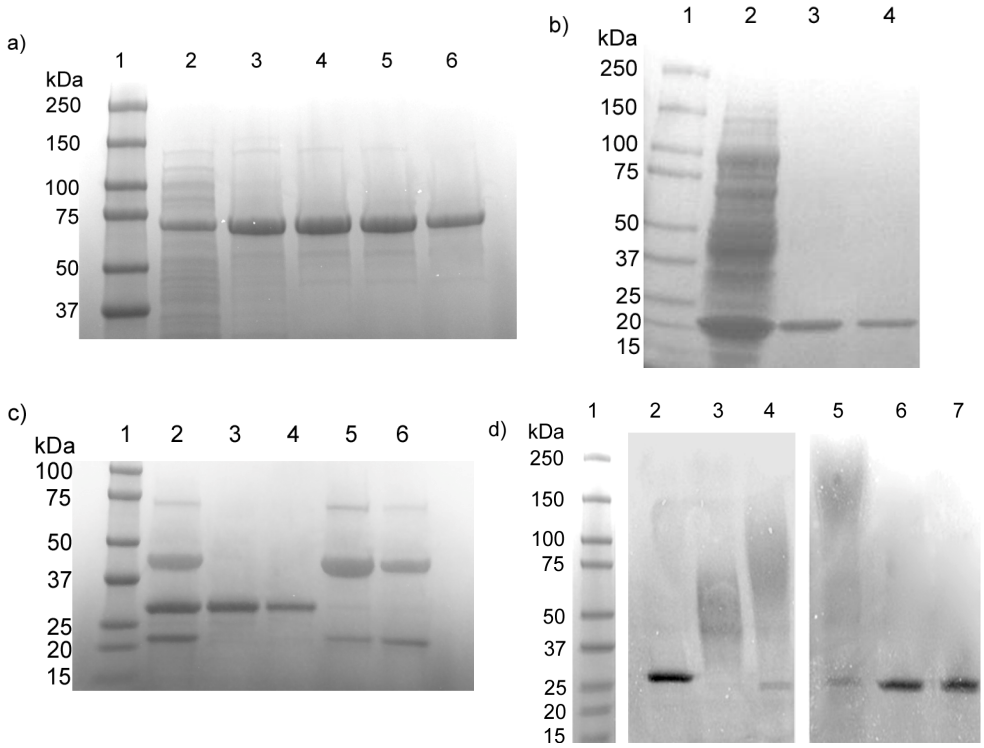


Figure S2. SDS-PAGE analysis of a) GFP-srt-ELP purified by ITC (yield: 240 mg L^{-1} of fermentation). Lane 1: marker, lane 2: *E. coli* lysate, lane 3: soluble protein after one ITC cycle, lane 4, after two ITC cycles, lane 5: after three ITC cycles, lane 6: after four ITC cycles. b) SrtA purified by His_6-tag purification (yield: 135mg L^{-1} of fermentation). Lane 1: marker, lane 2: *E. coli* lysate, lane 3: first elution wash with imidazole, lane 4: second elution wash with imidazole. c) GFP-C-Br purified by reverse His_6-tag purification. Lane 1: marker, lane 2: SCIA reaction mixture, lane 3: GFP-C-Br (without His_6-tag) in first elution without imidazole, lane 4: second elution without imidazole, lane 5: all other His_6-tagged components in first elution with imidazole, lane 6: second elution with imidazole. d) ATRP reaction products. Lane 1: marker, lane 2: GFP-C-Br macroinitiator before ATRP, lane 3: GFP-C-poly(OEGMA) conjugate from Rxn 1, lane 4: conjugate from Rxn 2, lane 5: conjugate from Rxn 3, lane 6: GFP-C-Gly_3 control after ATRP using Rxn 3 conditions, lane 7: GFP-C-Br physically mixed with free poly(OEGMA) synthesized using Rxn 3 conditions. Free poly(OEGMA) does not stain due to lack of charge.

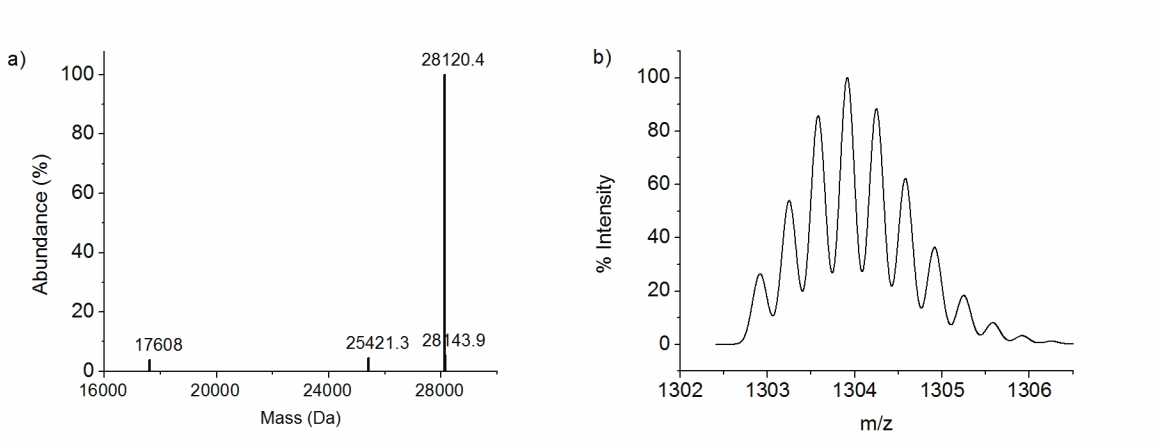


Figure S3. a) Deconvoluted LC/ESI-MS spectra of GFP-C-Br macroinitiator. Major peak at 28,120.4 Da agrees well with theoretical mass of 28,123.8 Da. b) Theoretical isotopic distribution of C-terminal peptide [DHMVLLEFVTAAGITHGMDELYNVDGGGSLPET-"AEBMP"]$^{3+}$ after tryptic digestion generated by Molecular Mass Calculator software (Pacific Northwest National Laboratory).

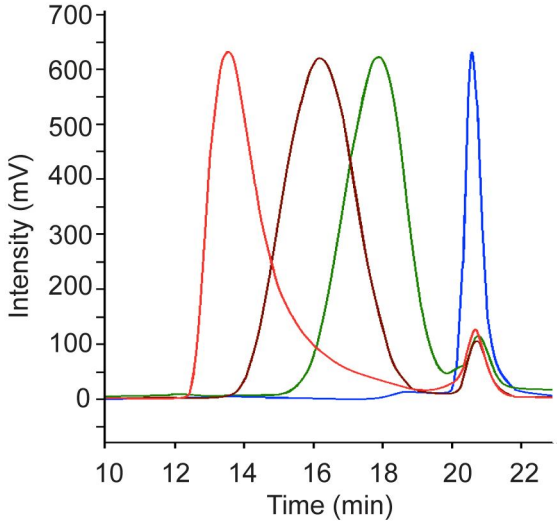


Figure S4. SEC traces of GFP-C-Br (blue), and conjugates from Rxn 1 (green), Rxn 2 (maroon), Rxn 3 (red) detected by fluorescence at 460 nm excitation and 507 nm emission.

Table S2. Initiator attachment efficiency (% transpeptidation) of SCIA determined by SDS-PAGE gel band quantification averaged across five SCIA reactions. Intensity of initial GFP-srt-ELP was determined by summing intensities of unreacted GFP-srt-ELP, cleaved ELP, and transpeptidized GFP-C-Br. % unreacted was calculated as the fraction of unreacted GFP-srt-ELP divided by sum of the three bands and multiplying by 100. % transpeptidized was calculated as 100% - % unreacted. A very faint GFP-SrtA intermediate band was also observed in all reactions, but only comprised of up to ~1% in all cases. Because the presence of SrtA in the intermediate alters its staining compared to the other three species, making direct quantitative manipulation difficult, its intensity was excluded from the calculation. % transpeptidation was 96.3 ±1.5 %.

	Intensity						**% Intensity**		
	Unreacted GFP-srt-ELP	**Cleaved ELP**	**GFP-C-Br**	**Total w/o intermediate**	**GFP-SrtA intermediate**	**Total w/ intermediate**	**% Interme-diate**	**% Unreacted**	**% Transpeptidation**
SCIA #1	203.7	1726.3	1810.2	3740.2	40.6	3780.8	1.1	5.4	94.6
SCIA #2	268.3	5899.2	6970.4	13137.9	87.4	13225.3	0.7	2.0	98.0
SCIA #3	505.6	4990.1	4971.3	10466.9	101.8	10568.7	1.0	4.8	95.2
SCIA #4	207.8	3783.8	4209.0	8200.6	61.1	8261.6	0.7	2.5	97.5
SCIA #5	538.9	6955.8	7421.7	14916.4	110.6	15026.9	0.7	3.6	96.4
Mean ± Std. Dev.									96.3 ±1.5

Table S3. Conjugation efficiency of in situ ATRP from *C* terminus of GFP determined by AUC of HPLC chromatograms of three independent reactions detected by UV-vis absorbance at 280 nm. Area % was calculated by dividing area of an individual peak by total area (sum of the two) and multiplying by 100. Averaging area % values of GFP-C-poly(OEGMA) conjugates from three reactions gives conjugation efficiency of 95.0 ± 2.2%.

	Area			**Area %**	
	GFP-C-Br	**GFP-C-poly(OEGMA)**	**Total**	**GFP-C-Br**	**GFP-C-poly(OEGMA)**
Rxn 1	59151.0	813750.0	872901.0	6.8	93.2
Rxn 2	70996.0	1202397.0	1273393.0	5.6	94.4
Rxn 3	29056.0	1133247.0	1162303.0	2.5	97.5
Mean ± Std. Dev.					95.0 ± 2.2

Table S4. Conjugation efficiency of in situ ATRP from the *C* terminus of GFP determined by AUC of HPLC chromatograms of three attempted reactions detected by Fluorescence at 460 nm excitation and 507 nm emission. Area % was calculated by dividing area of individual peak by total area (sum of the two peaks) and multiplying by 100. Averaging the area % values of GFP-C-poly(OEGMA) conjugates from three reactions yielded a conjugation efficiency of 93.6 ± 1.9%.

	Area			**Area %**	
	GFP-C-Br	**GFP-C-poly(OEGMA)**	**Total**	**GFP-C-Br**	**GFP-C-poly(OEGMA)**
Rxn 1	3235695.0	74156445.0	77392140.0	4.4	95.8
Rxn 2	7885455.0	95738118.0	103623573.0	8.2	92.4
Rxn 3	6046562.0	77048389.0	83094951.0	7.8	92.7
Mean ± Std. Dev.					93.6 ± 1.9

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