Supporting Information for Chemical Communications

Multivalent *Giant Amphiphiles* via simultaneous copper(I)-catalyzed azide–alkyne cycloaddition and living radical polymerization

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1. Materials

Chemicals were purchased from commercial sources and were used as received, unless otherwise stated. Copper bromide (Cu(I)Br) was purified by stirring in glacial acetic acid, filtration and rinsing with ethanol and diethyl ether before drying overnight at 70 ˚C under a vacuum line. Bovine serum albumin (BSA) was purchased from Sigma (> 99%). *N*-(*n*-propyl)-2-pyridylmethanimine was prepared as described earlier and stored at 4 ºC.[1] Dialysis bags (Spectra/Por® Biotech Regenerated Cellulose Dialysis Membranes, MWCO 10, 25 and 50 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, an SIL-10AD auto-injector, a RID- 10A refractive index detector and a SPD-10A Shimadzu UV-Vis spectrometer. The system was equipped with a Polymer Laboratories 30×7.8mm 5μm BioBasic SEC 60 guard column followed by a 300×7.8mm 5μm BioBasic SEC 300 Polymer Laboratories column, using a mixture of 0.1% TFA, 30% MeCN in MilliQ water or 10% MeCN in MilliQ water as the eluent at room temperature (flow rate: 0.5 $mL \cdot min^{-1}$). Chromatograms were simultaneously acquired at 254 nm and 280 nm wavelength and were processed with the EZStart 7.3 chromatography software.

Native Polyacrylamide Gel Electrophoresis (PAGE). Discontinuous Native PAGE electrophoresis was run using a 4% stacking gel and a 10% resolving gel under standard denaturing conditions. Samples were dissolved in TRIS buffer containing Bromophenol Blue and were visualized using Coomassie Brilliant Blue or Silver Staining.

Agarose Gel Electrophoresis was run using several percentages of gels (0.1 to 2%) using TRIS/Acetate/EDTA buffer (TAE). Samples were dissolved in TRIS buffer containing Bromophenol Blue, were typically resolved with 0.7 % agarose gels and were visualized using Coomassie Brilliant Blue.

Matrix-Assisted Laser Desorption Ionization Spectroscopy (**MALDI-TOF).** MALDI-TOF MS measurements were performed in the SVS-MS Mass Spectrometry Core Facility of the University of Geneva using an Axima CFR+ MALDI-TOF (Shimadzu Biotech, Manchester, UK) in positive ionization mode and sinapinic acid as the matrix. All samples were mixed with the matrix (1:1 volume ratio, sinapinic acid, 10 $mg \cdot mL^{-1}$) and air dried before analysis.

UV-Vis Spectroscopy. UV-Vis spectra were recorded using a JASCO V650 spectrophotometer equipped with a temperature controller.

Confocal Fluorescence Microscopy experiments were performed with a Leica TCS SP2 AOBS confocal microscope using a 100× oil immersion objective. Fluorescein was excited with the 488 line of the Argon-Krypton laser. Unidirectional scanning was done at 400 Hz with an image format of 512 by 512 pixels.

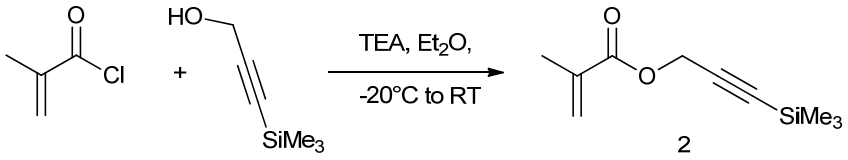
Transmission Electron Microscopy experiments were performed using a FEI Tecnai G2 Electron Microscope. Micrographs were taken using a Tietz CCD camera at a 2048 by 2048 pixel resolution.

The **FT-IR** spectra were recorded on a Thermo-Electron Nicolet 6700 FT-IR optical spectrometer with a DTGS KBr detector at a resolution of 2 cm^{-1}.

Field-emission Scanning Electron Microscopy (FESEM) imaging was performed using a JEOL 7000F FESEM.

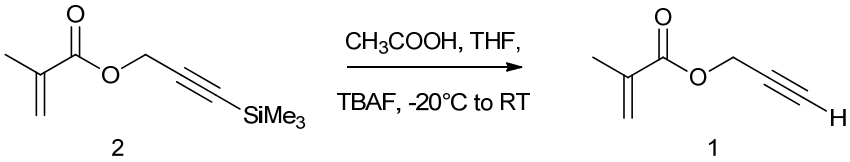
3. Experimental

i. Synthesis of protected alkyne monomer (2):



A solution of trimethylsilylpropyn-1-ol (5.0 g, 39.0 mmol) and Et_3N (7.1 mL, 50.65 mmol) in Et_2O (50 mL) was cooled to -20°C and a solution of methacryloyl chloride (4.4 mL, 46.5 mmol) in Et_2O (50 mL) was added dropwise over *ca.* 1 h. The mixture was stirred at this temperature for 30 min, then at ambient temperature overnight, the ammonium salts were removed by filtration and the volatiles removed under reduced pressure. ^{1}H NMR analysis of the yellow oily residue did not reveal the presence of substantial amount of any impurity, but two additional faint spots were observed by TLC analysis (petroleum ether/Et_2O 20:1), the crude product was therefore purified by flash column chromatography (CC, SiO_2, petroleum ether/Et_2O 50:1; R_f = 0.67 in petroleum ether/Et_2O 20:1) to obtain the pure product as a colorless oil (5.45 g, yield 71%). ^{1}H NMR (400 MHz, $CDCl_3$, 298 K) δ = 0.19 (s, 9H, $Si(CH_3)_3$); 1.97-1.98 (m, 3H, $CH_3C=CH_2$); 4.77 (s, 2H, OCH_2); 5.62-5.63 (m, 1H, C=C*H*H); 6.18 (m, 1H, C=CH*H*). ^{13}C{1H} (100 MHz, $CDCl_3$, 298 K) δ = –0.2 (3C, $Si(CH_3)_3$); 18.4 (1C, $CH_3C=CH_2$); 53.0 (1C, OCH_2); 92.0 (1C, C≡*C*Si$(CH_3)_3$); 99.2 (1C, *C*≡CSi$(CH_3)_3$); 126.5 (1C, $CH_3C=CH_2$); 135.8 (1C, $CH_3C=CH_2$); 166.6 (1C, CO_{ester}). Anal. Calcd. for $C_{10}H_{16}O_2Si$: C, 61.18; H, 8.21; N, 0.00; Found: C, 60.89; H, 8.22; N, 0.00; Mass Spectrometry (+ESI-MS) *m/z* (%): 219 ([M+Na], 100), 197 ([MH+], 40).

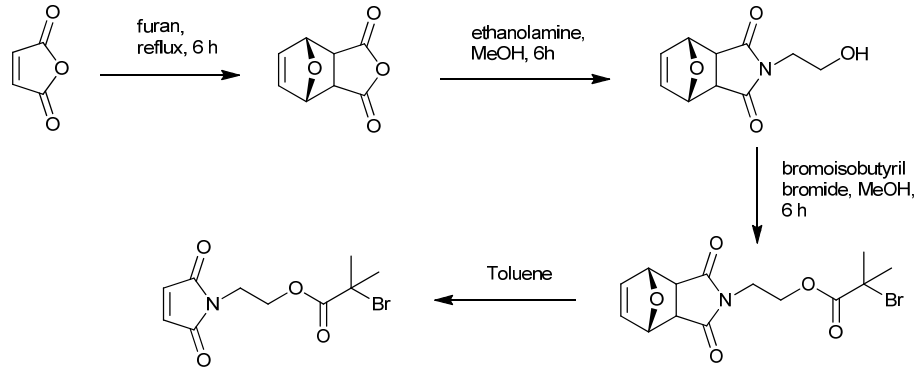
ii. Synthesis of the deprotected prop-2-ynyl methacrylate monomer (1):



500 mg (2.55 mmol) of 3-(trimethylsilyl)prop-2-ynyl methacrylate (**2**) were dissolved in THF (25 mL). 3.825 mL of 1 M acetic acid solution (3.825 mmol) were added and the resulting solution was cooled down to −20°C with an ice-acetone bath and bubbled with nitrogen for *ca.* 10 min before the addition of TBAF·$3H_2O$ (1.207 g, 3.825 mmol). The resulting solution was left to warm to room temperature and was stirred overnight. The solution was then passed through a pad of silica and the pad was washed with another 25 mL of THF. Removal of the volatiles under reduced pressure afforded the pure product **1** as colourless oil (307 mg, 2.47 mmol, 97% yield). ^{1}H NMR ($CDCl_3$, 400 MHz, 298 K) δ 6.17-6.15 (m, 1H, C=CH*H*), 5.59- 5.58 (m, 1H, C=C*H*H), 4.63 (d, 2H, *J* = 2.5 Hz, OCH_2), 2.52 (t, 1H, *J* = 2.3 Hz, C≡C*H*), 1.94- 1.93 (m, 3H, $CH_3C=CH_2$), 13C{1H} NMR ($CDCl_3$, 100 MHz, 298 K) δ 18.4 (1C, $CH_3C=CH_2$), 53.0 (1C, OCH_2), 92.0 (1C, C≡*C*H),

99.2 (1C, *C*≡CH), 126.5 (1C, CH_3C=*C*H_2), 135.8 (1C, CH_3*C*=CH_2), 166.6 (1C, CO_{ester}). ESI-MS (MeOH, +EI) *m/z* (%): 147 ([M+Na]$^+$, 100), 125 ([MH$^+$], 41), 55 (31).

iii. Synthesis of the maleimido-ATRP initiator 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 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 (400 MHz, $CDCl_3$, 298 K): δ = 3.17 (s, 2H, CH), 5.45 (t, 2H, *J* =1.0Hz, 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: 2.00 g, (12.0 mmol) of 4,10-dioxatricyclo[5.2.1.02,6]dec-8-ene-3,5-dione were 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 *ca.* 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 3×100 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 column chromatography to give the product (1.04 g, 5.00 mmol, 42% yield) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$, 298 K): δ = 1.90 (bs, 1H, OH), 2.90 (s, 2H, CH), 3.69-3.72 (m, 2H, NCH_2), 3.76-3.78 (m, 2H, OCH_2), 5.28 (t, 2H, *J* = 0.9 Hz, CH), 6.52 (t, 2H, *J* = 0.9 Hz, CH_{vinyl}).

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: A solution of the alcohol 4-(2-hydroxyethyl)-10-oxa-4-aza-tricyclo[5.2.1.0]dec-8-ene-3,5-dione (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 h 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 column chromatography (CC, SiO_2, petroleum ether/ethyl acetate 1:1). 3.54 g (9.88 mmol, 93% yield) of **3** was obtained as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$, 298 K): δ = 1.86 (s, 6H, CH_3), 2.84 (s, 2H, CH), 3.78 (t, 2H, *J* = 5.3 Hz, NCH_2), 4.30 (t, 2H, *J* = 5.3 Hz, OCH_2), 5.23 (t, 2H, *J* = 1.0 Hz, CHO), 6.49 (t, 2H, *J* =1.0 Hz, CH_{vinyl}).

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, 0.15 gr, 0.419 mmol) was suspended in dry toluene (5 mL) and heated to reflux under nitrogen atmosphere for 8 hours. The solvent was removed under reduced pressure to give a pale-yellow residue which was subsequently purified by flash column chromatography (SiO_2, petroleum ether/ethyl acetate 4:1) to yield **I** as a slightly yellow solid (90% yield). ^{1}H NMR (400 MHz, $CDCl_3$, 298 K): δ = 1.88 (s, 6H, CH_3), 3.84 (t, 2H, *J* = 5.3 Hz, NCH_2); 4.32 (t, 2H, *J* = 5.3 Hz, OCH_2); 6.72 (t, 2H, *J* = 1.0 Hz, CH_{vinyl}).

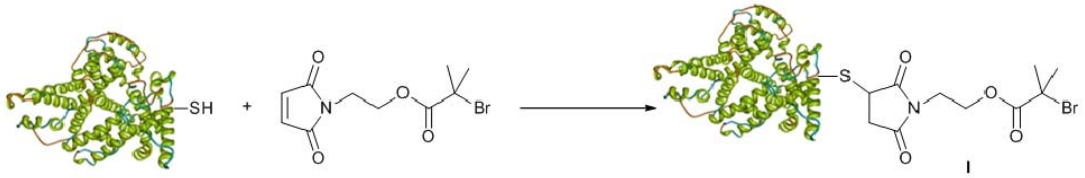
iv. Synthesis of Azides:

Azidation of Benzyl bromide: In a round bottom flask, benzyl bromide (0.51 g, 3.0 mmol) was added to a 0.5 M solution of NaN_3 (0.25 g, 3.8 mmol) in DMSO (8 mL) at ambient temperature. The solution was stirred for 24 hours at ambient temperature and then quenched with 10 mL H_2O (reaction slightly exothermic). After cooling down to ambient temperature, the mixture was extracted with Et_2O. The organic layers were washed with brine, dried over $MgSO_4$, filtered off and the solvent removed by extraction to afford the pure benzyl azide as a pale yellow oil which was used for the "*click*" reaction steps without any further purification. IR (neat): *ν* = 3032, 2929, 2089 (C−N_3 absorption band), 1738, 1496, 1455, 1349, 1252, 1202, 1078, 1029, 875, 735, 695 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$, 298 K) δ = 4.36 (s, 2H, CH_2-N_3), 7.34-7.40 (m, 5H, $CH_{aromatic}$). ^{13}C NMR (400 MHz, $CDCl_3$, 298 K) δ = 54.85 (1C, CH_2-N_3), 128.28 (2C, CH $_{aromatic}$), 128.37 (1C, CH $_{aromatic}$), 128.89 (2C, CH $_{aromatic}$).

2-(2-(2-azidoethoxy)ethoxy)ethanol: A solution of 1.1 g (7.33 mmol) of dry triethylene glycol, 1 mL of dry TEA, and 10 mL of dry ether was cooled down to 0°C under a nitrogen atmosphere. Methanesulfonyl chloride (0.42 g, 3.665 mmol) was added over a 1-hour period, after which the solution was allowed to warm slowly to room temperature overnight. The reaction contents were concentrated in vacuo, and 15 mL of 95% ethanol and 0.524 g (8.06 mmol) of sodium azide were added. The mixture was heated at reflux for 24 h, cooled down to ambient temperature, and concentrated in vacuo. The remaining mixture was diluted with 10 mL diethyl ether, washed with 5 mL brine, and dried over $MgSO_4$. Concentration in vacuo afforded the crude product, which was purified by silica gel column chromatography eluting with a gradient of 1:1 to 3:1 ethyl acetate/cyclohexane to afford 565 mg (3.22 mmol, 44% yield) of pure compound **21** as a slight yellow oil. IR (neat): *ν* = 3597, 3062, 2873, 2111 (C−N_3 absorption band), 1737, 1455, 1346, 1267, 1121, 1062, 930, 888, 715 cm^{-1}. ^{1}H NMR ($CDCl_3$, 400 MHz, 298 K): δ 3.80-3.60 (m, 8H, −CH_2O−), 3.59 (t, 2H, *J* = 5.1 Hz, C*H*$_2$OH), 3.37 (t, 2H, *J* = 5.3 Hz,

$CH_2{-}N_3$). ^{13}C NMR ($CDCl_3$, 100 MHz, 298 K) δ 72.7 (1C, CH_2), 70.9 (1C, CH_2), 70.6 (1C, CH_2), 70.3 (1C, CH_2), 62.0 ($CH_2{-}OH$), 50.9 ($CH_2{-}N_3$). ESI-MS (MeOH, +EI) *m/z* (%): 198 ($[M+Na]^+$, 2), 176 ($[MH^+]$, 5), 119 (10), 89 (31), 75 (18), 63 (18), 45 (100).

v. Synthesis of the Bovine Serum Albumin Macroinitiator I:

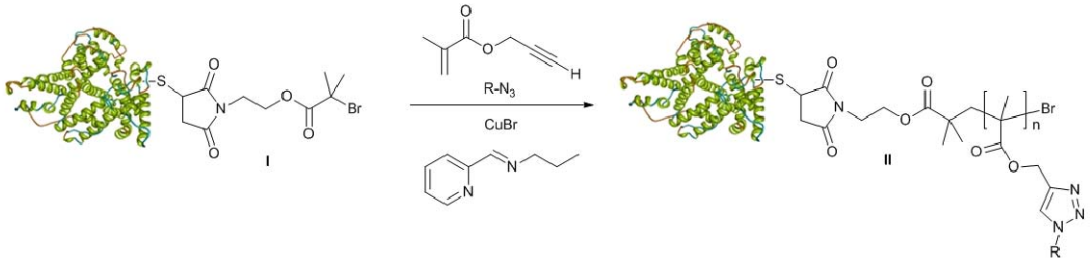


A solution of the maleimide functionalized ATRP initiator (2-bromo-2-methyl-propionic acid 2-(2,5-dioxo-2,5-dihydro-pyrrol-1-yl)-ethyl ester, 126 mM, 0.8 mL) in DMSO was slowly added to 9.0 mL of a 0.35 mM solution of BSA in 20 mM phosphate buffer (pH 7.4). The reaction mixture was gently shaken for 48 hours at 7°C. To eliminate the excess of the ATRP initiator, the mixture was then extensively dialyzed initially against 10% DMSO in 20 mM phosphate buffer, 2% EDTA and then against 20 mM phosphate buffer using 10 kDa regenerated cellulose dialysis membranes. The resulting solution of BSA-macroinitiator **I** was subsequently analyzed by aqueous SEC, native gel electrophoresis, agarose electrophoresis and MALDI-TOF.[2] The BSA-macroinitiator **I** was freeze-dried prior to ATRP-mediated *in situ* polymerization and stored at −20˚C. It is worth mentioning that the same reaction can be successfully performed in the absence of organic co-solvent or by using THF as co-solvent.

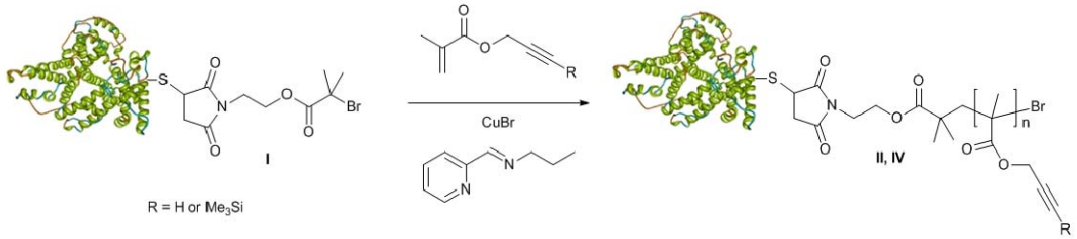
General procedure for the determination of free thiols by Ellman's assay:[3,4] 4 mg of 5,5'-dithio-bis-(2-nitrobenzoic acid) (Ellman's reagent) was dissolved in 1 mL of buffer solution (0.1 M sodium phosphate, pH 8.0, containing 1mM EDTA) to prepare Ellman's reagent solution (10.09 mM). 0.250 μL of a BSA or BSA-macroinitiator **I** conjugate solution (0.3 mM), 50 μL of Ellman's reagent and 2.50 mL of buffer solution were mixed for 15 min at room temperature. The absorbance at 412 nm was measured by a UV-Vis spectrophotometer. The thiol concentration was calculated using the Beer-Lambert's law (molar extinction coefficient of 2-nitro-5-thiobenzoic acid = 14,150 $M^{-1}{\cdot}cm^{-1}$ at 412 nm). The initiator itself does not absorb in UV at 412 nm and does not affect the Ellman's assay. The quantification of the bioconjugation reaction by the colorimetric Ellman's assay revealed 47 % of available cysteine conjugation sites on native BSA and practically no free conjugation sites on the isolated BSA-macroinitiator bioconjugate **I**, indicating the quantitative conjugation of the maleimido-ATRP-macroinitiator with the free thiol of BSA.

vi. Polymerization Reactions:

The polymerization experiments were performed using the optimum monomer to BSA-macroinitiator **II** ratio at 2000:1, as well as the ratio BSA-macroinitiator **I:**CuBr**:**ligand at 1**:**41**:**70 in all experiments.[2] Control reactions in the absence of the biomacroinitiator **I**, or the monomer were also performed.



Simultaneous CuAAC and ATRP (Route A): The propargyl methacrylate monomer **1** (150 mg, 1.215 mmol), the azide (1.35 mmol), and *N*-(propyl)-2-pyridylmethanimine (~8 mg, 0.057 mmol) were placed in a Schlenk tube and dissolved in 10 mL of a 20 mM sodium phosphate buffer pH 7.4 aqueous solution. The mixture was de-oxygenated by 5 freeze-pump-thaw cycles and was then canulated in another Schlenk tube containing the previously degassed BSA macroinitiator **I** (~62 mg, 0.93 μmol) and CuBr (4.7 mg, 33 μmol) under N_2 atmosphere. This canulation triggered the beginning of the polymerization (an increase of the opacity in the dark brown colour of the reaction medium was observed). The mixture was stirred under inert atmosphere for *ca.* 8 h upon which time the reaction was quenched with atmospheric oxygen. The reaction mixture was then dialyzed using a 25 kDa MWCO membrane initially against 2% EDTA 10% DMSO 20 mM phosphate buffer and then against 20 mM phosphate buffer pH 7.4. The resulting solutions were analyzed by means of SEC-HPLC, electrophoresis, MALDI-TOF analysis and IR. This reaction was found to proceed well in the presence and absence of organic co-solvent.



General polymerization procedure with monomer 1 (Route B): The propargyl methacrylate monomer **1** (150 mg, 1.215 mmol) and *N*-(propyl)-2-pyridylmethanimine (~8 mg, 0.057 mmol) were placed in a Schlenk tube and dissolved in 10 mL of 20 mM sodium phosphate buffer pH 7.4. The mixture was de-oxygenated by 5 freeze-pump-thaw cycles and was then canulated in another Schlenk tube containing the previously degassed BSA macroinitiator **I** (~62 mg, 0.93 μmol) and CuBr (4.7 mg, 33 μmol) under N_2 atmosphere. This canulation triggered the beginning of the polymerization (an increase of the opacity in the dark brown colour of the reaction medium was observed). The mixture was stirred under inert atmosphere for *ca.* 8 h upon which time the reaction was quenched with atmospheric oxygen. The reaction mixture was then dialyzed using a 25 kDa MWCO membrane initially against 2% EDTA 10% DMSO 20 mM phosphate buffer and then against 20 mM phosphate buffer pH 7.4. The resulting solutions were analyzed by means of SEC-HPLC, electrophoresis, MALDI-TOF analysis and IR. The reaction was found to proceed well in the presence and absence of organic co-solvent.

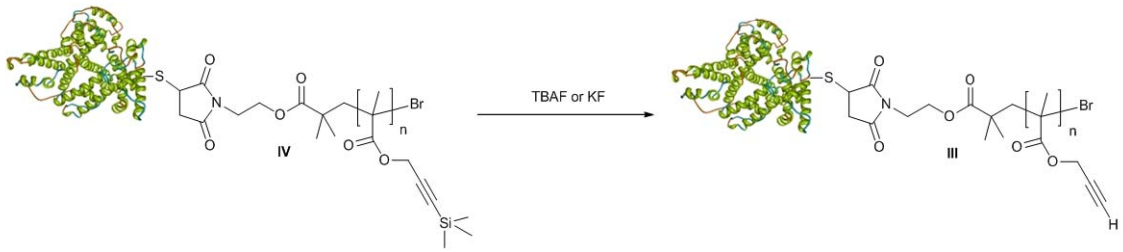
General polymerization procedure with monomer 2 (Route C): The trimethyl-silyl protected alkyne monomer **1** (238 mg, 1.215 mmol) and *N*-(propyl)-2-pyridylmethanimine (~8 mg, 0.057 mmol) were placed in a Schlenk tube and dissolved in 10 mL of 20 mM sodium phosphate buffer pH 7.4. The mixture was de-oxygenated by 5 freeze-pump-thaw cycles and was then canulated in another Schlenk tube containing the previously degassed BSA macroinitiator **I** (~62 mg, 0.93 μmol) and CuBr (4.7 mg, 33 μmol) under N_2 atmosphere. This canulation triggered the beginning of the polymerization (an increase of the opacity in the dark brown colour of the reaction medium was observed). The mixture was stirred under inert atmosphere for *ca.* 8 h upon which time the reaction was quenched with atmospheric oxygen. The reaction mixture was then dialyzed using a 25 kDa MWCO membrane initially against 2% EDTA 10% DMSO 20 mM phosphate buffer and then against 20 mM phosphate buffer pH 7.4. The resulting solutions were analyzed by means of SEC-HPLC, electrophoresis, MALDI-TOF analysis and IR.

BSA digestion: Samples of the polymerization mixture (400 μL of both non-purified crude polymerization mixtures and purified bioconjugate solutions obtained after extensive dialysis) were incubated at 80°C with 6 N HCl for *ca.* 8 hrs. The resulting mixtures were neutralized and polymers were then extracted in CH_2Cl_2 and analyzed by MALDI-TOF. No polymer was detected in the digested control experiment samples. The results of the digestion of crude and dialyzed bioconjugate solutions were in full agreement.

General polymerization procedure in the presence of carboxyfluorescein: 3.2 mg of carboxy fluorescein (0.01 mmol) were added in the polymerization feed under the general polymerization conditions described above.

BSA promoted hydrolysis.[5] The standard assay for the BSA-catalyzed rearrangement of 5-nitrobenzisoxazole was performed by measuring spectrophotometrically the absorbance increase at 380 nm as a function of time. The reaction was initiated by the addition of 30 μL of the BSA-conjugate solution in 970 μL solution containing 0.96 mM substrate (5-nitrobenzisoxazole) in 40 mM phosphate, 100 mM NaCl, pH 7.4.
All samples were found to be positive for hydrolytic activity nevertheless, their activity could not be directly compared due to background scattering. Representative data are presented in the manuscript.

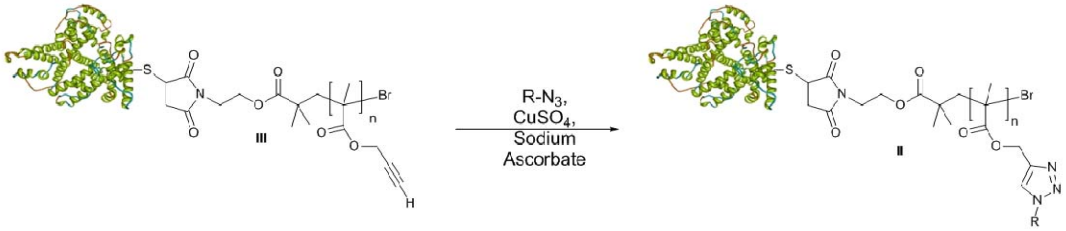
Deprotection of IV to afford III:



BSA-pPA IV deprotection with KF: 465 µL of a 1 M solution of KF in 20 mM sodium phosphate buffer pH 7.4 were added to a solution of BSA-pPA **IV** (2.5 mL, C~$9.3 \cdot 10^{-5}$ M in 20 mM sodium phosphate buffer pH 7.4) . The reaction mixture was gently stirred for 24 hours at 7°C and then purified by dialysis (MWCO 10 kDa) against 20 mM sodium phosphate buffer pH 7.4 before analysis by SEC-HPLC, electrophoresis, MALDI-TOF, TEM and CFM microscopy.

BSA-pPA IV deprotection with TBAF: 121.6 mg of TBAF were added to a solution of BSA-pPA **IV** (3 mL, C~$9.3 \cdot 10^{-5}$ M in 20 mM sodium phosphate buffer pH 7.4). The reaction mixture was gently stirred for 24 hours at 7°C and then dialyzed against 20 mM sodium phosphate buffer pH 7.4 before analysis by SEC, electrophoresis, MALDI-TOF, TEM and CFM microscopy.

Standard reaction for the [3+2] Huisgen catalyzed cycloaddition (*multi-click*) of azides with the BSA-Polyalkyne III:



20 mg of benzyl azide or 26 mg of triethylene glycol mono-azide (0.1488 mmol, 2000 equiv.) were added to a solution of the BSA-Polyalkyne (0.8 mL, C~$9.3 \cdot 10^{-5}$ M) in 20 mM phosphate buffer pH 7.4. $CuSO_4$ (56 µL of a 0.1 M solution in 20 mM sodium phosphate buffer pH 7.4) and sodium ascorbate (56 µL of a 0.2 M solution in 20 mM sodium phosphate buffer pH 7.4) were successively added and the reaction mixture was gently stirred for 2 days at 7°C. The mixture was dialyzed with Microcon 10 kDa dialysis cups against 20 mM sodium phosphate buffer pH 7.4 and analyzed by SEC, MALDI-TOF and elctrophoresis while aggregation was studied by TEM and CFM microscopy.

References

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