Supplementary Information

Precision Anisotropic Brush Polymers by Sequence Controlled Chemistry

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I. Experimental

1.1. Materials

Human serum albumin (HSA, 96%), tris(2-carboxyethyl) phosphine hydrochloride (TCEP, ≥ 98%), 2-bromoisobutanoic acid *N*-hydroxysuccinimide ester (NHS-BiB, 98%), *O*-[(*N*-succinimidyl)succinyl-aminoethyl]-*O′*-methylpolyethylene glycol (NHS-PEG, M_n ~ 2000), *N*-(3-Dimethylaminopropyl)-*N′*-ethylcarbodiimide hydrochloride (EDC·HCl, ≥ 98%), *N*-(2-aminoethyl)maleimide trifluoroacetate salt (MI-NH_2, 95%), trypsin (Type I from bovine pancreas, ~10,000 BAEE units/mg protein), tris(2-pyridylmethyl)amine (TPMA, 98%), copper(II) bromide ($CuBr_2$, 99%), and L-ascorbic acid (≥ 99%) were purchased from Sigma-Aldrich and used without further treatment. Ethylendiamine (> 99%), ethylenediaminetetraacetic acid (EDTA, 98%) and urea (99.5%) were purchased from Acros Organics and used as received. Oligo(ethylene glycol) methyl ether methacrylate (MOEGMA, Sigma-Aldrich, M_n ~300) was purified by passing a basic alumina column before use. Alexa Fluor 647 C_2 maleimide (AF647-MI) and Alexa Fluor™ 647 NHS ester (Succinimidyl ester) (AF647-NHS, 1250 g mol^{-1}) were purchased from Thermo Fisher Scientific and used under dark. Poly(ethylene glycol) [N-(2-maleimidoethyl)carbamoyl]methyl ether 2-(biotinylamino)ethane (biotin-PEG-maleimide or biotin-PEG-MI, M_n ~ 5400) was obtained from Sigma-Aldrich. Streptavidin (SA, buffer composition after restoration: 20 mM potassium phosphate, pH 6.5; Specific activity: 20.6 μg/mg protein), Alexa Fluor™ 568 labeled streptavidin (AF568-SA), and Alexa Fluor™ 647 labeled streptavidin (AF647-SA) were purchased from Thermo Fisher Scientific and used as received. Streptavidin-conjugated gold nanoparticles (Au-SA, particle size 5 nm, concentration 0.5 mg mL^{-1}, 2.5×10^{14} particles/mL) were purchased from NANOCS. Biotinylated somatostatin (Biotin-SST) was synthesized according to the literature.[1] Dulbecco's Modified Eagle's Medium (DMEM) high glucose, fetal bovine serum, penicillin/streptomycin were purchased from GE Healthcare. All other solvents and salts were obtained from commercial suppliers and used as received.

1.2. Preparation of protein-templated brush polymers (brush-1 to brush-4)

1.2.1. Synthesis of cationic HSA (cHSA)

HSA (150 mg, 2.26 μmol) was dissolved in 15 mL of degassed ethylenediamine solution (2.5 M) and the pH was tuned to 4.75 by using HCl. After adding EDC·HCl (4 mmol, 766 mg) and stirring for two hours at room temperature, acetate buffer (1 mL, 4 M, pH 4.75) was added to terminate the reaction. The obtained reaction solution was purified twice with acetate buffer (100 mM, pH 4.75) and thrice with deionized water by ultracentrifugation using a Vivaspin 20 concentrator (MWCO 30 kDa). The resulting solution was lyophilized to afford the product as a white fluffy solid (154 mg, yield: 94%, MALDI-ToF MS: 72.3 kDa).

1.2.2. Synthesis of PEGylated cHSA (PEG-cHSA)

cHSA (100 mg, 1.4 μmol) was firstly dissoleved in degassed phosphate buffer (30 mL, 50 mM, pH 8.0). NHS-PEG (105 mg, 52.4 μmol) was dissolved in 0.4 mL of DMSO and then added into the cHSA solution. After stirring at room temperature for four hours, the reaction solution was purified with deionized water for five times by ultracentrifugation using a Vivaspin 20 concentrator (MWCO 30 kDa). The resulting solution was lyophilized to obtain the product as a white fluffy solid (151 mg, yield: 80 %). The MALDI-ToF MS indicates a molecular weight of 136.6 kDa which means on average 32 PEG chains were conjugated into each cHSA backbone.

1.2.3. Synthesis of denatured PEG-cHSA (PEG-dcHSA)

Preparation of the urea-phosphate buffer (urea-PB)

Urea (150.15 g, 2.5 mol), EDTA (292.24 mg, 1 mmol), $Na_2HPO_4·7H_2O$ (5.4276 g, 25 mmol) and NaH_2PO_4 (0.5699 g, 25 mmol) were dissolved in 0.5 L of deionized water. The urea-PB with 50 mM phosphate buffer, 5 M urea and 2 mM EDTA was then obtained by adjusting the pH to 7.4.

Synthesis of PEG-dcHSA

To a 50 mL flask, 20 mL of urea-PB was added and then degassed via bubbling for five minutes. Followed PEG-cHSA (27.2 mg, 200 nmol) was dissolved and further stirred for 15 min. TCEP (5.8 mg, 20 μmol) was added and stirred for 30 min under argon flow. Lastly, MI-NH_2 (15.24 mg, 60 μmol) was added and stirred overnight under argon protection. The obtained reaction solution was purified with urea-PB for three times and with deionized water for five times by ultracentrifugation using a Vivaspin 20 concentrator (MWCO 30 kDa). The resulting solution was lyophilized to afford the product as a white fluffy solid (24.1 mg, yield: 86%, MALDI-ToF MS: 140.4 kDa).

1.2.4. Synthesis of macroinitiators PEG-dcHSA-Br_{39} and PEG-dcHSA-Br_{61}

PEG-dcHSA-Br_{39} and PEG-dcHSA-Br_{61} were synthesized by attaching different amounts of ATRP initiators to PEG-dcHSA. In a typical experiment for the synthesis of PEG-dcHSA-Br_{39}, PEG-dcHSA (5.0 mg, 36 nmol) was dissolved in 10 mL of $NaHCO_3$ (0.1 M, pH 8.5). NHS-BiB (15.3 mg, 0.058 mmol) dissolved in 1 mL of DMSO was then added dropwise into the PEG-dcHSA solution. The reaction solution was stirred overnight at room temperature and purified with deionized water for eight times by ultracentrifugation using a Vivaspin 20 concentrator (MWCO 50 kDa). The product was obtained as a white fluffy solid after lyophilizing (4.3 mg, yield: 83%, MALDI-ToF MS: 146.2 kDa).

1.2.5. Synthesis of brush-1 to brush-4

Preparation of the stock solution of $CuBr_2$/TPMA catalyst

$CuBr_2$ (4.47 mg, 0.02 mmol) and TPMA (46.5 mg, 0.16 mmol) was dissolved in 1 mL mixture solution of water:DMF (1:1 v/v) and stored at 4 °C prior to use. Therefore, the concentration of the effective Cu^{2+} in the stock solution was 20 nmol μL^{-1}.

Preparation of the ascorbic acid solution

To a 10 mL Schlenk flask, L-ascorbic acid (4.4 mg, 25 μmol) was firstly added under argon flow. Degassed deionized water (5 mL) was then added to dissolve L-ascorbic acid. The solution was then stirred under argon flow for 40 min. Therefore, the concentration of L-ascorbic acid was 5 nmol μL^{-1}.

Atom-transfer radical polymerization (ATRP)

Protein-templated brush polymers (**brush-1** to **brush-4**) were synthesized via activator regenerated by electron transfer (ARGET) ATRP under different conditions. As a typical protocol, PEG-dcHSA-Br_{61} (0.449 mg, 3 nmol) was firstly dissolved in 0.449 mL of deionized water in a 5 mL flask. MOEGMA (52.3 μL, 183 μmol), the $CuBr_2$/TPMA catalyst solution (11 μL, 220 nmol of Cu^{2+}) and 0.5 mL of deionized water were then added. Subsequently, the mixture solution was degassed through three freeze-pump-thaw cycles and the flask was refilled with argon. The L-ascorbic acid solution was then added with a syringe pump at a speed of 0.6 $\mu L\ min^{-1}$ at room temperature for two hours. The brush polymer solution was purified with deionized water for five times by ultracentrifugation using a Vivaspin 6 concentrator (MWCO 30 kDa).

1.3. Preparation of the protein-templated brush polymer site-specifically functionalized with a dye (brush-5)

1.3.1. Synthesis of fluorescent HSA (f-HSA) via site-specific labeling

The site-specific labelling of HSA with a dye was conducted under dark. HSA (10 mg, 0.15 μmol) was dissolved in 10 mL of degassed PBS (pH 6.5). Alexa Fluor 647 C_2 maleimide (AF647-MI, 0.465 mg, 0.37 μmol) dissolved in 30 μL of DMSO was then added dropwise and the reaction was allowed to proceed under dark for 18 h at room temperature. The reaction solution was washed with deionized water by ultracentrifugation using a Vivaspin 6 concentrator (MWCO 30 kDa) for several times until no color was observed in the filtered

solution. The resultant solution was lyophilized to afford the product as a light blue fluffy solid (8.3 mg, yield: 76%, MALDI-ToF MS: 67.2 kDa).

1.3.2. Synthesis of cationic f-HSA (f-cHSA)

f-HSA (8.1 mg, 0.12 μmol) was dissolved in 1 mL of degassed ethylenediamine solution (2.5 M) and the pH was tuned to 4.75 by using HCl. EDC·HCl (0.24 mmol, 45 mg) was then added. After stirring for two hours at room temperature, acetate buffer (0.1 mL, 4 M, pH 4.75) was added to terminate the reaction. The obtained reaction solution was purified twice with acetate buffer (100 mM, pH 4.75) and thrice with deionized water by ultracentrifugation using a Vivaspin 6 concentrator (MWCO 30 kDa). The resulting solution was lyophilized to afford the product as a light blue fluffy solid (8.4 mg, yield: 96%, MALDI-ToF MS: 72.5 kDa).

1.3.3. Synthesis of PEGylated f-cHSA (f-PEG-cHSA)

f-cHSA (8.1 mg, 0.11 μmol) was firstly dissoleved in 8 mL of degassed phosphate buffer (50 mM, pH 8.0). NHS-PEG (6.9 mg, 3.45 μmol) was dissolved in 0.1 mL of DMSO and then added into the cHSA solution. After stirring overnight at room temperature, the reaction solution was purified with deionized water for eight times by ultracentrifugation using a Vivaspin 6 concentrator (MWCO 30 kDa). The resulting solution was lyophilized to obtain the product as a light blue fluffy solid (11.6 mg, yield: 96%). The MALDI-ToF MS indicates a molecular weight of 107.9 kDa which means on average 18 PEG chains were conjugated into each f-cHSA backbone.

1.3.4. Synthesis of denatured f-PEG-cHSA (f-PEG-dcHSA)

The reaction and purification were conducted under dark. To a 25 mL flask, 10 mL of urea-PB (50 mM phosphate buffer, 5 M urea and 2 mM EDTA, pH 7.4) was added and then degassed via bubbling for ten minutes. f-PEG-cHSA (11.3 mg, 105 nmol) was then dissolved and stirred for 15 min. Subsequently, TCEP (2.9 mg, 10 μmol) was added and stirred for 30 min under argon flow. Lastly, MI-NH_2 (7.62 mg, 30 μmol) was added and stirred overnight under argon

atmosphere. The obtained reaction solution was purified with urea-PB for three times and with deionized water for five times by ultracentrifugation using a Vivaspin 6 concentrator (MWCO 30 kDa). The product was obtained as a light blue fluffy solid after lyophilizing (11.6 mg, yield: 99%, MALDI-ToF MS: 110.9 kDa).

1.3.5. Synthesis of the fluorescent macroinitiator f-PEG-dcHSA-Br

The reaction and purification were conducted under dark. f-PEG-dcHSA (11.5 mg, 104 nmol) was dissolved in 15 mL of $NaHCO_3$ (0.1 M, pH 8.5). NHS-BiB (147 mg, 0.56 mmol) dissolved in 1.5 mL of DMSO was then added dropwise into the f-PEG-dcHSA solution. The reaction solution was stirred overnight at room temperature and then washed with deionized water for eight times by ultracentrifugation using a Vivaspin 20 concentrator (MWCO 30 kDa). The purified solution was then lyophilized to afford the product as a light blue fluffy solid (11.8 mg, yield: 91%, MALDI-ToF MS: 125.2 kDa).

1.3.6. Synthesis of brush-5

The brush polymer site-specifically functionalized with AF647 (**brush-5**) was synthesized by ARGET ATRP under dark. Briefly, f-PEG-dcHSA-Br (0.48 mg, 3.83 nmol) was firstly dissolved in 0.48 mL of deionized water in a 5 mL flask. MOEGMA (80 μL, 280 μmol), $CuBr_2$/TPMA catalyst solution (10 μL, 200 nmol of Cu^{2+}) and 0.5 mL of deionized water were then added. The mixture solution was then degassed through three freeze-pump-thaw cycles and the flask was refilled with argon. The L-ascorbic acid solution was then added with a syringe pump at a speed of 0.6 μL min^{-1} at room temperature for two hours. The fluorescent brush polymer was purified with deionized water for five times by ultracentrifugation using a Vivaspin 6 concentrator (MWCO 30 kDa).

1.4. Preparation of protein-templated brush polymers site-specifically functionalized with biotin (biotin-brush-6 and biotin-brush-7) and their assembly

1.4.1. Synthesis of biotin-functionalized HSA (biotin-HSA) via site-specific conjugation

HSA (100 mg, 1.5 μmol) was dissolved in 50 mL of degassed PBS (pH 5.6). Poly(ethylene glycol) [*N*-(2-maleimidoethyl) carbamoyl] methyl ether 2-(biotinylamino) ethane (biotin-PEG-MI, 10.5 mg, 1.95 μmol) dissolved in 0.3 mL of DMSO was added dropwise into the HSA solution and the reaction was allowed to proceed for 15 h at room temperature. The reaction solution was washed with deionized water by ultracentrifugation using a Vivaspin 20 concentrator (MWCO 30 kDa) for six times to remove unreacted biotin-PEG-MI. The resultant product was intensively purified by a column composed of Thermo Scientific Pierce Monomeric Avidin Agarose to remove unreacted HSA. The product was lyophilized and obtained as a white fluffy solid (6.5 mg, yield: 6%, MALDI-ToF MS: 71.8 kDa).

1.4.2. Synthesis of cationic biotin-HSA (biotin-cHSA)

Biotin-HSA (6 mg, 84 nmol) was dissolved in 1 mL of degassed ethylenediamine solution (2.5 M) and the pH was tuned to 4.75 by using HCl. EDC·HCl (0.15 mmol, 28 mg) was then added. After stirring for two hours at room temperature, acetate buffer (0.1 mL, 4 M, pH 4.75) was added to terminate the reaction. The obtained reaction solution was purified twice with acetate buffer (100 mM, pH 4.75) and thrice with deionized water by ultracentrifugation using a Vivaspin 6 concentrator (MWCO 30 kDa). The resulting solution was lyophilized to afford the product as a white fluffy solid (6.2 mg, yield: 97%, MALDI-ToF MS: 76.6 kDa).

1.4.3. Synthesis of PEGylated biotin-cHSA (biotin-PEG-cHSA)

Biotin-cHSA (6.2 mg, 81 nmol) was firstly dissoleved in 6 mL of degassed phosphate buffer (50 mM, pH 8.0). NHS-PEG (4.8 mg, 2.4 μmol) was dissolved in 0.1 mL of DMSO and then added into the cHSA solution. After stirring overnight at room temperature, the reaction solution was purified with deionized water for eight times by ultracentrifugation using a Vivaspin 6 concentrator (MWCO 30 kDa). The resulting solution was lyophilized to obtain the product as a white fluffy solid (8.4 mg, yield: 94%). The MALDI-ToF MS indicates a molecular weight of

110.6 kDa which means in average 17 PEG chains were conjugated into each Biotin-cHSA backbone.

1.4.4. Synthesis of denatured biotin-PEG-cHSA (biotin-PEG-dcHSA)

To a 15 mL flask, 6 mL of urea-PB (50 mM phosphate buffer, 5 M urea and 2 mM EDTA, pH 7.4) was added and then degassed via bubbling for 15 minutes. Biotin-PEG-cHSA (8.3 mg, 75 nmol) was then dissolved and stirred for 15 min. Subsequently, TCEP (2.15 mg, 7.5 μmol) was added and stirred for 30 min under argon flow. Lastly, MI-NH_2 (5.72 mg, 22.5 μmol) was added and stirred overnight under argon atmosphere. The obtained reaction solution was purified with urea-PB for three times and with deionized water for five times by ultracentrifugation using a Vivaspin 6 concentrator (MWCO 30 kDa). The product was obtained as a white fluffy solid after lyophilizing (8.5 mg, yield: 99%, MALDI-ToF MS: 113.7 kDa).

1.4.5. Synthesis of biotin-functionalized macroinitiator biotin-PEG-dcHSA-Br

Biotin-PEG-dcHSA (8.5 mg, 75 nmol) was dissolved in 10 mL of $NaHCO_3$ (0.1 M, pH 8.5). NHS-BiB (160 mg, 0.61 mmol) dissolved in 1.6 mL of DMSO was then added dropwise into the biotin-PEG-dcHSA solution. The reaction solution was stirred overnight at room temperature and then washed with deionized water for eight times by ultracentrifugation using a Vivaspin 20 concentrator (MWCO 30 kDa). The purified solution was then lyophilized to afford the product as a white fluffy solid (6.3 mg, yield: 64%, MALDI-ToF MS: 131.7 kDa).

1.4.6. Synthesis of biotin-brush-6 and biotin-brush-7

Protein-templated brush polymers site-specifically functionalized with biotin (**biotin-brush-6** and **biotin-brush-7**) were synthesized by ARGET ATRP. In a typical example for the synthesis of **biotin-brush-6**, biotin-PEG-dcHSA-Br (0.53 mg, 4 nmol) was firstly dissolved in 0.53 mL of deionized water in a 5 mL flask. MOEGMA (139.4 μL, 488 μmol), $CuBr_2$/TPMA catalyst solution (14 μL, 280 nmol of Cu^{2+}) and 0.7 mL of deionized water were then added. The mixture solution was then degassed through three freeze-pump-thaw cycles and the flask was refilled with argon. The L-ascorbic acid solution was then added with a syringe pump at a speed of 0.6

μL min^{-1} at room temperature for one hour. The biotin-functionalized brush polymer was purified with deionized water for five times by ultracentrifugation using a Vivaspin 6 concentrator (MWCO 30 kDa).

1.4.7. Assembly with AF647-labeled streptavidin (AF647-SA)

AF647-SA (1.8 mg mL^{-1}, 10 μL) was mixed with **biotin-brush-6** (1 mL, ~ 0.3 nmol) and stirred overnight. The sample was then sent for FCS measurement.

1.4.8. Assembly with streptavidin-conjugated Au nanoparticles (Au-SA)

Streptavidin-conjugated gold nanoparticles (Au-SA, 0.096 mL, 5 nm, 0.5 mg mL^{-1}, 2.5×10^{14} particles mL^{-1}) was mixed with a biotin-containing brush polymer (0.1 mL, 0.04 nmol) with a molar ratio of 1:1 in water. The solution was gently shaken for two hours and then used for TEM characterization.

1.5. Preparation of the protein-templated fluorescent brush polymer site-specifically functionalized with biotin (biotin-brush-8) and its assembly

1.5.1. Synthesis of biotin-functionalized fluorescent macroinitiator biotin-PEG-f-dcHSA-Br

Biotin-PEG-dcHSA-Br (2.74 mg, 20 nmol) was dissolved in 4 mL of $NaHCO_3$ (0.1 M, pH 8.5). AF647-NHS (0.25 mg, 200 nmol) dissolved in 0.1 mL of DMSO was then added dropwise into the biotin-PEG-dcHSA-Br solution. The reaction solution was stirred overnight under dark at room temperature and then washed with deionized water for eight times by ultracentrifugation using a Vivaspin 6 concentrator (MWCO 30 kDa). The purified solution was then lyophilized to afford the product as a blue fluffy solid (2.3 mg, yield: 84 %, MALDI-ToF MS: 132.1 kDa).

1.5.2. Synthesis of biotin-brush-8

The protein-templated fluorescent brush polymer site-specifically functionalized with biotin (**biotin-brush-8**) was synthesized by ARGET ATRP. Briefly, biotin-PEG-f-dcHSA-Br (0.54 mg, 4 nmol) was firstly dissolved in 0.54 mL of deionized water in a 5 mL flask. MOEGMA (139.4 μL, 488 μmol), $CuBr_2$/TPMA catalyst solution (14 μL, 280 nmol of Cu^{2+}) and 0.7 mL of deionized water were then added. The mixture solution was then degassed through three freeze-pump-thaw cycles and the flask was refilled with argon. The L-ascorbic acid solution was then added with a syringe pump at a speed of 0.6 μL min^{-1} at room temperature for one hour. The biotin-functionalized fluorescent brush polymer was purified with deionized water for five times by ultracentrifugation using a Vivaspin 20 concentrator (MWCO 30 kDa).

1.5.3. Assembly with biotinylated somatostatin (biotin-SST) and streptavidin (SA)

SA or AF568-SA (32 μg, 0.6 nmol) was firstly dissolved in Milli-Q water to 1 mg mL^{-1} and then mixed with **biotin-brush-8** (2 mL, 0.3 μM) in a 5 mL tube. After stirring at room temperature for 8 h, biotin-SST (2.964 μL, 1 mg mL^{-1}) was added into the tube, and the mixture was stirred further for 10 h. The final solution was purified with PBS for five times by ultracentrifugation using a Vivaspin 6 concentrator (MWCO 30 kDa) and the final construct concentration was tuned to 2 μM.

1.5.4. Assembly with biotinylated aDEC205 antibody (biotin-aDEC) and streptavidin (SA)

Self-assembly of **biotin-brush-8** with biotin-aDEC was performed by dissolving 0.1 mg (1.8 nmol, 1 equiv) SA in 500 μL phosphate buffer (50 mM, pH 7.4) and subsequent addition of 0.27 mg (1.8 nmol, 1 equiv) biotin-aDEC and 0.78 mg (1.8 nmol, 1 equiv) **biotin-brush-8**. The mixture was incubated for 30 min under shaking and purified by rigorous ultrafiltration using a Vivaspin 500 (MWCO 30kDa, 3 × 500 μL of 50 mM phosphate buffer pH 7.4).

II. Characterization

2.1. Matrix-assisted laser desorption/ionization (MALDI) mass spectrometry

MALDI time-of-flight (MALDI-ToF) mass spectrometry was performed on Bruker rapifleX spectrometer. Saturated solution of sinapinic acid dissolved in a 50:50 water/acetonitrile with 0.2% TFA (trifloroacetic acid) was used as the matrix solution.

2.2. Gel permeation chromatography (GPC)

The molecular weight and molecular weight distribution were determined by GPC. Deionized water containing 0.1 M $NaNO_3$ was used as eluent at a flow rate of 1 mL min^{-1}. Shodex RI 101 detector was used. Linear PEG standards were used for calibration.

2.3. Dynamic light scattering (DLS)

Dilute solutions of the macroinitiator PEG-dcHSA-Br_{61} and brush polymers (**brush-1** to **brush-4**) were prepared in water and filtered through a 450 nm filter (Millipore HA) into optical cells with 20 mm diameter. The experiment was performed on an ALV5000 setup with a 194 mW laser at λ = 532 nm, at different scattering angles θ defining the scattering wave vector $q = \frac{4\pi n}{\lambda}\sin\frac{\theta}{2}$ with n = 1.333 being the water refractive index. The relaxation function, $C(q,t) = [G(q,t_0^{-1})]^{1/2}$ computed from the experimental scattering intensity autocorrelation function $G(q,t)$ was represented either by an inverse Laplace transform (ILT) analysis using the CONTIN algorithmor the stretched exponential function of the form: α

$$C(q,t) = \alpha e^{-(\frac{t}{\tau})^{\beta}} \qquad \text{(S1)}$$

where α is the amplitude, τ the relaxation time and $\beta \leqslant 1$ the stretching parameter. ILT was employed in the case of more than one relaxation process in $C(q,t)$, whereas Equation S1 can amount for the presence of one process deviating from the ideal single exponential shape. In dilute solutions, the relaxation rate $\Gamma(q) = 1/\tau(q)$ is usually diffusive defining the diffusion coefficient $D = \Gamma(q)/q^2$. For species with small size R i.e., both the scattering intensity $I(q)$ and

$D = D_0$ are q-independent with $I \sim cM$ and $D_0 = \frac{k_B T}{6\pi\eta_0 R_h}$ where c, M, R_h, η_0, k_B, and T are the probed species concentration, its molecular weight and hydrodynamic ratio, the solvent viscosity, the Boltzmann constant and the absolute temperature, respectively. For $qR \sim 1$, both $I(q)$ and $D(q)$ depend on q defining the probing length ($2\pi/q$). The former, known as the form factor, yields (at low qR_g) the radius of gyration R_g,

$$I(q)^{-1}=I(0)^{-1}(1+q^2R_g^2/3) \quad \text{(S2)}$$

whereas the effective D is given by,

$$D = D_0(1 + Aq^2) \quad \text{(S3)}$$

with A is a parameter characterizing the shape of the diffusing species.

Equations for prolate ellipsoids (Perrin)[2]

$$R_h = a/f(b/a) \quad \text{(S4)}$$

$$f(b/a) = \frac{\ln\left[\frac{1+(1-b^2/a^2)^{1/2}}{b/a}\right]}{(1-b^2/a^2)^{1/2}} \quad \text{(S5)}$$

for $a > b$ (prolate)

Static scattering:

$$I(q) = \frac{9\pi}{2}\int_0^{\pi/2} \frac{[J_{3/2}(u)]^2}{u^3} cos\beta d\beta \quad \text{(S6)}$$

$$u = qa(cos^2\beta + \frac{b^2}{a^2}sin^2\beta)^{1/2} \quad \text{(S7)}$$

$$J_{\frac{3}{2}}(u) = \left(\frac{2u}{\pi}\right)^{1/2}\left(\frac{sinu}{u^2} - \frac{cosu}{u}\right) \quad \text{(S8)}$$

Equation for Gaussian coils (Pecora)[3]

$$P(x) = \frac{2}{x^4}\left(e^{-x^2} - 1 + x^2\right) \quad \text{(S9)}$$

2.4. Stability test against trypsin

Trypsin was dissolved in deionized water to prepare a 3 mg mL^{-1} solution and then passed through a syringe filter with the pore size of 220 nm. The solution (1 mL) was then mixed with 2 mL of brush polymer solution (0.3 μM) in a clean hood at room temperature. Therefore, the final concentrations of trypsin and the brush polymer were 1 mg mL^{-1} and 0.2 μM, respectively.

DLS was then used to track the size change of the mixture solution immediately and after different time intervals until six hours.

2.5. Cell viability test

A549 mammalian lung adenocarcinoma cells were cultured in standard T-75 flasks using high glucose DMEM fortified with 10% fetal bovine serum, 1% penicillin/streptomycin and 1% MEM non-essential amino acids. The cells were split at near confluency and incubated at 37 °C, 5% CO_2 prior to each experiment.

Pre-cultured A549 cells were seeded (6,500 cells per well) in a white 96-well plate (half area, Greiner BioOne®) and allowed to adhere overnight at 37 °C, 5% CO_2. The brush polymers (**brush-1** to **brush-4**) were prepared at various concentrations (12.5 – 200 mg L^{-1}) in DMEM and introduced as independent replicates for 24 h at 37 °C, 5% CO_2. CellTiter-Glo® (Promega) was employed following manufacturer's protocol and the resultant luminescence was detected using Glomax® Multi 96-well plate reader (Promega). Data is presented in Figure 2D as mean ± SEM, n = 3.

2.6. Transmission electron microscopy (TEM)

TEM samples were prepared by adding 4 μL of the brush polymer solution onto a carbon-coated copper grid. After drying in air for 10 min, the remained solution was removed by a filter paper. The samples were then stained with 2% uranyl acetate solution and dried in air. The measurement was conducted on a JEOL JEM-1400 TEM operating at an accelerating voltage of 120 kV. ImageJ software was used for the analysis of length and width of brush polymer samples.

2.7. Fluorescent spectroscopy

Fluorescent emission spectra were collected using a TECAN (Spark 20M) microplate reader at room temperature. The excitation wavelength was set as 594 nm and the emission wavelength

was monitored from 600 to 800 nm. Excitation and emission bandwidths were both maintained at 20 nm and the emission wavelength step size was 1 nm.

2.8. Fluorescent correlation spectroscopy (FCS)

Fluorescence correlation spectroscopy (FCS) experiments were performed on a commercial confocal microscope, LSM 880 (Carl Zeiss, Jena, Germany) equipped with a C-Apochromat 40x/1.2W water immersion objective. The AF647 molecules and were excited by a HeNe laser (633 nm) fiber-coupled to the microscope. Emitted fluorescence light was collected with the same objective, passed through a confocal pinhole and directed to a spectral detection unit (Quasar, Carl Zeiss). In this unit emission is spectrally separated by a grating element on a 32 channel array of GaAsP detectors operating in a single photon counting mode. In all experiments the emission in the spectral range 640-700 nm was considered. An eight-well polystyrene-chambered coverglass (Laboratory-Tek, Nalge Nunc International, Penfield, NY) was used as a sample cell for the studied solutions. For each sample series of measurements with a total duration of 5 min were performed. The time-dependent fluctuations of the fluorescent intensity $\delta I(t)$ were recorded and analyzed by an autocorrelation function $G(\tau) = 1+<\delta I(t)\delta I(t+\tau)>/<I(t)>^2$. As has been shown theoretically for an ensemble of m different types of freely diffusing identical fluorescence species, $G(\tau)$ has the following analytical form:[4]

$$G(\tau) = 1 + \frac{1}{N}\left[1 + \frac{f_T}{1-f_T}e^{-t/\tau_T}\right]\sum_{i=1}^{m}\frac{F_i}{\left[1+\frac{\tau}{\tau_{Di}}\right]\sqrt{1+\frac{\tau}{S^2\tau_{Di}}}} \qquad \text{(S10)}$$

Here, N is the average number of diffusing fluorescence species in the observation volume, f_T and τ_T are the fraction and the decay time of the triplet state, $S = z_0/r_0$ is the so-called structure parameter with z_0 and r_0 representing the axial and radial dimensions of the confocal volume. F_i is the fraction of the i-th species and τ_{Di} is their diffusion time through the observation volume that is related to their diffusion coefficient, D_i, through $D_i = r_0^2/4\tau_{Di}$. The experimentally obtained $G(\tau)$ were fitted with Equation S10, yielding the corresponding diffusion times and subsequently the diffusion coefficients of the fluorescent species. Finally, the hydrodynamic radii R_h were calculated (assuming spherical particles) using the Stokes-Einstein relation: $R_h =$

$\frac{k_B T}{6\pi\eta D}$ where k_B is the Boltzmann constant, T is the temperature, and η is the viscosity of the solvent. Furthermore, FCS yielded also the fluorescent brightness (*FB)* of the studied species defined as the ratio between the detected average fluorescent intensity and the mean number of fluorescent species in the observation volume, $FB=\langle I(t)\rangle/N$. As the value of r_0 depends strongly on the specific characteristics of the optical setup a calibration was done using the reference value[5] of the AF647 diffusion coefficient 3.3×10^{-10} m^2/s at 25°C.

2.9. Confocal laser scanning microscopy (CLSM)

Cell culture was performed with human lung adenocarcinoma cell line, A549, pre cultured in high glucose DMEM, supplemented with 10% Fetal Bovine Serum, 1% Penicillin/Streptomycin, 1% MEM. Cell passages used within the experiment are between 7-12.

Cells were seeded at a density of 15,000 cells/well in an Ibidi® µ-slide 8-well confocal microscopy chamber and left to adhere for 24 h. Stock solutions, prepared at 2 µM, were diluted with DMEM to a final concentration of 500 nM. From the cells, DMEM was aspirated and the samples were introduced into each individual well. The cells were then incubated for 24 h. Subsequently, the cell nuclei were stained with Hoechst for 20 min before imaging live on a Leica SP5 confocal microscope system.

For the time lapse studies at 2 h, 6 h and 24 h, the cells were seeded identically as above with the samples introduced into the cells in such a way that all three time intervals end at the same time for imaging. Each well was likewise washed and stained with Hoechst for 20 min before imaging live on a Leica SP5 confocal microscope system.

2.10. SDS-PAGE and Western blot

Electrophoresis was carried out using 10% Bis-Tris polyacrylamide resolving gels with 6% Bis-Tris polyacrylamide stacking gel. Sample preparation was performed under denaturing conditions: 3 µL of LDS sample buffer (NuPAGE, Invitrogen, Carlsbad, CA, USA) and 1 µL

of 1 M Dithiothreitol (DTT) where mixed with 8 µL of a 2 µM protein solution and incubated for 10 min at 95 °C prior to gel.

As a reference 2 µL of AppliChem Protein Marker VI® protein ladders were loaded to the gel. The gel was run using 1 × MES SDS Running Buffer (NuPAGE, Invitrogen, Carlsbad, CA, USA) with a constant 150 V for about 50 min. The bioconjugation was confirmed using Western blot analysis and immunoblot detection with a Streptavidin-Horseradish peroxidase (Strep-HRP) conjugate to detect the presence of biotin. Western Blot was performed after SDS-PAGE by blotting on a nitrocellulose membrane using Mini Trans-Blot® Cell Module (Bio-Rad Laboratory-Inc. Hercules, USA). The nitrocellulose membrane was rinsed with MeOH and equilibrated with filter paper, sponges, and gel in a blotting buffer for 10 minutes. A blotting sandwich was prepared according to the transfer charge by stacking a sponge, two filters, gel, the membrane, followed by two filters and a sponge. The transfer was performed using 90 mA overnight at 4 °C, revealing a successful transfer to the membrane, visualized by Ponceau S. Following this, the membrane was blocked using 5 % skim milk in 1 × Tris-buffered saline with Tween20 (TBS-T) for 60 minutes. After washing with TBS-T, the membrane was incubated with Strep-HRP (1:4000 in 5% skim milk/TBST) for one hour. Chemiluminescence was detected using enhanced chemiluminescence (ECL, Western Lightning Plus, PerkinElmer, Waltham, USA).

III. Supplementary Data and Discussion

3.1. The amino acid sequence and solvent accessibility of HSA

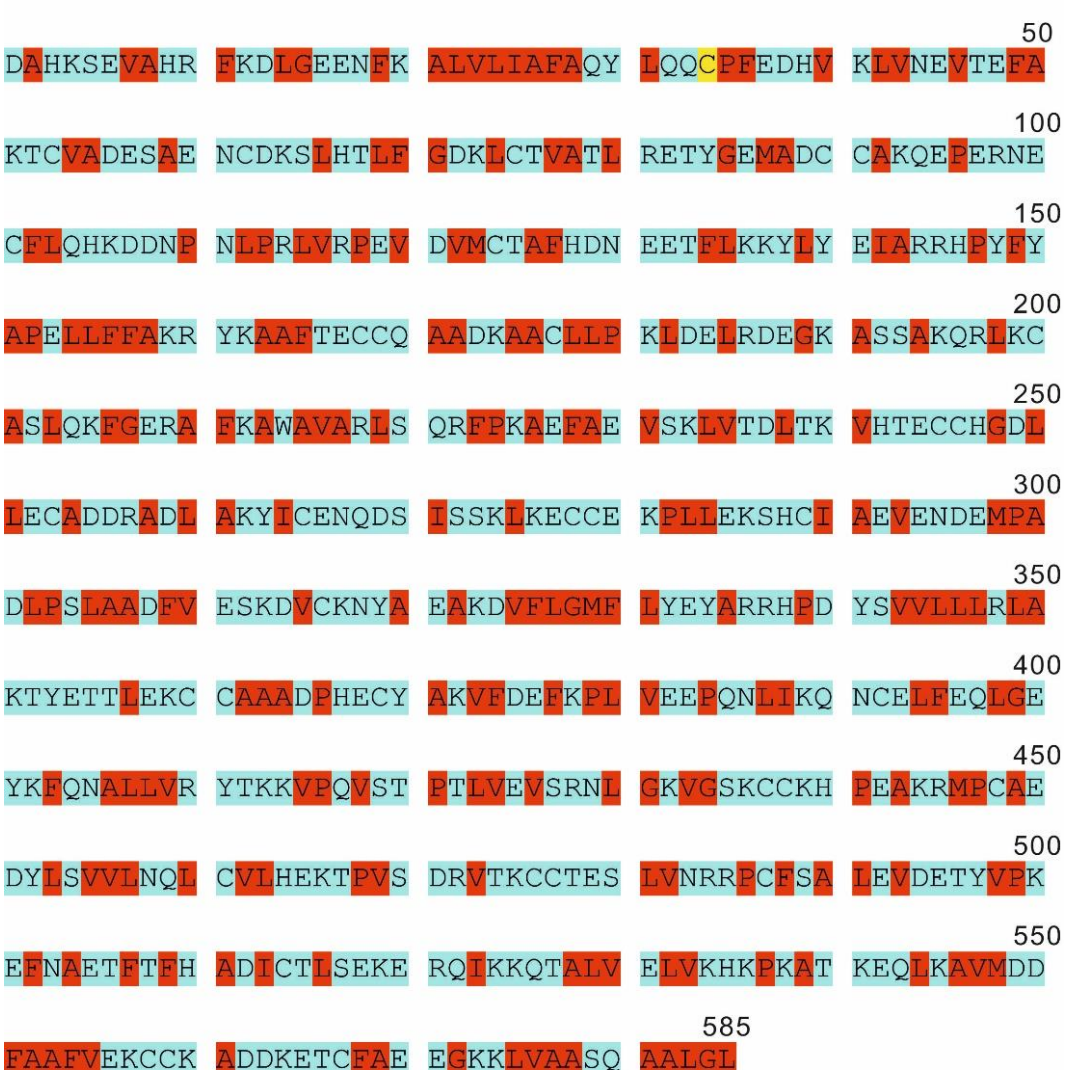

Figure S1. The full amino acid sequence of HSA. The one-letter abbreviations for amino acids are as follows: R: Arginine; K: Lysine; D: Aspartic acid; E: Glutamic acid; Q: Glutamine; N: Asparagine; H: Histidine; S: Serine; T: Threonine; Y: Tyrosine; C: Cysteine; W: Tryptophan; A: Alanine; I: Isoleucine; L: Leucine; M: Methionine; F: Phenylalanine; V: Valine; P: Proline; G: Glycine.

Table S1. Solvent accessibility of HSA (PDB: 1AO6) obtained with Pymol 2.03.

ASP`1 0% | |	LYS`41 54% |===== |	ARG`81 74% |======= |
ALA`2 0% | |	LEU`42 2% | |	GLU`82 99% |========= |
HIS`3 0% | |	VAL`43 6% | |	THR`83 82% |======== |
LYS`4 0% | |	ASN`44 48% |==== |	TYR`84 11% |= |
SER`5 23% |== |	GLU`45 32% |=== |	GLY`85 41% |==== |
GLU`6 35% |=== |	VAL`46 6% | |	GLU`86 73% |======= |
VAL`7 1% | |	THR`47 4% | |	MET`87 15% |= |
ALA`8 1% | |	GLU`48 71% |======= |	ALA`88 18% |= |
HIS`9 51% |===== |	PHE`49 28% |== |	ASP`89 68% |====== |
ARG`10 14% |= |	ALA`50 0% | |	CYS`90 0% | |
PHE`11 11% |= |	LYS`51 44% |==== |	CYS`91 39% |=== |
LYS`12 63% |====== |	THR`52 48% |==== |	ALA`92 78% |======= |
ASP`13 52% |===== |	CYS`53 0% | |	LYS`93 47% |==== |
LEU`14 23% |== |	VAL`54 34% |=== |	GLN`94 79% |======= |
GLY`15 36% |=== |	ALA`55 82% |======== |	GLU`95 31% |=== |
GLU`16 38% |=== |	ASP`56 48% |==== |	PRO`96 73% |======= |
GLU`17 85% |======== |	GLU`57 46% |==== |	GLU`97 54% |===== |
ASN`18 31% |=== |	SER`58 86% |======== |	ARG`98 7% | |
PHE`19 0% | |	ALA`59 35% |=== |	ASN`99 2% | |
LYS`20 34% |=== |	GLU`60 94% |========= |	GLU`100 57% |===== |
ALA`21 16% |= |	ASN`61 33% |=== |	CYS`101 5% | |
LEU`22 1% | |	CYS`62 0% | |	PHE`102 1% | |
VAL`23 0% | |	ASP`63 84% |======== |	LEU`103 16% |= |
LEU`24 5% | |	LYS`64 32% |=== |	GLN`104 75% |======= |
ILE`25 0% | |	SER`65 54% |===== |	HIS`105 27% |== |
ALA`26 10% | |	LEU`66 8% | |	LYS`106 11% |= |
PHE`27 1% | |	HIS`67 18% |= |	ASP`107 26% |== |
ALA`28 1% | |	THR`68 25% |== |	ASP`108 28% |== |
GLN`29 3% | |	LEU`69 14% |= |	ASN`109 51% |===== |
TYR`30 6% | |	PHE`70 10% |= |	PRO`110 20% |= |
LEU`31 5% | |	GLY`71 5% | |	ASN`111 74% |======= |
GLN`32 7% | |	ASP`72 45% |==== |	LEU`112 22% |== |
GLN`33 44% |==== |	LYS`73 26% |== |	PRO`113 79% |======= |
CYS`34 3% | |	LEU`74 2% | |	ARG`114 79% |======= |
PRO`35 46% |==== |	CYS`75 25% |== |	LEU`115 62% |====== |
PHE`36 8% | |	THR`76 59% |===== |	VAL`116 87% |======== |
GLU`37 73% |======= |	VAL`77 19% |= |	ARG`117 38% |=== |
ASP`38 36% |=== |	ALA`78 1% | |	PRO`118 53% |===== |
HIS`39 0% | |	THR`79 59% |===== |	GLU`119 63% |====== |
VAL`40 20% |== |	LEU`80 56% |===== |	VAL`120 12% |= |

ASP`121 41% |==== |	TYR`161 12% |= |	ALA`201 5% | |
VAL`122 50% |==== |	LYS`162 39% |=== |	SER`202 10% |= |
MET`123 13% |= |	ALA`163 42% |==== |	LEU`203 15% |= |
CYS`124 5% | |	ALA`164 5% | |	GLN`204 50% |==== |
THR`125 64% |====== |	PHE`165 0% | |	LYS`205 74% |======= |
ALA`126 43% |==== |	THR`166 44% |==== |	PHE`206 33% |=== |
PHE`127 2% | |	GLU`167 40% |==== |	GLY`207 26% |== |
HIS`128 59% |===== |	CYS`168 0% | |	GLU`208 51% |===== |
ASP`129 85% |======== |	CYS`169 16% |= |	ARG`209 55% |===== |
ASN`130 49% |==== |	GLN`170 70% |====== |	ALA`210 24% |== |
GLU`131 33% |=== |	ALA`171 13% |= |	PHE`211 1% | |
GLU`132 66% |====== |	ALA`172 77% |======= |	LYS`212 35% |=== |
THR`133 41% |==== |	ASP`173 43% |==== |	ALA`213 31% |=== |
PHE`134 16% |= |	LYS`174 29% |== |	TRP`214 30% |=== |
LEU`135 19% |= |	ALA`175 19% |= |	ALA`215 1% | |
LYS`136 28% |== |	ALA`176 40% |==== |	VAL`216 11% |= |
LYS`137 43% |==== |	CYS`177 30% |== |	ALA`217 0% | |
TYR`138 16% |= |	LEU`178 0% | |	ARG`218 20% |== |
LEU`139 9% | |	LEU`179 13% |= |	LEU`219 9% | |
TYR`140 5% | |	PRO`180 57% |===== |	SER`220 0% | |
GLU`141 18% |= |	LYS`181 32% |=== |	GLN`221 2% | |
ILE`142 27% |== |	LEU`182 10% | |	ARG`222 16% |= |
ALA`143 0% | |	ASP`183 29% |== |	PHE`223 3% | |
ARG`144 12% |= |	GLU`184 63% |====== |	PRO`224 3% | |
ARG`145 29% |== |	LEU`185 6% | |	LYS`225 49% |==== |
HIS`146 29% |== |	ARG`186 60% |===== |	ALA`226 1% | |
PRO`147 2% | |	ASP`187 60% |===== |	GLU`227 71% |======= |
TYR`148 14% |= |	GLU`188 31% |=== |	PHE`228 20% |= |
PHE`149 3% | |	GLY`189 12% |= |	ALA`229 66% |====== |
TYR`150 9% | |	LYS`190 39% |=== |	GLU`230 15% |= |
ALA`151 0% | |	ALA`191 38% |=== |	VAL`231 0% | |
PRO`152 7% | |	SER`192 20% |== |	SER`232 28% |== |
GLU`153 5% | |	SER`193 13% |= |	LYS`233 41% |==== |
LEU`154 1% | |	ALA`194 27% |== |	LEU`234 2% | |
LEU`155 11% |= |	LYS`195 45% |==== |	VAL`235 3% | |
PHE`156 8% | |	GLN`196 5% | |	THR`236 54% |===== |
PHE`157 2% | |	ARG`197 14% |= |	ASP`237 30% |=== |
ALA`158 12% |= |	LEU`198 20% |= |	LEU`238 18% |= |
LYS`159 39% |=== |	LYS`199 17% |= |	THR`239 5% | |
ARG`160 30% |== |	CYS`200 4% | |	LYS`240 54% |===== |

VAL`241 1% | |	LYS`281 55% |===== |	GLU`321 69% |====== |
HIS`242 2% | |	PRO`282 62% |====== |	ALA`322 47% |==== |
THR`243 39% |=== |	LEU`283 50% |===== |	LYS`323 46% |==== |
GLU`244 14% |= |	LEU`284 18% |= |	ASP`324 59% |===== |
CYS`245 11% |= |	GLU`285 28% |== |	VAL`325 51% |===== |
CYS`246 2% | |	LYS`286 20% |= |	PHE`326 6% | |
HIS`247 38% |=== |	SER`287 9% | |	LEU`327 14% |= |
GLY`248 17% |= |	HIS`288 20% |= |	GLY`328 16% |= |
ASP`249 16% |= |	CYS`289 28% |== |	MET`329 36% |=== |
LEU`250 5% | |	ILE`290 8% | |	PHE`330 1% | |
LEU`251 3% | |	ALA`291 39% |=== |	LEU`331 5% | |
GLU`252 18% |= |	GLU`292 53% |===== |	TYR`332 7% | |
CYS`253 0% | |	VAL`293 18% |= |	GLU`333 22% |== |
ALA`254 14% |= |	GLU`294 76% |======= |	TYR`334 6% | |
ASP`255 6% | |	ASN`295 40% |=== |	ALA`335 0% | |
ASP`256 24% |== |	ASP`296 10% | |	ARG`336 8% | |
ARG`257 10% |= |	GLU`297 69% |====== |	ARG`337 20% |= |
ALA`258 25% |== |	MET`298 51% |===== |	HIS`338 24% |== |
ASP`259 53% |===== |	PRO`299 26% |== |	PRO`339 35% |=== |
LEU`260 11% |= |	ALA`300 95% |========= |	ASP`340 45% |==== |
ALA`261 5% | |	ASP`301 96% |========= |	TYR`341 12% |= |
LYS`262 54% |===== |	LEU`302 29% |== |	SER`342 0% | |
TYR`263 27% |== |	PRO`303 67% |====== |	VAL`343 1% | |
ILE`264 7% | |	SER`304 71% |======= |	VAL`344 8% | |
CYS`265 24% |== |	LEU`305 11% |= |	LEU`345 0% | |
GLU`266 69% |====== |	ALA`306 22% |== |	LEU`346 1% | |
ASN`267 32% |=== |	ALA`307 44% |==== |	LEU`347 11% |= |
GLN`268 21% |== |	ASP`308 65% |====== |	ARG`348 5% | |
ASP`269 95% |========= |	PHE`309 7% | |	LEU`349 0% | |
SER`270 57% |===== |	VAL`310 15% |= |	ALA`350 19% |= |
ILE`271 2% | |	GLU`311 74% |======= |	LYS`351 56% |===== |
SER`272 10% | |	SER`312 35% |=== |	THR`352 19% |= |
SER`273 63% |====== |	LYS`313 77% |======= |	TYR`353 0% | |
LYS`274 23% |== |	ASP`314 57% |===== |	GLU`354 13% |= |
LEU`275 0% | |	VAL`315 1% | |	THR`355 49% |==== |
LYS`276 59% |===== |	CYS`316 29% |== |	THR`356 11% |= |
GLU`277 72% |======= |	LYS`317 65% |====== |	LEU`357 1% | |
CYS`278 3% | |	ASN`318 53% |===== |	GLU`358 58% |===== |
CYS`279 29% |== |	TYR`319 6% | |	LYS`359 76% |======= |
GLU`280 83% |======== |	ALA`320 78% |======= |	CYS`360 2% | |

CYS`361 43% |==== |	TYR`401 28% |== |	PRO`441 63% |====== |
ALA`362 82% |======== |	LYS`402 55% |===== |	GLU`442 82% |======== |
ALA`363 44% |==== |	PHE`403 0% | |	ALA`443 56% |===== |
ALA`364 96% |========= |	GLN`404 2% | |	LYS`444 51% |===== |
ASP`365 71% |======= |	ASN`405 11% |= |	ARG`445 20% |= |
PRO`366 19% |= |	ALA`406 34% |=== |	MET`446 6% | |
HIS`367 46% |==== |	LEU`407 5% | |	PRO`447 20% |== |
GLU`368 84% |======== |	LEU`408 0% | |	CYS`448 42% |==== |
CYS`369 47% |==== |	VAL`409 20% |= |	ALA`449 4% | |
TYR`370 1% | |	ARG`410 34% |=== |	GLU`450 0% | |
ALA`371 60% |===== |	TYR`411 10% |= |	ASP`451 20% |== |
LYS`372 61% |====== |	THR`412 1% | |	TYR`452 19% |= |
VAL`373 2% | |	LYS`413 23% |== |	LEU`453 13% |= |
PHE`374 32% |=== |	LYS`414 7% | |	SER`454 19% |= |
ASP`375 61% |====== |	VAL`415 4% | |	VAL`455 15% |= |
GLU`376 52% |===== |	PRO`416 6% | |	VAL`456 3% | |
PHE`377 2% | |	GLN`417 35% |=== |	LEU`457 3% | |
LYS`378 62% |====== |	VAL`418 6% | |	ASN`458 2% | |
PRO`379 59% |===== |	SER`419 56% |===== |	GLN`459 20% |== |
LEU`380 27% |== |	THR`420 10% | |	LEU`460 2% | |
VAL`381 12% |= |	PRO`421 68% |====== |	CYS`461 11% |= |
GLU`382 50% |==== |	THR`422 12% |= |	VAL`462 10% | |
GLU`383 44% |==== |	LEU`423 3% | |	LEU`463 14% |= |
PRO`384 0% | |	VAL`424 3% | |	HIS`464 8% | |
GLN`385 47% |==== |	GLU`425 41% |==== |	GLU`465 55% |===== |
ASN`386 51% |===== |	VAL`426 4% | |	LYS`466 49% |==== |
LEU`387 15% |= |	SER`427 1% | |	THR`467 70% |======= |
ILE`388 8% | |	ARG`428 18% |= |	PRO`468 66% |====== |
LYS`389 56% |===== |	ASN`429 15% |= |	VAL`469 53% |===== |
GLN`390 67% |====== |	LEU`430 6% | |	SER`470 4% | |
ASN`391 17% |= |	GLY`431 0% | |	ASP`471 80% |======= |
CYS`392 7% | |	LYS`432 30% |== |	ARG`472 24% |== |
GLU`393 57% |===== |	VAL`433 12% |= |	VAL`473 0% | |
LEU`394 32% |=== |	GLY`434 2% | |	THR`474 41% |==== |
PHE`395 18% |= |	SER`435 43% |==== |	LYS`475 54% |===== |
GLU`396 74% |======= |	LYS`436 50% |===== |	CYS`476 2% | |
GLN`397 71% |======= |	CYS`437 1% | |	CYS`477 12% |= |
LEU`398 18% |= |	CYS`438 17% |= |	THR`478 69% |====== |
GLY`399 43% |==== |	LYS`439 81% |======== |	GLU`479 77% |======= |
GLU`400 41% |==== |	HIS`440 31% |=== |	SER`480 32% |=== |

LEU`481 23% |== |	ARG`521 6% | |	ALA`561 50% |===== |
VAL`482 23% |== |	GLN`522 24% |== |	ASP`562 85% |======== |
ASN`483 44% |==== |	ILE`523 37% |=== |	ASP`563 34% |=== |
ARG`484 9% | |	LYS`524 51% |===== |	LYS`564 86% |======== |
ARG`485 5% | |	LYS`525 6% | |	GLU`565 68% |====== |
PRO`486 22% |== |	GLN`526 0% | |	THR`566 48% |==== |
CYS`487 38% |=== |	THR`527 33% |=== |	CYS`567 27% |== |
PHE`488 0% | |	ALA`528 4% | |	PHE`568 1% | |
SER`489 26% |== |	LEU`529 4% | |	ALA`569 52% |===== |
ALA`490 74% |======= |	VAL`530 2% | |	GLU`570 60% |====== |
LEU`491 9% | |	GLU`531 25% |== |	GLU`571 39% |=== |
GLU`492 76% |======= |	LEU`532 10% | |	GLY`572 10% | |
VAL`493 49% |==== |	VAL`533 2% | |	LYS`573 69% |====== |
ASP`494 14% |= |	LYS`534 10% | |	LYS`574 77% |======= |
GLU`495 80% |======= |	HIS`535 21% |== |	LEU`575 22% |== |
THR`496 80% |======== |	LYS`536 23% |== |	VAL`576 16% |= |
TYR`497 24% |== |	PRO`537 21% |== |	ALA`577 57% |===== |
VAL`498 95% |========= |	LYS`538 96% |========= |	ALA`578 55% |===== |
PRO`499 47% |==== |	ALA`539 58% |===== |	SER`579 17% |= |
LYS`500 72% |======= |	THR`540 19% |= |	GLN`580 62% |====== |
GLU`501 82% |======== |	LYS`541 83% |======== |	ALA`581 78% |======= |
PHE`502 38% |=== |	GLU`542 79% |======= |	ALA`582 35% |=== |
ASN`503 45% |==== |	GLN`543 39% |=== |	LEU`583 0% | |
ALA`504 60% |===== |	LEU`544 6% | |	GLY`584 0% | |
GLU`505 87% |======== |	LYS`545 52% |===== |	LEU`585 0% | |
THR`506 64% |====== |	ALA`546 54% |===== |	
PHE`507 3% | |	VAL`547 18% |= |	
THR`508 64% |====== |	MET`548 8% | |	
PHE`509 9% | |	ASP`549 61% |====== |	
HIS`510 63% |====== |	ASP`550 46% |==== |	
ALA`511 28% |== |	PHE`551 2% | |	
ASP`512 58% |===== |	ALA`552 26% |== |	
ILE`513 5% | |	ALA`553 39% |=== |	
CYS`514 32% |=== |	PHE`554 3% | |	
THR`515 37% |=== |	VAL`555 2% | |	
LEU`516 30% |== |	GLU`556 52% |===== |	
SER`517 39% |=== |	LYS`557 66% |====== |	
GLU`518 45% |==== |	CYS`558 4% | |	
LYS`519 60% |===== |	CYS`559 49% |==== |	
GLU`520 23% |== |	LYS`560 65% |====== |	

3.2. Synthesis and characterization of brush-1 to brush-4

3.2.1. MALDI-ToF mass spectra

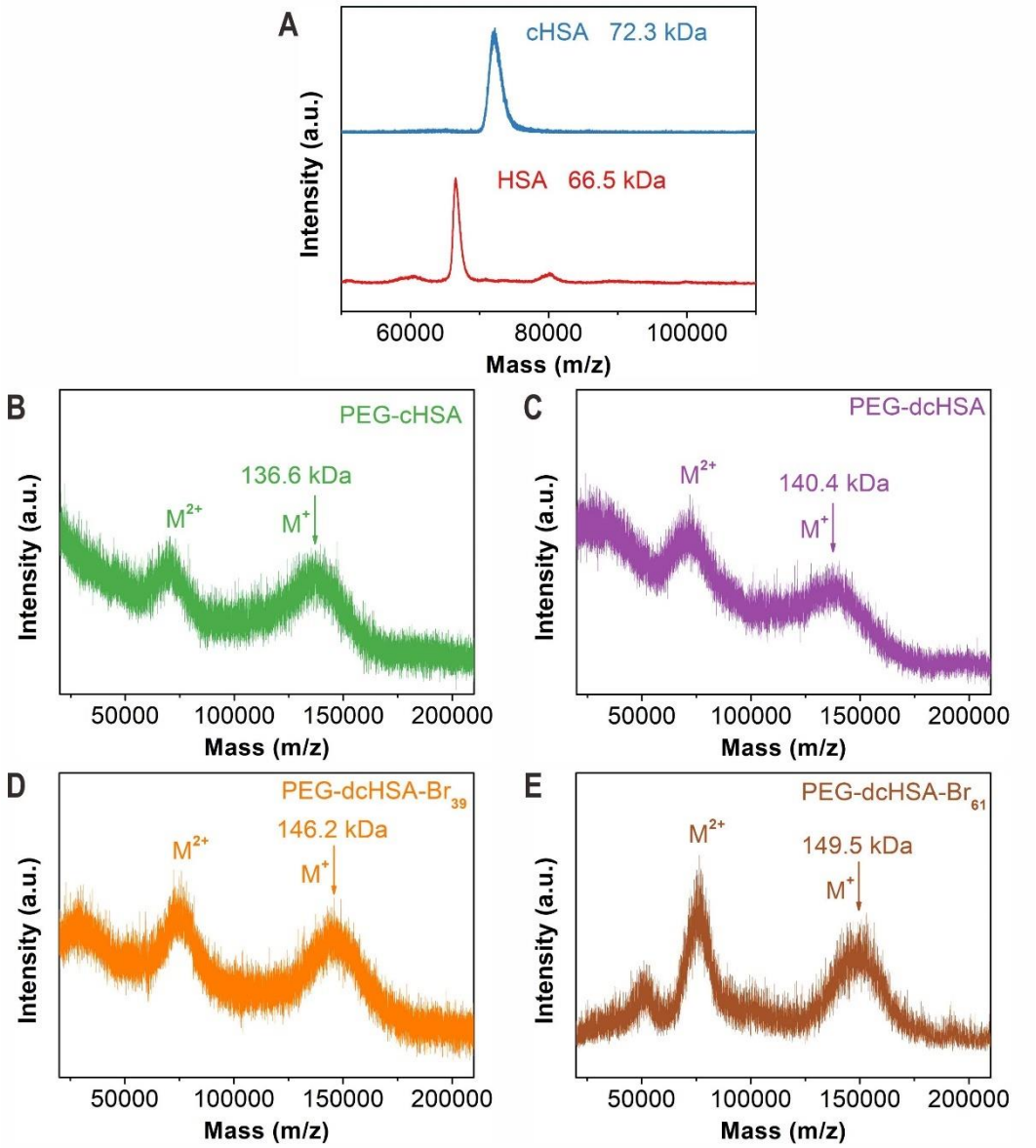

Figure S2. MALDI-ToF mass spectra of (A) HSA and cHSA, (B) PEG-cHSA, (C) PEG-dcHSA, as well as two macroinitiators (D) PEG-dcHSA-Br$_{39}$ and (E) PEG-dcHSA-Br$_{61}$.

3.2.2. Stability of brush-1 to brush-4

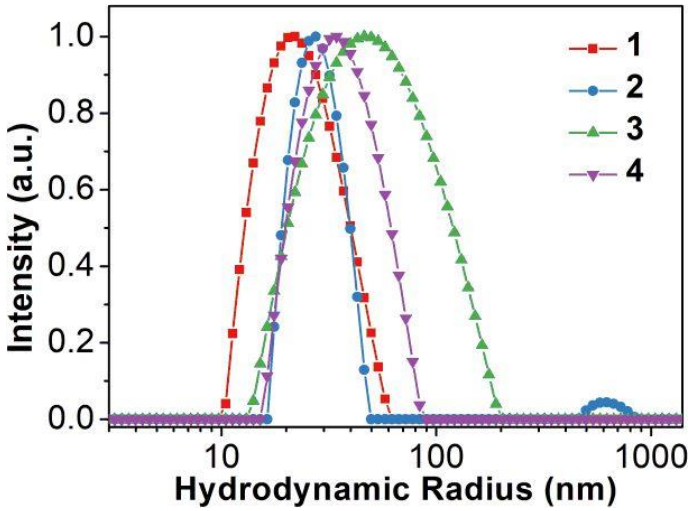

Figure S3. Size distribution of **brush-1** to **brush-4** after storage at 4 °C for two months measured by DLS.

As shown in Figure S3, the sizes of brush polymers (**brush-1** to **brush-4**) did not change during storage and they remained stable even in water solution for more than two months at 4 °C suggesting that they could be stored at low and elevated temperatures without the formation of stable aggregates and precipitates.

3.2.3. Thermo-responsiveness of the brush polymer

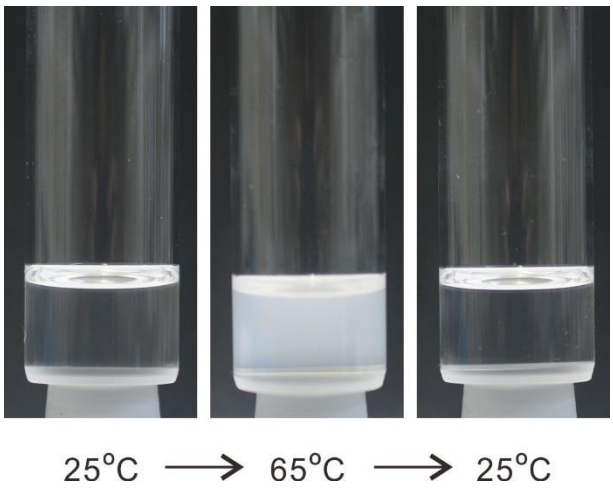

Figure S4. Digital images showing the thermal responsiveness of **brush-3**.

Brush-3 reveals high solubility in water at 25 °C. Upon temperature increase to 65 °C, the solution turned turbid immediately (Figure S4). This lower critical solution temperature (LCST) is consistent with other PMOEGMA polymers based on the monomer with a molecular weight of 300 g mol^{-1}.[6] Importantly, this behavior is fully reversible and the solution became transparent when the temperature was again decreased to 60 °C underlining that individual brush polymers were recovered and no permanent aggregation occurred. Such reversible responsiveness of the brush polymers also provides interesting future opportunities, e.g. to control their self-assembly.

3.2.4. Supplementary TEM characterization of brush polymers

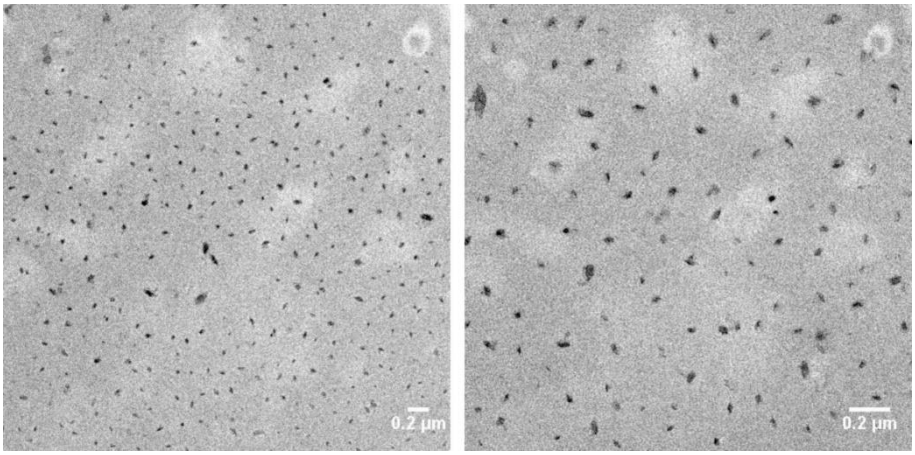

Figure S5. Additional TEM images for the statistical analysis of **brush-1**.

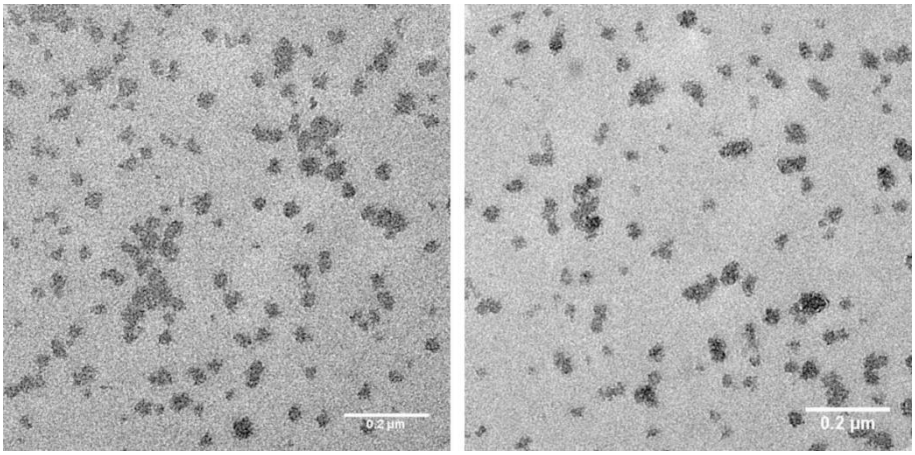

Figure S6. Additional TEM images for the statistical analysis of **brush-2**.

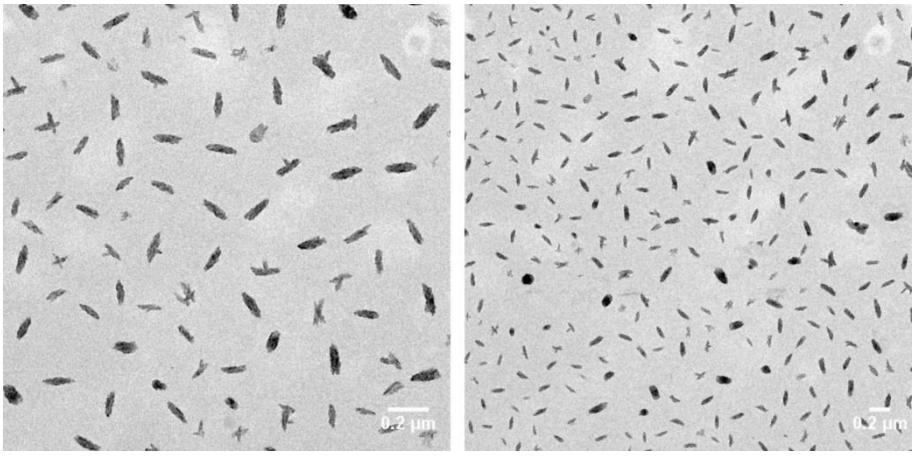

Figure S7. Additional TEM images for the statistical analysis of **brush-3**.

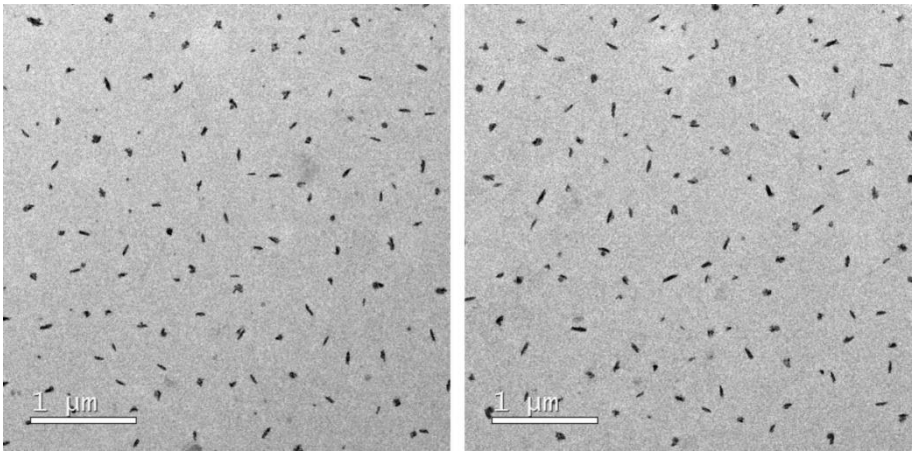

Figure S8. Additional TEM images for the statistical analysis of **brush-4**.

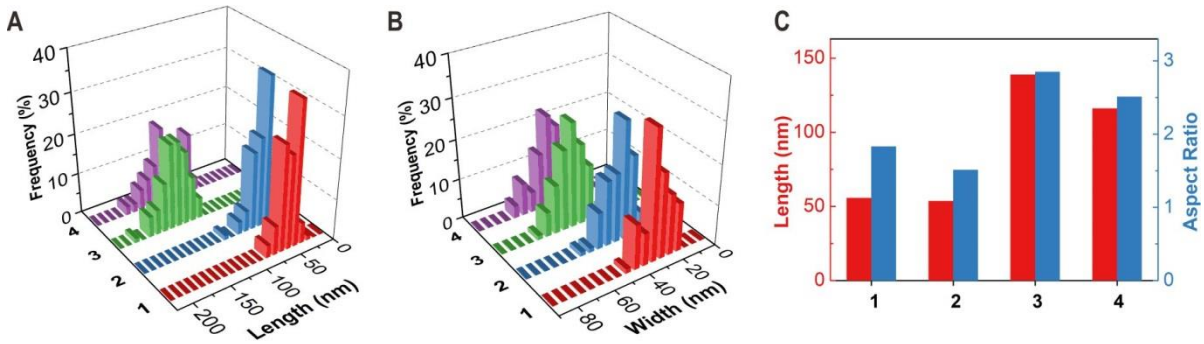

Figure S9. (A, B) Statistic analysis of length distribution (A) and width distribution (B) of **brush-1** to **brush-4**. (C) Comparison of the average length and the aspect ratio of **brush-1** to **brush-4**.

Based on TEM images in Figure 2E-J and Figures S5-S8, statistical analysis was performed for **brush-1** to **brush-4** using ImageJ software. For each polymer sample, the length and width of 100 brush polymers were analyzed based on their length (Figure S9A) and width (Figure S9B) distributions. The average lengths and aspect ratios of these structures were plotted in Figure S9C. From this graph, **brush-3** with the highest density and length of the PMOEGMA side chains displayed an average length of 139 nm and an aspect ratio of 2.85:1. **Brush-4** providing comparable PMOEGMA side chain length but lower chain density reveals an average length of 116 nm and a slightly lower aspect ratio of 2.51:1. In comparison, much lower values were obtained for **brush-1** and **brush-2** with shorter side chains, revealing average lengths of ~55 nm and aspect ratios of ~1.7:1. These results clearly indicate that architectures, molecular weights and contour lengths of bottlebrush polymers could be controlled within a wide range by simply tuning initiator densities along the polypeptide backbone or varying the polymerization conditions.

3.2.5. Supplementary light scattering analysis of brush polymers

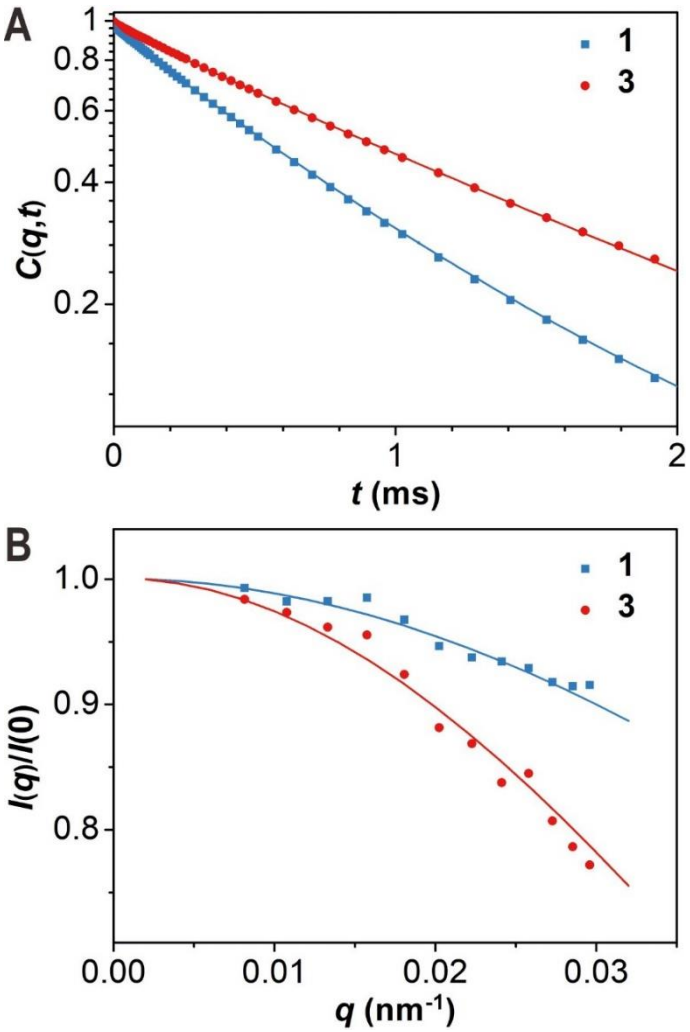

Figure S10. (A) Relaxation functions $C(q,t)$ for the translational diffusion dynamics in aqueous solution of **brush-1** (blue filled squares) and **brush-3** (red filled circles) at 20°C at two scattering wave vectors (q = 0.011 nm^{-1}). The intensities of **brush-1** (blue filled squares) and **brush-3** (red filled circles) are shown in (B). The solid lines denote the representation of the form factor of a solid prolate ellipsoid (**brush-1**: a = 26 nm, b = 20 nm; **brush-3**: a = 38 nm, b = 34 nm).

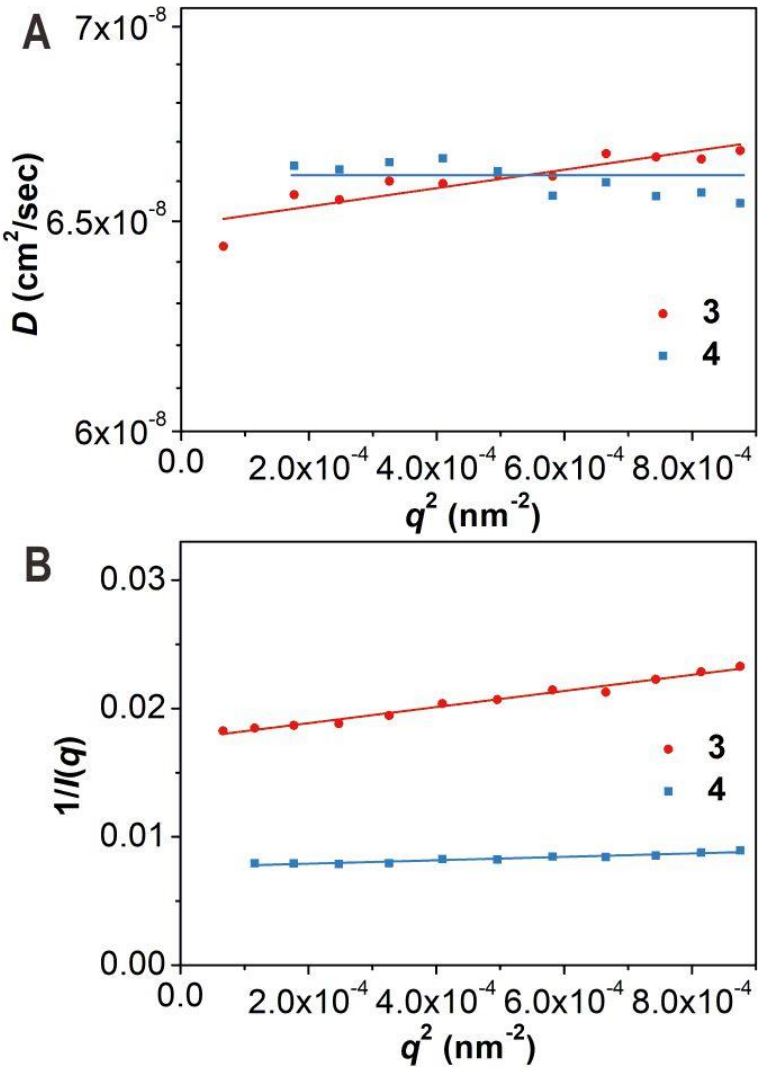

Figure S11. Diffusion coefficients and $1/I(q)$ versus q^2 for **brush-3** (red filled circles) and **brush-4** (blue filled squares) are shown in the (A) and (B), respectively.

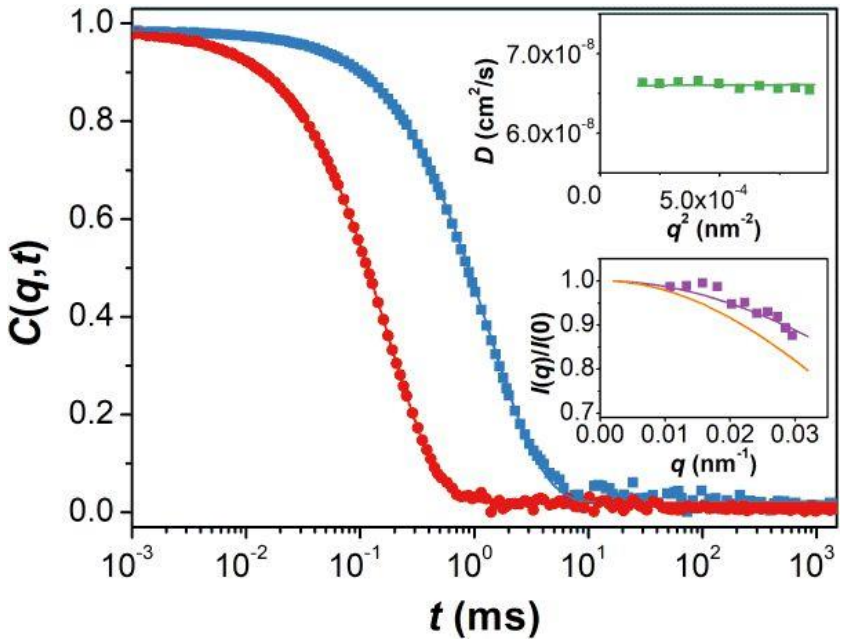

Figure S12. Relaxation functions $C(q,t)$ for the translational diffusion dynamics in aqueous solution of **brush-4** (1 mg mL^{-1}) at 293 K at two scattering wave vectors (blue filled squares, q = 0.011 nm^{-1} and red filled circles, q = 0.030 nm^{-1}) represented by a single exponential decay (solid lines). Upper inset: The diffusion coefficient, D as a function of q^2 with the solid green line indicating a linear representation. Lower inset: Normalized light scattering intensity $I(q)/I(0)$ as a function of q where the purple solid line denotes the representation by the form factor of a solid prolate ellipsoid with semi-axes, a = 28 nm and b = 20 nm. The yellow line indicates the ellipsoid form factor using a = 35 nm and b = 29 nm in case **brush-4** would conform to a brush configuration (Figure S13).

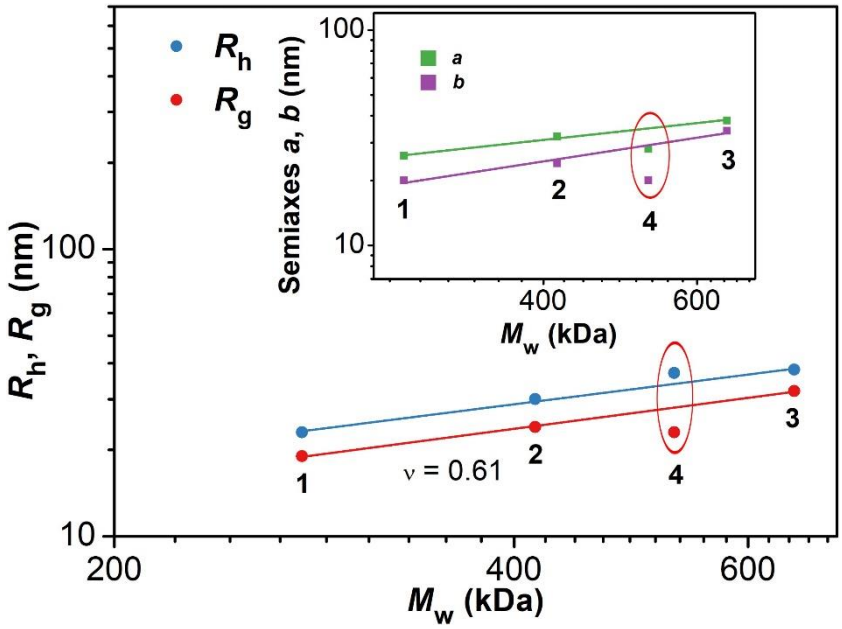

Figure S13. Experimental DLS/SLS data: double logarithmic plot of the hydrodynamic radius, R_h (blue filled circles) and the radius of gyration, R_g (red filled circles) for **brush-1** to **brush-4** as a function of the corresponding molecular weight M_w. Solid lines represent the scaling $R_h \sim M_w^{0.6}$. Inset: The semi-axes, *a* (green filled squares), *b* (purple filled squares), of a prolate ellipsoid as a function of M_w. The scaling for *a* and *b* is 0.44 and 0.62, respectively. The encircles points in the main plot and the inset deviate from the reported scaling behavior.

3.3. Simulation details and further analysis

To study the structural behavior of brush polymers with different side chain lengths and grafting densities (as shown in Table S2), we have performed molecular dynamics simulation of an implicit solvent generic bead-spring model.[7] In this model, individual monomers of in a polymer interact with each other via a 6–12 Lennard–Jones (LJ) potential. Furthermore, to model the bonded connectivity, adjacent monomers in a polymer are connected via an additional finitely extensible nonlinear elastic potential (FENE). The parameters are chosen such that a reasonably large time step can be chosen. The results are presented in units of the LJ interaction energy ε, LJ length unit σ and unit of mass m. This leads to a time unit of $\tau = \sigma(\frac{m}{\varepsilon})^{1/2}$. The specific choice of the model is motivated by the fact that we want to address rather generic polymer structural properties, without attempting to complicate our model system with specific chemical details.

Table S2. Details for molecular simulation.

brush polymer	M_w/kDa	backbone number	side chain number	repeating units of side chains
brush-1	277	585	61	7
brush-2	415	585	61	15
brush-3	650	585	61	27
brush-4	528	585	39	33

We use simplified replica of the experimental brush polymer system. Here the experimental backbone sequence of hydrophobic and hydrophilic residues (see Figure S14) are modeled with the standard Lennard-Jones (LJ) interactions, where interaction between two hydrophobic residues is chosen as attractive with interaction strength of $2k_BT$ and a cutoff of 2.5σ. The interaction between hydrophilic units is modeled as the repulsive LJ with a cutoff of $2^{1/6}\sigma$ and interaction strength of k_BT. This ensures that the native structure of bare backbone is well reproduced in our generic simulations, as known from experimental backbone structure. The backbone sequence used as an input in our simulations is shown in Figure S14. To model the

hydrophilic side chains, we again use repulsive LJ interaction. Note that in our simplified model, a one-to-one monomer mapping is done for the backbone chain.

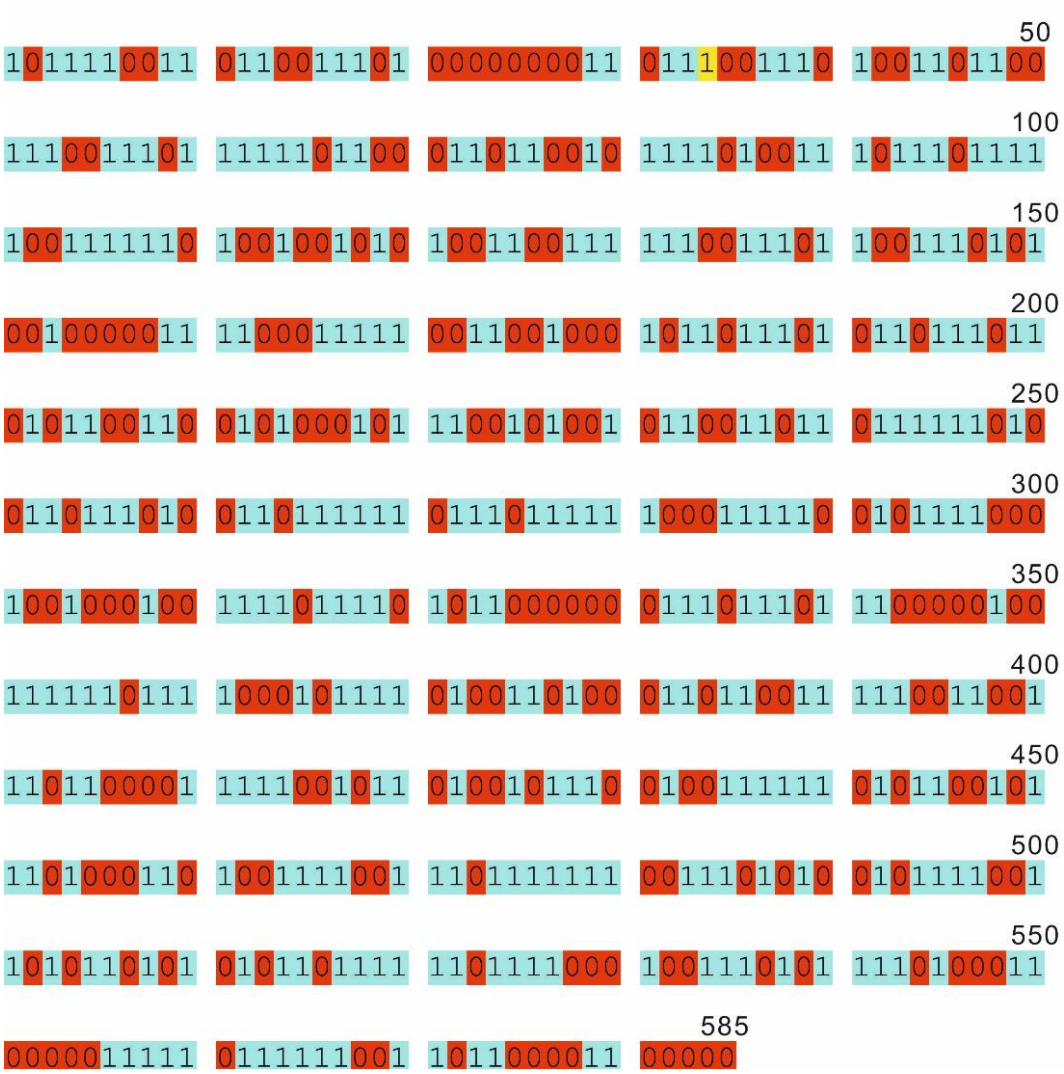

Figure S14. The hydrophobic-hydrophilic pattern of amino acid sequence of HSA for molecular simulation. Hydrophilic amino acids are represented as number "1" and hydrophobic acids are number "0" in the simulation.

We consider a chain of length $N_l = 585$, the same as number of residues in the backbone of the experimental system. The side chain lengths and the grafting densities are again taken to be the same as the experimental system. The equations of motion are integrated using a velocity Verlet algorithm with a time step $\delta t = 0.01\tau$. The simulations were usually equilibrated for 10^7 MD time steps. The measurements are typically observed over another 5×10^9 MD steps. These values are at least one order of magnitude larger than the typical

chain end-to-end relaxation time. During this time, observables such as the gyration radius R_g and static structure factor $S(q)$ are calculated. The temperature is set using a Langevin thermostat with damping constant $\gamma = 1.0\tau^{-1}$ and the temperature is set to 1 ε/k_B.

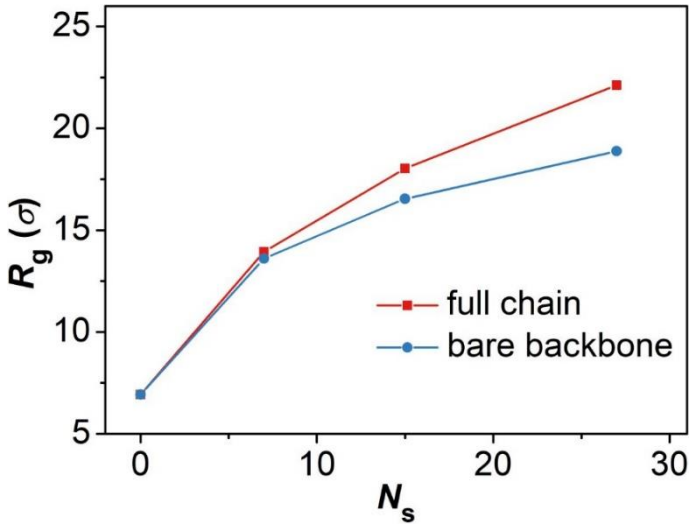

Figure S15. Polymer gyration radius R_g as a function of side chain length N_s for a given grafting density where a backbone is grafted with 61 side chains (see Table S2). Results are shown for bare backbone and also for the full brush polymer chain.

In Figure S15 we show simulation results of R_g values as a function of side chain length N_s for a given grafting density, where a backbone is grafted with 61 side chains. The data is shown for only bare backbone size and also for the full chain. As expected, a chain becomes more swollen with increasing N_s.

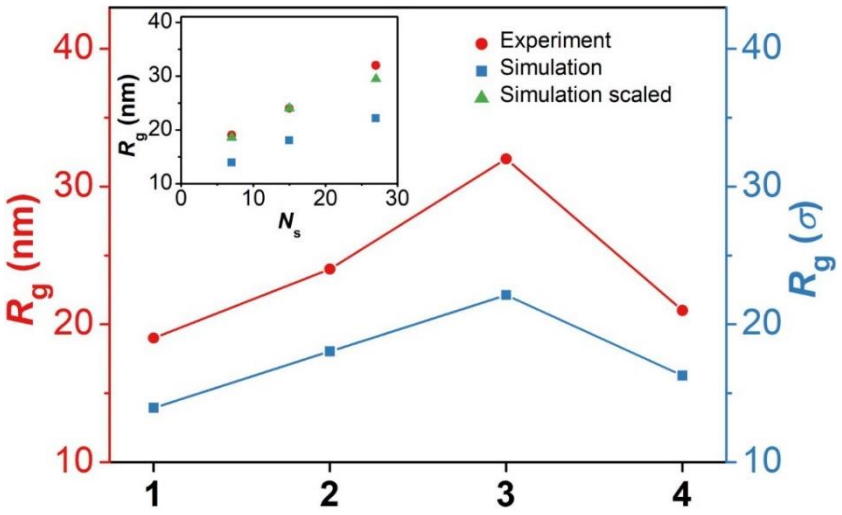

Figure S16. A comparative plot of gyration radius R_g obtained from experiments and simulations for all four brush polymer samples (**brush-1** to **brush-4**). Inset shows the same R_g with changing side chain length N_s for a given grafting density where the backbone is grafted with 61 side chains.

In the main panel of Figure S16 we present a comparative experimental and simulation data of R_g for four different bottle brush systems. It can be appreciated that rather simplified simulation model reproduces the correct trend observed in experiments. Not only that we have the same trend, simulations also give same scaling of total R_g with the side chain length N_s (see the inset of Figure S16). Interestingly, a simple shifting of the simulation data (green triangle) by a factor of ~1.33 gives a rather convincing master curve, i.e. $R_g^* \sim 1.33\ R_g$. While we do not have a direct evidence of this shifting parameter, we use it as a match between chemical specific experimental and chemically independent generic simulation data.

3.4. Synthesis and characterization of brush-5

3.4.1. Schematic illustration for the synthesis of brush-5

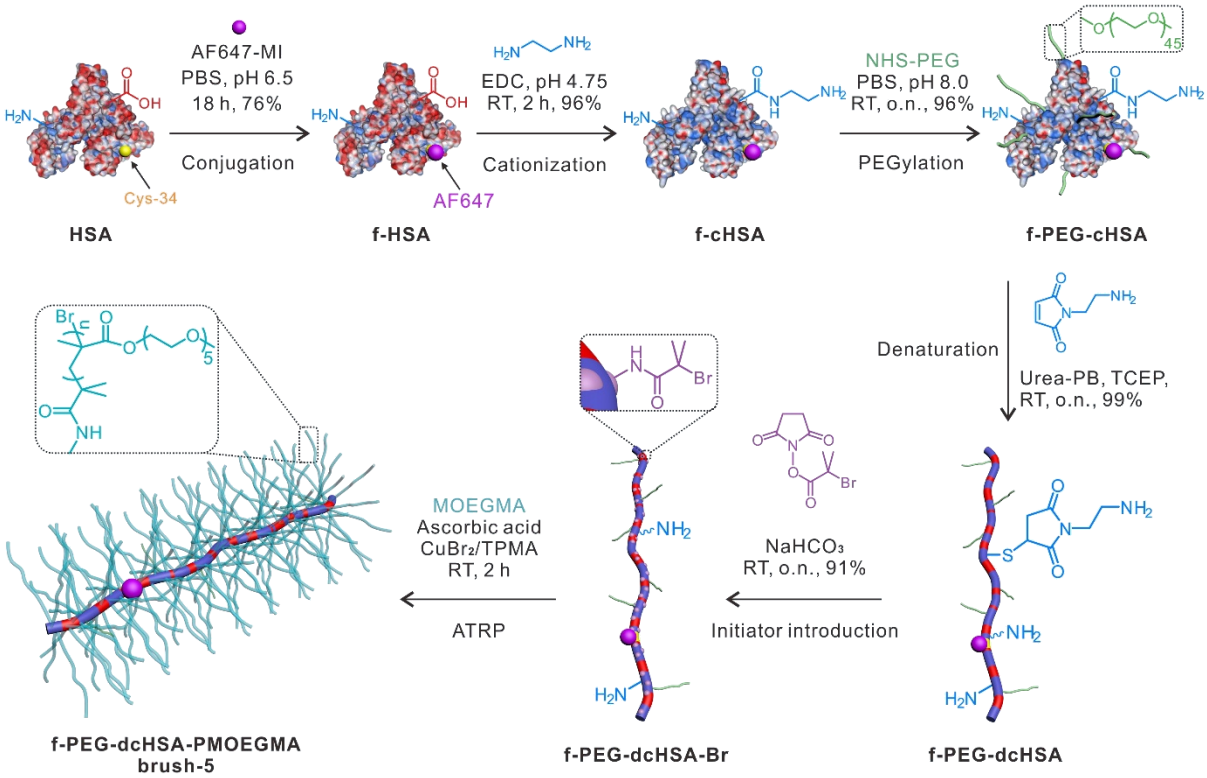

Figure S17. Schematic illustration for the synthesis of the brush polymer site-specifically functionalized with AF647 (**brush-5**).

3.4.2. MALDI-ToF mass spectra

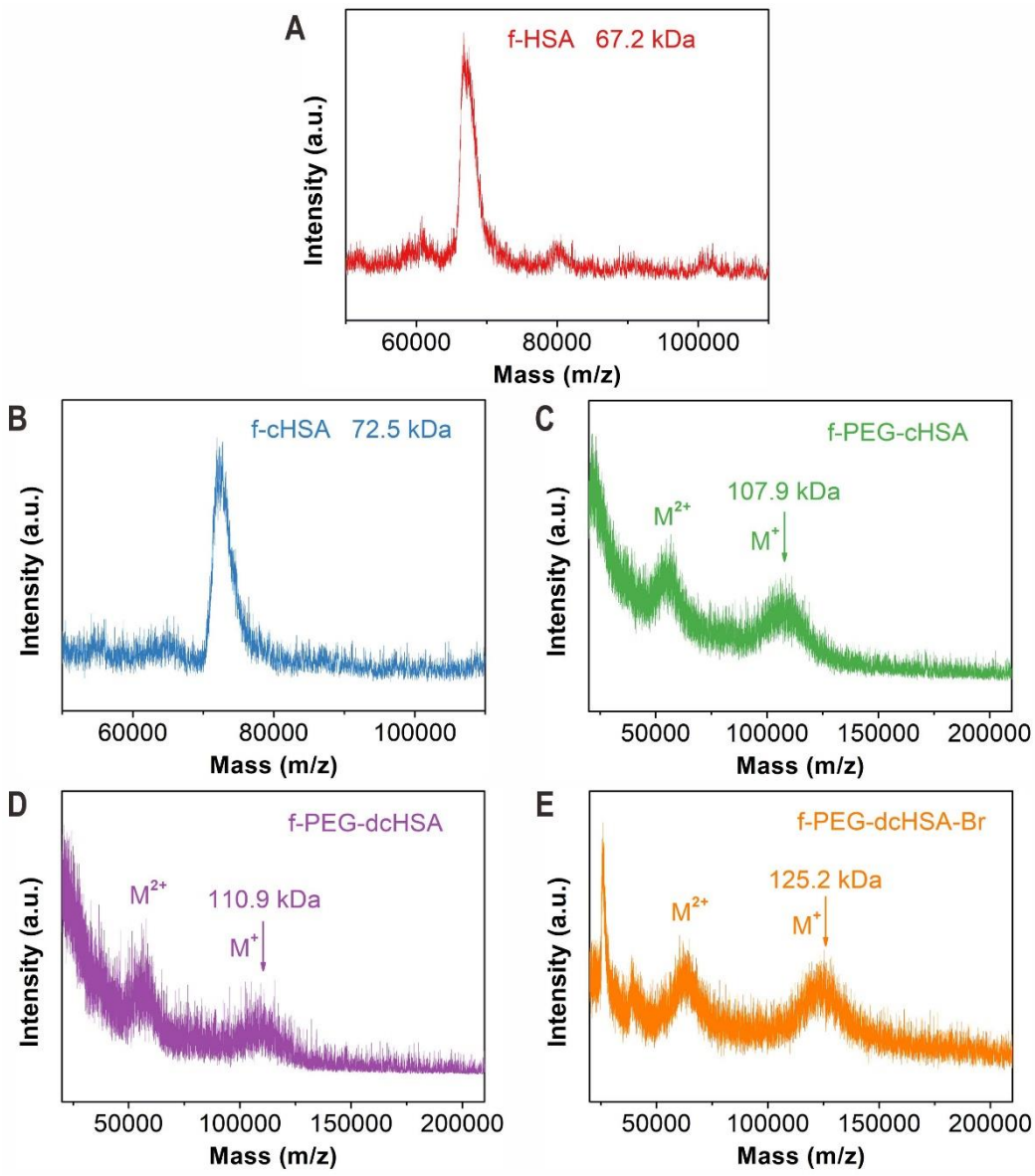

Figure S18. MALDI-ToF mass spectra of (A) f-HSA, (B) f-cHSA, (C) f-PEG-cHSA, (D) f-PEG-dcHSA, and (E) the AF647-labeled fluorescent macroinitiator f-PEG-dcHSA-Br.

3.4.3. Characterization of brush-5

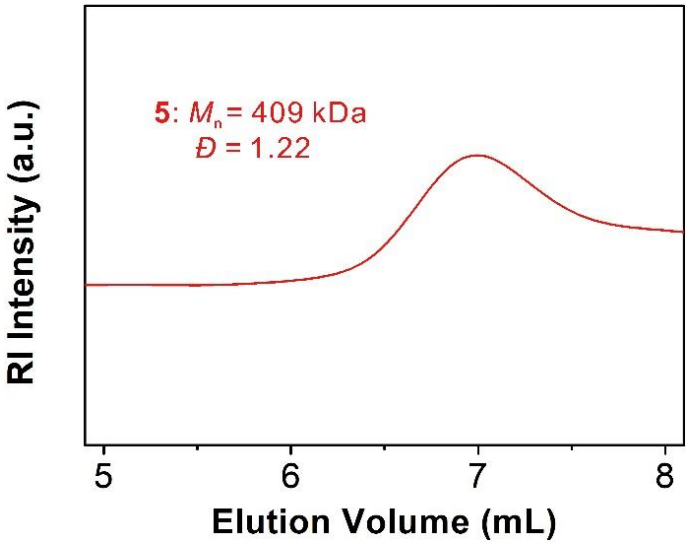

Figure S19. GPC elution curve of **brush-5**.

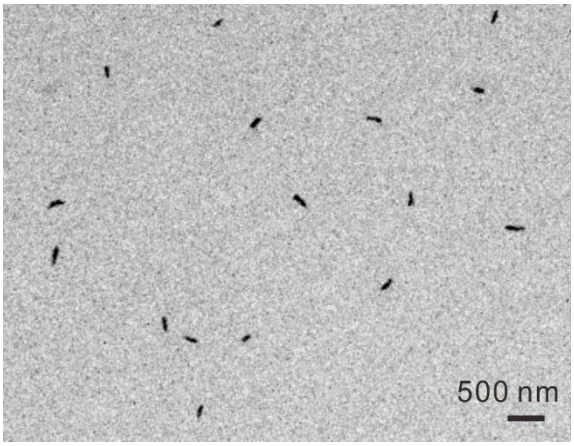

Figure S20. TEM image of **brush-5**.

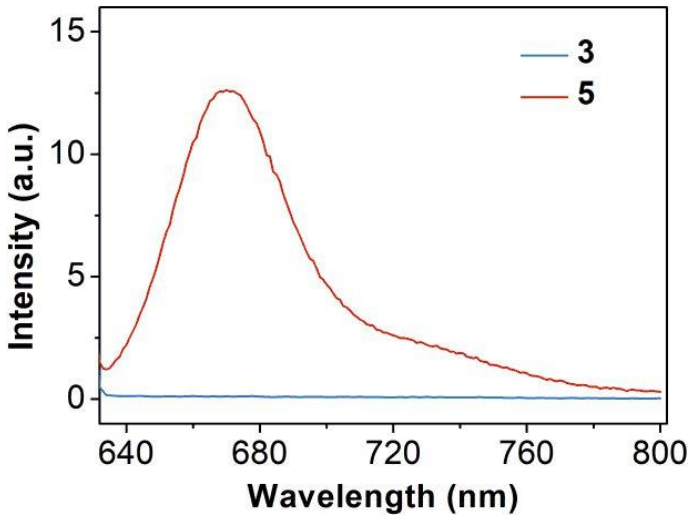

Figure S21. Comparison of fluorescent spectra of **brush-3** and **brush-5**. The characteristic peak of AF647 in the spectrum of **brush-5** confirms the successful introduction of the fluorescent dye.

3.4.4. FCS characterization and analysis

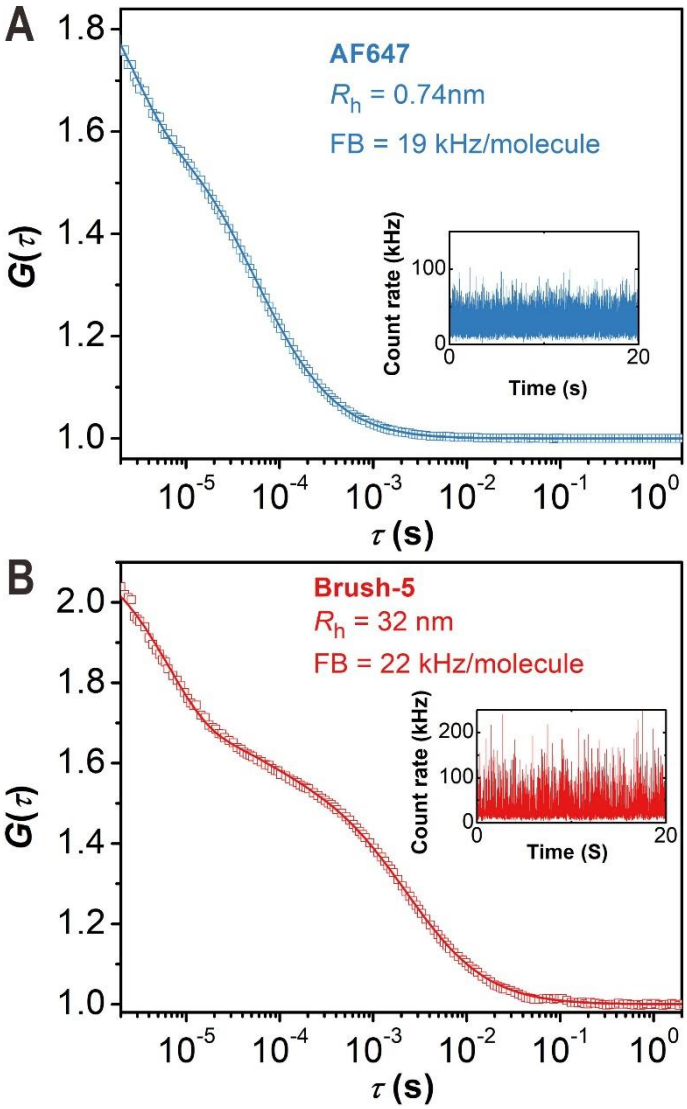

Figure S22. FCS autocorrelation curves (symbols) measured in ~2 nM aqueous solutions of AF647 (A) and the AF647-labeled polymer **brush-5** (B). The solid lines represent the corresponding fits with Equation S10. The insets show the respective intensity time trace plots.

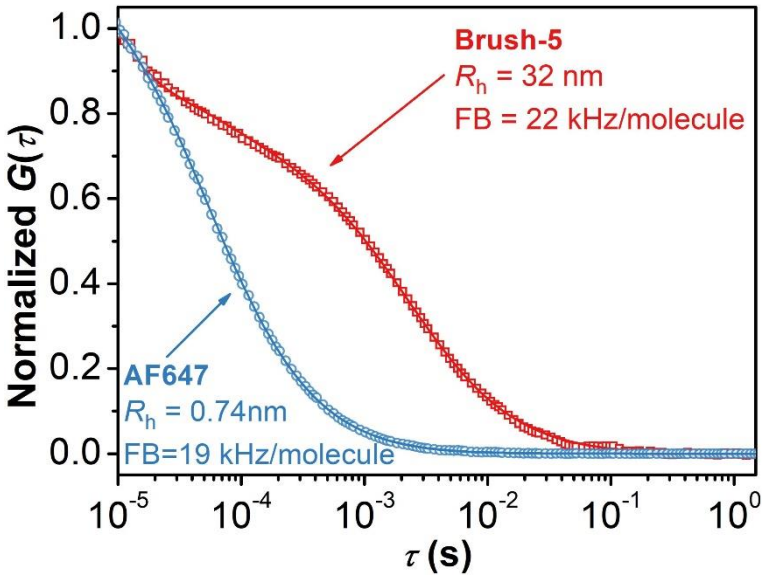

Figure S23. Normalized FCS autocorrelation curves measured in ~ 2 nM aqueous solutions of AF647 (blue circles) and the **brush-5** (red squares). The solid lines represent the corresponding fits with Equation S10. The fits yield values of $R_{h,\ AF647} \approx 0.74$ nm and $FB_{AF647} = 19$ kHz/molecule for the hydrodynamic radius and the fluorescent brightness of the individual AF647 molecules. The corresponding values for **brush-5** were $R_{h,\ \mathbf{brush\text{-}5}} \approx 32$ nm and $FB_{\mathbf{brush\text{-}5}} = 22$ kHz/molecule. The very similar fluorescent brightness values for both systems indicate that only one AF647 molecule is attached to each brush molecule.

3.5. Synthesis and characterization of biotin-brush-6 and biotin-brush-7

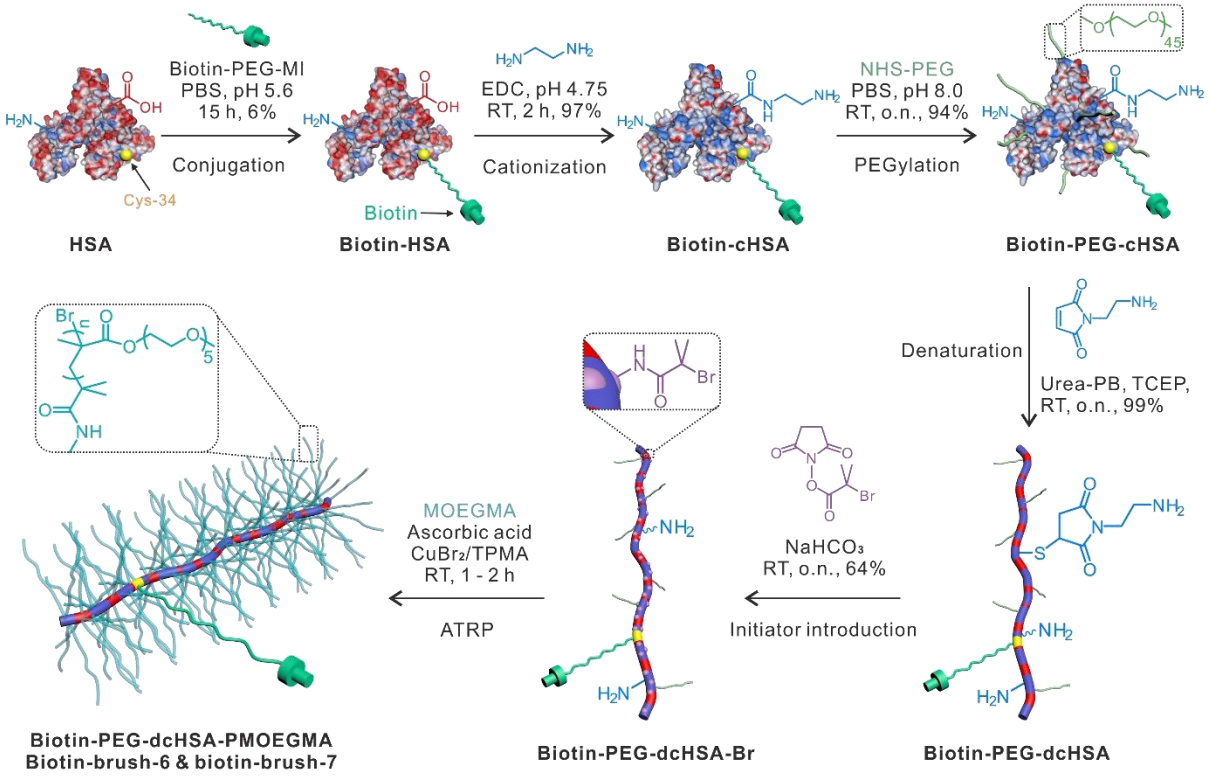

Figure S24. Schematic illustration for the synthesis of protein-templated brush polymers site-specifically functionalized with biotin (**biotin-brush-6** and **biotin-brush-7**).

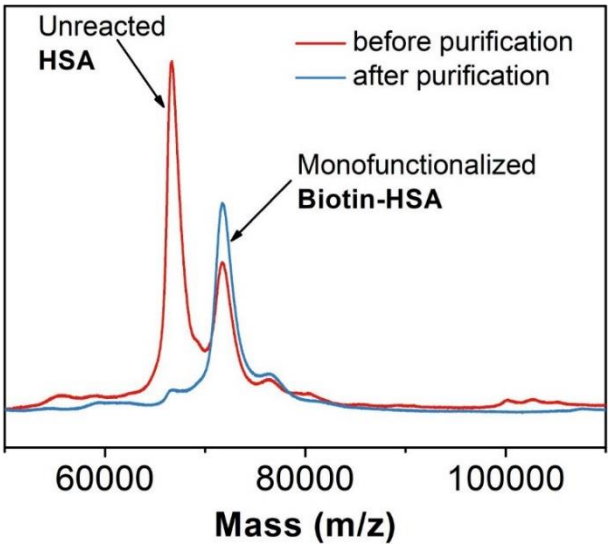

Figure S25. Comparison of the MALDI-ToF mass spectra of biotin-HSA before and after purification by a column composed of Thermo Scientific Pierce Monomeric Avidin Agarose.

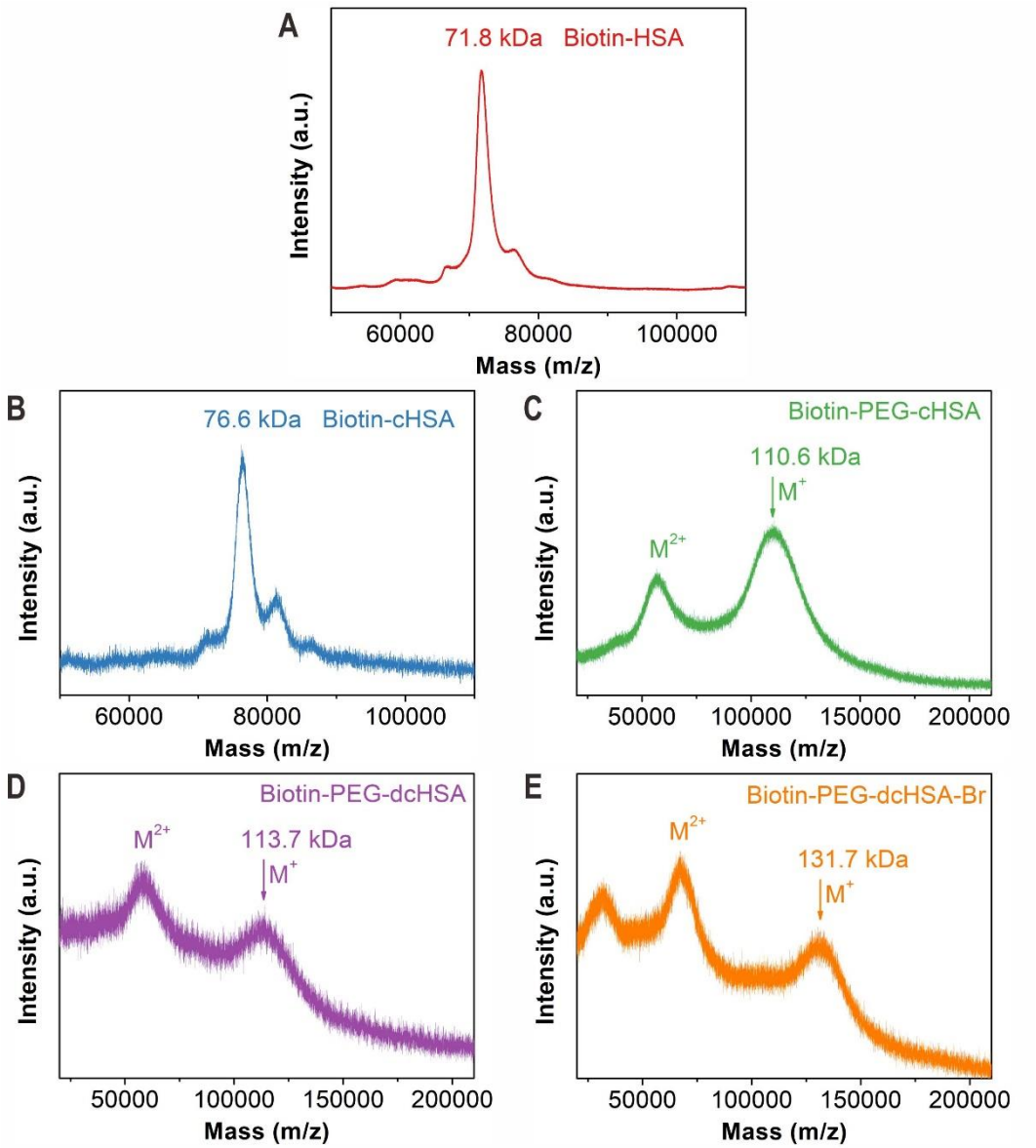

Figure S26. MALDI-ToF mass spectra of (A) biotin-HSA, (B) biotin-cHSA, (C) biotin-PEG-cHSA, (D) biotin-PEG-dcHSA, and (E) the biotin-functionalized macroinitiator biotin-PEG-dcHSA-Br.

3.6. Synthesis and characterization of biotin-brush-8

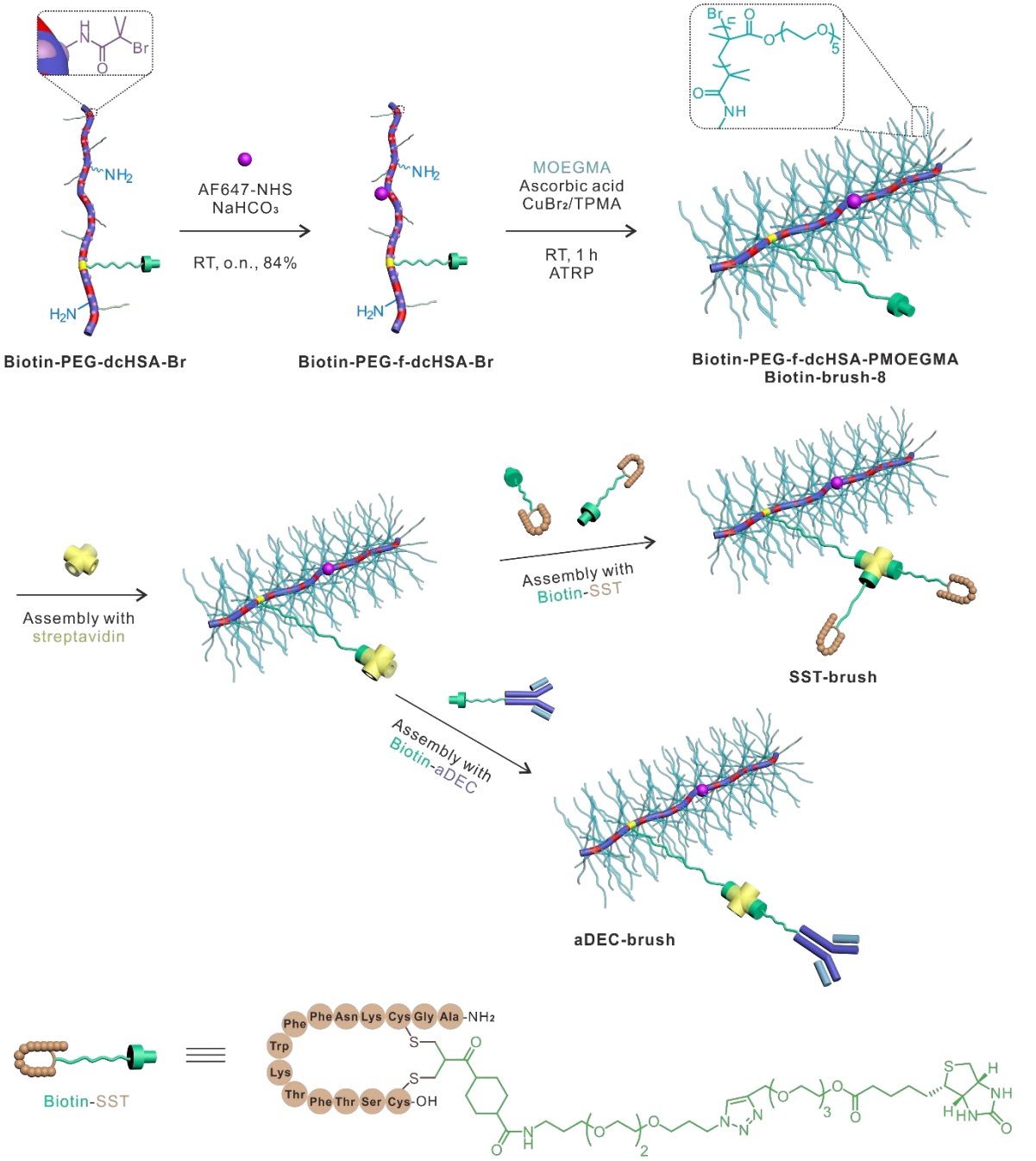

Figure S27. Synthesis of the protein-templated fluorescent brush polymer site-specifically functionalized with biotin (**biotin-brush-8**) and its stepwise assembly with (1) streptavidin and (2) biotin-SST or biotin-aDEC.

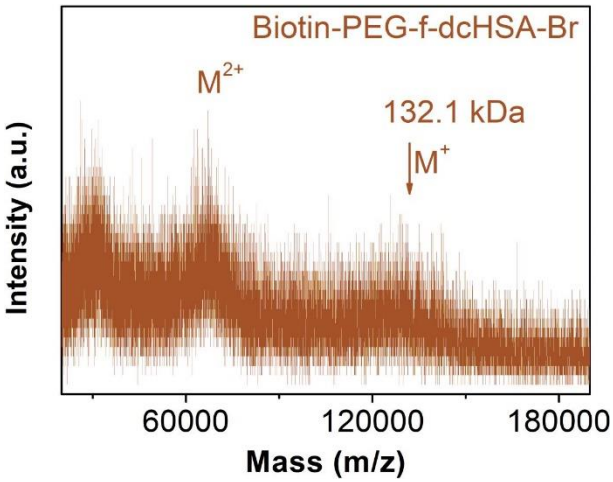

Figure S28. MALDI-ToF mass spectrum of biotin-functionalized fluorescent macroinitiator biotin-PEG-f-dcHSA-Br.

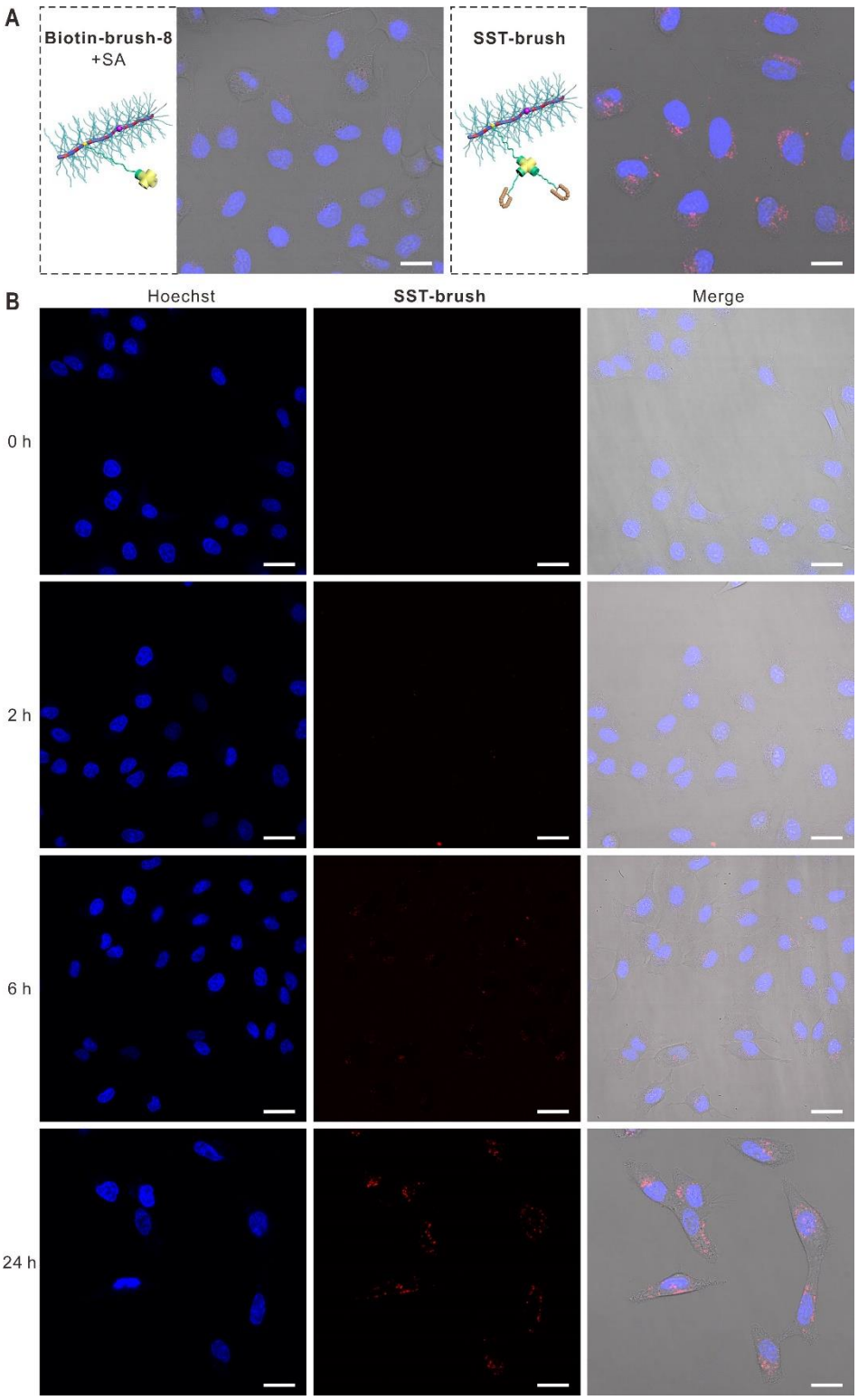

Figure S29. (A) Confocal laser scanning microscopy images showing the cellular uptake of **biotin-brush-8** after assembly with SA (left) and SA/Biotin-SST yielding SST-brush (right) after 24 h. Cellular uptake by A549 cells is only observed after the assembly of Biotin-SST. (B) Time lapse study at 2 h, 6 h and 24 h of 500 nM of SST-brush at 37 °C, 5% CO_2. Nuclei are visualized by Hoechst. Scale bars: 20 µm.

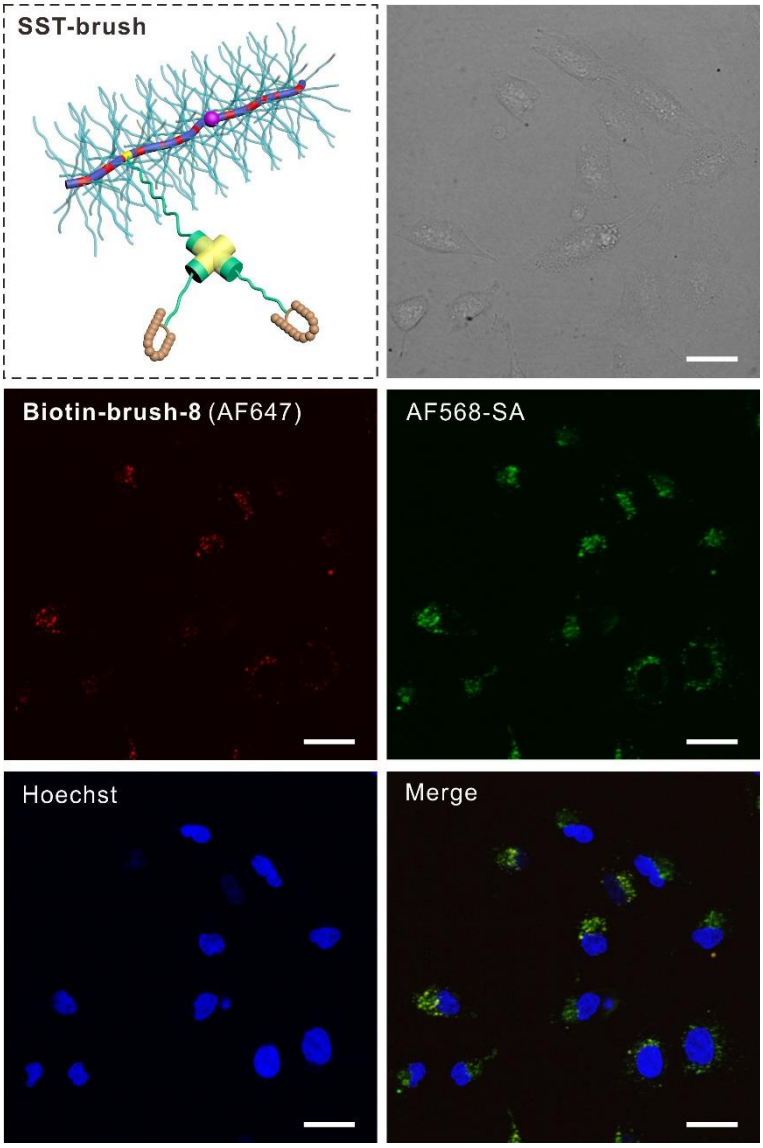

Figure S30. Confocal laser scanning microscopy images showing the cell internalization of SST-brush that has been labeled with AF647 (**biotin-brush-8** labeled with AF647) at the polypeptide backbone. Colocalization of AF568-SA and the brush polymer after 24 h were observed indicating that the SST-brush remained intact inside the A549 cells. Scale bars: 20 μm.

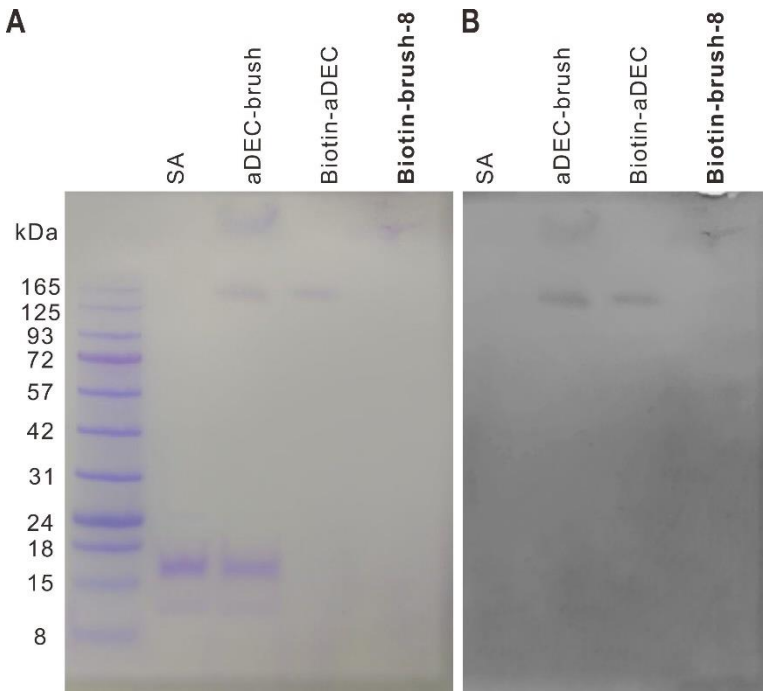

Figure S31. SDS-PAGE with coomassie staining (A) and western blot with chemiluminescence detection of SA-HRP (B) results, showing the assembly of **biotin-brush-8** with a biotinylated model antibody (biotin-aDEC) and SA generating aDEC-brush. Under heating conditions, the aDEC-brush complex can be separated in its consisting components to be visualized by coomassie stain (A) or SA-HRP (B) to detect biotinylated molecules.

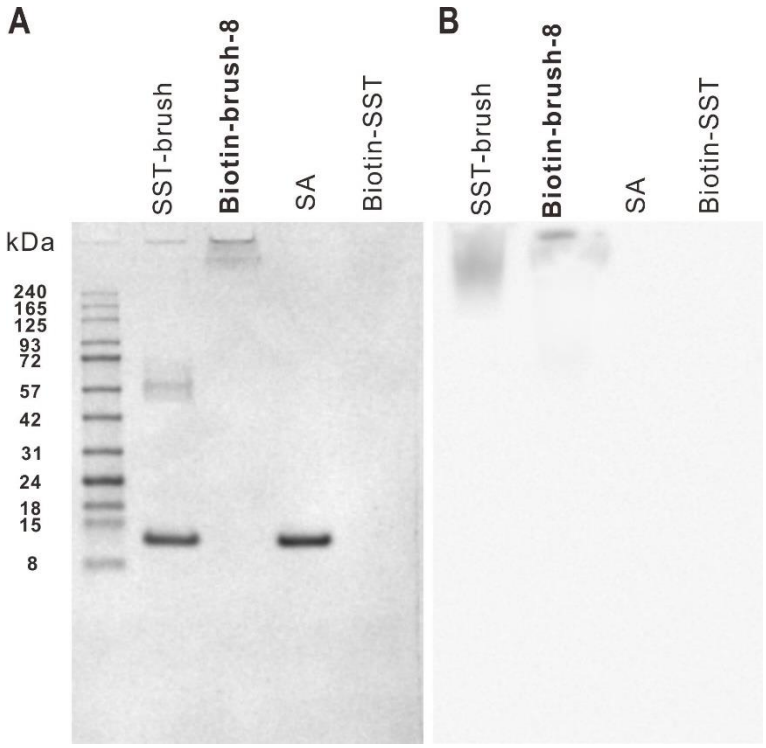

Figure S32. SDS-PAGE after coomassie staining (A) and western blot with chemiluminescence detection of SA-HRP (B) results, showing the assembly of **biotin-brush-8** with biotinylated SST and SA generating SST-brush. Under heating conditions, the SST-brush complex can be separated in its consisting components to be visualized by coomassie stain (A) or SA-HRP (B) to detect biotinylated molecules. The cyclic peptide SST consist of only 14 amino acids, which were not enough for a sufficient staining with coomassie dye. Additionally, the small size of the SST prevented retention on the nitrocellulose membrane with a pore size of 0.2 µm.

IV. References

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