Supporting Information

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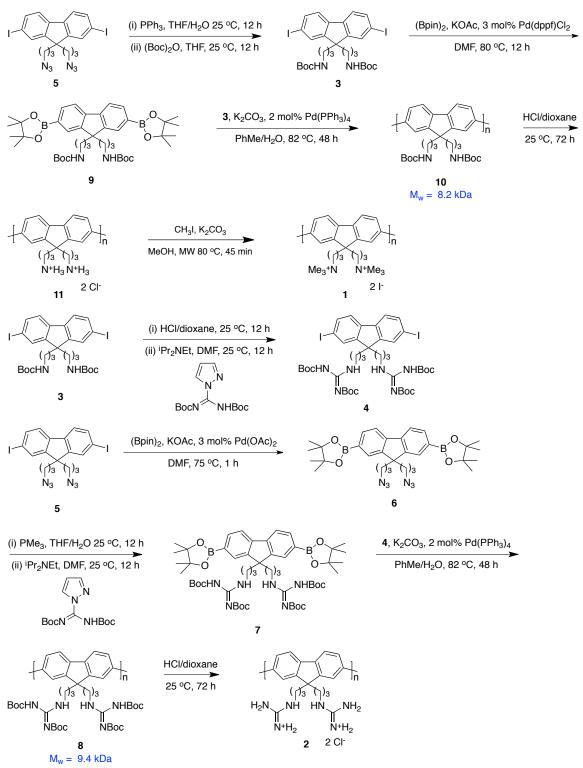
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1. General Procedures

Dry DMF, (<50 ppm water) was purchased from Acros. THF was dried with molecular sieves and Et₃N was distilled from CaH₂. Other solvents and reagents were used as received. All reactions were carried out under an atmosphere of dry nitrogen. Unless otherwise indicated, common reagents or materials were obtained from commercial source and used without further purification.

NMR spectra were recorded on a VXP-300 MHz and Inova-500 MHz spectrometers (¹H at 300 MHz or 500 MHz, and ¹³C at 75 or 125 MHz) at room temperature unless other mentioned. Chemical shifts of ¹H NMR spectra were recorded and chemical shifts are reported in ppm from the solvent resonance (CDCl₃ 7.26 ppm, CD₃OD 3.30 ppm, CD₃SOCD₃ 2.50 ppm). Data are reported as follows: chemical shift, multiplicity (s = singlet, br = broad, d = doublet, t = triplet, q = quartet, m = multiplet), coupling constants, and number of protons. Proton decoupled ¹³C NMR spectra were also recorded in ppm from tetramethylsilane resonance (CDCl₃ 77.0, CD₃OD 49.1, DMSO-d₆ 39.5 ppm). Analytical thin layer chromatography (TLC) was performed on EM Reagents 0.25 mm silica-gel 60-F plates, and visualized with UV light. Flash chromatography was performed using silica gel 60 (230–400 mesh). MS were measured under ESI or MALDI conditions.

2. Outline Of Syntheses Of Polymers 1 and 2



3. Experimental Procedures and Characterization Of Compounds 1 - 11

9,9-*bis*(3-Azidopropyl)-2,7-diiodo-9*H*-fluorene (5). 9,9-*bis*(3-Bromopropyl)-2,7-diiodo-9*H*-fluorene (1.5 g, 2.3 mmol), prepared by alkylation of 2,7-diiodo-9*H*-fluorene and 1,3-dibromopropane (Liu, H. *et al. Chem. Eur. J.* 2009, **15**, 2289-2295), NaN₃ (533 mg, 8.20 mmol) and 40 mL DMSO were added into a 250 mL round bottom flask. The mixture was stirred at 70 °C for 12 h. The reaction mixture was extracted with Et₂O (100 mL) then water (50 mL). Aqueous layer was re-extracted with Et₂O (50 mL x 2). The combined organics were washed with water (50 mL x 3), brine (50 mL x 2), then dried over MgSO₄. The product was obtained without further purification (1.3 g, 98%) as yellow solid. ¹H NMR (300 MHz, CDCl₃), δ 7.71-7.67 (m, 4H), 7.42 (d, *J* = 8.1 Hz, 2H), 3.03 (t, *J* = 6.6 Hz, 4H), 2.06-2.01 (m, 4H), 0.90-0.84 (m, 4H); ¹³C NMR (75 MHz, CDCl₃), δ 150.6, 139.7, 136.8, 131.9, 121.8, 93.6, 54.6, 51.3, 37.0, 23.3. FT-IR (NaCl): N₃ band at 2101.72 cm⁻¹.

Di-tert-butyl ((2,7-diiodo-9H-fluorene-9,9-diyl)bis(propane-3,1-

diyl))dicarbamate (3). Compound **5** (1.3 g, 2.2 mmol) was dissolved in THF (40 mL) and H₂O (5 mL) in a 250 mL flask. Triphenyl phosphine (1.56 g, 5.94 mmol) was added to the solution and the mixture was stirred at 25 °C for 12 h. The solvents were removed under reduced pressure. The residue and di-*tert*-butyl dicarbonate (1.07g, 4.90 mmol) were dissolved in THF (45 mL). The mixture was stirred at 25 °C for 12 h. The solvent was removed under reduced pressure. The residue and stirred at 25 °C for 12 h. The solvent was removed under reduced pressure. The residue was purified by flash chromatography eluting with CH₂Cl₂ and MeOH (98:2) to give desired product (1.5 g, 92%) as white solid. ¹H NMR (300 MHz, CDCl₃), δ 7.67-7.62 (m, 4H), 7.39 (d, *J* = 7.8 Hz, 2H), 4.30-4.18 (br, 2H), 2.89-2.85 (m, 4H), 2.02-1.85 (m, 4H), 1.39 (s, 18H), 0.78-0.67 (m, 4H); ¹³C NMR (75 MHz, CDCl₃), δ 155.8, 151.2, 139.7, 136.5, 131.8, 121.7, 93.4, 54.7, 40.4, 37.1, 28.4, 24.5 MS (MALDI) calcd for C₂₉H₃₈l₂N₂NaO₄ (M+Na)⁺ 755.08, found 755.30.

Di-tert-butyl ((2,7-bis(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-9Hfluorene-9,9-diyl)bis(propane-3,1-diyl))dicarbamate (9). In a 100 mL flask,

compound **3** (500 mg, 0.680 mmol), KOAc (400 mg, 4.08 mmol), bis(pinacolato)diborane (433 mg, 1.70 mmol) in degassed DMF (30 mL) were stirred at 25 °C. The catalyst, Pd(dppf)Cl₂ was added in one portion. The mixture was deoxygenated for 3 min. The reaction mixture was stirred at 80 °C under N₂ for 12 h. Water (50 mL) was added to the reaction mixture, and the product was extracted from water with CH₂Cl₂ (50 mL x 3). The combined organics were washed with water (50 mL x 3) and brine (50 mL x 2), then dried over MgSO₄. After removing the organic solvent under reduced pressure, the crude product was purified by flash chromatography eluting with CH₂Cl₂ and MeOH (99:1) to give desired product (400 mg, 80 %) as brown solid. ¹H NMR (300 MHz, CDCl₃), δ 7.84 (d, *J* = 7.5 Hz, 2H), 7.74 (d, *J* = 7.5 Hz, 4H), 4.21-4.18 (br, 2H), 2.90-2.80 (m, 4H), 2.10-2.05 (m, 4H), 1.40 (s, 18H), 1.31 (s, 24H), 0.80-0.65 (m, 4H). ¹³C NMR (75 MHz, CDCl₃), δ 155.8,149.2, 143.9, 134.2, 128.8, 119.7, 83.9, 83.6, 54.4, 40.6, 28.4, 25.1, 25.0, 24.5. MS (MALDI) calcd for C₄₁H₆₂B₂N₂NaO₈⁺ (M+Na)⁺, 755.46, found, 755.66.

Polymer 10. In a 5 mL flask, compounds **3** (50 mg, 0.068 mmol), **9** (50 mg, 0.068 mmol), K_2CO_3 (116 mg, 0.840 mmol), and *tetra-N*-butylammonium bromide (0.45 mg, 0.0014 mmol) in degassed 4:1 toluene/H₂O (3 mL) were stirred at 25 °C. The catalyst Pd(PPh₃)₄ (2.0 mg, 0.0014 mmol) was added. The mixture was deoxygenated for 5 min. The reaction was then heated to 82 °C with stirring under N₂. After 48 h, 2 drops of iodobenzene were added and the reaction was stirred for an additional 12 h at 82 °C. After cooling to 25 °C, water (10 mL) was added to the reaction mixture, and the product was extracted with CHCl₃ (10 mL x 3). The combined organics were washed with water (10 mL x 3), brine (10 mL x 2), then dried over MgSO₄. After removal of the solvent under reduced pressure, the residue was added to petroleum ether to give a precipitate. The precipitate was transferred to a vial equipped with a stir bar and was stirred in 5mL of acetone at 25 °C. After 24 h, the acetone was decanted off and the solid was stirred into an additional 5 mL of acetone. This was repeated for a total of 3 times (72 h). Polymer **10** was obtained as a yellow powder (37 mg). ¹H NMR

S5

(300 MHz, CDCl₃): δ 7.93-7.82 (br, 4H), 7.82–7.60 (br, 2H), 3.16-2.85 (br, 4H), 2.93-1.50 (br, 4H), 1.41(s, 18H), 1.03-0.78 (br, 4H). GPC (THF): M_n = 6762, M_w = 8187, PDI = 1.21 based on GPC analysis. FT-IR (NaCl): NH band at 3345.77 and 1506.48 cm⁻¹; C=O band at 1700.23 cm⁻¹.

Polymer 11. Polymer **10** (30 mg) was dissolved in dioxane (10 mL) in a 25 mL flask, then HCl in dioxane (4 M, 2 mL) was added in one portion and the solution mixture was stirred at 25 °C for 72 h. After removal of the solvent under reduced pressure, 5 mL of acetone was added to give a precipitate; this was filtered and washed with chloroform to give polymer **11** as a yellow powder (18 mg). ¹H NMR (300 MHz, CD₃OD): δ 8.20–7.70 (m, 6H), 2.91–2.69 (m, 4H), 2.59-2.40 (m, 4H), 1.23-0.97 (m, 4H). FT-IR (NaCl): NH (NH₃⁺Cl⁻) band at 3448.16 and 1656.36 cm⁻¹; C=O band at 1700.23 cm⁻¹.

Polymer 1. In a sealed tube, polymer **11** (8 mg), MeI (0.20 mL, 3.2 mmol), and K_2CO_3 (442 mg, 3.20 mmol) were added in MeOH (2 mL). The mixture was heated to 80 °C in a CEM Discover microwave for 45 min. Polymer **1** was obtained as a brown powder (10 mg). ¹H NMR (300 MHz, DMSO-d₆): δ 8.39-7.70 (br), 3.20-2.80 (br), 2.60-2.40 (br), 1.10-0.78 (br). FT-IR (NaCI) shows the absence of a N-H stretch in the region 3300-3000 cm⁻¹.

BOC-Protected Guanidine 4. In a 250 mL flask, compound **3** (800 mg, 1.09 mmol) was dissolved in dioxane (50 mL), then HCl in dioxane (4M, 8 mL) was added and the solution mixture was stirred at 50 °C for 12 h. After cooling to 25 °C, the solvent was removed and the residue was dissolved in MeOH (10 mL). An aqueous solution of KOH (10 % wt, 10 mL) was added. The mixture was stirred for 15 min and the solvents were removed. The residue was extracted with CH_2Cl_2 . The organic layer was washed with H_2O and brine, then dried over

MgSO₄. The solvent was removed and the yellow oil product was dissolved in DMF (12 mL). *N,N'*-bis(*tert*-Butoxycarbonyl)1*H*-pyrrazole-1-carboxamidine (744 mg, 2.40 mmol), diisopropylethylamine (12 mL) were added to the solution. The reaction mixture was stirred at 25 °C for 12 h. The solvent was removed under reduced pressure. The residue was purified by flash chromatography eluting with CH₂Cl₂ and MeOH (99:1) to give desired product (930 mg, 85%) as yellow solid. ¹H NMR (300 MHz, CDCl₃): δ 11.43 (s, 1H), 8.32 (s, 1H), 7.62-7.69 (m, 4H), 7.41 (d, *J* = 8.1 Hz, 2H), 6.42 (s, 1 H), 3.16 (q, *J* = 5.4 Hz, 4H), 1.98 (m, 4H), 1.45 (s, 36H), 0.83-0.75 (m, 4H). ¹³C NMR (75 MHz, CDCl₃): δ 163.4, 155.9, 153.2, 150.7, 142.7, 139.7, 139.1, 136.7, 131.7, 128.9, 121.8, 109.8, 93.6, 83.1, 79.2, 54.6, 40.6, 37.2, 29.7, 28.3, 28.1, 28.0, 23.2. MS (ESI) calcd for C₄₁H₅₉l₂N₆O₈⁺ (M+H)⁺ 1017.25, found 1017.27.

Diborate Ester 6. In a 250 mL flask, compound **5** (500 mg, 0.860 mmol), KOAc (500 mg, 5.16 mmol), bis(pinacolato)diborane (524 mg, 2.06 mmol) in degassed DMF (50 mL) were stirred at 25 °C. The Pd(OAc)₂ catalyst (2 mg, 0.01 mmol) was added in one portion, and the mixture was deoxygenated for 3 min. The reaction mixture was stirred at 75 °C under N₂ for 1 h. The solution was filtered through celite and the solvent was removed under reduced pressure. The product was obtained without further purification (500 mg, quantitative) as brown solid. ¹H NMR (300 MHz, CDCl₃), δ 7.83-7.59 (m, 6H), 3.05-2.83 (m, 4H), 2.22-2.07 (m, 4H), 1.21 (s, 24H) 1.01-0.78 (m, 4H). ¹³C NMR (125 MHz, CD₃Cl₃), δ 148.6, 143.8, 134.6, 128.7, 119.8, 83.9, 83.5, 83.1, 83.0, 54.4, 54.3, 51.5, 51.4, 37.0, 29.7, 25.0, 24.9, 24.8, 24.5, 24.1, 23.4. MS (ESI) calcd for C₃₁H₄₃B₂N₆O₄³⁺ (M+H)⁺ 585.35, found 585.35.

Boc-Protected Diguanidine 7. Compound **6** (300 mg, 0.51 mmol) was dissolved in THF (22 mL) and H₂O (3 mL) in a 100 mL flask. Then PMe₃ (1M in THF; 1.28 mL, 1.28 mmol) was added to the solution and the mixture was stirred at 25 °C for 12 h. The solvents were removed under reduced pressure, then the residue was dissolved in DMF (6 mL) and *N*,*N*'-bis(*tert*-butoxycarbonyl)1*H*-

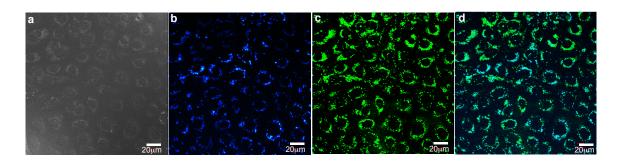
pyrrazole-1-carboxamidine (348 mg, 1.12 mmol), and diisopropylethylamine (6 mL) were added. The reaction mixture was stirred at 25 °C for 12 h. The solvent was removed under reduced pressure. The residue was purified by flash chromatography eluting with CH₂Cl₂ and MeOH (99:1) to give desired product (360 mg, 69%) as yellow solid. ¹H NMR (300 MHz, CDCl₃): δ 11.40-11.35 (br, 2H), 7.92-7.35 (m, 6H), 3.18-2.98 (m, 4H), 2.18-1.87 (m, 4H), 1.41-1.35 (m, 36H), 1.19 (s, 24H), 0.85-0.58 (m, 4H). ¹³C NMR (75 MHz, CDCl₃, ppm): δ 155.7, 153.0, 150.9, 132.2, 132.0, 131.9, 128.6, 128.6, 128.4, 83.8, 82.9, 79.1, 41.1, 37.3, 31.9, 29.7, 29.3, 28.3, 28.0, 25.0, 22.7. MS (ESI) calcd for C₄₁H₅₉l₂N₆O₈⁺ (M+H)⁺ 1017.25, found 1017.27.

Boc-Protected Oligoguanidine 8. In a 5 mL flask, compound 7 (50 mg, 0.05 mmol), compound 4 (50 mg, 0.05 mmol), K_2CO_3 (83 mg, 0.6 mmol), and tetrabutylammonium bromide (0.3 mg, 0.001 mmol) in degassed 4:1 toluene/H₂O (3 mL) were stirred at 25 °C. Catalytic Pd(PPh₃)₄ (1.2 mg, 0.001 mmol) was added, and the mixture was deoxygenated for 5 min. The reaction was heated to 80 °C with stirring under N₂. After 48 h, 2 drops of iodobenzene were added and the reaction was stirred for an additional 1 h at 80 °C. The mixture was then precipitated by addition of MeOH, filtered, and thoroughly washed with MeOH. The precipitate was transferred to a vial equipped with a stir bar and was stirred in 5 mL of acetone at 25 °C under N2. After 24 h, the acetone was decanted off and the solid was stirred into an additional 5 mL of acetone. This was repeated for a total of 3 times (72 h). Polymer 8 was obtained as a yellow powder (15 mg). ¹H NMR (300 MHz, CD₃OD): δ 7.98–7.56 (m, 6H), 3.11-2.65 (br, 4H), 2.23-1.78 (br, 4H), 1.37(br, 36H), 0.93-0.54 (br, 4H). GPC (THF): $M_n = 7558$, $M_w = 9446$, PDI = 1.25 based on GPC analysis. FT-IR (NaCl): NH band at 3393.31 cm⁻¹; C=N band at 1623.46 cm⁻¹; C=O band at 1700.23 cm⁻¹.

Oligoguanidine 2. In a 25 mL flask, polymer 8 (10 mg) was dissolved in

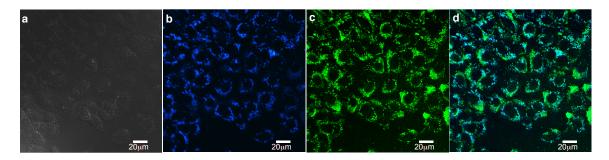
dioxane (10 mL). HCl in dioxane (4 M, 2 mL) was added and the solution mixture was stirred at 25 °C for 72 h. Polymer **2** was obtained as a yellow powder (5 mg). ¹H NMR (500 MHz, CD₃OD): δ 9.35-9.25 (br), 8.10–7.30 (br), 3.25-2.85 (br), 2.23-1.98 (br), 1.03-0.58 (br). FT-IR (NaCl): NH (amine) band at 3329.74 cm⁻¹; NH (imine) band at 3161.91 cm⁻¹; C=N band at 1651.51 cm⁻¹.

4. Delivery Of Avidin-FITC and β -Galactosidase-Alexa Fluor[®] 488 (at 37 °C mediated by polymer 1 and 2 into Clone 9 cells)

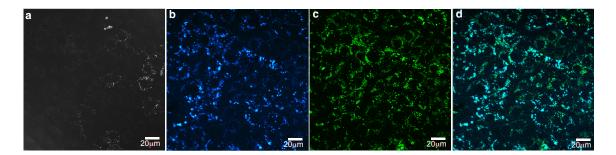


(1-avidin-FITC)

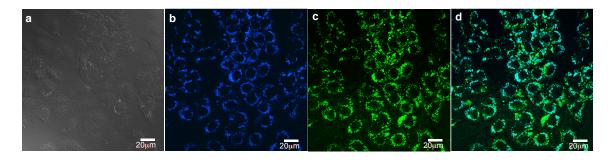
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(2-avidin-FITC)



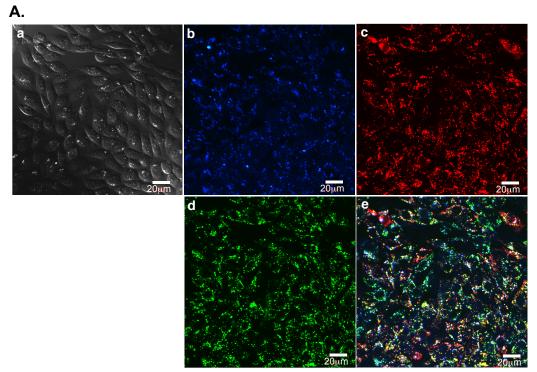
$(1-\beta$ -galactosidase-Alexa Fluor[®] 488)



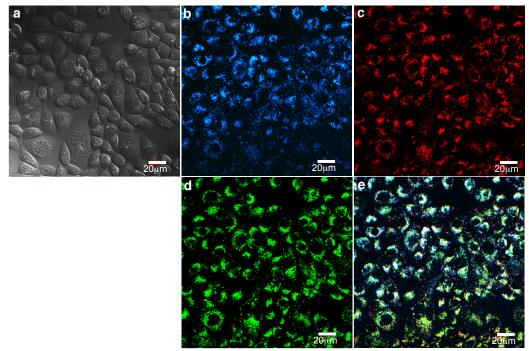
$(2-\beta$ -galactosidase-Alexa Fluor[®] 488)

Figure S1. Delivery of avidin-FITC (**A**) and β -galactosidase-Alexa Fluor[®] 488 (**B**) into Clone 9 cells at 37 °C mediated by polymers **1** and **2**; **a** channels represent differential interference contrast (DIC) images, **b** channels showing fluorescence by polymer; **c** channel showing avidin-FITC import (**A**) and β -galactosidase-Alexa Fluor[®] 488 (**B**) mediated by **1** or **2**; **d** combined fluorescence for protein and polymer inside the cells. The Clone 9 cells were incubated at 37 °C for 12 h; the cells were then washed 3x with PBS buffer and 3x heparin and analyzed by fluorescence microscopy. *Note;* the polymer **2** was added in 1 % MeOH, and a blank (no nanoparticles) was performed in parallel to check for the effects of MeOH.

5. Delivery Of Streptavidin-Texas Red, Avidin-FITC, β -Galactosidase-Alexa Fluor[®] 488, and GFP (at 37 °C mediated by 1 and by 2 in CHO-K1 cells)

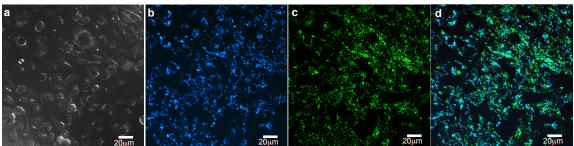


(1- streptavidin-Texas Red[®])

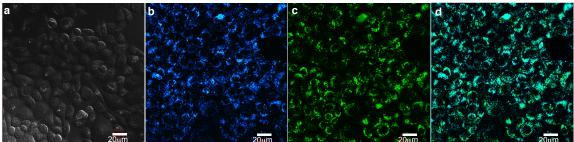


(2-streptavidin-Texas Red[®])

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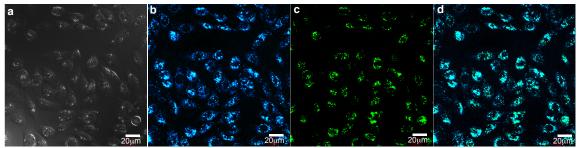


(1-avidin-FITC)

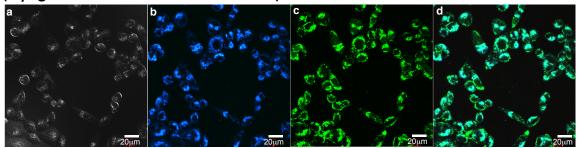


(2-avidin-FITC)

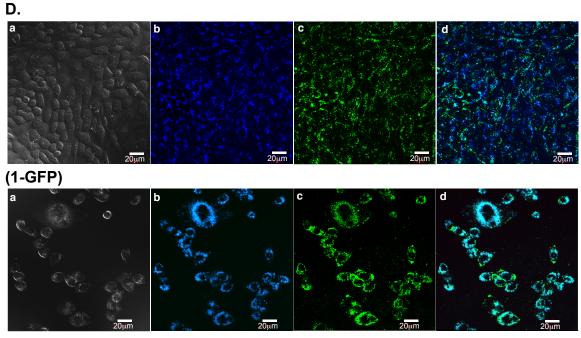
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(1-β-galactosidase-Alexa Fluor[®] 488)



(2-β-galactosidase-Alexa Fluor[®] 488)



(2-GFP)

Figure S2. Delivery of streptavidin-Texas Red[®] (**A**), avidin-FITC (**B**), β galactosidase-Alexa Fluor[®] 488 (**C**), and GFP (**D**) into CHO K1. Data for streptavidin-Texas Red[®] transport: (**a**) polymer channel; (**b**) streptavidin-Texas Red[®] channel; (**c**) LysoTracker[®] Green; (**d**) colocalization shows mostly distinct blue, red, and green areas for polymer **1** system while colocalization shows mainly white areas where all three labels coexist for polymer **2** system. Data for other proteins transport: **a** channels represent differential interference contrast (DIC) images, **b** channels showing fluorescence by polymer; **c** channel showing avidin-FITC import (**B**), β -galactosidase-Alexa Fluor[®] 488 (**C**) and GFP (**D**) mediated by **1** or **2**; **d** combined fluorescence for protein and polymer inside the cells. The CHO K1 cells were incubated at 37 °C for 12 h; the cells were then washed 3x with PBS buffer and 3x heparin and analyzed by fluorescence

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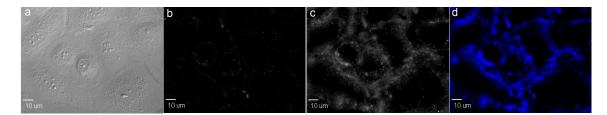
spectroscopy. *Note;* the polymer **2** was added in 1 % MeOH, and a blank (no nanoparticles) was performed in parallel to check for the effects of MeOH.

6. Delivery Of BSA-Texas Red[®] at 4 ^oC Mediated By Polymer 1 in Clone 9 and CHO-K1 Cells

Fluorescence Microscopy

Subcellular protein localization was measured on living Clone 9 and CHO K1 cells using a Zeiss Stallion Dual Detector Imaging System consisting of an Axiovert 200M inverted fluorescence microscope, CoolSnap HQ digital cameras and Intelligent Imaging Innovations (3I) software. Digital images of Texas Red-tagged BSA and **1** (blue polymer) were captured with a C-APO 63X/1.2 W CORR D=0.28M27 objective with the following filter sets: Exciter BP560/40, Dichroic FT 585, Emission BP 630/75 for Texas Red; and Exciter G 365, Dichroic FT 395, Emission BP 445/50 for **1**. Sequential optical sections (Z-stacks) from the basal-to-apical surfaces of the cell were acquired. Digital image acquisition was initiated approximately 1 μ m below the basal surface of the cells and optical slices were collected at 0.5 μ m steps through the apical surface of the cells with a high numerical objective lens (C-APO 63X/1.2 W CORR D=0.28M27). These wide-field images were subjected to deconvolution using 3I software.

The protein:carrier complexes were pre-formed at room temperature for 1 h by mixing (in a mol:mol ratio) the protein and the carrier in Ham's Nutrient Mixture F-12 with 10% fetal bovine serum (FBS) and DMEM supplemented with 10% fetal bovine serum (FBS) for Clone 9 cells and CHO K1 cells respectively. A 1:2 mol:mol ratio of protein:carrier was used. To study the cellular uptake of the proteins, the culture medium was removed, the preformed protein:carrier complex was added, and the cells were incubated for another hour at 4 or 37 °C. After the incubation period, the cells were washed with phosphate-buffered saline (PBS, pH 7.4) and heparin solution several times before imaging. For experiments at 4 °C, cells were pre-incubated at 4 °C for 30 min before being incubated with the protein solution for 1.5 h.



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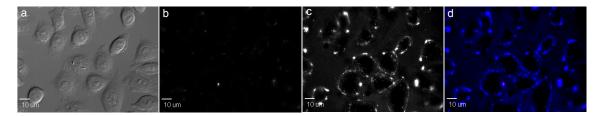


Figure S3. Delivery of BSA-Texas Red[®] into Clone 9 (**A**) and CHO-K1 (**B**) cells at 4 °C mediated by polymers **1**; **a** channels represent differential interference contrast (DIC) images, **b** channels showing no BSA-Texas Red[®] is imported mediated by **1**; **c** channel showing polymer **1** fluorescence; **d** combined fluorescence for protein and polymer.

7. Effect Of Pyrenebutyrate

Clone 9 and CHO K1 cells were first incubated with pyrenebutyrate (1pyrenebutyric acid) (50 μ M) in serum-free Ham's medium for 2 min, and then the protein:polymer complex in culture media was added to yield the final concentration of fetal bovine serum (FBS) 5%.

Kinetic Study

Clone 9 cells (1K cells, 1 mL, in Ham's medium) were suspended in a centrifuge tube. After centrifugation and removal of the medium, the protein:polymer complex in Ham's medium was added. The cells were incubated at 37 °C in 5% CO₂ and 95% air. After 5 min, 100 μ L of the cell suspension was transferred to a new centrifuge tube, and the remained for the suspension was incubated for an additional minute (15, 30, and 60). The cells were washed thoroughly by centrifugation with PBS (2x) then heparin (3x).

Thereafter, the cells were resuspended in 500 μ L of serum-free Ham's media, and placed on a Lab-Tek chambered coverglass slides.

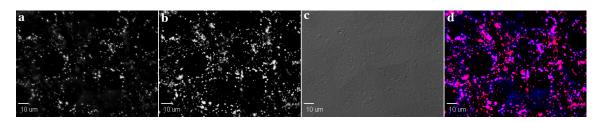
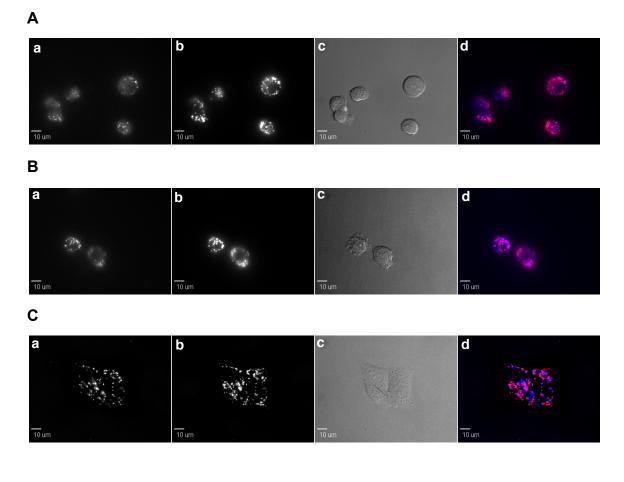


Figure S4. Delivery of BSA-Texas Red[®] in Clone 9 cells at 37 °C mediated by **1** in the presence of pyrenebutyrate; **a** channel showing polymer **1** fluorescence; **b** channels showing BSA-Texas Red[®] import mediated by **1**; **c** represents differential interference contrast (DIC) images; **d** combined fluorescence for protein and polymer.

Kinetic Studies



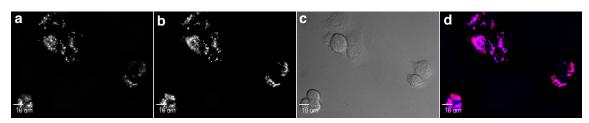
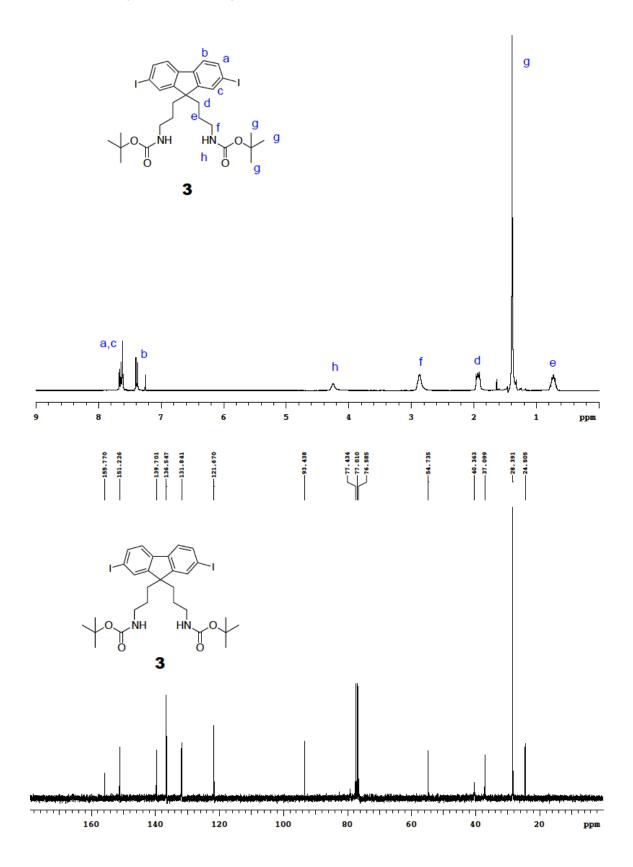
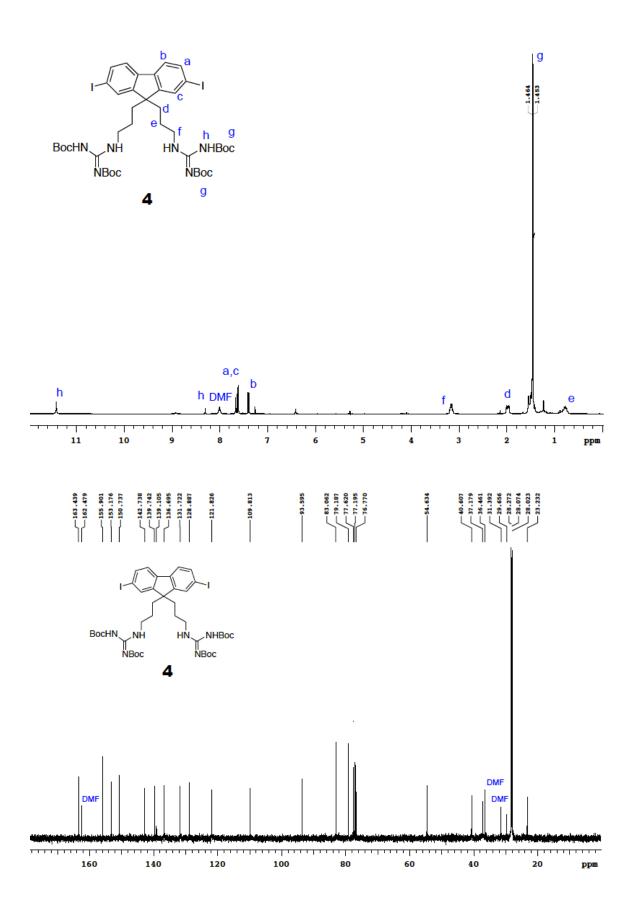
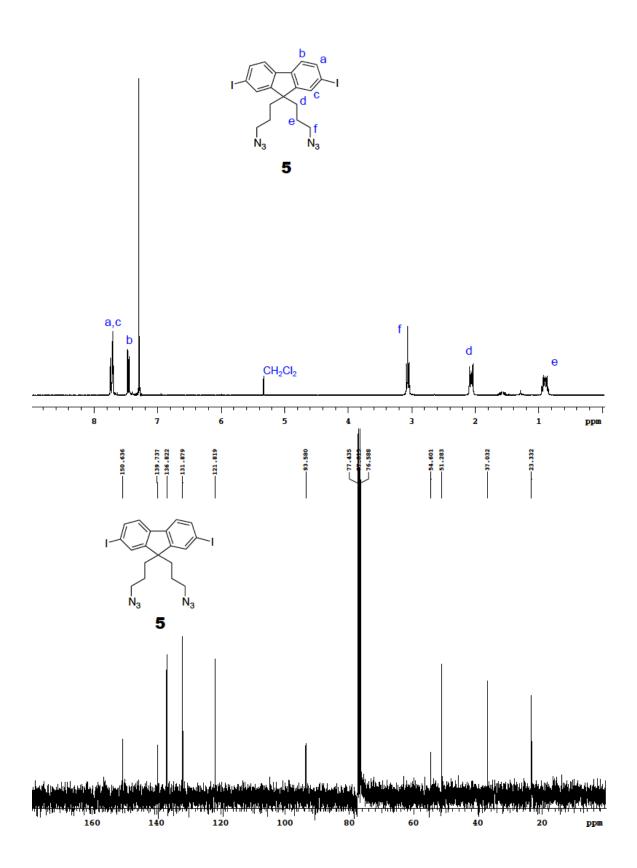


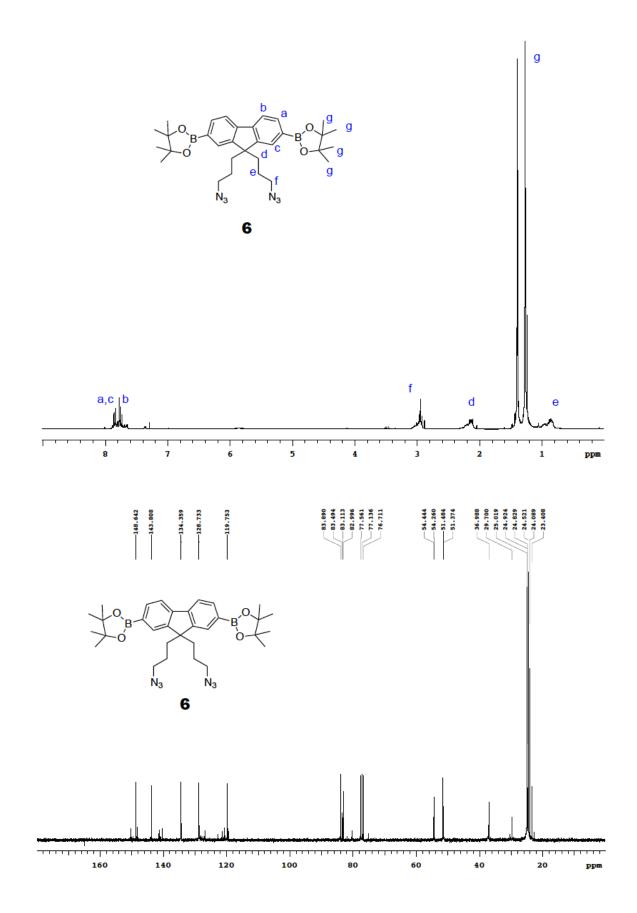
Figure S5. Delivery of BSA-Texas Red[®] in Clone 9 cells at 37 °C mediated by 1 in the presence of pyrenebutyrate. The cells were incubated for different times, **A** 5 min, **B** 15 min, **C** 30 min and **D** 60 min; **a** channel showing polymer 1 fluorescence; **b** channels showing BSA-Texas Red[®] import mediated by 1; **c** represent differential interference contrast (DIC) images; **d** combined fluorescence for protein and polymer. Throughout the carrier (1.0 μ M), BSA-Texas Red[®] (0.5 μ M) and the CHO K1 cell suspensions were incubated at 37 °C with PBS; the cells were then washed 3x with PBS buffer and 3x heparin and analysed by fluorescence microscopy. Images shown are after deconvolution.

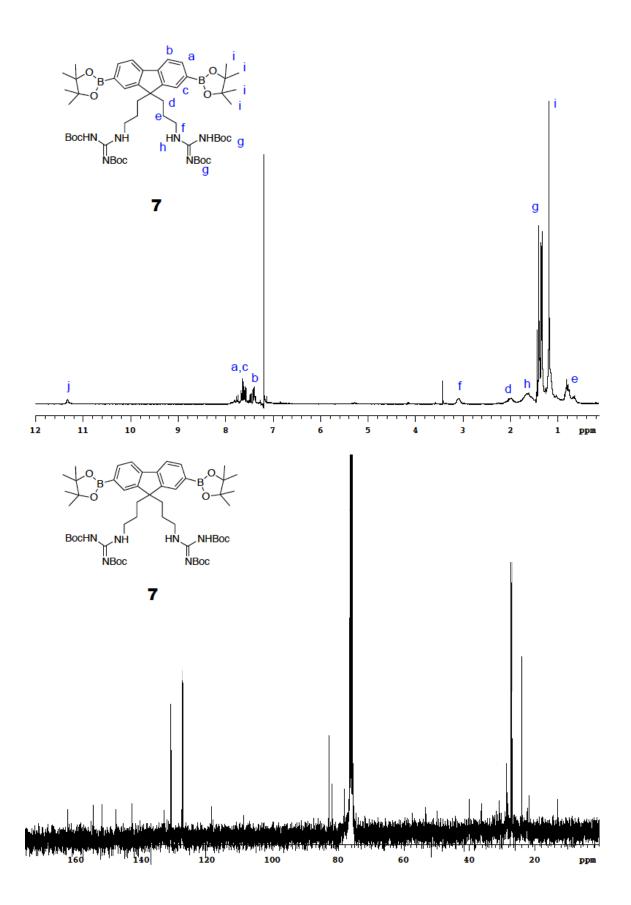
8. NMR Spectra Of Compounds 1-11

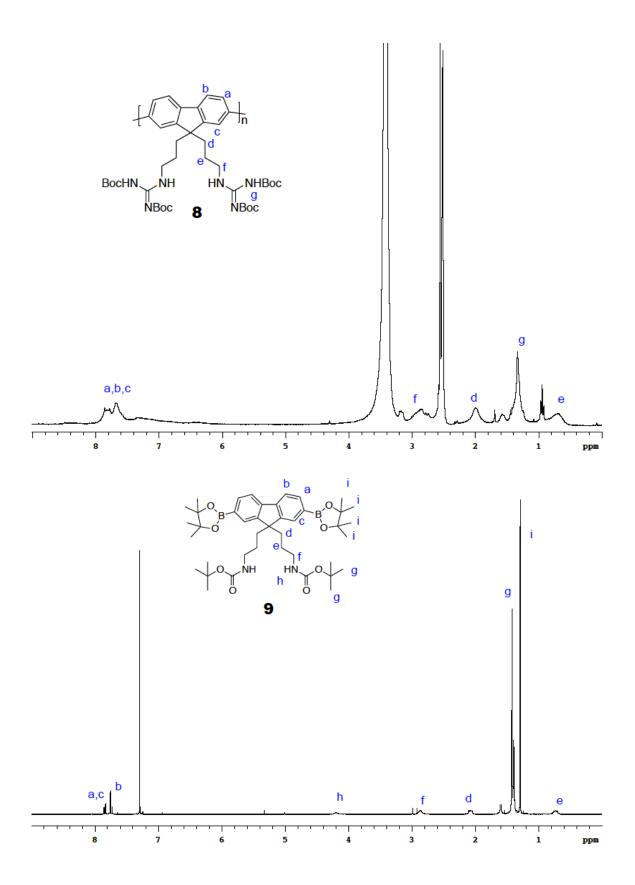












S23

