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Supporting Information

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Supporting Information

for

Design of Cell-Surface-Retained Polymers for Artificial Ligand Display

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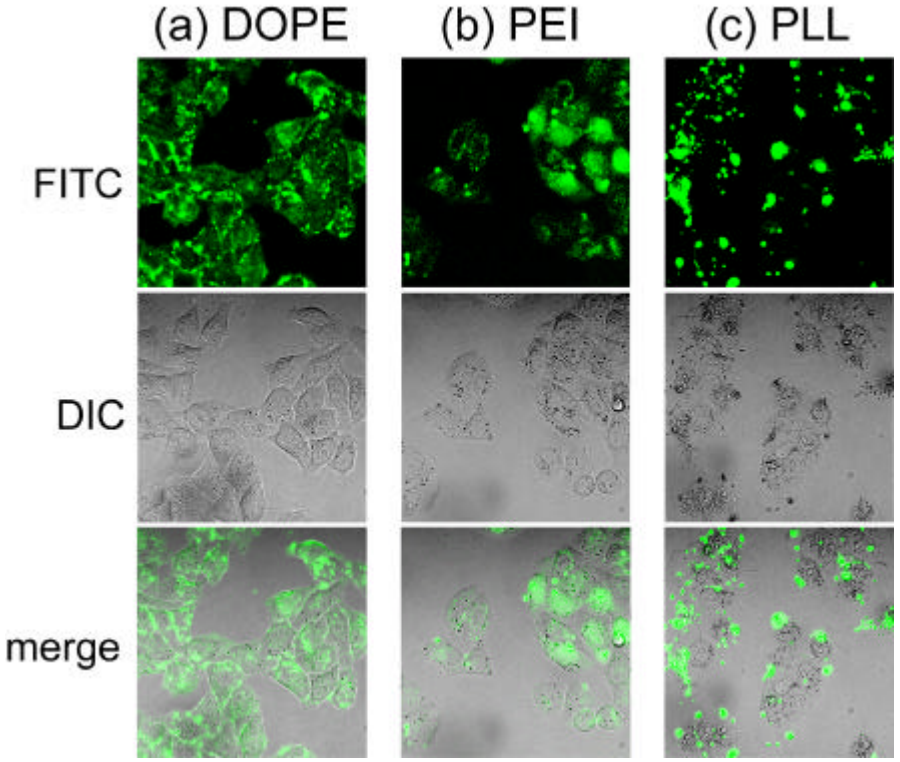


Figure S1. CLS microscope images of live HeLa cells treated with a) FITC-DOPE (1 μM), b) FITC-labeled PEI (25 μg/mL) or c) FITC-labeled PLL (25 μg/mL) for 10 min.

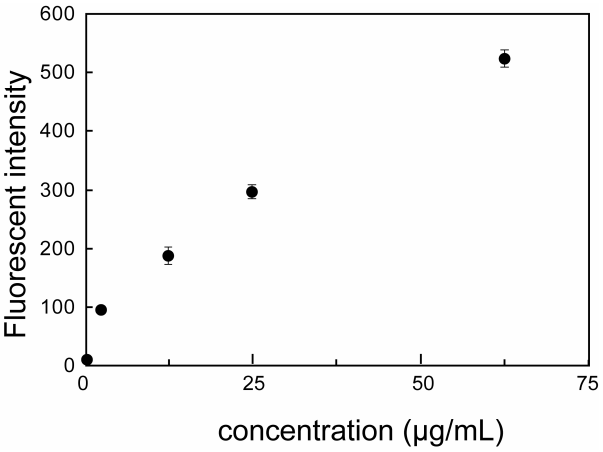


Figure S2. Flow cytometry analysis of HeLa cells treated with the polymer **5** at various concentrations.

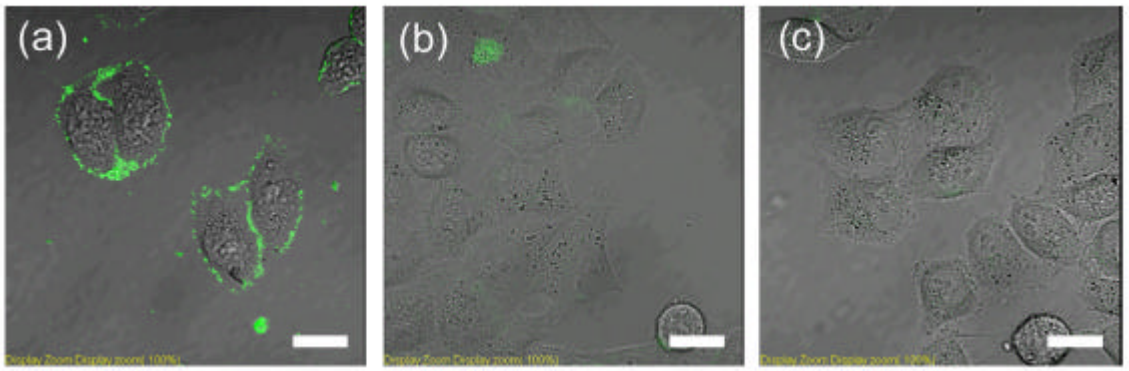


Figure S3. CLS and DIC microscope images of HeLa cells. a) Polymer **10a** modified-cells were treated with FITC-avidin. b) Cells were treated with FITC-avidin after incubation with biotin for 10 min. c) Native HeLa cells after the addition of FITC-avidin (control experiment). White bars are 20 μm.

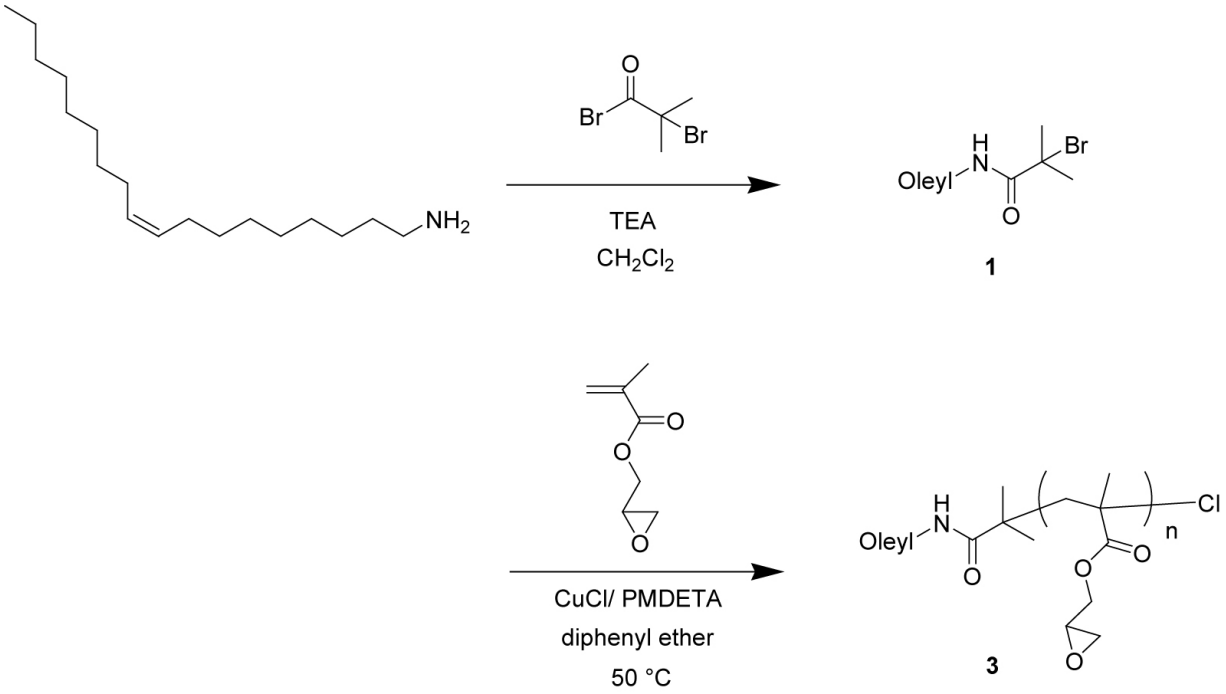
Table S1. Cytolytic activities of polymer **5** and **10a**. LDH assay was performed according to the standard protocol using a LDH-Cytotoxic kit (Wako, Japan). As a positive control, 0.2 % Tween-20 was added to the cells. The mean values and standard deviations were obtained from 3-5 independent experiments.

Sample	polymer		DOPE	non-treated	Tween 20 (0.2 %)
	5	**10a**			
Absorbance (570 nm)	0.195 ± 0.04	0.168 ± 0.03	0.179 ± 0.05	0.180 ± 0.01	0.881 ± 0.05
Cell toxicity(%)	2.1	0	0	0	100

Experimental Section

General Methods and Materials

The glycidyl methacrylate monomer (GMA) (98%, Aldrich) was purified by vacuum distillation before use. Copper chloride (CuCl) was purified by washing with the acetic acid and diethyl ether, and stored under a nitrogen atmosphere. *N*,*N*,*N*',*N*',*N*"-pentamethyldiethylenetriamine (PMDETA) (99%, Wako), diphenylether and other chemical reagents and solvents were used as received. Reactions were monitored by TLC on 250 μm silica gel plates (E. Merck, 60F254) using UV light and a cerium molybdate solution (10% Cerium(IV) Sulfate, 15% H_2SO_4 aqueous solution). NMR spectra were recorded on a JEOL 400 spectrometer. MALDI-TOF-MS spectra were measured with a Voyager-DE STR-H spectrometer (Applied Bio Systems) using 2,5-dihydroxybenzoic acid (Bluker, Germany) as the matrix. Gel permeation chromatography (GPC) analysis was carried out at 40 °C on the HLC-8220 GPC system (TOSOH, Japan) equipped with the TSK gel Super HM-M column (TOSOH, Japan). Chloroform was used as an eluent at a flow rate of 0.3 mL/min. Polystyrene standards (Polymer Laboratories, USA) were used to calibrate the GPC system. Infrared reflection absorption spectra were measured using an FT/IR-660 spectrometer (JASCO, Japan) equipped with a MCT detector. Single-channel transmittance spectra (1000 scans) were collected at a spectral resolution of 4 cm^{-1}. Flow cytometry analyses were performed with FACS canto (Becton Dickinson, USA). For each analysis, 25 000 events were monitored. Confocal laser scanning microscopy (CLSM) employed an Olympus FV300 microscope. Hoechst 33342 was excited with a 405 nm argon ion laser and emitted photons were collected through 445/15 nm band pass filter. Fluorescein isothiocyanate isomer I (FITC) was excited with a 488 nm argon ion laser and emitted photons were collected through 510 nm long pass filter. AlexaFluor 546 was excited with a 543 nm HeNe laser and emitted photons were collected through 610 nm long pass filter. Images were processed with Adobe Photoshop 7.0.



Scheme S1. Synthesis of oleyl-PGMA **3**.

2-bromo-2-methyl-*N*-oleyl propanamide (1): A solution of 2-bromo-2-methylpropanoyl bromide (2.08 mL, 16.8 mmol) and triethylamine (TEA) (2.34 mL, 16.8 mmol) in dichloromethane (10 mL) was cooled to 0 °C and oleylamine (1.50 g, 5.6 mmol) was added dropwise in ca. 10 min. After stirring overnight at room temperature, 100 mL chloroform was added and the solution were washed with brine (3 x 100 mL) and dried with Na_2SO_4. After filtration the solvent was removed under reduced pressure and the oily residue was purified by column chromatography (silica gel, hexane/ $CHCl_3$ 9:1) to give 1.50 g (64.2 %) of **1** as a colorless liquid; ^{1}H-NMR (400 MHz, $CDCl_3$): d = 6.72 (br, 1H, N*H*), 5.35 (m, 2H, C*H*=C*H*), 3.25 (dd, 2H, *J*=5.6 and 12.9 Hz, NHCH_2), 2.00 (m, 4H, CH_2-CH=CH-CH_2), 1.96 (s, 6H, -C-CH_3 × 2), 1.52 (m, 2H, NHCH$_2$CH_2), 1.38-1.23 (m, 22H, CH_2), 0.88 (t, 3H, *J*=6.6 Hz, -CH$_2$-CH_3); MALDI-TOF-MS (pos) calcd. for $C_{22}H_{43}BrNO$ $[M+H]^+$: 416.24; found 416.32.; HR-FAB-MS calcd. for $C_{22}H_{43}BrNO$: 416.2528, found: 416.2515.

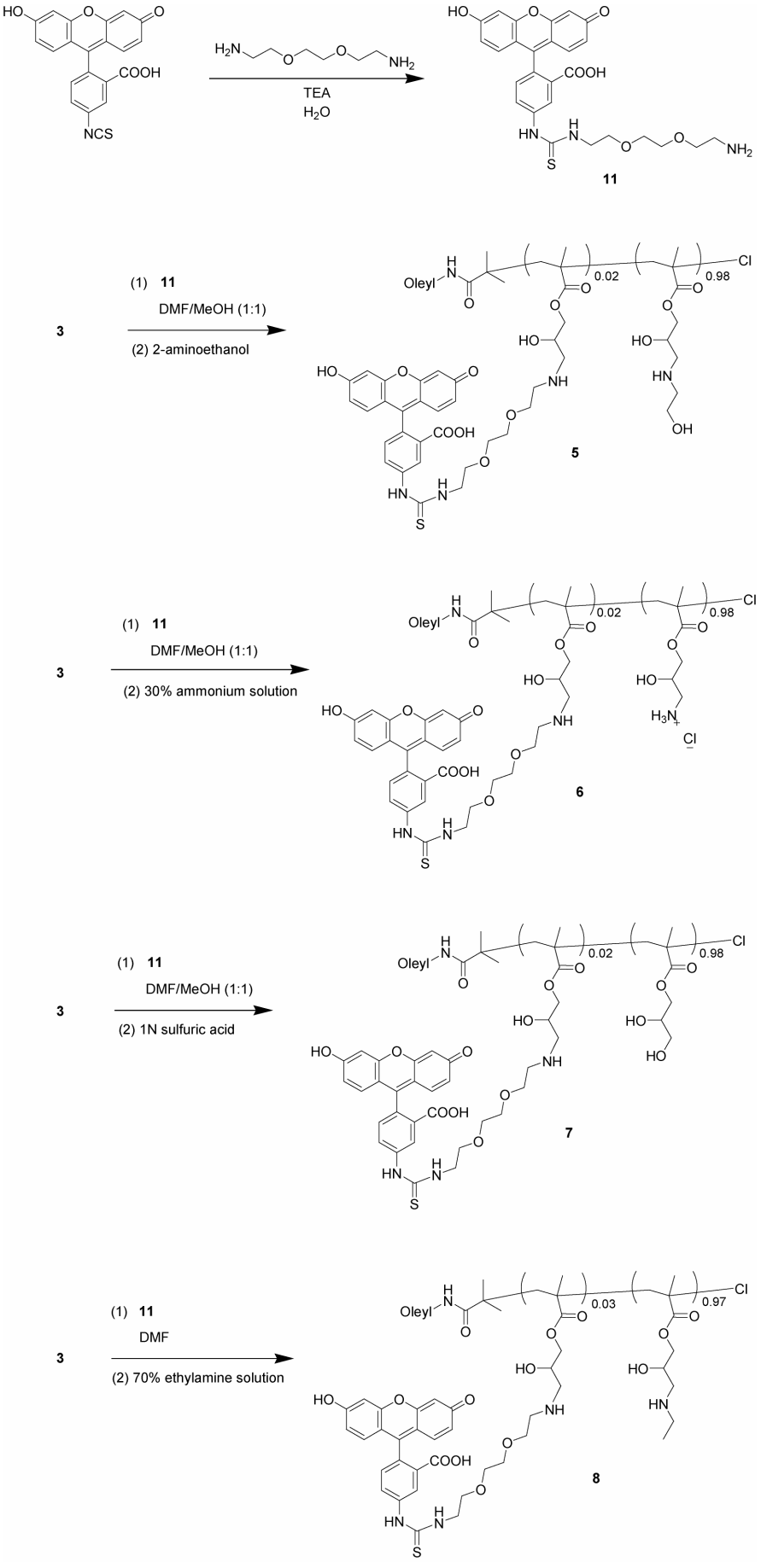
Oleyl-PGMA (3): GMA monomer (2.50 mL, 18.8 mmol) was dissolved diphenyl ether (2.5 mL) and the solution was bubbled with dry nitrogen gas for 15 min. Purifed CuCl (9.4 mg, 95 µmol) and PMDETA (19.9 µL, 95 µmol) were added. After bubbling with dry nitrogen gas for 30 min, the initiator **1** (33.3 mg, 95 µmol) was added to start the polymerization. Reaction was carried out for the desired times (15, 30, 45, 60, 75, 90, 105 and 120 min) at 50 °C. After filtration through a short neutral alumina column to

remove the copper complexes, the polymer **3** was precipitated from ethanol. Precipitation was collected by centrifugation and dried under reduced pressure; ^{1}H NMR (400 MHz, $CDCl_3$, d/ppm, see Figure S6(a)); IR (KBr, cm^{-1}, see Figure S5(a)): 3002, 2938, 1730, 1486, 1267, 1150, 994, 907, 846, 759; GPC ($CHCl_3$ eluent, polystylene standard, see Figure S4); Anal. calcd. for $(C_7H_{10}O_3)_n$: C, 59.14; H, 7.09. found: C, 59.20; H, 7.32.

5-(3-(2-(2-(2-aminoethoxy)ethoxy)ethyl)thioureido)-2-(3-hydroxy-6-oxo-6H- xanthen-9-yl)benzoic acid (11): FITC isomer-I (100 mg, 257 µmol), 2-(2-(2-aminoethoxy)ethoxy)ethanamine (376 µL, 2.57 mmol) and TEA (7.2 µL, 51.4 µmol) were dissolved in distilled water (1.0 mL). Reaction was carried out for 10 h at room temperature. The reaction was checked by TLC ($CHCl_3$/MeOH 1:1). The solvent was evaporated and acetonitrile (2.0 mL) was added. The precipitation was collected by filtration and dried under reduced pressure to give 84.6 mg (61.1 %) of **11** as orange powder; ^{1}H-NMR (400 MHz, $CDCl_3$): d = 7.69 (s, 1H), 7.51 (br, 1H), 7.31 (d, 1H, *J*= 8.12 Hz), 7.22 (d, 2H, *J*= 9.32 Hz), 6.64 (s, 2H), 6.61 (s, 1H), 3.80 (br, 1H, N*H*), 3.77-3.60 (m, 10H), 3.04 (m, 2H), 2.96 (m, 4H); MALDI-TOF-MS (m/z) calcd. for $C_{27}H_{27}N_3NaO_7S$: 560.15 $[M+Na]^+$, found 560.53; HR-ESI-MS calcd. for $C_{27}H_{27}N_3NaO_7S$: 560.1467, found: 560.1487.

Polymer (5): To a solution of oleyl-PGMA **3** (20.0 mg) in DMF/MeOH (1:1 *v/v*, 1.0 mL) were added compound **11** (15.2 mg, 28.3 µmol). The mixture was stirred for12 h at 50 °C. 2-aminoethanol (100 µL, 3.31 mmol) was added and stirred for 1 day at 50 °C. The solution was dialyzed using a 11,000 MWCO membrane in distilled water for 3 days and lyophilized to give 25.3 mg of **5** as a yellow solid; ^{1}H-NMR (400 MHz, $CDCl_3$, d/ppm, see **Figure S6(b)**); IR (KBr, cm^{-1}, see **Figure S5(b)**): 3418, 2987, 2955, 1727, 1631, 1581, 1453, 1274, 1159, 1064; Anal. found: C, 49.11; H, 7.75; N, 5.81; S, N.D.

Polymer (6): To a solution of oleyl-PGMA **3** (20.0 mg) in DMF/MeOH (1:1 *v/v*, 1.0 mL) were added compound **11** (15.2 mg, 28.3 µmol). The mixture was stirred for12 h at 50 °C. 30% ammonium solution (2 mL) was added and stirred for 1 day at room temperature. The solution was dialyzed using a 11,000 MWCO membrane in distilled water for 3 days and lyophilized to give 20.3 mg of **6** as a yellow solid; ^{1}H-NMR (400 MHz, $CDCl_3$): d = 4.60-3.75 (br, 3H, OCH_2CHOH), 3.40-2.62 (br, 2H, C$H_2$$NH_3^+$), 2.38-1.77 (br, 1H, CH$HCCH_3$), 1.52-0.60 (br, 4H, C$HHCCH_3$); IR (KBr, cm^{-1}): 3429,

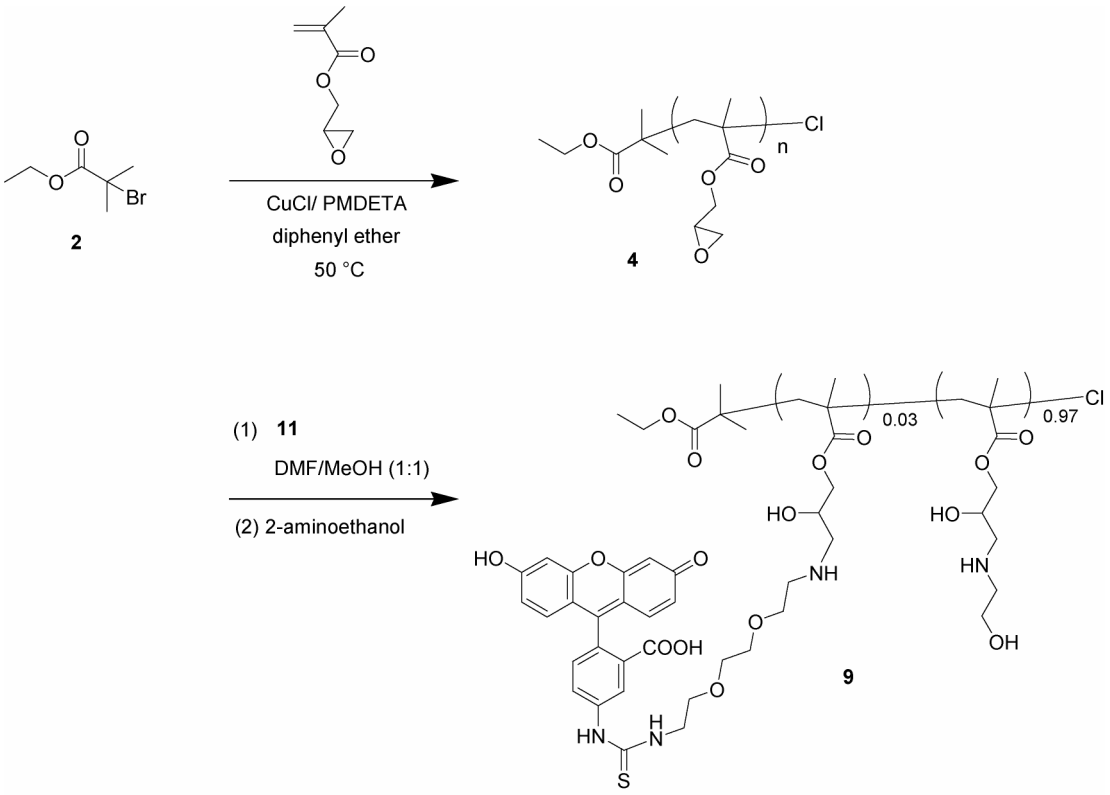


Scheme S2. Synthesis of FITC-derivative **11**, polymer **5**, **6**, **7** and **8.**

2993, 2950, 1723, 1635, 1577, 1462, 1391, 1328, 1277, 1166; Anal. found: C, 45.96; H, 7.33; N, 4.36; Cl, 5.16.

Polymer (7): To a solution of oleyl-PGMA **3** (20.0 mg) in DMF/MeOH (1:1 *v/v*, 1.0 mL) were added compound **11** (15.2 mg, 28.3 µmol). The mixture was stirred for12 h at 50 °C. 1N sulfuric acid (1 mL) was added and stirred for 1 day at 50 °C. The solution was dialyzed using a 11,000 MWCO membrane in distilled water for 3 days and lyophilized to give 18.0 mg of **7** as a yellow solid; IR (KBr, cm^{-1}): 3420, 2948, 2888, 1726, 1636, 1455, 1388, 1268, 1161, 1121, 1056; Anal. found: C, 46.79; H, 6.79; N, 0.50; S, N.D.

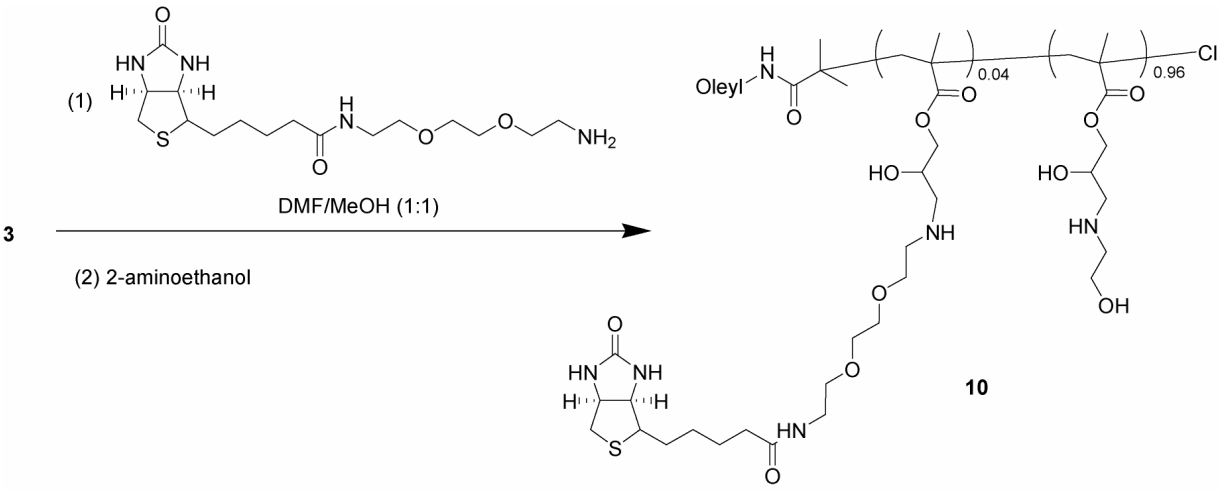
Polymer (8): To a solution of oleyl-PGMA **3** (14.8 mg) in DMF (2.0 mL) were added compound **11** (2.71 mg, 5.04 µmol). The mixture was stirred for12 h at 60 °C. 70% ethylamine solution (2 mL) was added and stirred for 1 day at 60 °C.. The solution was dialyzed using a 11,000 MWCO membrane in distilled water for 3 days and lyophilized to give 5.43 mg of **8** as a yellow solid; ^{1}H-NMR (400 MHz, $CDCl_3$): d = 4.41-3.99 (br, 3H, OC*H*$_2$C*H*OH), 3.11-2.80 (br, 4H, C*H*$_2$NHC*H*$_2$CH$_2$), 2.29-1.73 (br, 1H, CH*H*CCH$_3$), 1.51-0.68 (br, 7H, NHCH$_2$C*H*$_3$ and C*H*HCC*H*$_3$); IR (KBr, cm^{-1}): 3364, 2961, 2941, 1730, 1663, 1634, 1577, 1464, 1388, 1328, 1274, 1158, 1107.



Scheme S3. Synthesis of ethyl-PGMA **4** and polymer **9**.

Polymer (9): GMA monomer (2.50 mL, 18.8 mmol) was dissolved diphenyl ether (2.5 mL) and the solution was bubbled with dry nitrogen gas for 15 min. Purifed CuCl (9.4 mg, 95 µmol) and PMDETA (19.9 µL, 95 µmol) were added. After bubbling with dry nitrogen gas for 30 min, the ethyl 2-bromo-2-methylpropanoate **2** (13.9 µL, 95 µmol) was added to start the polymerization. Reaction was carried out for the 45 min at 50 °C. After filtration through a short neutral alumina column to remove the copper complexes, the ethyl-PGMA **4** was precipitated from ethanol. Precipitation was collected by centrifugation and dried under reduced pressure; GPC analysis, M_n = 9000, M_w = 11400, PDI= 1.27.

To a solution of ethyl-PGMA **4** (18.0 mg) in DMF/MeOH (1:1 *v/v*, 1.0 mL) were added compound **11** (15.2 mg, 28.3 µmol). The mixture was stirred for12 h at 50 °C. 2-aminoethanol (100 µL, 3.31 mmol) was added and stirred for 1 day at 50 °C. The solution was dialyzed using a 11,000 MWCO membrane in distilled water for 3 days and lyophilized to give 22.3 mg of **9** as a yellow solid; ^{1}H-NMR (400 MHz, $CDCl_3$): d = 7.30-6.83 (br, fluorescein), 4.23-3.88 (br, 3H, OC*H*$_2$C*H*OH), 3.88-3.60 (br, 2H, NHCH$_2$C*H*$_2$-OH), 3.04-2.62 (br, 4H, C*H*$_2$NHC*H*$_2$CH$_2$OH), 2.25-1.79 (br, 2H, C*H*$_2$CCH$_3$), 1.25-0.65 (br, 3H, CH$_2$CC*H*$_3$); IR (KBr, cm^{-1}):3367, 2943, 1727, 1635, 1576, 1461, 1387, 1328, 1275, 1163, 1058; Anal. found: C, 48.75; H, 7.83; N, 5.83; S, N.D.



Scheme S4. Synthesis of polymer **10**.

Polymer (10b): To a solution of oleyl-PGMA **2** (20.0 mg) in DMF/MeOH (1:1 *v/v*, 1.0 mL) were added biotin PEO-amine (7.03 mg, 18.8 µmol, PIERCE, USA). The mixture was stirred for12 h at 80 °C. 2-aminoethanol (100 µL, 3.31 mmol) was added and

stirred for 1 day at 50 °C. The solution was dialyzed using a 11 000 MWCO membrane in distilled water for 3 days and lyophilized to give 19.5 mg of **10b** as a white solid; ^{1}H-NMR (400 MHz, $CDCl_3$, d/ppm, see Figure S6c); IR (KBr, cm^{-1}, see Figure S5c): 3388, 2951, 2851, 1727, 1653, 1557, 1456, 1274, 1161, 1058; Anal. found: C, 49.56; H, 7.88; N, 5.13; S, 0.58.

Cell culture: Human HeLa cells were grown in a monolayer in Dulbecco's modified Eagle medium (DMEM) supplemented with 10 % fetal bovine serum (FBS), penicillin (500 units/mL), and streptomycin (500 µg/mL). The cultures were kept at 37 °C in a humidified incubator under 5 % CO_2.

Incubation of HeLa Cells with FITC-labeled Polymer (5, 6, 7, 8 or 9) for CLS microscopy: HeLa cells were seeded at a density of 1.25 × 10^5 cells/dish into a glass-bottomed culture dishes (ASAHI GLASS, Japan) and allowed to grow in the above mentioned conditions. Hoechst 33342 dye (1 µg/mL, DOJINDO, Japan) in serum-free culture medium (Opti-MEM I, GIBCO, USA) was added for 10 min. The cells were rinsed with PBS (2 × 1.0 mL) and polymer **5**, **6**, **7**, **8** or **9** dissolved in serum-free culture medium to a compound concentration of 25 µg/mL was added to the cells. The cells were incubated in the presence of polymer at 37 °C for 10 min, washed with PBS (2 × 1.0 mL) to remove unanchored compound, added 2.0 mL fresh serum-free medium and observed in a CLS microscope.

Flow cytometry analysis of polymer (5 or 9)-treated cells: HeLa cells were grown at a density of 3.7 × 10^5 cells/dish in the cell culture dishes and treated with polymer **5** or **9** by the above mentioned method. Then cells were treated with Trypsin/EDTA (0.05%) at 37 °C for 3 min. After centrifugation at 200 G for 3 min, the fluorescence of cells was measured by flow cytometry.

Endocytosis assay of transferrin in HeLa cells: HeLa cells were seeded at a density of 1.25 × 10^5 cells/dish into a glass-bottomed culture dishes and allowed to grow in the above mentioned conditions. After treatement with polymer **5**, HeLa cells were incubated with 25 µg/mL AlexaFluor 546-transferrin dissolved in serum-free medium for 10 min at 37 °C. The cells were rinsed with PBS (2 × 1.0 mL), added 2.0 mL fresh serum-free medium and observed in a CLS microscope.

Incubation of HeLa Cells with polymer 10a for CLS microscopy: HeLa cells were cultured under the same conditions as for polymer **10a** treatment. Polymer **10a** dissolved in serum-free culture medium to a compound concentration of 25 µg/mL was

added to the cells. The cells were incubated in the presence of polymer at 37 °C for 10 min, washed with PBS (2 × 1.0 mL) to remove unanchored compound. Then cells were incubated in serum-free medium with 10 μg/mL fluorescein-labeled avidin at 4 °C for 10 min. After washing with PBS (2 × 1.0 mL), 2.0 mL fresh serum-free medium was added.

Flow cytometry analysis of polymer 10-treated cells: HeLa cells treated with polymer **10** prepared by the above mentioned method were treated with Trypsin/EDTA (0.05%) at 37 °C for 3 min. After centrifugation the cells were incubated in serum-free medium with 10 μg/mL fluorescein-labeled avidin at 4 °C for 10 min. After washing with PBS (2 × 1.0 mL), the cells were resuspended in PBS (500 μL) and analyzed by the flowcytometer.

HABA assay: 2-(4'-hydroxyazobenzene)-2-carboxylic acid (HABA) assay was performed accordingly the previously reported method.[1] 40 μL of the polymer **10a** treated cells (3.2×10^5 cells/mL) were added in the HABA/avidin solution (2.0 mL, pH 7.4 PBS contained 25 μg/mL avidin, 0.3 mM HABA, 0.3 mM NaOH). After incubation for 5 min at room temperature, the absorbance at 500 nm was measured using the UV-visible spectrometer (UV-1650PC, SHIMADZU, Japan). Quantification of the number of biotin moieties of the single cell was calculated from the standard plot using the biotin solution.

LDH cytotoxicity assay: HeLa cells were seeded into each well of the 96-well microplate (5,000 cells/well) and incubated at 37 °C in a humidified incubator under 5 % CO_2 for 24 h. After washing with PBS (2 × 200 μL), the cells were incubated with 25 μg/mL polymer **5** or **10a** in serum-free medium (100 μL/well) for 15 min at 37 °C. Each supernanant (80 μL) was transferred to a 96-well microplate and the absorbance at 570 nm was measured.

Fabrication of cell patterning of polymer 10a-treated HeLa cells: Avidin-immobolized plate were prepared accordingly the previously reported method.[2] HeLa cells were treated with Trypsin/EDTA (0.05%) at 37 °C for 3 min. After centrifugation the cells (3.2×10^5 cells in 2.0 mL serum-free medium) were treated with polymer **10a** for 10 min and collected by the centrifugation. The cells were seeded to the FITC-avidin immobilized substrate and incubated at 37 °C in a humidified incubator under 5 % CO_2 for 5 min. The substrate was gently washed with PBS (3 × 1.0 mL) to remove the extra cells on the pattern and observed with fluorescent microscopy.

References

(1) M. D. Savage, G. Mattson, S. Desai, G. W. Nielander, S. Morgensen, E. J. Conklin, in *Avidin-Biotin Chemistry: A Handbook*, Rockford, Illinois: Poerce Chemical Company, **1992**, 16-19.

(2) M.-C. Shao, *Anal. Biochem.* **1992**, *205*, 77-82.

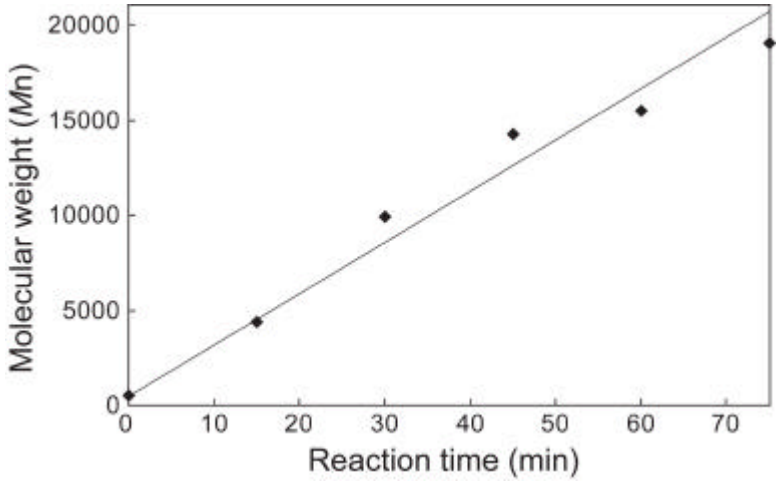


Figure S4. Controlled molecular weight of the oleyl-PGMA **3** by the polymerization reaction time. The number-average molecular weight (*M*n) were determined using the GPC.

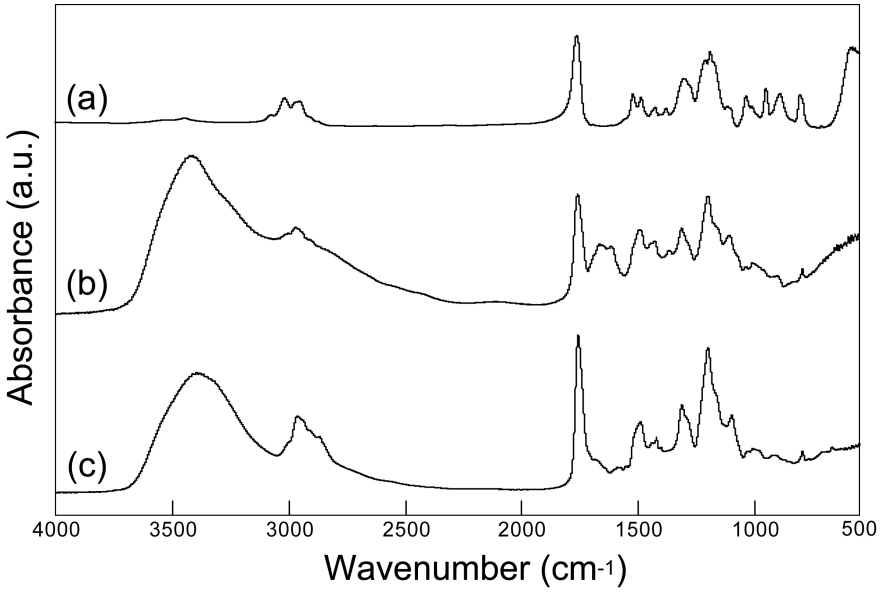
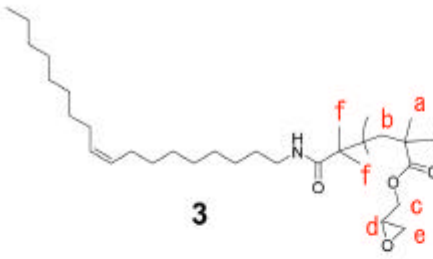


Figure S5. FT-IR spectroscopy of a) oleyl-PGMA **3**, b) polymer **5** and c) polymer **10b**. In (a), the characteristic peaks of epoxide groups appeared at 910 and 850 cm^{-1}. After the reaction with biological molecules, the epoxide peaks were diminished and a secondary amine band arose at 1640 cm^{-1}. In addition, the starching band of -CNH- at 1580 cm^{-1} confirmed that the each polymer have objective functional groups via a covalent bond.

(a)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3

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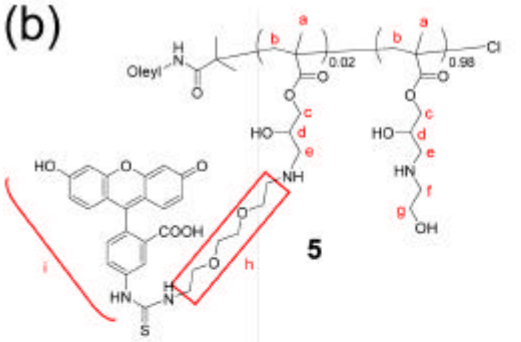
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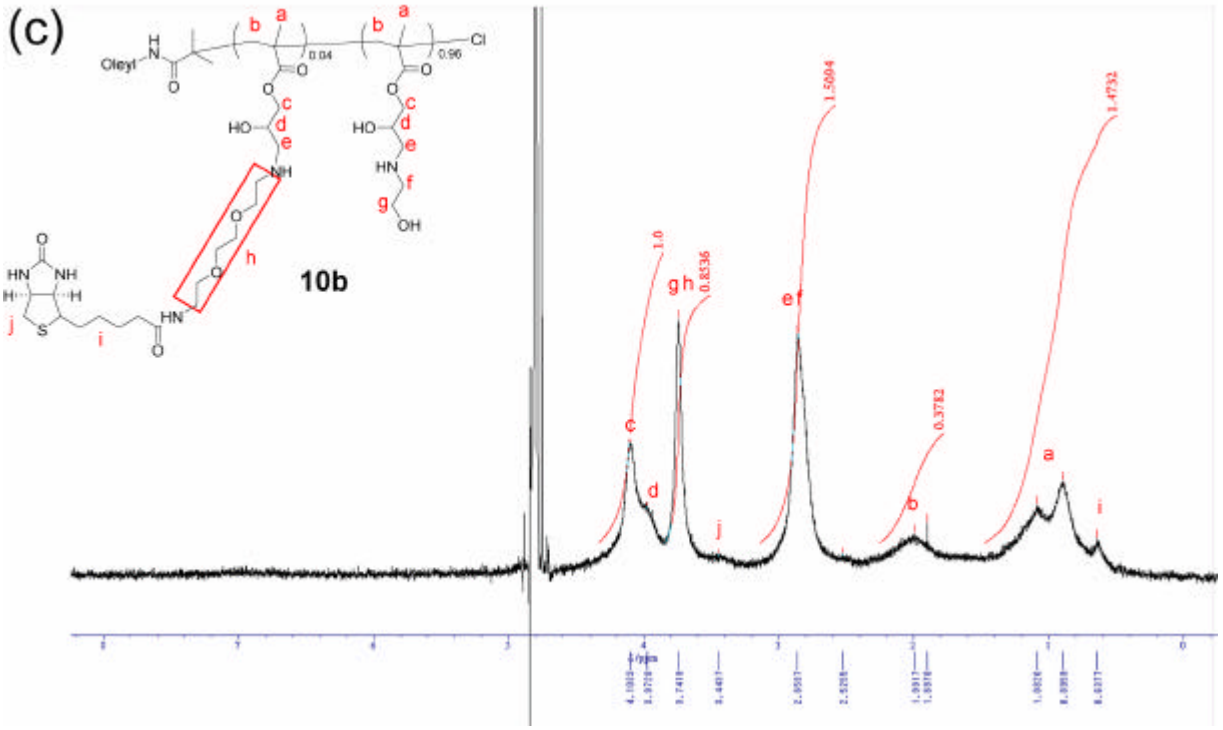

Figure S6. NMR spectrum of a) oleyl-PGMA **3**, b) polymer **5** and c) polymer **10b**