ADVANCED MATERIALS

Supporting Information

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By Kangwon Lee, Jiseok Lee, Eun Jeong Jeong, Adam Kronk, Kojo S. J. Elenitoba-Johnson, Megan S. Lim, and *Jinsang Kim**

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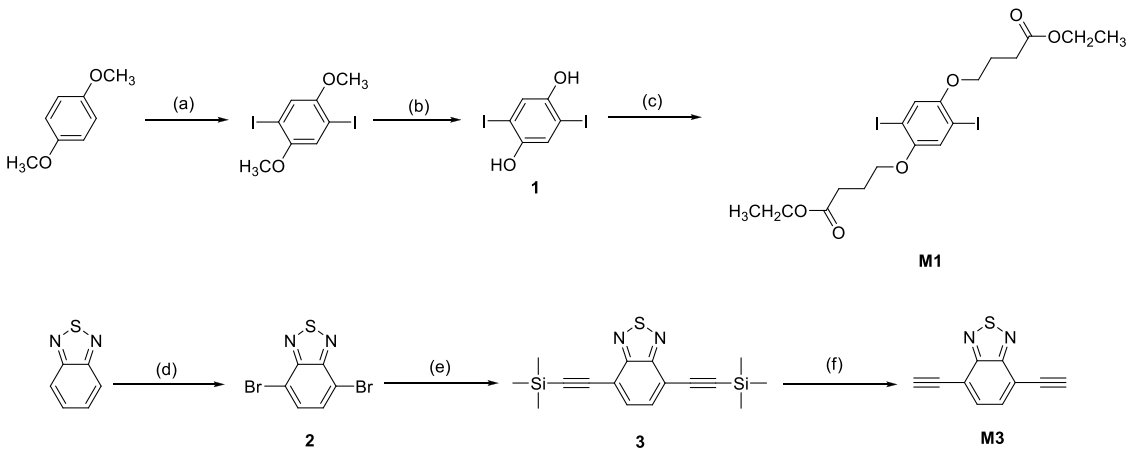
PPE1 and PPE2 Preparation For M1 synthesis for PPE1 copolymer, 2,5-diiodohydroquinone starts by reacting 1,4-dimethoxybenzene with iodine through the acid based electrophilic aromatic iodination in 85 % yield as previously reported in the literature (Scheme S1).[1] Demethylation reaction was achieved by means of borontribromide. The resulting 2,5-diiodohydroquinone was then reacted with ethyl 4-bromobutyrate by Williamson-ether synthesis to give M1 having the ethyl-protected carboxylic group. In monomer M3 synthesis for PPE2, benzothiadiazole compound was reacted with bromine, followed by trimethylsilylacetylene and a subsequent deprotection reaction in base to give M3.[2] M3 turned out to be very unstable so it was immediately used for polymerization upon preparation. The copolymerization of M1 and M2 for PPE1 was carried out using conventional palladium-catalyzed Sonogashira-Hagihara copolymerization method (Scheme S2).

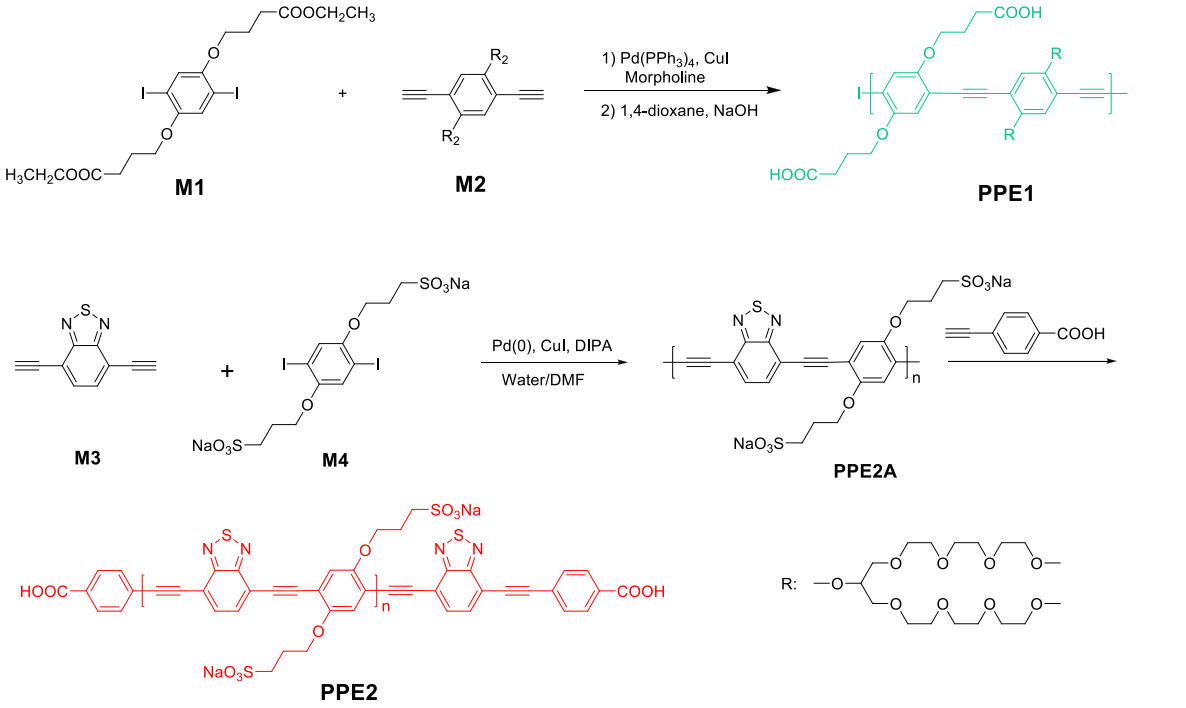
To be a good sensory reporter of a biosensor, a conjugated polymer should be completely water-soluble or at least highly hydrophilic and have an appropriate functional group for conjugation with biological moieties because any biological target should be handled in aqueous environment. Conventionally, when there is a problem in water-solubility the hydrophobic reagents are dissolved in a polar organic solvent such as dimethyl sulfoxide or methanol and the solution is diluted in an aqueous buffer. However, this method can give a detrimental effect on the biological system due to the potential toxicity of organic solvents. We recently systematically investigated the correlation between the chemical structure of conjugated polyelectrolytes and their water-solubility and photophysical property through a series of PPEs containing carboxylic acid side chains. It has been found that a bulky bifurcated ethylene oxide side chain provides an excellent solubility of PPEs in water and a highly emissive property.[1a,3] Our conjugated polyelectrolyte, PPE1, showed an excellent water solubility exceeding 10 mg/mL in deionized water.

Copolymerization of M3 and M4 for the red emissive PPE2A was also conducted using the Pd-catalyzed method. Originally we designed a red-emissive polymer in such a way that the polymer has a large portion of the bulky ethylene oxide side chains and carboxylic acid group to give a good water-solubility like PPE1. However, our systematic investigation about the correlation between the emission color of PPE2 derivatives (PPE-BT_x, Figure S1 and S2 in the supporting information) and the mole fraction of the benzothiadiazole unit in the polymer backbone revealed that the polymer must have a large mole fraction of the benzothiadiazole unit to have a pure red emission. In this case, however, the abundant benzothiadiazole units in the polymer backbone reduced the water-solubility of the CPE. By putting a carboxylic acid group-containing unit and the benzothiadiazole unit alternatingly in the CPE backbone we could achieve very good water solubility. Unfortunately, however, the bioconjugation of the CPE with an antibody reduced the water-solubility of the CPE due to the consumption of the carboxylic group for the bioconjugation, resulting in a low quantum yield. Therefore, we redesigned a red-emissive CPE to have alternating benzothiadiazole unit and non-reactive sulfonic acid unit as an ionic group.[4] Reactive carboxylic acid groups were introduced at the two ends of the CPE for bioconjugation with an antibody, instead. The *in-*

situ end-modification of PPE2A with 4-ethynylbenzoic acid and additional palladium catalyst gave PPE2 having carboxylic acid groups at the two chain ends of the polymer backbone. The resulting PPE2 was fairly water-soluble (> 3 mg/mL in deionized water) and showed relatively bright red-emission (quantum yield of 1%).



Scheme S1. Monomer synthesis (a) I_2, H_2SO_4, acetic acid, water, 100 ºC (b) BBr_3, dichloromethane, -60 ºC → r. t., 48 h (c) K_2CO_3, ethyl 4-bromobutyrate, dimethylformamide (DMF), 80 ºC, 48 h (d) Bromine, hydrobromic acid, 100 ºC overnight (e) trimethylacetylene, $Pd_3(PPh_3)_4$, CuI, triethylamine, toluene. 65 ºC, 7 h. (f) potassium hydroxide, methanol:tetrahydrofuran (1:1 v/v), room temperature, 1 h.



Scheme S2. Synthesis of PPE1 and PPE2.

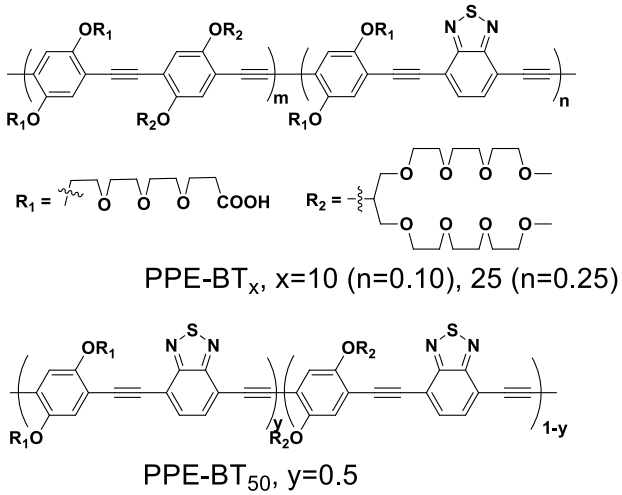


Figure. S1. Chemical structure of PPE2 derivatives containing benzothiadiazole unit.

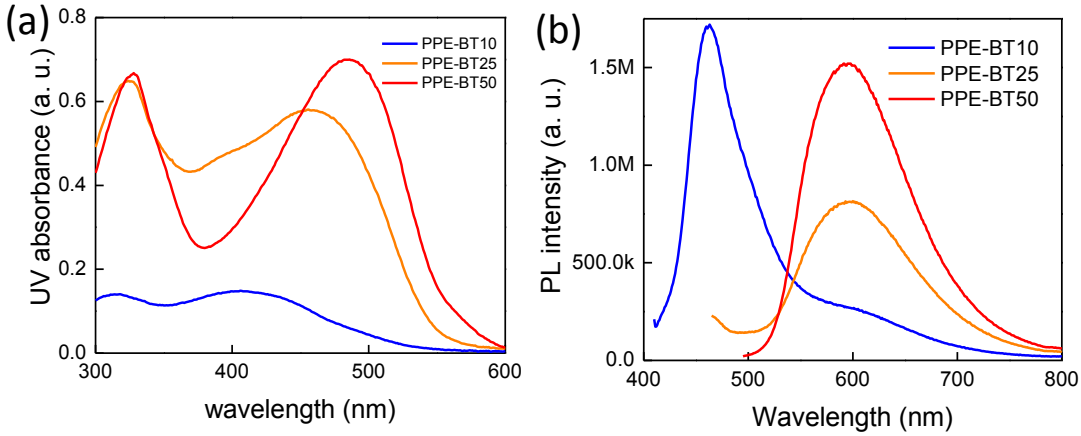


Figure. S2. The Effect of the Mole Fraction of the Benzothiadiazole Unit in the Polymer Backbone on the Emission Color (a) UV absorption and (b) photoluminescence spectra of PPE-BT_x.

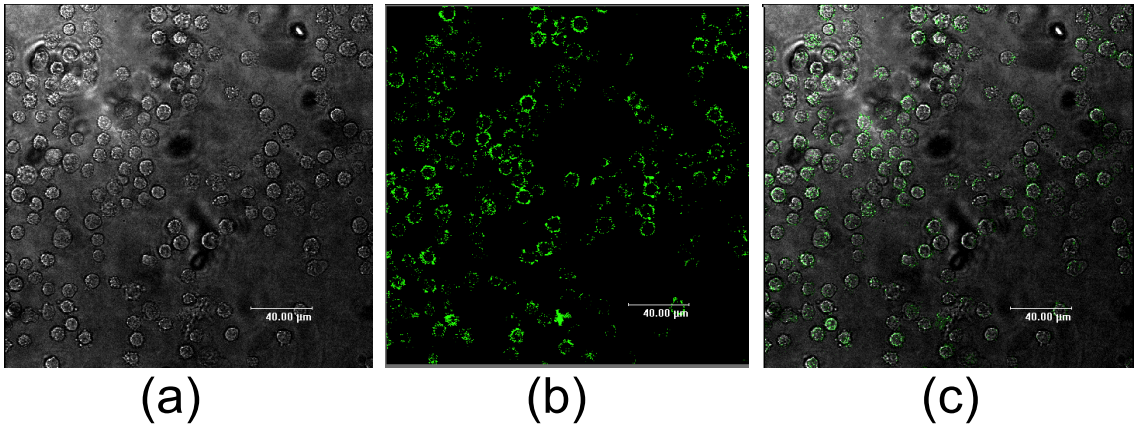


Figure S3. Confocal Microscope Image of Cells Stained with CPE-antibody. Representative confocal laser scanning microscope images of Jurkats stained with PPE1-CD3; (a) DIC, (b) fluorescence, and (c) overlay images.

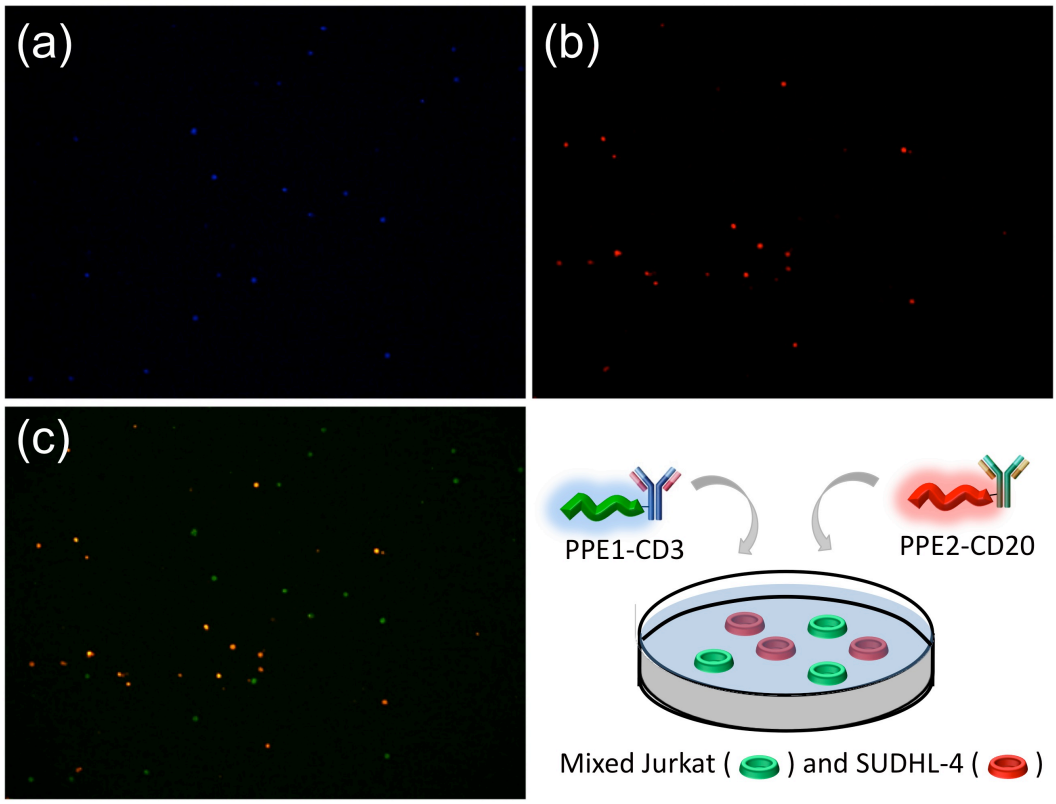
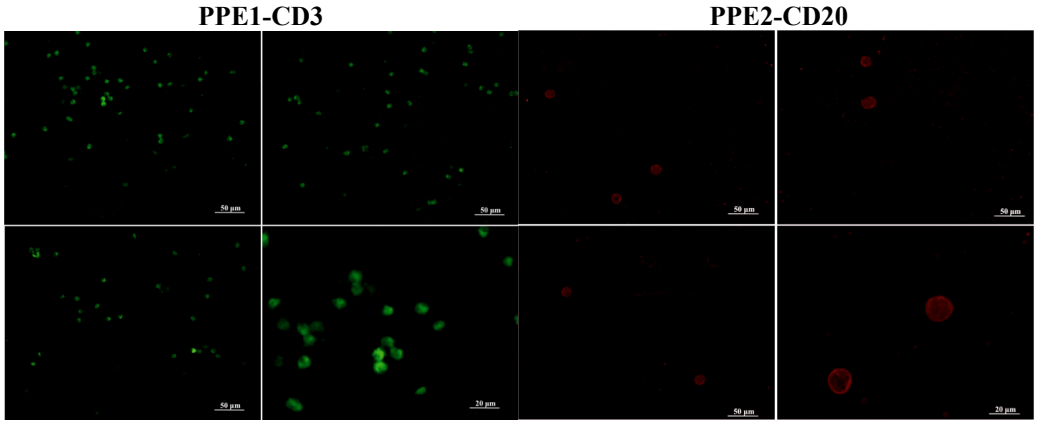


Figure S4. Fluorescence microscope images of the mixed Jurkat and SUDHL-4 after incubation with PPE1-CD3 (1.0 μM) and PPE2-CD20 (1.0 μM) together. Figure 5(a) shows blue emission (excitation: 395-415 nm, emission: 435-485 nm) of PPE1-CD3-stained Jurkat. Figure (b) shows PPE2-CD20-stained SUDHL-4 having red emission (excitation: 540-580 nm, emission: 590-650 nm). The image in Figure 5(c) is like an overlay image of Figures 5(a) and 5(b) and was obtained by using a wideband emission filter (> 500 nm), exposure of wide excitation filter (450-490 nm), and an external incandescent light, showing both Jurkat and SUDHL-4 having different emission colors. The image size is 700 μm x 527 μm.

Human Blood Test To demonstrate the performance characteristics of the CPEs on peripheral blood lymphocytes, we used peripheral blood samples of healthy normal volunteers (University of Michigan Institutional Review Board Study HUM00023256). PPE1-CD3 staining highlighted a mean number of 92.5 cells/mm^2 of CD3-positive T cells in peripheral blood while PPE2-CD20 staining highlighted a mean number of 4.3 B-cells/mm^2 in peripheral blood (Figure S5). This is consistent with the relative prevalence of T-cells (90%) and B-cells (5%) as determined by immunocytochemistry or flow cytometry.[5]



Areas	PPE1-CD3	PPE2-CD20
1	95 cells/mm^2	5 cells/mm^2
2	100 cells/mm^2	3 cells/mm^2
3	95 cells/mm^2	4 cells/mm^2
4	85 cells/mm^2	3 cells/mm^2
5	95 cells/mm^2	6 cells/mm^2
6	85 cells/mm^2	5 cells/mm^2
Mean #	92.5 cells/mm^2	4.3 cells/mm^2

Figure S5. Fluorescence microscope images of peripheral blood sample stained with (a) PPE1-CD3 and (b) PPE2-CD20. (c) Mean number of B-cells and T-cells stained with PPE1-CD3 and PPE2-CD20. Scale bar: 50 μm.

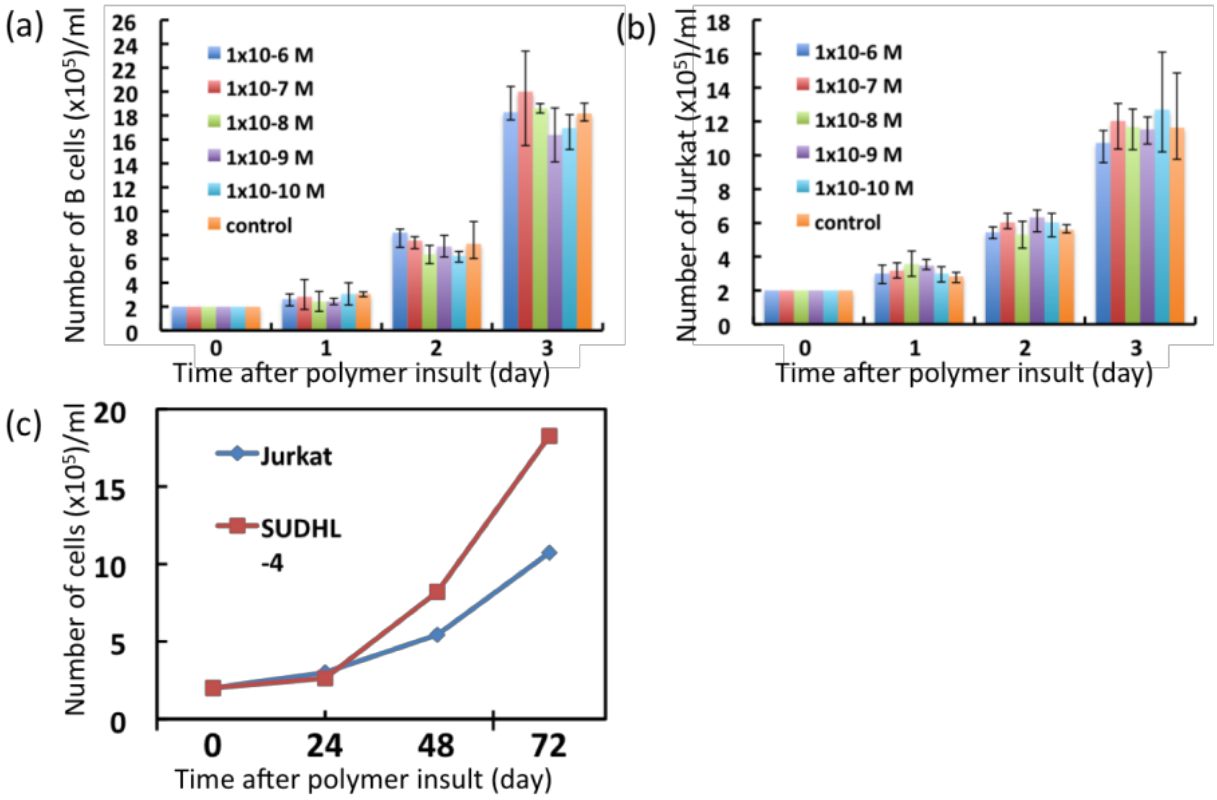


Figure S6. Cell viability and proliferation of (a) SUDHL-4 and (b) Jurkat incubated with different concentrations of PPE2 for 3 days. Trypan blue reagents were added to a small aliquot of the samples, and the number of dead cells and live cells were counted by a hemacytometer in every 24 hours after the incubation with PPE2. (c) A cell proliferation curve of SUDHL-4 (black) and Jurkat (red) upon incubation with the highest concentration of PPE2 (500 μg/mL).

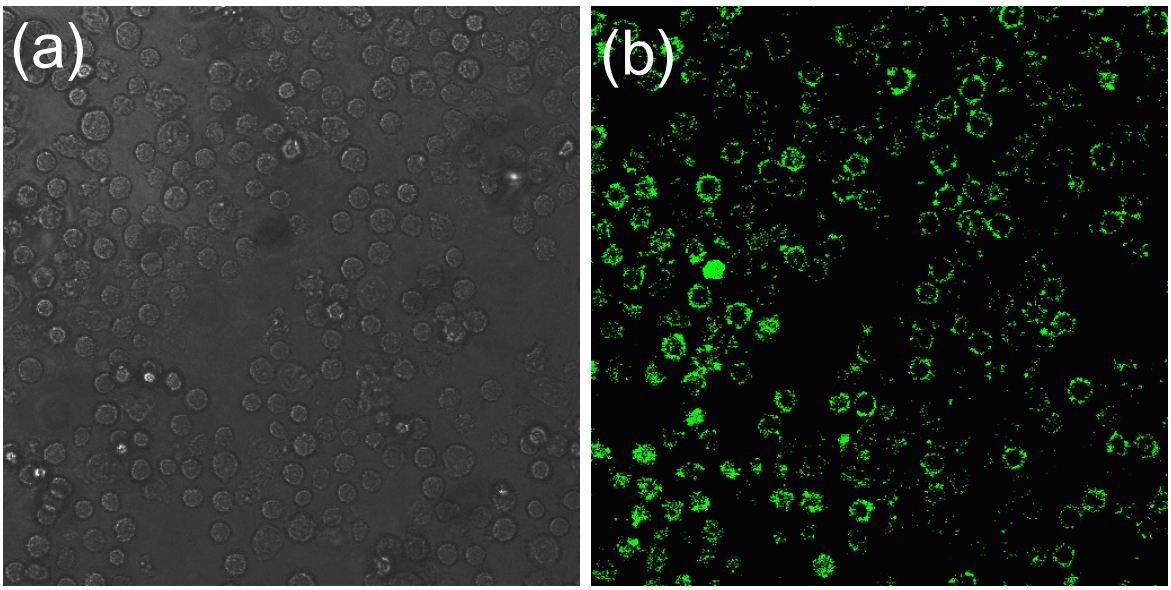


Figure S7. Confocal microscope Image of prefixed jurkat cells after staining with PPE1-CD3. Cells were fixed with 4 % formaldehydes solution; (a) DIC and (b) fluorescence images.

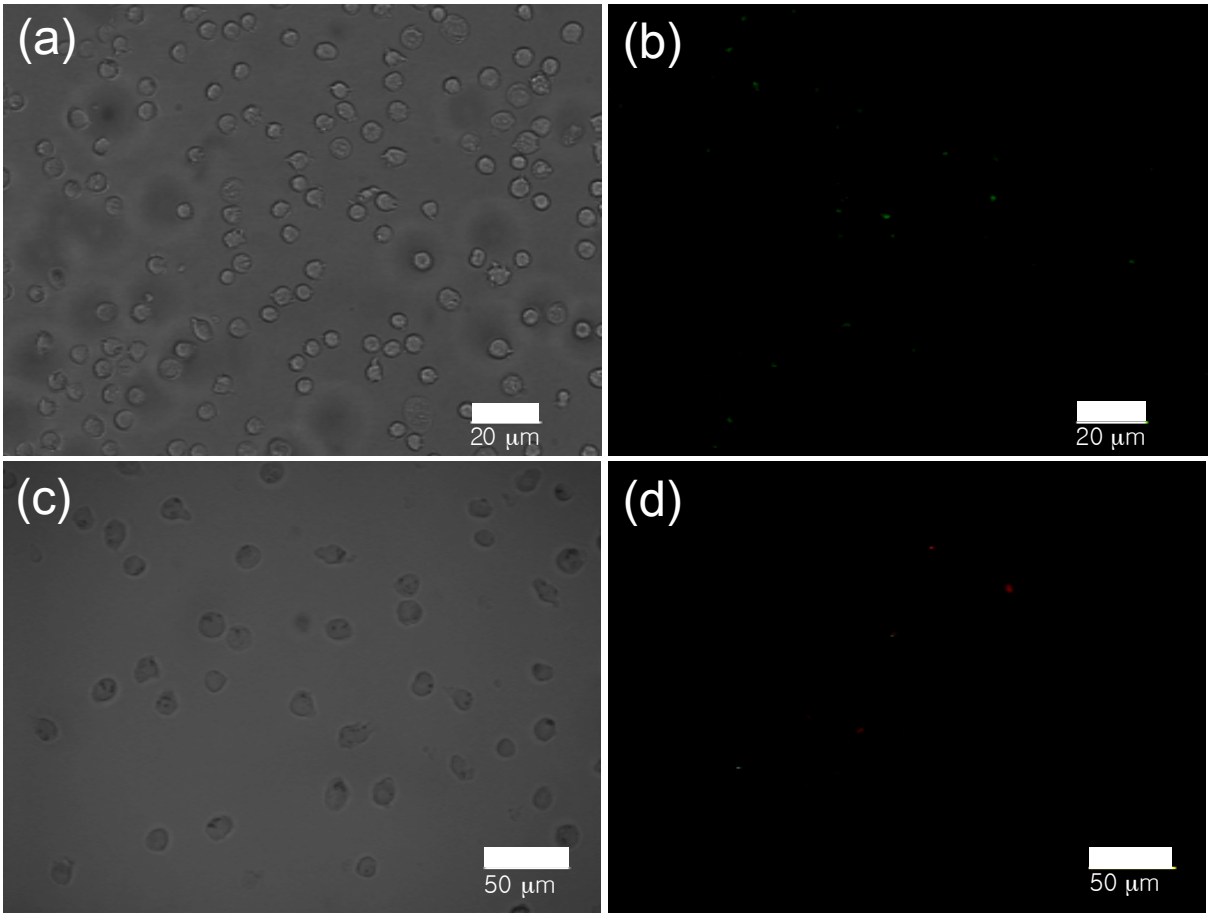


Figure S8. DIC (a, c) and the corresponding fluorescence (b, d) images of live cells after incubation with antibody-free PPE-1: b and d are the fluorescence image after incubation with PPE and PPE-2 for 30 min respectively.

Experimental

Materials and Methods All solvents and reagents for polymer preparation were used without further purification as purchased from Fisher Scientific or Sigma-Aldrich Chemical Co. Detailed synthetic routes for 2,5-diiodo-1,4-hydroquinone (1), 4,7- dibromo-2,1,3-benzothiadiazole (2), M2 and M4 were previously published.[24,34] NMR characterization of polymers was conducted by Varian Inova 500 (11.7 Tesla, oxford magnet). The following materials and chemicals for conjugation and cell study were used as received. 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC), N-hydroxysulfosuccinimide (Sulfo-NHS), 2-(N-morpholino)ethanesulfonic acid (MES) buffer and phosphate buffer saline (PBS) buffer were purchased from Pierce Biotechnologies for bioconjugation. Mouse monoclonal anti-CD3 and CD20 were purchased from GeneTex, Inc. and BD Biosciences,

respectively. Functional grade purified anti-human CD3 and FITC anti-human CD20 were purchased from eBioscience, Inc. Human anaplastic large cell lymphoma (SUDHL-1andthe Human B cell lymphoma (SUDHL-4) and human T cell leukemia (Jurkat) cell lines were purchased from Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ) GmbH (Braunschweig, Germany). RPMI1640 for cell culture experiment to grow SUDHL-1 (T cell), SUDHL-4 (B cell), or Jurkat cells and HyQ PBS buffer (pH=7.0) for cell staining using polymer-antibody conjugates were purchased from HyClone, Thermo Fisher Scientific Inc.

Synthesis of Diethyl 4,4'-(2,5-diiodo-1,4-phenylene)bis(oxy)-dibutanoate (M1) To a solution of 2,5-diiodo-1,4-hydroquinone (**1**, 1.0 g, 2.76 mmol) were added a potassium carbonate (1.615 g, 8.28 mmol), ethyl 4-bromobutyrate (1.615 g, 8.28 mmol) and dimethylformamide (DMF, 15 mL). The reaction mixture was stirred at 80 ºC for 48 h. After the reaction, the reaction mixture was cooled down to room temperature and filtered through a filter paper. DMF was removed by rotary evaporator at a reduced pressure. Crude mixture was re-dissolved in chloroform and extracted twice with deionized water. After drying over $MgSO_4$ and filtering, chloroform was removed in vacuo. Further purification was done by column chromatography (ethyl acetate : hexane = 1 : 1 v/v) and the following recrystallization in methanol at –18 ºC gave white waxy powder (yield: 0.65 g, 41 %). ^{1}H-NMR (500 MHz, $CDCl_3$): δ/ppm 7.10 (s, 2H, aromatic), 4.20 (m, 4H, -OC$\underline{H_2}$CH$_3$), 4.01 (t, 4H, -OC$\underline{H_2}$-), 2.60 (t, 4H, -C$\underline{H_2}$COO-), 2.15 (m, 4H, -C$\underline{H_2}$-), 1.27 (t, 6H, -CH$_3$). ^{13}C-NMR (125 MHz, $CDCl_3$) δ/ppm 173.1, 152.7, 122.5, 86.1, 67.7, 60.3, 30.6, 24.4, 141.1.

Synthesis of 4,7-bis((trimethylsilyl)ethynyl)benzothiadiazole (3) To a 50 mL Schlenck flask with a stir bar were added 4,7- dibromo-2,1,3-benzothiadiazole (**2**, 1.55 g, 5.27 mmol), trimethylacetylene (1.79 mL, 12.65 mmol), $Pd(PPh_3)_4$ (61 mg, 52.7 μmol) and CuI (10.0 mg, 52.7 μmol). After purging with Ar for 2 min, 20 mL of toluene and 5 mL of diisopropylamine were added respectively. The mixture was stirred at 65 ºC for 7 h after cycles of argon

purging and degassing by vacuum several times. Solvent was evaporated at 32 ºC with reduced pressure and the crude mixture was purified by a short column of silica gel with ether as an eluent. Further purification was done by column chromatography (methylene chloride : hexane = 2 : 3 v/v). Recrystallization in methylene chloride and hexane (2:7) at – 18 ºC gave yellow-white fluffy powder (yield: 1.03 g, 60 %) ^{1}H-NMR (500 MHz, $CDCl_3$): δ/ppm 7.71 (s, 2H, aromatic), 0.341 (s, 18H, -Si($C\underline{H}_3$)$_3$). ^{13}C-NMR (125 MHz, $CDCl_3$) δ/ppm 154.22, 133,16, 117.26, 103,65, 99.99, 0.11. HRMS (Voltage ES+, electrospray with Na+ added): calculated m/z of [M+Na]+ 351.0783; measured m/z 351.0777.

Synthesis of 4,7-diethynylbenzodthiadiazole (M3) In a 100 mL 2-neck round bottom flask with Ar purging was added compound **3** (0. 361 g, 1.098 mmol) and tetrahydrofuran (5 mL). After stirring for 5 min for complete dissolution, potassium hydroxide (0. 247 g, 4.4 mmol) in methanol (5 mL) was dropwise added and the solution became brown immediately. The solution was left at room temperature with stirring for 1 h and the reaction completion was confirmed by thin layer chromatography. The solvent was evaporated at 32 ºC with reduced pressure and the crude compound was redissolved in chloroform and purified by silica gel-based flash column chromatography (methylene chloride : hexanes = 1 : 1 v/v) to give M3 as a yellow powder (air unstable, 0.20 g, 98 %) ^{1}H-NMR (500 MHz, $CDCl_3$): δ/ppm 7.77 (s, 2H, aromatic), 3.70 (s, 2H, C≡C-$\underline{H}$) ^{13}C-NMR (125 MHz, CDCl3): δ/ppm 154.3, 133,2, 116.7, 102,4, 99.5. HRMS (EI+ voltage): calculated m/z of [M+] 184.0095; measured m/z 184.0098.

Polymerization of PPE1 Monomer M1 (40.8 mg, 69.1 69.1e, monomer M2 (61.6 mg, 69.1 9.1 e, toluene (1.0 mL), morpholine (1 ml), and diisopropylamine (2 mL) were placed into a 50 mL Schlenck flask. After complete dissolution of the two monomers, the solution was degassed by three times of vacuum and argon purging. In a separate Schlenck flask, tetrakistriphenylphosphine palladium (0) (3 mol % of total monomers) and copper (I) iodide (3 mol % of total monomers) were transferred under a nitrogen atmosphere of a glove box and argon was purged in the Schlenck flask for 10 min. Two catalysts were dissolved in toluene

(1.0 mL) and degassed by three times of vacuum and argon purging. The degassed solution containing catalyst was cannulated into the monomer solution. After transfer of the catalyst solution to the monomer solution, three cycles of degassing of the polymer solution were finally applied. The polymer solution was allowed to stir under argon purging at 55 ºC for 48 h. The reaction mixture was filtered through a 0.8 micrometer membrane syringe. The mixture solution was concentrated at reduced pressure and precipitated in diethylether (15 mL). The crude polymer was redissolved in 15 mL of 1,4-dioxane and the solution was mixed with 10 % aqueous NaOH solution (15 mL). The solution was stirred under argon atmosphere at room temperature for 12 h. Polymer solution was centrifuged to remove insoluble impurity and dialyzed (Spectra/Por, Spectrum Laboratories, Inc., 12,000-14,000 MWCO) against deionized water for 2 days (10 × 4 L water exchanges). The polymer solution was lyophilized to yield a yellow solid (51 mg, 60 %). ^{1}H-NMR (500 MHz, D_2O): δ/ppm 7.27 (s, 2H, aromatic), 7.15 (s, 2H, aromatic), 4.03 (broad m, 6H, -$CH_2C\underline{H}_2O$-, -$OC\underline{H}$-), 3.81-3.21 (broad m, 56H, -$OC\underline{H}_2C\underline{H}_2$), 3.18 (broad s, 12H, -$OC\underline{H}_3$), 2.25 (broad t, 4H, -$CH_2C\underline{H}_2COO$-), 1.87 (broad m, 4H, -$CH_2C\underline{H}_2CH_2$-), ^{13}C-NMR (125 MHz, THF-d_8, before deprotection) δ/ppm 173.7, 153.6, 118.4, 117.1, 116.8,114.6, 102.4, 91.5, 91.3, 81.8, 72.0, 70.1, 70.6, 70.4, 69.6, 69.3, 68.2, 67.6, 64.2, 58.9, 33,3, 24.9, 22.0. GPC (THF-based, it was measured before deprotection of an ethyl group) M_n= 73,100 $gmol^{-1}$, M_w= 214,200 $gmol^{-1}$, PDI = 2.93. Molar extinction coefficient at 424 nm : 1.56 x 10^{-6} $M^{-1}cm^{-1}$. Quantum yields (QYs) of PPE-1 (concentration: 10^{-4}, 10^{-5}, 10^{-6}, and 10^{-7} M) at DI water are 37.7, 49.9, 51.3 and 56.7 % respectively. QY of PPE-1 (1.0 x 10^{-6} M) in 1 X PBS (pH = 7.4) = 45.3 %.

Polymerization of PPE2 To a 25 mL Schlenck flask with a stir bar were added M3 (68.5 mg, 0.372 mmol) and M4 (230.2 mg, 0.354 mmol). The flask was placed under argon atmosphere and 3.5 mL of dimethylformamide, 4 mL of deionized water, and 1 mL of diisopropylamine (DIPA) were added to the flask after degassing. To a separate flask were added tetrakis(triphenylphosphine)palladium, Pd(0) (17.2 mg, 11.5 μmol), and CuI (2.84 mg, 11.5

μmol) and the flask was also degassed. Pd(0) catalyst was successively added to the monomer mixture by cannular transfer and degassed by argon purging and vacuum recycles several times. The mixture was stirred at 55 ºC for 24 h. 4-ethynylbenzoic acid (54.4 mg, 0.372 mmol), Pd catalyst (8.6 mg), CuI (1.4 mg), DIPA (0.5 mL) were added to the solution and further reacted at 55 ºC for additional 24 h for the end-capping reaction. The cooled polymer solution was filtered, concentrated, precipitated in acetone (40 mL), and filtered again. The polymer was redissolved in water and precipitated in acetone/ether/methanol (3:3:1, total 63 mL), filtered, washed with tetrahydrofuran, and dried in vacuo. The polymer powder was dissolved in 1 M NaOH solution (50 mL) and, dialyzed (Spectra/Por, Spectrum Laboratories, Inc., 12,000-14,000 MWCO) against several changes of deionized water for 2 days. Lyophilization of the resulting red solution gave PPE2 as a red fiber. Yield: 37 %, ^{1}H NMR (500MHz, D_2O) δ /ppm 8.2-7.2 (broad, aromatic C-H), 4.2-3.3 (broad, aliphatic broad C-H), 2.90-2.50 (broad, aliphatic C-H). ^{13}C-NMR (125 MHz, DMSO, before end-capping) δ /ppm 156.1, 153.9, 125.3, 116.7, 113.9, 110.1, 91.1, 67.8, 46.7, 25.5. GPC (DMF- based) M_n = 49,500 g/mol, PDI = 3,81, Molar extinction coefficient at 542 nm : 1.31 x 10^{-6} $M^{-1}cm^{-1}$. Quantum yield of PPE-1 (1.0 x 10^{-6} M) in 1 X PBS (pH = 7.4) = 1.0 %.

Photophysical Analysis of CPEs UV/Vis absorption spectra were recorded with a Varian Cary50 UV/Vis spectrophotometer in various solvents. Photoluminescence spectra were taken on a PTI QuantaMaster™ spectrofluorometer, QM4 (Photon Technology International, Birmingham, NJ).

Measurement of quantum yield Absolute quantum yields (QYs) of PPE1 and PPE2 were obtained from an integrating sphere as an accessory of QM4. First ,the following four types (Scan A, B, C, and D) of values are obtained. Scan A: emission scan of a blank media looking at the emission range of your material, which should just be background. Scan B: emission scan of the blank looking at the emission range of your excitation beam which should be a big peak showing the total excitation light. Scan C: emission scan of the polymer sample

looking at the emission range of your excitation beam which should be similar to Scan B but lower because now your sample is absorbing some of the excitation light. Scan D: emission scan of the sample looking at the emission range which should be your sample emission (plus the background as seen in Scan D). We typically used a neutral density filter in scan B and C. The detector becomes inaccurate over 1 million counts so the filter cuts the emission of scan B and C to be < 1 million. Then we needed to run two scans to calculate the filter factor: one emission scan with the filter out and the intensity < 1 million and then another emission scan with the filter In. Filter Factor (FF=filter Out / filter In) was calculated. In the end, QY can be calculated with the following equation if the filter is used.

QY = (Scan D - Scan A, total emission) / (FF * (Scan B - Scan C, total absorption))

Polymer-Antibody Bioconjugation (PPE1-CD3 and PPE2-CD20) All reagents are immediately handled and used before bioconjugation. Bioconjugation between an antibody and a polymer was conducted by standard carbodiimde chemistry. 1 mg of PPE1 was dissolved in 100 μl MES buffer (0.1 M, pH=4.7). 200 mM (or 50 mM in PPE2-CD20 case) of EDC (100 μl) and 200 mM (50 mM in PPE2-CD20 case) of sulfo-NHS (100 μl) (Pierce Biotechnologies, Thermo-scientific, Inc) in MES buffer were prepared respectively. 10 μl (final concentration 18 mM) of EDC was directly added to 100 μl of PPE1 solution, which was based on a 13 kDa PPE1-CD3, results in a 30-fold molar excess of EDC to polymer. To the reaction mixture 25 μl of sulfo-NHS was also added to the final concentration of 37 mM. Reaction components were mixed well and stirred for 15 minutes at room temperature for reaction. Activated PPE1 or PPE2 was separated from excess EDC, EDC-byproducts, and sulfo-NHS using Zeba^{TM} Desalt Spin Columns (5 mL) and the medium buffer (final volume: 400 μl) was exchanged to phosphate-buffered saline (PBS, 0.1 M sodium phosphate, 0.15 M NaCl, pH 7.4). Purification of solution containing sulfo-NHS after the separation was fractionally confirmed by UV absorbance peak at 280 nm that was significantly decreased.

Different amount of activated polymer solution (67 μl, 33 μl, 7 μl, and 3 μl) was added to 100 μl (1 mg/mL) of an antibody, respectively (final volume of each sample: 500 μl). The solution was mixed well and then reaction was allowed to proceed for 2 h at room temperature. Reaction was quenched by adding base to raise the pH above 8 to promote autohydrolysis of the NHS esters, thereby regenerating the original carboxylic groups. Medium was finally exchanged to cell buffer (PBS, pH=7.0, HyQ^{TM}, HyClone, UT) and used for cell staining immediately (final concentration: 3.3 μM based on a 150 kDa antibody). The synthesis of CPE-antibody conjugates was verified by 10.0 % Tris-HCl (PPE1) (stacking gel pH=6.8, separating gel pH=8.8) and 4-12% Bis-Tris (PPE2) SDS PAGE at denaturating conditions after boiling in the SDS loading buffer for 5 min and stained with coomassie blue dye (Figure 2). Images were obtained from Fotodyne Foto/convertible Dual transilluminator with Foto/Analyst software with relevant filter sets (PPE1) and from the Visioneer 7100 scanner. Dot intensity from an image was measured by ImageJ software provided by National Institutes of Health (NIH) and the mean intensity value of a certain area was calculated.

Cell Culture Human B cell lymphoma (SUDHL-4) and human T cell leukemia (Jurkat) cell line were cultured in 75 cm^2 flasks at 37 ºC in a humidified atmosphere with 5 % CO_2. The medium contained 10 % fetal bovine serum (FBS, 50mLs heat deactivated) in RPMI-1640 supplemented with a proprietary brand of Glutamine called Glutamax^{TM}-I Supplement (Invitrogen^{TM}, 5 mL of this stuff dissolved in 500 mL of RPMI - 10%) and antibiotic-antimycotic mix for antibiotics (InvitrogenTM, 100x, liquid). It contains 10,000 units of penicillin (base), 10,000 μg of streptomycin (base), and 25 μg of amphotericin B/mL utilizing penicillin G (sodium salt), streptomycin sulfate, and amphotericin B as Fungizone® Antimycotic in 0.85% saline. Before use, 5mL of this stuff was diluted in 500mL of RPMI 1640 with 10% FBS. The medium was changed every third day and cell viability was checked every day with trypan blue staining.

Fluorescence Microscopy and Laser Scanning Confocal Microscopy Fluorescence images were acquired by Olympus BX41 fluorescence microscope (Optical Analysis corporation, Nashua, NH 03063) equipped with metal halide lamp, various optical filters (approximate excitation/emission in nm = 400/420, 420/475, 470/500 or 560/620 with narrow or wideband emission) DP71 digital camera, and Microsuite5 biological suite software. For actual comparison of the intensity of CPE-antibody conjugates with FITC-labeled antibody, the microscope was used with the same setup condition. The amount of antibody adsorbed on cell surfaces was quantified by fluorescence intensity measurements. Confocal Images were obtained from a Leica TCS SP2 confocal microscope operating with a 63× oil immersion objective (numerical aperture 1.4).

Cell Imaging with CPE-Antibody Conjugate 100 μl of each cell suspension (ca. 1 million cells /each tube) was prepared in PBS (pH=7) and 10 μl of polymer-antibody conjugates (final concentration: 0.3 μM, based on 150kDa antibody) prepared were incubated to suspension cell at room temperature for 30 min. Unbound antibody was removed by spin-down of cell (HyQ^{TM} PB, RCF, 400 × g, 7 min) for three recycling times. Cells were resuspended in 50 μl of PBS (Hyclone, UT) and 10 μl cells suspension stained was diluted with PBS (1 to 10 times) and placed on a glass slide and a cover slip was mounted on the slide. Labeled cells were immediately visualized by fluorescence microscope or preserved in refrigerator (4 ºC) for 48 h with/without fixation with formaldehyde (final concentration: 4 %). Dilution test to check the selectivity of polymer-antibody conjugates was also conducted in the same manner with the cell staining test. 20 μl of PPE1-CD3 and PPE2-CD20 conjugates were incubated to one millions of cells per 100 μl for 30 minutes respectively and images were obtained from fluorescence microscope in the same setup condition. The same concentration of Jukat and B cell were prepared and the two solutions were mixed in different ratios (100:0, 90:10, 80:20, 70:30, 60:40, 50:50, 40:60, 30:70, 20:80, 10:90, 0:100 in Jurkat : SUDHL-4 v/v). PPE1-CD3

and PPE2-CD20 conjugates were then incubated in each of the cell mixtures. After the unbound polymer-antibody conjugates were removed, images were obtained from a fluorescence microscope with two different optical filters having an emission wavelength cut-off of 475 and 620 nm, respectively. Control test using FITC-labeled antibody was performed to compare the sensitivity of polymer-antibody conjugated prepared. Antibody used in this study is primary antibody and concentration of FITC-antibody used to stain the cells (10,000 cell/ μl, total staining volume: 100 μl) in PBS (pH = 7) was 0.3 μM. The FITC-antibody was incubated at room temperature for 30 min and unbound dyes was washed by spinning-down of cells (3 times). We used same microscope condition as the case in polymer-antibody for precise comparison of fluorescence intensity.

Cytotoxicity and Proliferation Assay of Cells against the CPEs The viability and proliferation of cells against the conjugated polyelectrolytes were evaluated in different concentrations. All cells were cultured in 75 cm^2 flasks briefly 96 h prior to the cytotoxicity test and confirmed 99 % viability of cells before use. Arbitrary concentrations (mM to nM range) of PPE1 solutions were prepared to find a kill curve. 2000 μl of RPMI buffer with 2 × 10^5 cells were replated on each well of a 24 well-plate (the plate in triplicate, which means 3 wells of cells for each concentration to try and control for any error) and 20 μl of a various concentrations of polymer solutions to this cell mixture was added. Additional control was also prepared by adding the same amount of water to cells as a diluent without the polymer solution. Cytotoxicity was evaluated every 24 h using a hemacytometer as a cell-counting method after dead cells were stained with trypan blue dye. Doubling time of the cultured cells is calculated by the following equation. It is assumed that cells grow exponentially to compute a doubling time.

$$N=N_0*2^{t/T}$$

N_0 and N: the initial and the final concentration of cells, t: the duration of culture, T: the doubling time, T and t have the same unit.

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