Electronic Supplementary Material (ESI) for Chemical Science.

Electronic Supporting Information

Polymeric dipicolylamine based mass tags for mass cytometry

Yefeng Zhang[a], Peng Liu[b], Daniel Majonis[b], Mitchell A. Winnik [a,c]*

[a] Department of Chemistry, University of Toronto, 80 St. George Street, Toronto, ON M5S 3H6, Canada

[b] Fluidigm Canada Inc.,1380 Rodick Road, Suite 400 Markham, ON L3R 4G5, Canada

[c] Department of Chemical Engineering and Applied Chemistry, University of Toronto, Toronto, ON M5S 3E5, Canada

* Corresponding author: m.winnik@utoronto.ca

TABLE OF CONTENTS

ADDITIONAL EXPERIMENTAL DETAILS

Materials and reagents

Triethylamine (cat. no. 471283), acryloyl chloride (cat. no. 549797), 2-(dodecylthiocarbonothioylthio)-2-methylpropanoic acid (DDMAT, cat. no. 723010), 2,2'-azobis(2-methylpropionitrile) (AIBN, cat. no. 44109), Nε-Boc-L-lysine (cat. no. 359661), sodium triacetoxyborohydride (STAB, cat. no. 316393), 2-pyridinecarboxaldehyde (cat. no. P62003), HCl (4M in dioxane, cat. no. 345547), Tris(2-carboxyethyl)phosphine hydrochloride solution (TCEP, cat. no. 646547), tetraethylammonium bromide (NEt_4Br, cat. no. 241059), bromopentacarbonylrhenium(I) ($ReBr(CO)_5$, cat. no. 327638), diethylene glycol dimethyl ether (diglyme, cat. no. 281662), azido-PEG_6-NH_2 (cat. no. 76172), DBCO-PEG_4-TFP (TFP = tetrafluorophenyl ester, cat. no. QBD11362), maleimide (cat. no. 129585), potassium tetrachloroplatinate (K_2PtCl_4, cat. no. 520853) were obtained from Sigma Aldrich. Pentafluorophenol was purchased from Matrix Scientific (cat. no. 006058). mPEG_6-NH_2 (cat. no. 281204) was obtained from ChemPep. The bis-maleimide Bis-Mal-PEG_6 (cat. no. BP-22152) was obtained from BroadPharm. 4-(4,6-dimethoxy-1,3,5-triazin-2-yl)-4-methylmorpholinium chloride (DMTMM, cat. no. D461245) was purchased from Toronto Research Chemicals. All organic solvents (anhydrous) were obtained from commercial sources and used without further purifications. CD8a (RPA-T8 clone), CD20 (2H7 clone), Rh- and Ir-intercalating agents, Maxpar® conjugates (^{162}Dy-CD8a, ^{154}Sm-CD45, ^{160}Gd-CD14, ^{170}Er-CD3, ^{147}Sm-CD20, ^{174}Yb-CD4, ^{148}Nd-CD16), Maxpar® Cell Staining Buffer (CSB), Maxpar® Fix and Perm Buffer, Maxpar® Cell Acquisition Solution (CAS), EQ™ four-element calibration beads were obtained from Fluidigm Canada. PBMCs were acquired from Cellular Technology Limited (CTL, Ohio, U.S.A.).

Synthesis of pentafluorophenyl acrylate (PFPA) monomer

Triethylamine (18.3 mL) was slowly added to pentafluorophenol solution (20 g in 130 mL of dichloromethane) at 0 ℃ under nitrogen followed by 10.6 mL of acryloyl chloride. The reaction mixture was stirred at 0 ℃ for 2 h and then at room temperature overnight. The salt was removed by filtration. The solution was concentrated by rotary evaporation and then purified using a silica gel column chromatography with hexane as the eluent. ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.71 (dd, J = 17.3, 1.0 Hz, 1H), 6.37 (dd, J = 17.3, 10.6 Hz, 1H), 6.17 (dd, J = 10.5, 0.9 Hz, 1H). ^{19}F NMR

(564 MHz, $CDCl_3$) δ -153.17 – -153.27 (m), -158.74 (t, *J* = 21.5 Hz), -163.11 (td, *J* = 23.0, 22.5, 5.4 Hz).

Synthesis of lysine-derived dipicolylamine (DPA) ligand 5

To a mixture of Nε-Boc-L-lysine (2 g) and STAB (4.5 g) in dichloroethane (45 mL) was added 2-pyridinecarboxaldehyde (1.85 g, dissolved in 5 mL dichloroethane) at 0 °C under nitrogen. The suspension was stirred at room temperature for ca. 5 h and became homogeneous bright yellow. The reaction mixture was decomposed with deionized water (30 mL) and diluted with dichloroethane (100 mL). The separated organic layer was washed with deionized water (30 mL ×4), dried with Na_2SO_4, and concentrated by rotary evaporation to obtain the tert-butyloxycarbonyl (Boc) protected ligand 5 (2.5 g). ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.53 (ddd, *J* = 5.1, 1.8, 0.9 Hz, 2H), 7.65 (td, *J* = 7.7, 1.8 Hz, 2H), 7.32 (dt, *J* = 7.9, 1.1 Hz, 2H), 7.20 (ddd, *J* = 7.6, 5.0, 1.2 Hz, 2H), 4.70 (s, 1H), 4.10 (d, *J* = 3.8 Hz, 4H), 3.47 (dd, *J* = 7.8, 6.5 Hz, 1H), 3.16 – 3.07 (m, 2H), 2.04 – 1.92 (m, 1H), 1.88 – 1.72 (m, 1H), 1.52 – 1.45 (m, 4H), 1.43 (s, 9H).

To remove the Boc group, hydrogen chloride solution (4M in dioxane, 7 mL) was slowly added to a solution of above compound (1.5 g) in DCM (5 mL) at 0 °C. The resulting solution was stirred for 24 h at room temperature. A yellow precipitate was formed during the reaction. The solvent was discarded, and the precipitate was dissolved in methanol (5 mL) and precipitated into diethyl ether (15 mL). The precipitate was collected by centrifugation (2700 ×g, 10 min). This dissolution-precipitation cycle was repeated once more. Finally, the precipitate was dried in a vacuum oven at room temperate for 24 h to obtain the Boc deprotected lysine-based rhenium chelator as an amine salt (1.3 g). ^{1}H NMR (600 MHz, D_2O) δ 8.68 (ddd, *J* = 6.0, 1.6, 0.7 Hz, 2H), 8.49 (td, *J* = 7.9, 1.6 Hz, 2H), 8.05 (dt, *J* = 8.0, 1.0 Hz, 2H), 7.92 (ddd, *J* = 7.5, 5.9, 1.3 Hz, 2H), 4.42 (q, *J* = 16.5 Hz, 4H), 3.57 – 3.51 (m, 1H), 2.96 (t, *J* = 7.7 Hz, 2H), 1.92 (ddd, *J* = 9.9, 8.4, 5.8 Hz, 1H), 1.89 – 1.82 (m, 1H), 1.69 – 1.61 (m, 2H), 1.52 – 1.44 (m, 2H).

To convert the amine salt to the free base, concentrated NaOH solution (5M) was slowly added to a solution of the amine salt (0.5 g dissolved in 2 mL of water) at 0 °C till a pH of ~ 13 was reached. The basified solution was then lyophilized to obtain a light brown solid. Dichloromethane (5 mL) was added to dissolve the free base. The undissolved solids were removed by centrifugation (16000 ×g, 10 min). The supernatant was collected, concentrated, and dried under vacuum at room temperature for 24 h to obtain the final lysine-based rhenium chelator as the free base (0.3 g). ^{1}H

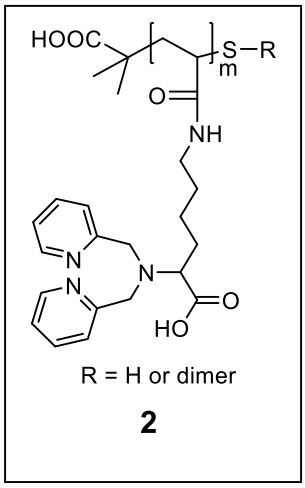
NMR (500 MHz, D_2O) δ 8.24 (ddd, *J* = 5.0, 1.8, 0.9 Hz, 2H), 7.60 (td, *J* = 7.7, 1.8 Hz, 2H), 7.37 (dt, *J* = 7.9, 1.2 Hz, 2H), 7.14 (ddd, *J* = 7.6, 5.0, 1.2 Hz, 2H), 3.95 (d, *J* = 14.6 Hz, 2H), 3.79 (d, *J* = 14.6 Hz, 2H), 3.21 (dd, *J* = 8.5, 6.3 Hz, 1H), 2.59 (t, *J* = 6.8 Hz, 2H), 1.75 – 1.64 (m, 2H), 1.45 – 1.26 (m, 4H).

RAFT polymerization of PFPA monomer

PFPA monomer (2.0 g, 8.4 mmol), RAFT agent 2-(dodecylthiocarbonothioylthio)-2-methylpropanoic acid (DDMAT, 102.0 mg, 0.28 mmol), AIBN (4.60 mg, 0.028 mmol) and dioxane (6.0 mL) were mixed in a Schlenk flask (25 mL) with a stir bar inside. The solution was degassed by three freeze-pump-thaw cycles. The flask was then sealed and put into a preheated oil bath (70 °C) for 9 hours. After polymerization, the solution was cooled to room temperature with tap water and was exposed to air. The polymer was precipitated into excess cold hexane (30 mL). The polymer obtained was dissolved in chloroform (5 mL) and precipitated again into hexane (30 mL). This dissolution-precipitation process was repeated for 3 times. The final polymer, poly(PFPA)$_{20}$ was obtained as yellow powder after drying in a vacuum at room temperature overnight.

Preparation of polymer 2 by aminolysis of poly(PFPA)$_{20}$ with DPA ligand 5

To a solution of DPA ligand **5** (220 mg in 5 mL DMF), poly(PFPA)$_{20}$ (100 mg in 1 mL DMF) was added. After stirring overnight at room temperature (13 h), 100 μL of ethanolamine was added to the reaction mixture and the reaction mixture was stirred for 3 h. The polymer was precipitated into diethyl ether (50 mL) and then dissolved in H_2O. Excess ligands were removed using a spin filter (Amicon, Ultra-15, 3 kDa), washed twice with H_2O, thrice with PBS buffer and thrice with H_2O. The polymer solution was lyophilized overnight to obtain the final product, polymer **2**, as a light brown powder.



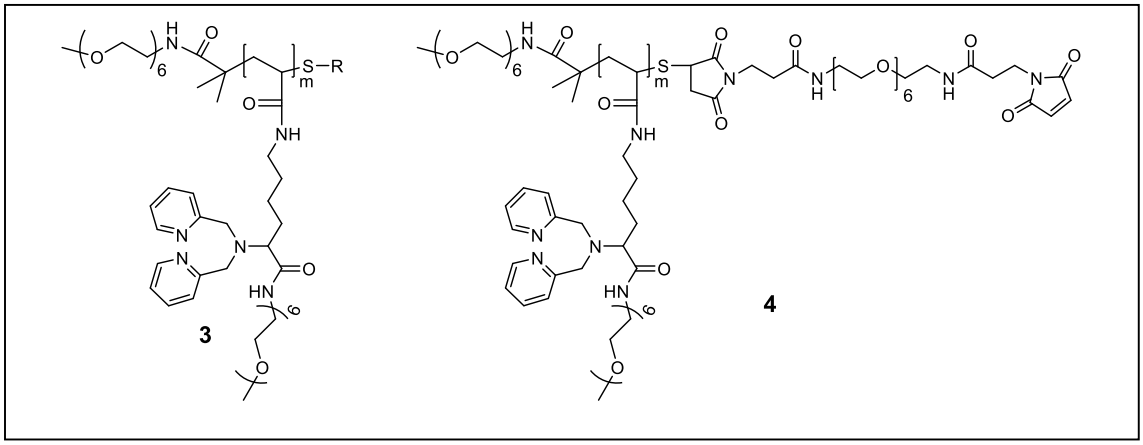
2

Preparation of polymer 3

To a solution of polymer **2** (50.3 mg) in PB buffer (4 mL, 0.2M, pH 8.0), DMTMM (288 mg in 1 mL H_2O, ca. 8 molar equivalents to each carboxylic acid group) was added. The reaction mixture was stirred at room temperature for 5 min for pre-activation. Then, mPEG$_6$-NH_2 (0.26 mL, ca. 7 molar equivalents to each carboxylic group) was added quickly with stirring. The reaction mixture was stirred overnight (15 h). The polymer was purified using a spin filter (Amicon, Ultra-

15, 10 kDa), washed thrice with H_2O, twice with PBS and thrice with H_2O. The final polymer, polymer **3**, was obtained as viscous brown solid after lyophilization.

Preparation of polymer 4



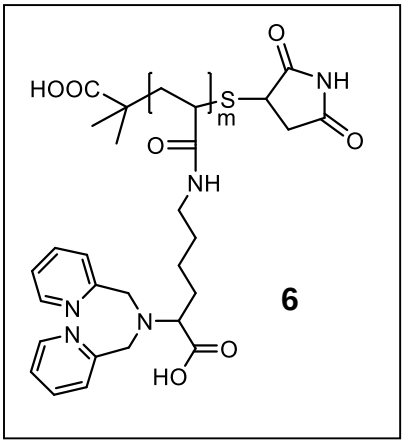
To a solution of polymer **3** (10 mg) in H_2O (1 mL), TCEP (100 µL, 0.5 M) was added. The concentration of TCEP in the reaction solution was ca. 50 mM. The reaction mixture was stirred at room temperature for 1h. Then, the polymer was washed twice with acetic acid solution (ca. 5 mM, pH 3.5) using a spin filter (Amicon, Ultra-15, 10 kDa) to remove excess TCEP. Immediately afterwards, the concentrated polymer solution (ca. 300 µL) was transferred to a 2-dram (~ 7 mL) glass vial, 0.5 mL of PB buffer (0.2 M, pH 7.0) was added followed by Bis-Mal-PEG_6 (22 mg in 120 µL DMF). The reaction solution was stirred at room temperature for 90 min. The polymer was then purified using a spin filter (Amicon, Ultra-15, 10 kDa), washed twice with H_2O, once with PB buffer (0.2 M, pH 7.0) and thrice with H_2O. After washing, the concentrated polymer solution was centrifuged at 12000 ×g for 10 min to remove undissolved solids. The supernatant was taken and lyophilized to obtain the final polymer **4** as viscous brown solid.

Preparation of Re-loaded polymer 4 (PolyRe)

To a solution of polymer **4** (2.1 mg in 2 mL anhydrous MeOH) in a 2-dram (~ 7mL) glass vial, the rhenium salt ($[NEt_4]_2Re(Br)_3(CO)_3$, 3 mg, 1.2-fold equivalents to each chelator, dissolved in 0.5 mL anhydrous MeOH) was added. The reaction mixture was incubated at 37 ℃ in an oil bath for 2 h without stirring. After incubation, the solution was poured into an Amicon spin filter (Ultra-15, 10 kDa) prefilled with H_2O (12 mL). The polymer was washed thrice with H_2O. After washing, the polymer solution was lyophilized to obtain the Re-loaded polymer **4**, denoted PolyRe.

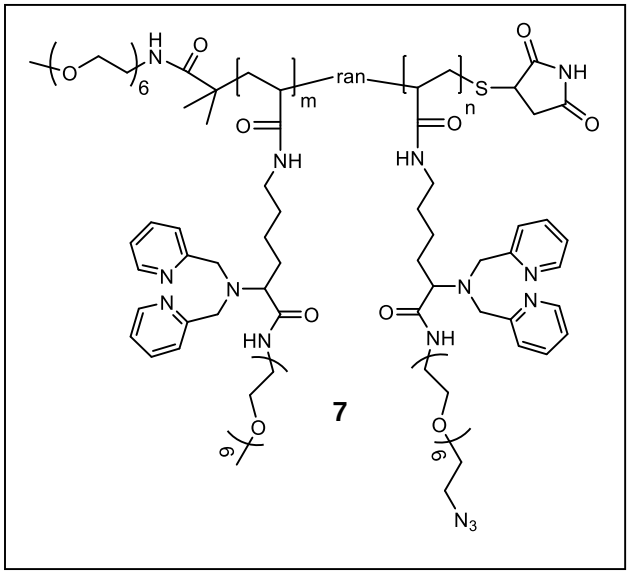
The rhenium salt was prepared by heating a mixture of NEt_4Br (1.69 g) and $ReBr(CO)_5$ (1.5 g) in 2,5,8-trioxanonane (diglyme) (300 mL) at 115 °C for 5 h under nitrogen. During the reaction, a white precipitate was formed. The precipitate was collected by filtration and washed with cold diglyme, diethyl ether, ethanol and dried in a vacuum oven overnight to obtain the product as a white powder.

Preparation of polymer 6



A TCEP solution (100 µL, 0.5 M) was added to a solution of polymer **2** (20 mg in 1 mL H_2O) to reduce any disulfide bonds. The resulting mixture was stirred at room temperature for 2 h. After reduction, the polymer was washed three times with acetic acid solution (5 mM) using a spin filter (Amicon, Ultra-5, 10 kDa). The concentrated polymer solution (200 µL) was then transferred to a 2-dram (~ 7 mL) glass vial containing maleimide (10 mg in 0.5 mL DMF). Additional PB buffer (0.5 mL, 0.2 M, pH 7.0) was then added. The reaction solution was stirred at room temperature for 3 h. The excess maleimide was removed using a spin filter (Amicon, Ultra-5, 10 kDa), washed five times with H_2O. The polymer solution was lyophilized overnight to obtain the polymer **6**.

Preparation of polymer 7



To a solution of polymer **6** (20 mg in 2 mL PB buffer, 0.2M, pH 8.0), a DMTMM solution (124 mg in 0.5 mL H_2O, ca. 8-fold to each COOH) was added. The solution was stirred at room temperature for 5 min to activate the COOH groups. After 5 min, a mixture of PEGs containing $mPEG_6$-NH_2 (54 mg, 3.5-fold equivalents to each COOH) and azide-PEG_6-NH_2 (64 mg, 3.5-fold equivalents to each COOH) was added. The reaction mixture was stirred at room temperature overnight. The polymer was purified using a spin filter (Amicon, Ultra-

5, 10 kDa), washed three times with H_2O, twice with PB buffer (0.2 M, pH 7.6) and three times with H_2O. The polymer solution was then lyophilized overnight to obtain polymer **7**.

Preparation of Pt-loaded polymer 7 (PolyPt)

To a solution of polymer **7** (5.8 mg in 5.5 mL anhydrous MeOH) in a 2-dram (~ 7 mL) glass vial, a solution of K_2PtCl_4 (200 µL, 50 mM in DMSO, 1.2-fold equivalents to each chelator) was added. The vial was wrapped with aluminum foil to avoid exposure to daylight. The reaction mixture was incubated at 45 °C in an oil bath for 2 h without stirring. After incubation, the solution was poured into an Amicon spin filter (Ultra-15, 10 kDa) prefilled with H_2O (10 mL). The polymer was washed three times with NaCl solution (20 mM) and once with H_2O. After washing, the polymer solution was lyophilized to obtain the Pt-loaded polymer **7**, denoted PolyPt.

Antibody labeling with PolyRe

The polymer-antibody conjugate was prepared following standard Maxpar® antibody labeling protocol. Briefly, the antibody was partially reduced by TCEP, washed in a spin filter, and then mixed with an excess of polymer, and the mixture was incubated at 37°C for 1 h. The antibody-polymer conjugate was washed three times with a spin filter (Amicon, Ultra-0.5, 50 kDa) and was further purified by fast-protein liquid chromatography to remove excess unconjugated polymers. The conjugate concentration was determined by the Bradford assay. Two polymer-Ab conjugates ,^{nat}Re-CD20 and ^{nat}Re-CD8a were prepared.

Preparation of DBCO modified anti-CD20 Ab

Before modification, the anti-CD20 Abs were washed five times with PBS (Amicon, Ultra-0.5, 30 kDa) to remove sodium azide. After wash, the concentrated Ab solution (ca. 2.8 mg/mL, 130 µL) was transferred to an Eppendorf tube and a solution of DBCO-PEG_4-TFP (0.5 µL, 140 mM in DMSO, 29-fold to Ab) was added. The reaction mixture was vortexed at room temperature for 1 h. Excess DBCO reagent was removed by washing three times with PBS using a spin filter (Amicon, Ultra-0.5, 30 kDa).

Antibody labeling with PolyPt

To a solution of DBCO modified anti-CD20 Ab (160 µg, 1.6 mg/mL), a solution of PolyPt was added (0.3 mg in 50 µL H_2O, ca. 13-fold molar excess to Ab). The reaction mixture was vortexed at room temperature for 2 h and then at 4 °C overnight. The conjugate was purified by

washing five times with PBS using a spin filter (Amicon, Ultra-0.5, 100 kDa). The conjugate concentration was determined by BCA assay.

Mass cytometry experiments with polymer-Ab conjugate

The antibody staining cocktails (70 µL) were prepared by mixing different Maxpar® MCP-Ab conjugates with the ^{nat}Re-Ab conjugate or the ^{nat}Pt-Ab conjugate. For immunoassays with the ^{nat}Re-CD20, five antibody staining cocktails were prepared. One cocktail contained only Maxpar® MCP-Ab conjugates (^{154}Sm-CD45, ^{160}Gd-CD14, ^{170}Er-CD3 and ^{147}Sm-CD20) and was used as a positive control. The other four cocktails contained both Maxpar® MCP-Ab conjugates (^{154}Sm-CD45, ^{160}Gd-CD14 and ^{170}Er-CD3) and the ^{nat}Re-CD20 conjugate, where the concentration of the ^{nat}Re-CD20 conjugate in each cocktail was different for titers of 0.1, 0.3, 0.5 and 1.0 µg/mL, respectively. For immunoassays with the ^{nat}Re-CD8a, four antibody staining cocktails were prepared. One cocktail contained only Maxpar® MCP-Ab conjugate (^{154}Sm-CD45, ^{160}Gd-CD14, ^{170}Er-CD3, ^{147}Sm-CD20, ^{174}Yb-CD4, ^{148}Nd-CD16 and ^{162}Dy-CD8a) and was used as a positive control. The other three cocktails contained both Maxpar® MCP-Ab conjugates (^{154}Sm-CD45, ^{160}Gd-CD14, ^{170}Er-CD3, ^{147}Sm-CD20, ^{174}Yb-CD4 and ^{148}Nd-CD16) and the ^{nat}Re-CD8a conjugate, where the concentration of the ^{nat}Re-CD8a conjugate in each cocktail was different for titers of 0.05, 0.1 and 0.2 µg/mL, respectively. For immunoassays with the ^{nat}Pt-CD20, six antibody staining cocktails were prepared. One cocktail contained only Maxpar® MCP-Ab conjugates (^{154}Sm-CD45, ^{160}Gd-CD14, ^{170}Er-CD3 and ^{147}Sm-CD20) and was used as a positive control. The other five cocktails contained both Maxpar® MCP-Ab conjugates (^{154}Sm-CD45, ^{160}Gd-CD14 and ^{170}Er-CD3) and the ^{nat}Pt-CD20 conjugate, where the concentration of the ^{nat}Pt-CD20 conjugate in each cocktail was different for titers of 0.1, 0.3, 0.5, 1.0 and 2.5 µg/mL, respectively.

For the staining process, a PBMC suspension (ca. 3 million cells in 30 µL Maxpar® cell staining buffer, Fc blocked) was added to the antibody cocktail (70 µL). The mixture was gently vortexed and incubated at room temperature for 30 min. After incubation, cells were washed twice with cell staining buffer and then fixed with 1.6% formaldehyde/PBS solution at room temperature for 10 min. The fixed cells were pelleted and cell intercalation solution (Ir-intercalator, 1 mL, final concentration: 125 nM) was added. The cells were then incubated at 4 °C overnight. After incubation, cells were washed twice with cell staining buffer and twice with Maxpar® cell acquisition solution. The pelleted cells were resuspended in cell acquisition solution (1 million

cells per mL) containing EQ™ Four Element Calibration Beads and subjected to mass cytometry analysis.

Ligand challenge experiments with PolyRe and PolyPt

To a PolyRe/Pt solution (1 mL, 0.1 mg/mL in PBS, corresponding to ca. 0.1 mM of the metal complex), a stock solution of glycine, histidine or cysteine (25 uL, 200 mM) was added. The final concentration of glycine, histidine or cysteine was ca. 4.9 mM. The mixture was incubated at room temperature for 3 days. After incubation, the polymer was washed three times with PBS using a spin filter (Amicon, Ultra-0.5, 3 kDa) to remove the excess amino acids. The UV-vis absorption of the concentrated polymer solution and the first filtrate solution was measured using a Nanodrop device.

INSTRUMENTATION

^{1}H NMR and **^{19}F NMR** experiments were performed on an Agilent DD2 500 MHz spectrometer or Agilent DD2 600 MHz spectrometer.

UV-vis measurements were performed on an Agilent Cary 300 UV-vis spectrophotometer.

FT-IR measurements were performed on a PerkinElmer Spectrum Two™ infrared spectrometer with an ATR accessory. All spectra were collected in the range of 500-4000 cm^{-1}at a resolution of 1 cm^{-1}.

GPC measurements were performed on a Waters 515 HPLC equipped with a Viscotek VE 3580 refractive index (RI) detector. Tetrahydrofuran (THF) containing 2.5 g/L tetra-n-butylammonium bromide (TBAB) was used as the eluent (35 °C, flow rate = 0.6 mL/min). The system was calibrated with PMMA standards.

FPLC experiments were performed on the AKTA pure 25L system. For the purification of the polymer-antibody conjugate, a Superdex™ 200 10/300 GL column was used.

Mass cytometry experiments were performed on a CyTOF® Helios™ system at Fluidigm Canada (Markham, ON). Data were obtained in FCS3.0 file format and analyzed using Cytobank.

SUPPORTING FIGURES AND SCHEME

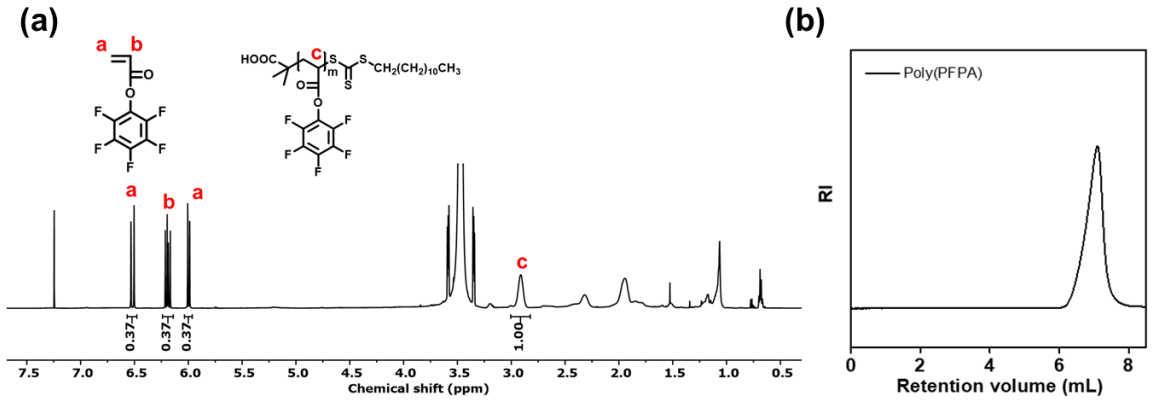


Figure S1. (a) ^{1}H NMR ($CDCl_3$) characterization of RAFT reaction mixture after 9 h. The monomer conversion was calculated to be 73% (1/1.37 = 73%) by comparing integrals of peak b of monomer and peak c of polymer backbone. (b) THF GPC trace of poly(PFPA) synthesized by RAFT polymerization of PFPA monomer. Based on PMMA standards, M_n = 9.4 kDa, PDI = 1.25.

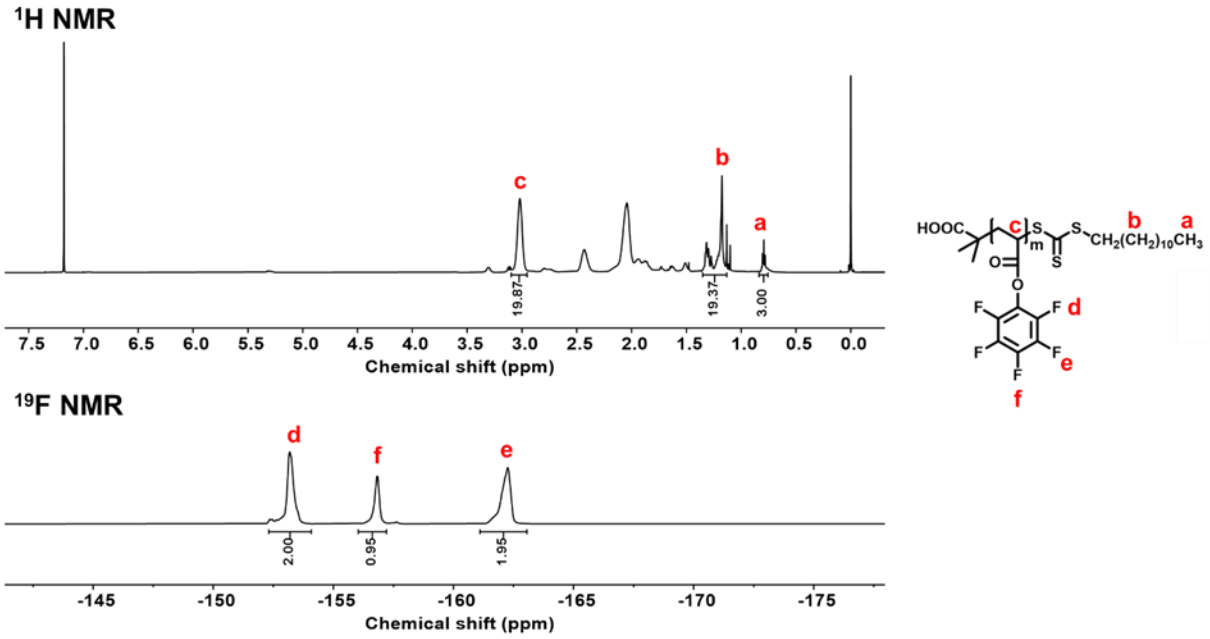
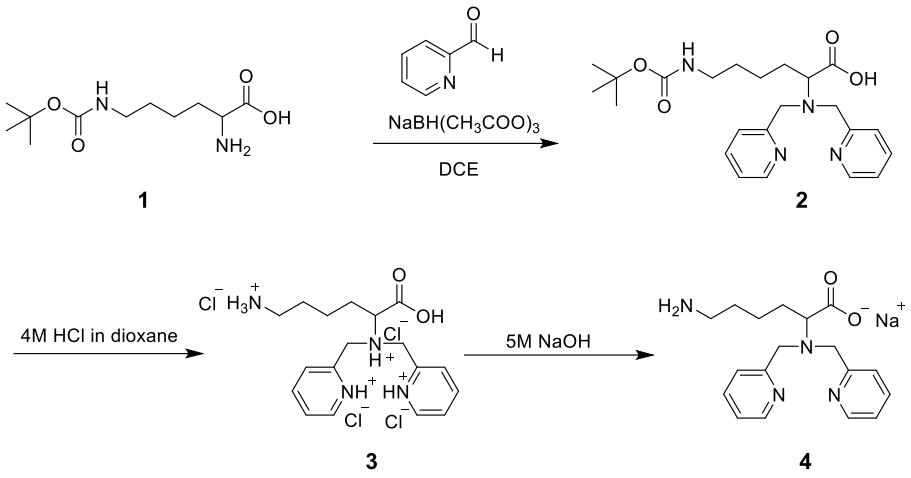


Figure S2. ^{1}H NMR ($CDCl_3$) characterization of poly(PFPA)$_{20}$. Top: ^{1}H NMR spectrum. Bottom: ^{19}F NMR spectrum. The DP of the polymer was determined to be ca. 20 by comparing the integration of peak c to that of peak a.



Scheme S1. Synthesis of lysine-derived dipicolylamine ligand.

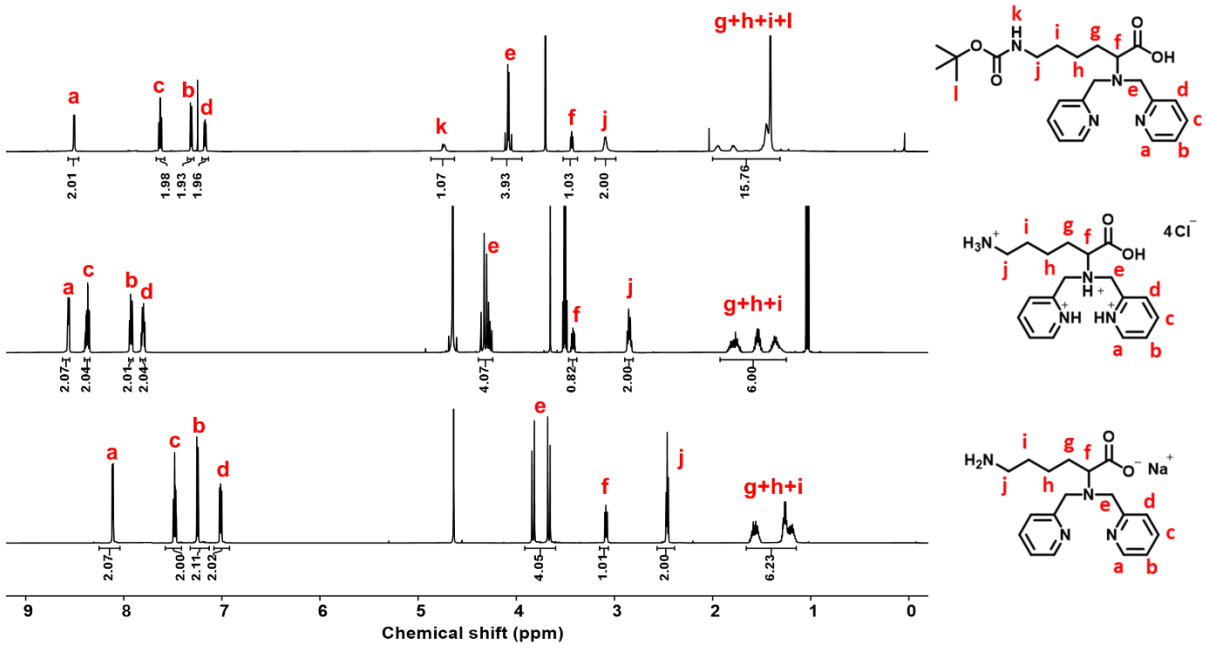


Figure S3. ^{1}H NMR spectra of compound **2** (top, $CDCl_3$), compound **3** (middle, D_2O) and compound **4** (bottom, D_2O) shown in Scheme S1.

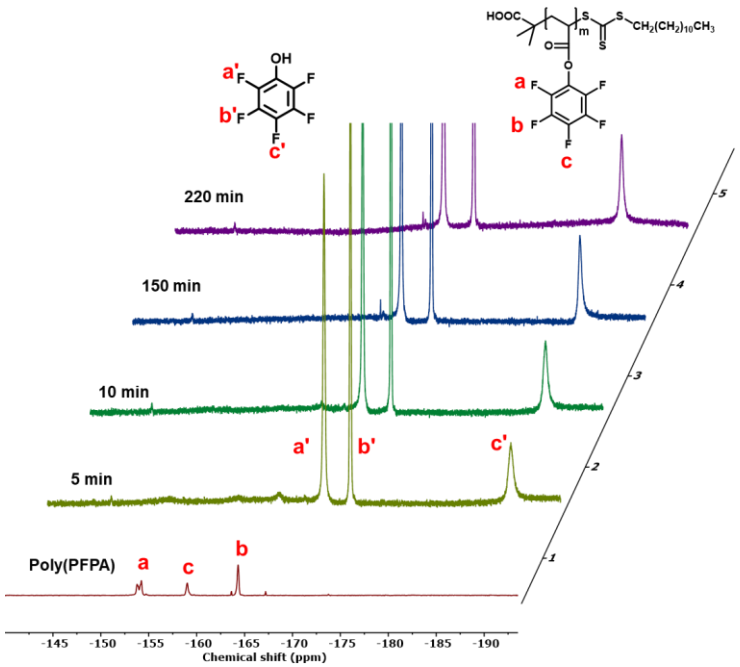


Figure S4. Aminolysis of poly(PFPA)$_{20}$ with the DPA ligand in DMF monitored by ^{19}F NMR at various time points. poly(PFPA)$_{20}$ had three broad peaks at −153.2, −156.8, and −162.3 ppm. During aminolysis, a set of new peaks at −168.9, −171.6, and −188.4 ppm appeared. These new peaks corresponded to the released pentafluorophenol. Note that the aminolysis reaction was so fast that nearly all poly(PFPA)$_{20}$ signals disappeared within 5 minutes.

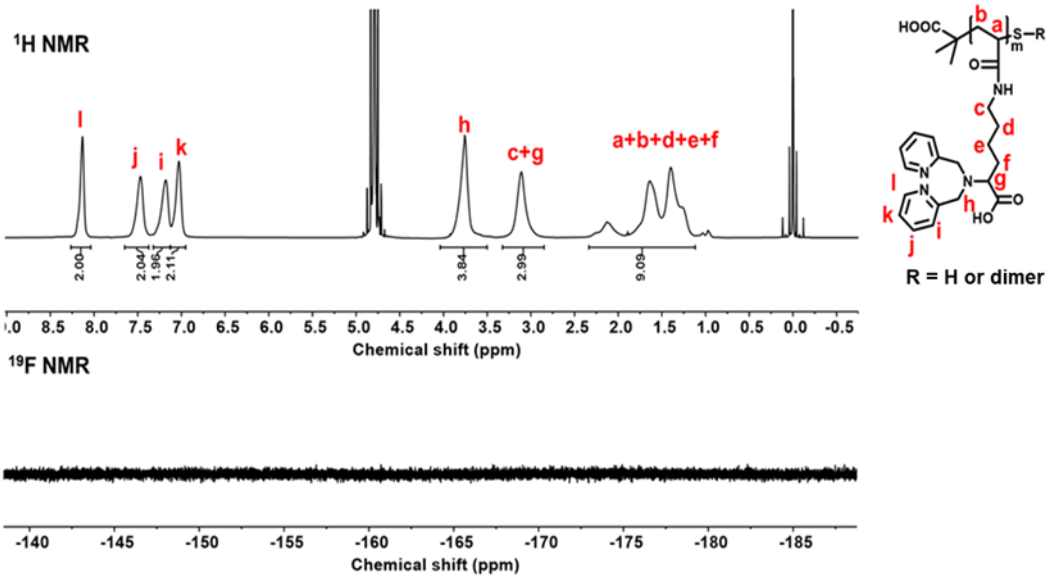


Figure S5. ^{1}H NMR (D_2O) characterization of polymer **2** shown in Scheme 1. Top: ^{1}H NMR spectrum. Bottom: ^{19}F NMR spectrum.

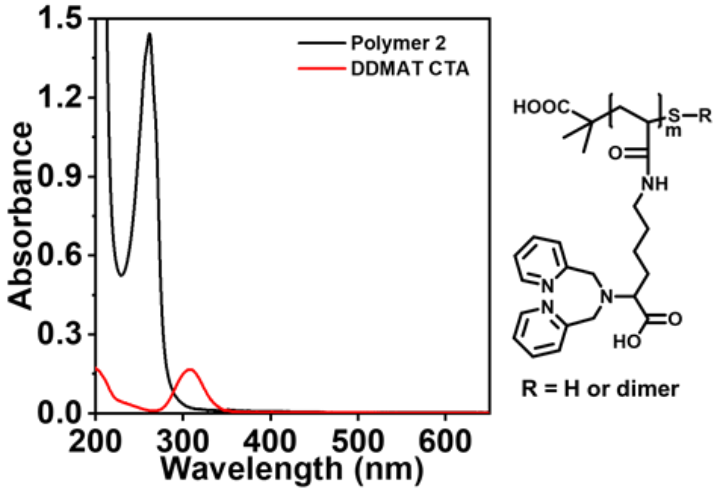


Figure S6. UV-vis spectra of polymer **2** (black trace) and DDMAT CTA (red trace) in MeOH. Concentration of polymer **2**: 11 μM. Concentration of DDMAT CTA: 10 μM.

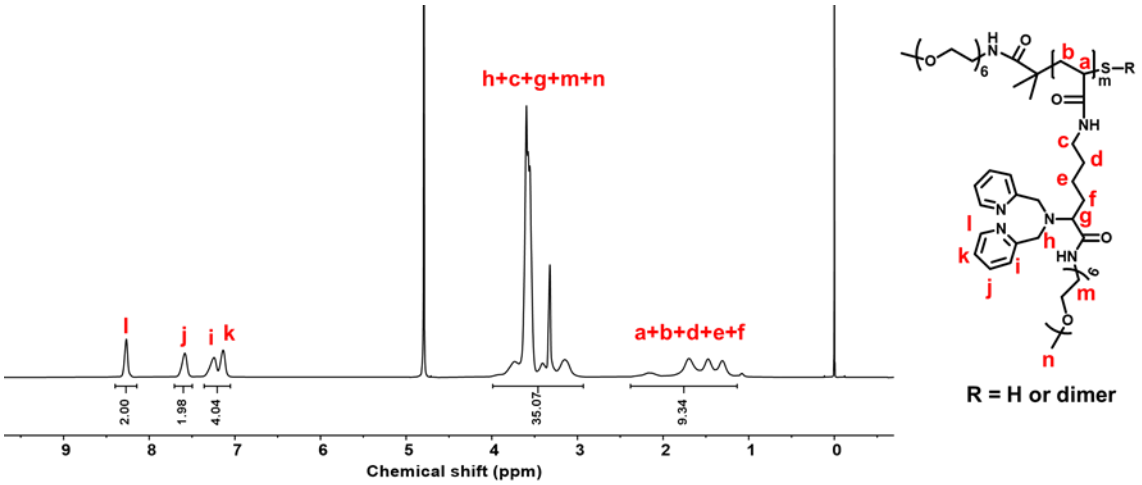


Figure S7. ^{1}H NMR spectrum of polymer **3** obtained by PEGylation of polymer **2**. PEGylation efficiency = (35.07−4−2−1)/(4×6+3)×100% = 104%.

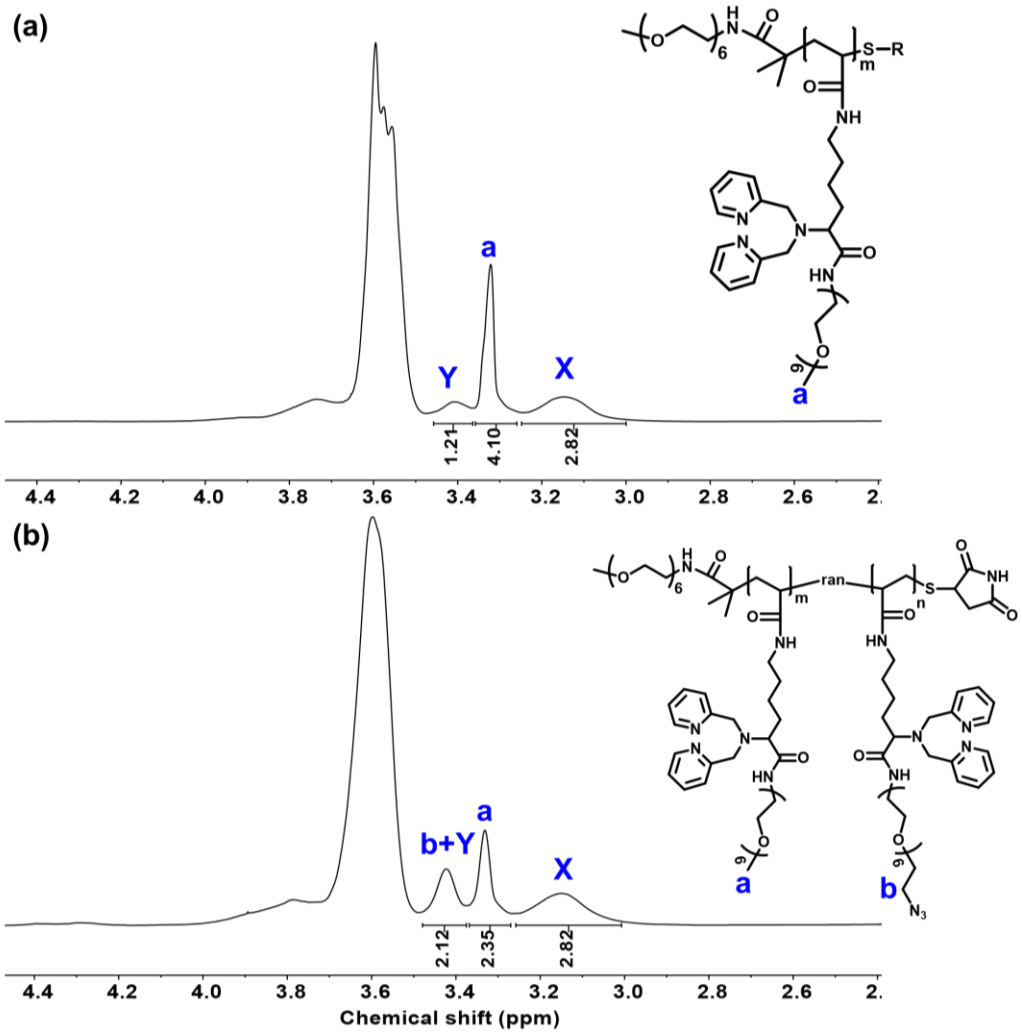


Figure S8. (a) Partial ^{1}H NMR spectrum of polymer **3**. (b) Partial ^{1}H NMR spectrum of polymer **7**. For the analysis of peak integrations, the integration of pyridyl protons at 8.27 ppm was set to 2 and used as the reference peak (Figure S7 and Figure 2a). In the PEGylation step of polymer **6** (Scheme 2), we used a mixture of $mPEG_6$-NH_2 and azide-PEG_6-NH_2 at a 1:1 molar ratio. Assuming the same reactivity of the two PEGs, the ratio of the two pendant PEGs in polymer **7** should be close to 1:1. While we obsvered the corresponding proton NMR peaks of the two PEGs (Figure S8b), it is difficult to accurately determine the ratio of the two PEGs from their peak integrals due to their spectra overlap with other proton signals of the polymer. Nevertheless, by using the ^{1}H NMR spectrum of polymer **3** as a reference, we deterimined the ratio of $mPEG_6$ to azide-PEG_6 in polymer **7** to be 1:1.1 as follows: for the $mPEG_6$ modified polymer **3** (Figure S8a), the integration of the methoxy peak **a** was 4.10 while the two neighbouring peaks X and Y had integration values of 2.82 and 1.21, respectively. Note the the integration of the methoxy peak **a** was not equal to 3, which might be due to the presence of other proton signals in the same region. For polymer **7**, the integration of the methoxy peak **a** was 2.35 while two neighbouring peaks X and b+Y had integration values of 2.82 and 2.12, respectively. The integration value of peak **b** was further determined to be 0.91 (2.12-1.21 = 0.91) by substracting the integral of peak Y. Finally, the ratio of $mPEG_6$ to azide-PEG_6 was determined by comparing the integration of peak **a** to that of peak **b [**((2.35-1.1)/3)/(0.91/2) = 0.91], which gave 1:1.1.

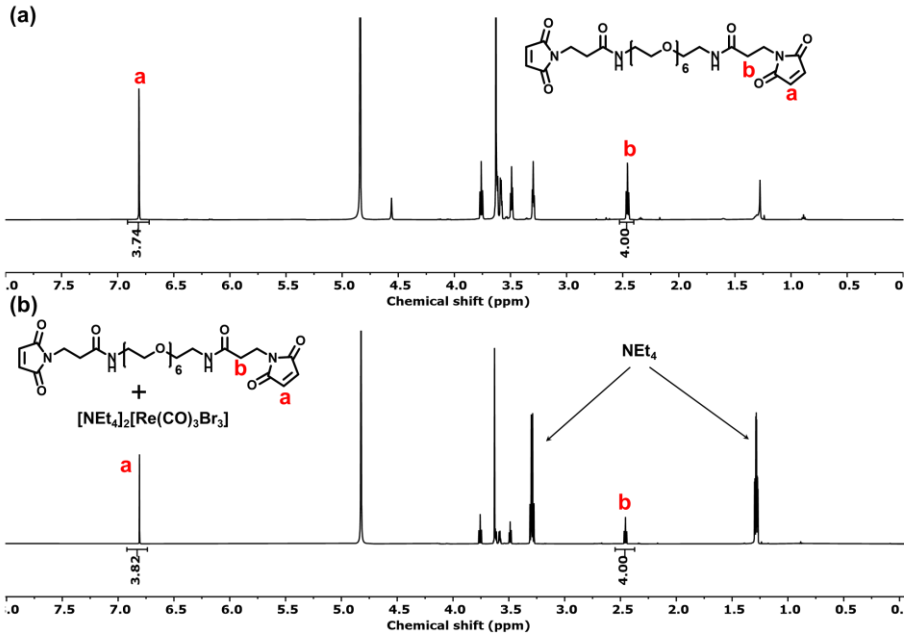


Figure S9. (a) ^{1}H NMR spectrum of fresh Bis-Mal-PEG$_6$ in deuterated methanol. (b) ^{1}H NMR spectrum of Bis-Mal-PEG$_6$ and rhenium salt mixture in deuterated methanol after incubating at 37 ºC for 2h. No significant difference in maleimide signals was observed under metal loading conditions.

Figure S10. A picture showing lyophilized PolyRe in PCR tubes (ca. 300 µg per tube).

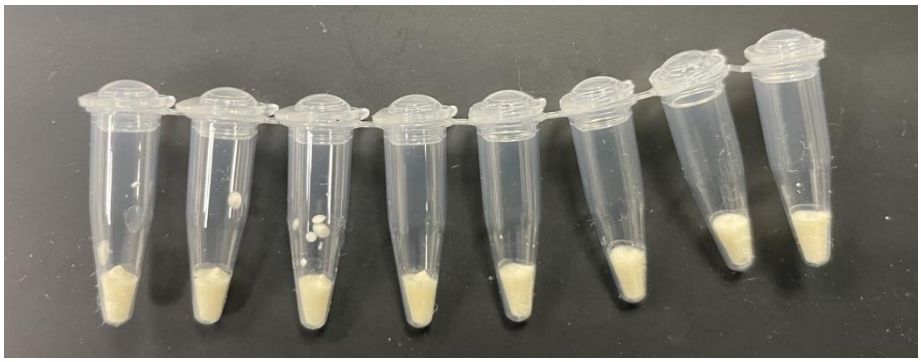


Figure S11. A picture showing lyophilized PolyPt in PCR tubes (ca. 300 µg per tube).

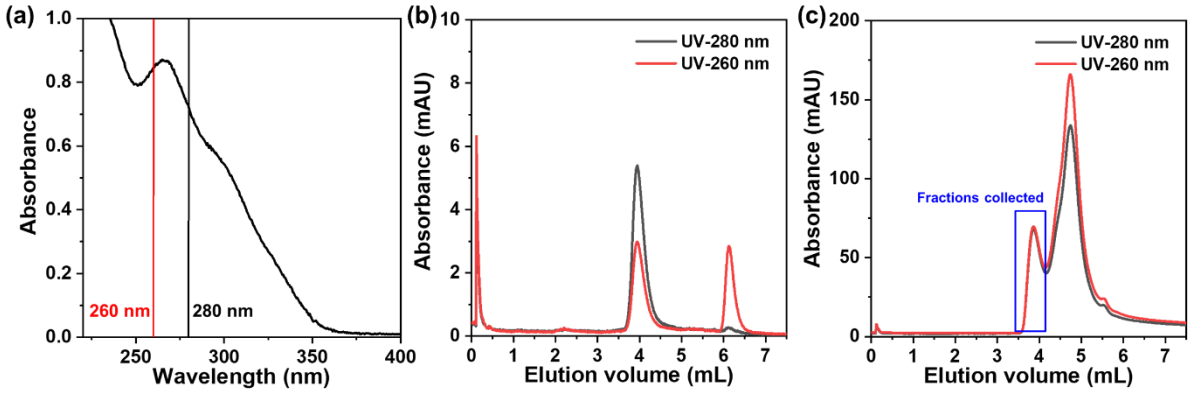


Figure S12. (a) UV-vis spectrum of PolyRe in PBS (0.1 mg/mL, pH 7.4). Note that the polymer extinction coefficient at 260 nm is larger than that at 280 nm. (b) FPLC chromatogram of pure CD20 antibody. Note that the antibody extinction coefficient at 260 nm is smaller than that at 280 nm. (c) FPLC chromatogram of CD20-polymer conjugate mixture after washing 4 times with Amicon 50 kDa filter. Significant amounts of free polymers were left after spin filtration purification. The conjugate was therefore further purified by FPLC. The blue box indicates fractions collected for the purified antibody-polymer conjugate. Note that the conjugate extinction coefficient at 260 nm is similar to that at 280 nm, suggesting successful labeling of antibody with the polymer.

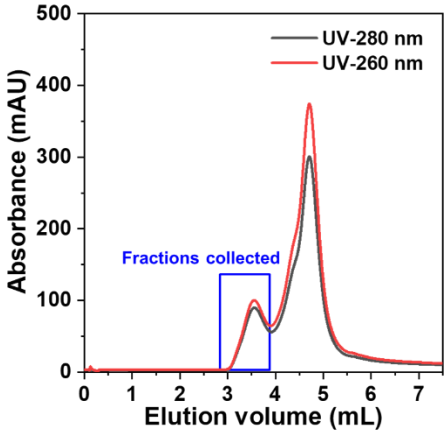


Figure S13. FPLC chromatogram of CD8a-polymer conjugate mixture. The blue box indicates fractions collected for the purified CD8a-polymer conjugate.

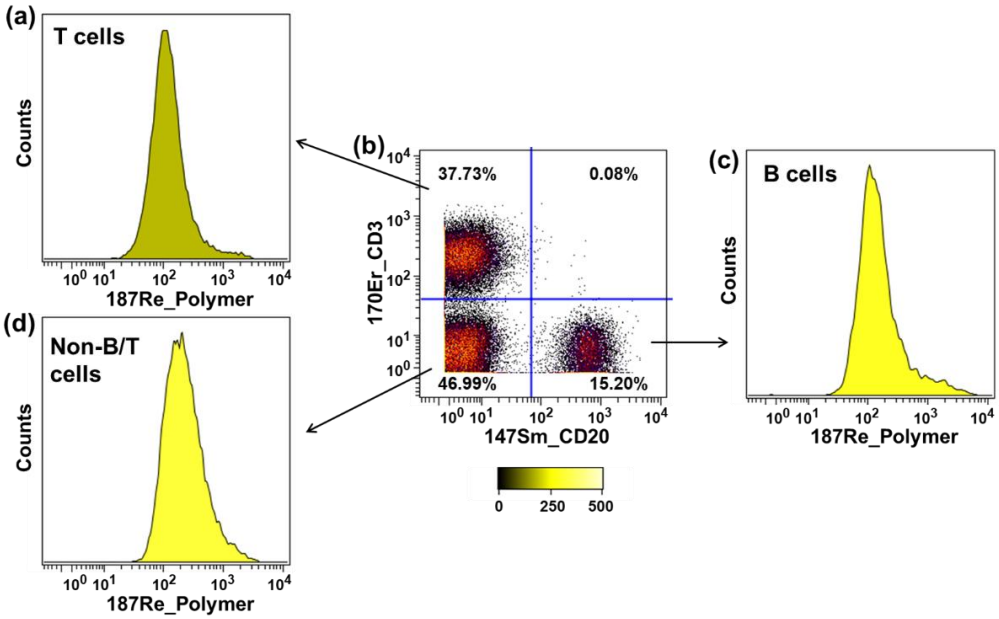


Figure S14. Non-specific binding test of PolyRe with human PBMC samples. In this experiment, we prepared a staining cocktail (50 µL) containing PolyRe (polymer itself) and Maxpar conjugates (^{147}Sm-CD20, ^{154}Sm-CD45, ^{160}Gd-CD14, ^{170}Er-CD3). The polymer concentration in the cocktail was 10 µg/mL. This cocktail was then used to stain PBMCs. Because the polymer was not conjugated to the Ab, any Re signal generated from the CyTOF measurements was considered as non-specific binding signal. The rhenium 187 channel was used for data analysis. (a) ^{187}Re signal distribution histogram for the CD45+CD20−CD3+ T-cells. Median ^{187}Re signal intensity: 117. (b) Biaxial scatter plot of ^{170}Er-CD3 vs. ^{147}Sm-CD20 within human PBMCs at the optimal titer. (c) ^{187}Re signal distribution histogram for the CD45+CD3−CD20+ B-cells. Median ^{187}Re signal intensity: 138. (d) ^{187}Re signal distribution histogram for the non-B/T cells (CD45+CD3−CD20−). Median ^{187}Re signal intensity: 211. PolyRe was stored in PBS for several days at 4 ºC (to hydrolyze the maleimide group) before added to the antibody cocktail for the staining.

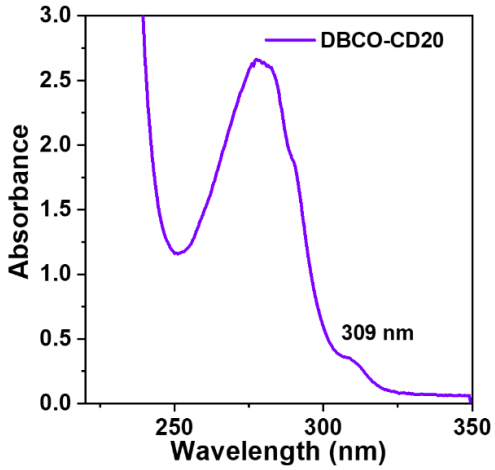


Figure S15. UV-vis spectrum of DBCO-modified anti-CD20 Ab in PBS.

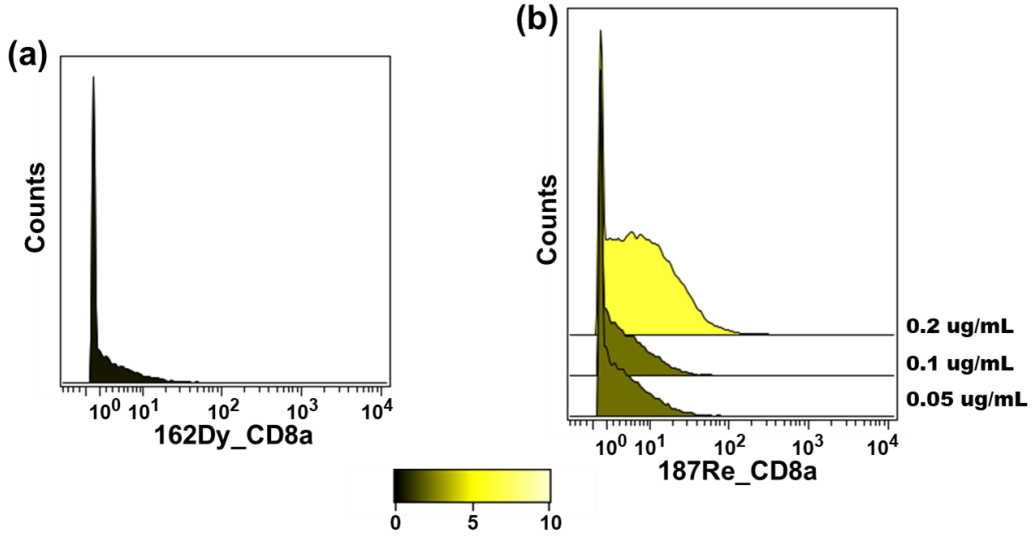


Figure S16. (a) Signal distribution histogram of ^{162}Dy signals obtained from B cells (CD45+CD3−CD20+) within PBMCs with the ^{162}Dy-CD8a conjugate at optimal titer. Median ^{162}Dy signal intensity: 0.5. (b) Signal distribution histograms of ^{187}Re signals obtained from B cells (CD45+CD3−CD20+) within PBMCs with the ^{nat}Re-CD8a conjugate at different titers. Median ^{187}Re signal intensity: 2.2 (0.05 ug/mL); 2.2 (0.1 ug/mL); 6.5 (0.2 ug/mL).