SUPPORTING INFORMATION

Synthesis of a Functional Metal-Chelating Polymer and Steps towards a Quantitative Mass Cytometry Bioassay

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Polymer Synthesis

Materials. All reagents and solvents, including carbon disulfide (≥99.9%, Sigma-Aldrich), cesium carbonate (99%, Aldrich), (1-bromoethyl)benzene (97%, Aldrich), trifluoroacetic acid (TFA) (Caledon Laboratories LTD), diethylenetriaminepentaacetic acid (DTPA) (98%, Aldrich), DL-dithiothreitol (DTT) (99%, Aldrich), and other compounds were used without further purification unless otherwise noted. Acetonitrile was dried over 4A molecular sieves. Tert-butyl acrylate (98%, Aldrich) was gravity-filtered over silica to remove the inhibitor before polymerization. Water was purified through a MilliQ water purification system (10 MΩcm). Sodium bicarbonate/carbonate buffer (pH 9.4, 200 mM) was purchased from Pierce Biotechnology. All other buffers were prepared in our laboratory. The Spectra/Por dialysis membrane (MWCO 1 kDA) was purchased from Spectrum Laboratories, Inc. The 4 mL and 15 mL, 3 kDa MWCO Millipore Amicon spin filters were purchased from Fisher Science, Canada. 2,2'-(Ethylenedioxy)bis(ethylmaleimide) was prepared according to a patent procedure,[1] although it is available commercially from Pierce Biotechnology. 2,2'-Azobis(2-methylbutyronitrile) (AMBN, Dupont USA), 4-(4,6-dimethoxy-1,3,5-triazin-2-yl)-4-methylmorpholinium chloride (DMTMM, Acros Organics, 99+%, from Fisher Science, Canada), and t-BOC-ethylenediamine (>97%, TCI America) were stored in a dessicator inside a freezer at -20 ^{0}C. Before use, their temperatures were equilibrated in a dessicator kept at room temperature.

Synthesis of Di-1-Phenylethyl Trithiocarbonate (CTA). The procedure to synthesize this CTA was taken from the literature.[2] To a 100 mL round-bottom flask was added carbon disulfide (1.51 mL, 25 mmol), cesium carbonate (8.15 g, 25 mmol) and dry acetonitrile (20 mL). After mixing for two hours at room temperature, the mixture had a pale red color. At this point, (1-bromoethyl)benzene (3.41 mL, 25 mmol) in dry acetonitrile (5 mL) was added, and the mixture was stirred for an additional 2 days. Afterwards, the yellow reaction mixture was poured into a 1 L separatory funnel containing ice water (500 mL), after which ethyl acetate (250 mL) was added. After agitation the water layer was removed, and then the ethyl acetate layer was washed with additional water (250 mL), dried over magnesium sulfate, and filtered over a fritted funnel containing celite. The ethyl acetate was removed by rotary evaporator, after which the crude product was purified by flash column chromatography, eluting with hexanes:dichloromethane (9:1 v:v), product TLC R_f = 0.20. Finally, all solvent was removed by rotary evaporator, followed by drying under vacuum (room temperature, $<10^{-3}$ torr) to yield an orange oil. Yield: 2.64 g (66%); ^{1}H NMR ($CDCl_3$): δ(ppm) 1.72 (t, 3H, CH_3), 5.30 (q, 1H, methine), 7.32 (m, 5H, aromatic)

Polymer Series. Two polymer samples were synthesized using the same chemistry, with number average degrees of polymerization (DP_n) of 67 and 79. Details are provided for the $DP_n = 67$ sample, but characterization data are provided for both samples. All reagents and solvent volumes scale with the amount of reactant.

RAFT polymerization of tBA. The polymerization was carried out using 80% monomer/20% acetone by volume as a diluent, a round-bottom flask fitted with a rubber septum, and purging with N_2 as the degassing method. The molar ratio of tBA:CTA:AMBN was 85:0.5:0.1. To a 50 mL round-bottom flask was added **CTA** (174 mg), AMBN (18.6 mg), tert-butyl acrylate (11.90 g), acetone (2.69 g), and a magnetic stir bar, after which the septum was secured and the contents purged with N_2 for 15 minutes. The flask was lowered into a 60 ^{0}C oil bath, and after 3.3 hours the solution was observed to be viscous. The septum was opened and an aliquot was removed and dissolved in $CDCl_3$ for ^{1}H NMR analysis. [Comparison of the ^{1}H NMR signals of the vinylic monomer (5.72, 6.03, and 6.30 ppm, 3H) to the methine polymer backbone signal (2.15-2.35 ppm, 1H) showed a monomer conversion of 78%.] Acetone (10 mL) was added to the polymerization mixture, after which the polymer was precipitated twice from a concentrated acetone solution (ca. 20 mL) into a mixture of water and methanol (1:1, v:v, 300 mL), transferred to a tared round-bottom flask as an acetone solution, and then dried by rotary evaporation using a water bath at 70 ^{0}C. Dissolution in acetone and rotary evaporation was repeated a total of seven times to remove any remaining monomer. Finally, the polymer was dried for 24 hours under ca. 10^{-3} torr vacuum at 70 ^{0}C, and then stored in a refrigerator at 12 ^{0}C; Yield = 8.69 g (73%); ^{1}H NMR (CD_2Cl_2): δ(ppm, integrated peak areas are reported are based on C_6H_5 = 5H as the reference) 1.1-1.9 (broad, 2H per monomer, backbone methylene, peak overlapped), 1.43 (s, 9H per monomer, -$C(CH_3)_3$ ester, peak overlapped), 2.1-2.5 (broad, 1H per monomer, backbone methine, integration = 66.1), 2.71 (s, 1H per polymer, (phenyl)(methyl)-C(H)- methine, integration = 0.9), 4.60 (s, 1H end group methine, integration = 0.9), 7.17 & 7.26 (broad doublet & triplet, 5H, end group phenyl, integration = 5.0); DP_n = 67, calculated by comparing the integration of the ^{1}H NMR signals at 7.17 and 7.26 ppm (end group) to that at 2.1-2.5 ppm (backbone methine) + 4.60 (end group methine); GPC (THF, RI) M_n = 16,000 Da, PDI = 1.11, strong corresponding peak observed in the UV/VIS trace monitored at 310 nm.

P(tBA) DP_n = 79. Synthesis: molar ratio tBA:CTA:AMBN = 105:0.5:0.1;, polymerization time = 3.5 hours, monomer conversion = 76%; Yield = 8.83 (69%). ^{1}H NMR (CD_2Cl_2): 1.1-1.9 (peak overlapped), 1.43 (peak overlapped), 2.1-2.5 (integration = 77.8), 2.71 (integration = 0.9), 4.60

(integration = 1.0), 7.17 & 7.26 (integration = 5.0); calculated DP_n = 79. GPC (THF, RI) M_n = 19,000 Da, PDI = 1.11, strong corresponding peak observed in the UV/VIS trace (310 nm).

Aminolysis of the Trithiocarbonate and Formation of a Polymeric Disulfide. PtBA (DP_n = 67, 3.37 g) was placed in a 500 mL round-bottom flask. THF (12.64 mL), ethanolamine (126 μL), and a magnetic stir bar were added, after which a glass stopper was secured with Apiezon H grease. After stirring the solution at room temperature overnight, the disulfide PtBA product was recovered by removing the solvent with a rotary evaporator using a water bath at room temperature. GPC (THF, RI) M_n = 14,400 Da, PDI = 1.16, no corresponding peak observed in the UV/VIS trace (310 nm).

DP_n = 79 sample: Synthesis reaction scale = 2.66 g; GPC (THF, RI) M_n = 17,000 Da, PDI = 1.16, no corresponding peak observed in the UV/VIS trace (310 nm).

Deprotection of PtBA to PAA. The PtBA-disulfide (DP_n = 67, entire sample) was dissolved in dichloromethane (20.2 mL). Trifluoroacetic acid (8.6 mL) was added and the solution was stirred overnight. The polymer precipitated as a large mass, which was then collected over a fritted funnel and washed with dichloromethane (30 mL) and then diethyl ether (30 mL). After being transferred to a round-bottom flask, the precipitate was dried for 30 minutes on a rotary evaporator using a water bath at room temperature. The polymer was dissolved in H_2O:MeOH (2:1 v:v, 25.3 mL), and then transferred to a Spectra/Por dialysis bag (MWCO 1 kDA). The polymer was dialyzed against water (4 L) for three days, with the water being changed twice daily. Afterwards the solution was transferred to a 50 mL Falcon centrifuge tube and then freeze-dried to yield PAA-disulfide. Yield = 1.78 g (94%); ^{1}H NMR (D_2O): δ(ppm, integrated peak areas reported based on C_6H_5 = 5H) 1.25 (b, 3H, end group –CH_3, integration = 3.0), 1.46 (s, 9H, 1% unremoved tert-butyl ester, integration = 3.2), 1.4-2.1 (b, 2H per monomer, backbone methylene, integration = 127.0), 2.2-2.7 (b, 1H per monomer, backbone methine, integration = 66.1), 7.2-7.45 (m, 5H, phenyl, integration = 5.0); GPC (aqueous, relative to poly(methacrylic acid) standards, RI) M_n = 17,400 Da, PDI = 1.19.

DP_n = 79 sample: Yield = 1.40 g (97%); ^{1}H NMR (D_2O): δ(ppm) 1.25 (integration = 2.8), 1.46 (integration = 6.9), 1.4-2.1 (integration = 152), 2.2-2.7 (integration = 78.5), 7.2-7.45 (integration = 5.0); GPC (Aq., RI) M_n = 21,100 Da, PDI = 1.17.

Coupling of t-BOC-ethylenediamine to PAA. PAA-disulfide (DP_n = 67, 50 mg) was added to a 50 mL round-bottom flask and dissolved in water (5 mL). A solution of t-BOC-ethylenediamine (277 mg) in 5 mL water was added to the polymer solution. A solution of DMTMM (480 mg) in water (8.75 mL) was added immediately, and the mixture was stirred at room temperature overnight. The solution took on the appearance of milk after one hour, and had precipitated by the next morning. The liquid was

poured off, and the polymer was rinsed with water (3 x 10 mL). To remove all water, the polymer product was dissolved in dichloromethane, and then the solvent was removed with a rotary evaporator using a water bath at room temperature. This dissolution in dichloromethane and rotary evaporation was repeated a total of seven times in order to azeotropically remove all water. The polymer product was not characterized, but was used directly in the deprotection step.

Deprotection of Boc Groups to Yield the Amino Polymer. Boc-protected polymer (DP_n = 67, entire sample) was dissolved in dichloromethane (9 mL), after which anisole (1.5 mL) and trifluoroacetic acid (4.5 mL) were added with stirring. The mixture was stirred for 30 min, after which the product polymer was observed to have precipitated on the walls of the flask as a transparent goo. After three hours of stirring, the mother liquor was poured off, and the precipitated polymer was rinsed with diethyl ether (2 x 10 mL). The product was dried on a rotary evaporator using a water bath at room temperature for five minutes, after which it was dissolved in water and then washed with water (5 x 11 mL) using a 15 mL, 3 kDA MWCO Millipore Amicon spin filter. Finally, the aqueous solution was freeze-dried to yield the Amino Polymer-disulfide as the trifluoroacetate salt.; Yield = 165 mg (100%); ^{1}H NMR (D_2O): δ(ppm, integrated peak areas reported based on C_6H_5 = 5H) 1.19 (broad t, 3H, end group –CH_3, integration = 3.4), 1.25-2.40 (broad, 2H per monomer, backbone methylene + 1H per monomer, backbone methine, integration = 194), 3.13 (broad s, 2H per monomer, ethylenediamine –C*H*$_2$-NH_3^+, integration = 126), 3.47 (broad s, 2H per monomer, ethylenediamine –CO-NH-C*H*$_2$-, integration = 132.0), 7.15-7.45 (m, 5H phenyl, integration = 5.0).

DP_n = 79 sample: Yield = 127 mg (80%); ^{1}H NMR (D_2O): δ(ppm) 1.19 (integration = 3.8), 1.25-2.40 (integration = 239), 3.13 (integration = 153), 3.47 (integration = 160), 7.15-7.45 (integration = 5.0).

Synthesis of P(DTPA). DTPA (3.5 g, ca. 80 equivalents to each polymeric amino group) and H_2O (5 mL) were added to a 100 mL round-bottom flask. Next, NaOH (5 M aq.) was added to dissolve the DTPA and bring the solution pH to 8.5 (monitored with a pH meter). DMTMM (250 mg, ca. 8 equivalents to each polymeric amino group) was dissolved in water (5 mL) with sonication and added quickly with stirring to the first solution. This solution was given 5 minutes to pre-react. Then a solution of the Amino Polymer (25 mg) in water (5 mL) was added quickly with stirring. The reaction solution was stirred for 1 hour, then transferred to a 15 mL 3kDA MWCO Millipore Amicon spin filter and washed with water (9 x 11 mL). Finally, the aqueous solution was freeze-dried to yield P(DTPA)-disulfide.; Yield = 52 mg (85%); ^{1}H NMR (D_2O): δ(ppm, integrated peak areas reported based on C_6H_5 = 5H) 1.0-2.4 (b, 3H per monomer, backbone, integration = 187), 2.7-4.0 (broad m, 4H ethylenediamine

and 18H DTPA per monomer, integration = 1400), 7.15 – 7.45 (broad t, 5H phenyl, integration = 5.0); GPC (Aqueous, relative to poly(methacrylic acid) standards, RI) M_n = 36,800 Da, PDI = 1.18.

DP_n = 79 sample: Yield = 56 mg (93%); ^{1}H NMR (D_2O): δ(ppm) 1.0-2.4 (integration = 225.4), 2.7-4.0 (integration = 1620), 7.15 – 7.45 (integration = 5.0); GPC (Aq., RI) M_n = 43,300 Da, PDI = 1.17.

Reduction of P(DTPA)-disulfide with DTT Followed by Functionalization with a Bismaleimide Linker. We followed the procedure described in ref 5. A solution of DTT (dithiothreitol, 20 mM) was freshly prepared in phosphate buffer (50 mM, pH 8.5). P(DTPA)-disulfide (10 mg) was dissolved in 300 μL of this DTT solution. The vial threads were wrapped with Teflon tape; the cap was secured, and the solution was stirred at 50 ^{0}C for one hour. Immediately afterwards the polymer solution was diluted with acetate buffer (3.5 mL, 50 mM, pH 3.0) and transferred to a 4 mL 3 kDA MWCO Millipore Amicon spin filter. The solution was spun through the filter, after which the polymer was washed (3 x 4 mL) with aqueous acetic acid (5 mM, pH = 3.5). Next, the resultant polymer solution (100 μL) was transferred to a 2 dram vial and diluted to a total volume of 300 μL with phosphate buffer (200 mM, pH 7.0 or pH 8.5). A freshly prepared solution of 2,2'-(ethylenedioxy)bis(ethylmaleimide) (10 mg) in DMF (150 uL) was quickly added with mixing to the polymer solution, and the solution was stirred for 1 hr at room temperature. Directly after this, the solution was diluted with water, transferred to a new 4 mL 3 kDA MWCO Millipore Amicon spin filter, and washed (7 x 11 mL) with water. Finally, the aqueous solution was freeze-dried to yield maleimide end-labeled DTPA polymer. Reaction at pH 7.0, yield = 6.3 mg (63%); Reaction at pH 8.5, yield = 5.0 mg (50%); ^{1}H NMR (D_2O): δ(ppm, integrated peak areas reported based on C_6H_5 = 5H) 1.0-2.4 (broad, 3H per monomer, backbone, integration = 203), 2.7-4.0 (broad m, 4H ethylenediamine and 18H DTPA per monomer, integration = 1580), 6.88 (s, 2H vinylic maleimide, integration = 1.84 (pH 7.0), integration = 1.62 (pH 8.5)), 7.15 – 7.45 (broad peaks, 5H phenyl, integration = 5.0). GPC (Aqueous, relative to poly(methacrylic acid) standards, RI) M_n = 22,500 Da, PDI = 1.15.

DP_n = 79 sample. Yield = 14.3 mg (75%) (pH 7.0), Yield = 5.0 mg (50%) (pH 8.5); ^{1}H NMR (D_2O): δ(ppm) 1.0-2.4 (integration = 237), 2.7-4.0 (integration = 1830), 6.88 (integration = 1.66 (pH 7.0), integration = 1.66 (pH 8.5)), 7.15 – 7.45 (integration = 5.0). GPC (Aq, RI) M_n = 26,100 Da, PDI = 1.17.

Table S1: ^{1}H NMR T_1 and T_2 Relaxation Constants of DP_n = 67 Polymer Samples

DP_n = 67 Samples	**PtBA-Trithiocarbonate (CD_2Cl_2)**	**PAA-Disulfide (D_2O)**	**Amino Polymer-Disulfide (D_2O)**	**P(DTPA)-Disulfide (D_2O)**	**P(DTPA)-Maleimide (D_2O)**
T_1 (seconds)					
2H Phenyl (Raft Agent)	2.12 ± 0.10	1.38 ± 0.02	1.60 ± 0.03	1.50 ± 0.16	1.4 ± 0.2
3H Phenyl (Raft Agent)	1.73 ± 0.06	1.152 ± 0.015	1.28 ± 0.03	1.22 ± 0.07	1.17 ± 0.09
2H Maleimide Group	---	---	---	---	4.38 ± 0.07
1H Methine Adjacent to Trithiocarbonate	1.22 ± 0.04	---	---	---	---
2H Ethylenediamine (Methylene adjacent to amide)	---	---	0.700 ± 0.002	---	---
2H Ethylenediamine (Methylene adjacent to protonated amine)	---	---	0.6366 ± 0.0017	---	---
Ethylenediamine + DTPA	---	---	---	0.51 ± 0.11	0.55 ± 0.02
1H Methine (Raft Agent)	0.71 ± 0.06	0.82 ± 0.04	---	---	---
1H Backbone Methine	0.704 ± 0.010	0.756 ± 0.018	0.728 ± 0.017	---	---
2H Backbone Methylene (overlapped with 9H tert-butyl)	0.42 ± 0.07	---	---	---	---
2H Backbone Methylene	---	0.505 ± 0.012	0.599 ± 0.002	---	---
3H Backbone	---	---	---	1.13 ± 0.04	1.12 ± 0.05
Residual tert-Butyl Ester	---	0.502 ± 0.006	---	---	---
3H Methyl (Raft Agent)	---	0.438 ± 0.012	0.429 ± 0.010	---	---
T_2 (seconds)					
2H Phenyl (Raft Agent)	0.97 ± 0.12	0.264 ± 0.011	0.42 ± 0.05	0.13 ± 0.03	0.19 ± 0.03
3H Phenyl (Raft Agent)	0.84 ± 0.07	0.236 ± 0.019	0.38 ± 0.03	0.14 ± 0.03	0.131 ± 0.019
2H Maleimide Group	---	---	---	---	2.2 ± 0.3
1H Methine Adjacent to Trithiocarbonate	0.025 ± 0.003	---	---	---	---
2H Ethylenediamine (Methylene adjacent to amide)	---	---	0.026 ± 0.004	---	---
2H Ethylenediamine (Methylene adjacent to protonated amine)	---	---	0.031 ± 0.007	---	---
Ethylenediamine + DTPA	---	---	---	0.037 ± 0.017	0.031 ± 0.009
1H Methine (Raft Agent)	0.0095 ± 0.0016	0.0146 ± 0.0015	---	---	---

1H Backbone Methine	0.027 ± 0.005	0.023 ± 0.004	0.0148 ± 0.0006	---	---
2H Backbone Methylene (overlapped with 9H tert-butyl)	0.10 ± 0.11	---	---	---	---
2H Backbone Methylene	---	0.014 ± 0.006	0.0123 ± 0.0007	---	---
3H Backbone	---	---	---	0.0074 ± 0.0017	0.008 ± 0.002
Residual tert-Butyl Ester	---	0.194 ± 0.012	---	---	---
3H Methyl (Raft Agent)	---	0.037 ± 0.005	0.035 ± 0.008	---	---

Table S2: ^{1}H NMR end-group analysis and Gel Permeation Chromatography data for all polymer samples

Polymer Sample		**67**	**79**
PtBA-trithiocarbonate	^{1}H NMR (CD_2Cl_2) end-group analysis (DP_n)	67	79
	THF GPC M_n (Da)	16,000	19,000
	THF GPC PDI	1.11	1.11
PtBA-disulfide	THF GPC M_n (Da)	14,400	17,000
	THF GPC PDI	1.16	1.16
PAA-disulfide	^{1}H NMR (D_2O) end-group analysis (DP_n)	67	79
	^{1}H NMR (D_2O) t-butyl deprotection	99%	99%
	Aq. GPC M_n (Da)	17,400	21,100
	Aq. GPC PDI	1.19	1.17
Amino Polymer-disulfide	^{1}H NMR (D_2O) end-group analysis (DP_n)	65	80
	^{1}H NMR (D_2O) acrylamide functionality	100%	99%
	^{1}H NMR (D_2O) BOC deprotection	100%	100%
P(DTPA)-disulfide	^{1}H NMR (D_2O) end-group analysis (DP_n)	62	75
	^{1}H NMR (D_2O) DTPA functionality	100%	97%
	Aq. GPC M_n (Da)	36,800	43,300
	Aq. GPC PDI	1.18	1.17
P(DTPA)-maleimide	^{1}H NMR (D_2O) end-group analysis (DP_n)	68	79
	^{1}H NMR (D_2O) maleimide functionality (reaction pH 8.5)	81%	83%
	^{1}H NMR (D_2O) maleimide functionality (reaction pH 7.0)	92%	83%
	Aq. GPC M_n (Da)	22,500	26,100
	Aq. GPC PDI	1.15	1.17

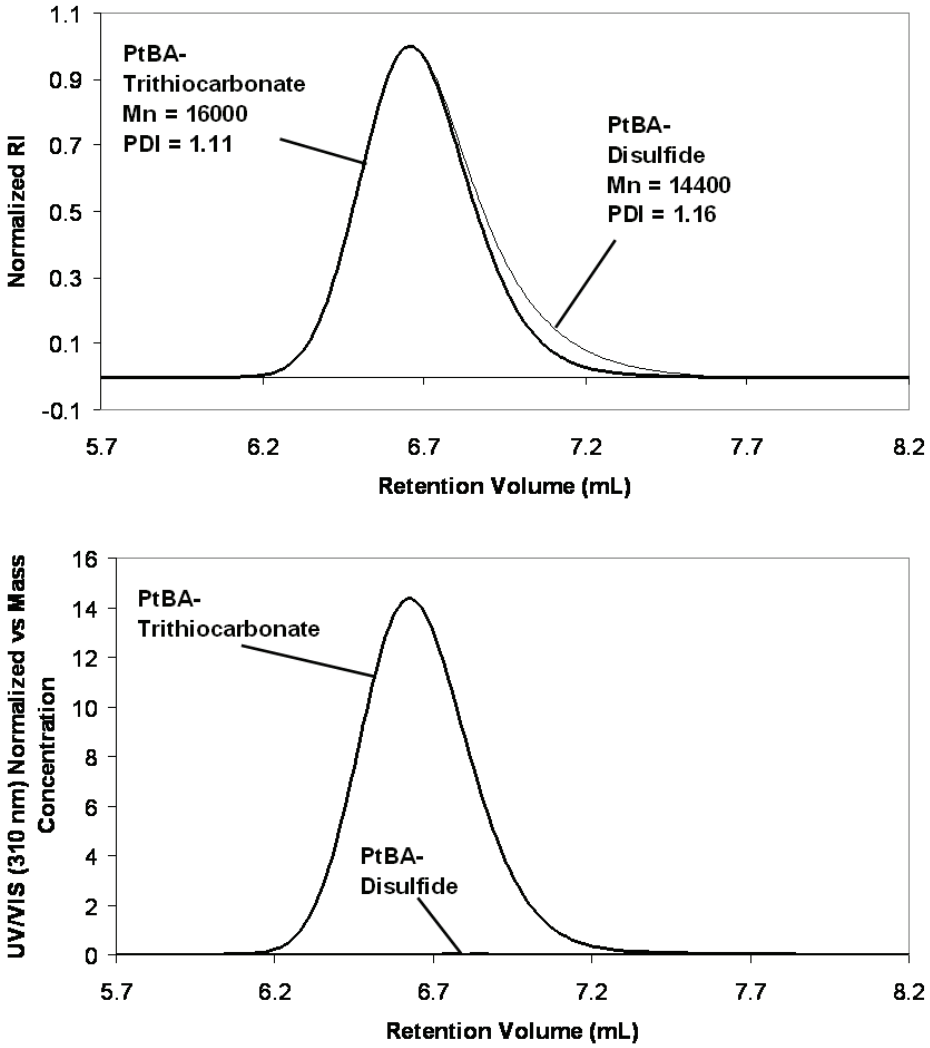

Figure S1. Top: THF GPC Chromatographs as Monitored by Refractive Index (RI) Detector of PtBA-Trithiocarbonate and PtBA-Disulfide ($DP_n = 67$). The PtBA-Trithiocarbonate is in the form of a dimer, linked through the trithiocarbonate moiety. After aminolysis, the majority of the chains have reformed as dimers linked through a disulfide bond. Bottom: THF GPC Chromatographs as Monitored by UV/VIS Detector at 310 nm of PtBA-Trithiocarbonate and PtBA-Disulfide. UV/VIS signal is normalized against mass concentration via dividing by the RI detector signal.

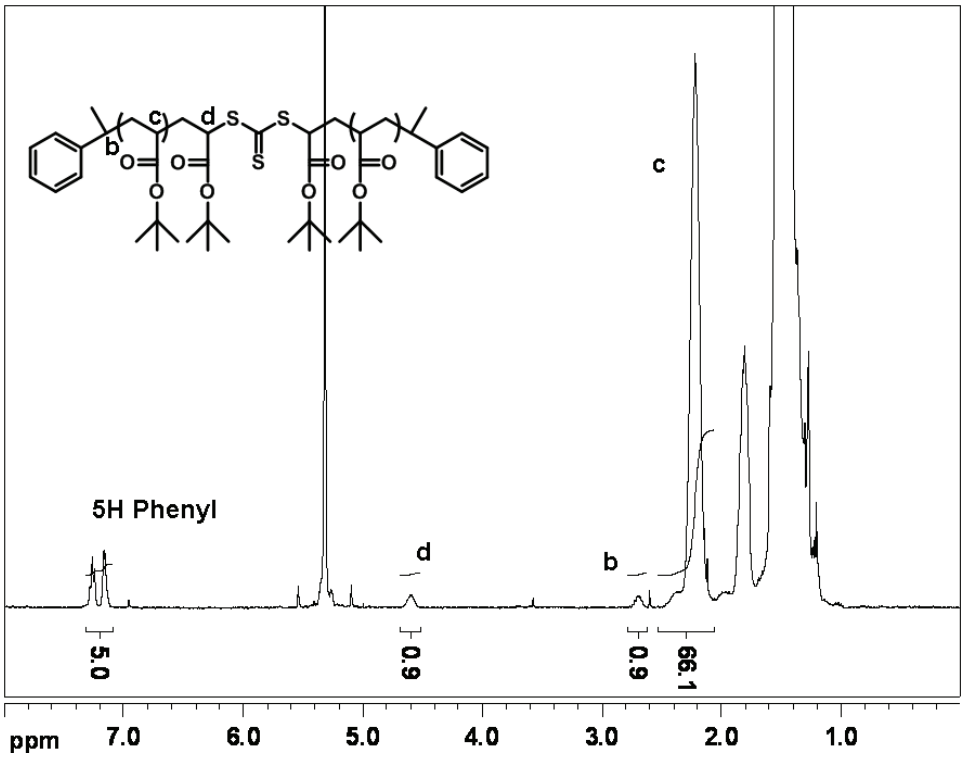

Figure S2. ^{1}H NMR Spectra (CD_2Cl_2) of PtBA-Trithiocarbonate (DP_n = 67). End-group analysis shows the degree of polymerization to be 67.

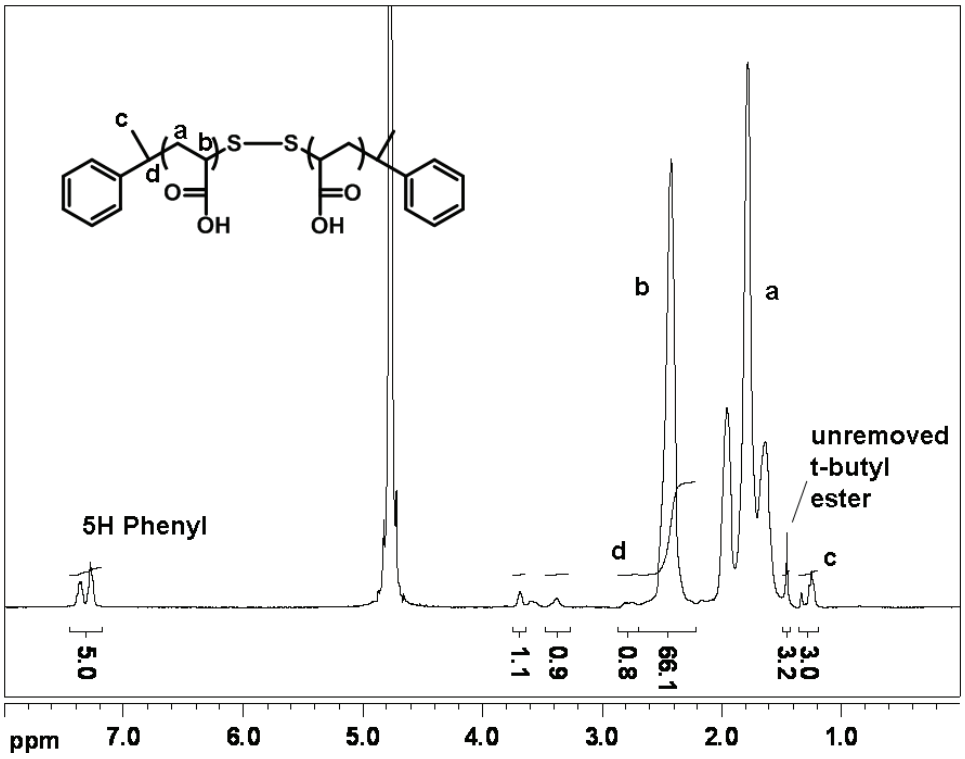

Figure S3. ^{1}H NMR Spectra (D_2O) of PAA-Disulfide (DP_n = 67). The degree of polymerization has not changed relative to the PtBA sample, remaining at 67. T-butyl ester deprotection is largely complete; the peak at 1.46 ppm has an integration of 3, representing ~0.5% of initial t-butyl groups present before deprotection.

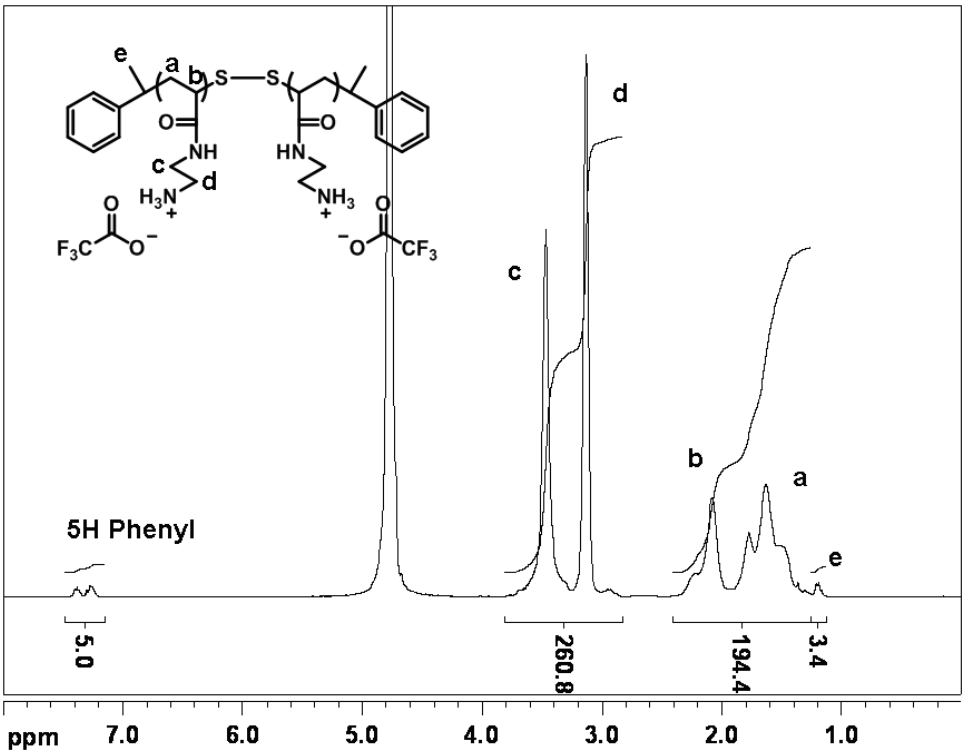

Figure S4. ^{1}H NMR Spectra (D_2O) of Amino Polymer-Disulfide (DP_n = 67). In this spectrum, DP_n is calculated by comparing the 5H Phenyl end-group to the *a* and *b* backbone signals, yielding DP_n = (194.4/3) = 65. Furthermore, within NMR error, the polymer is fully functionalized with ethylenediamine groups. This is calculated by comparing the *a* and *b* backbone signals to the *c* and *d* ethylenediamine signals, where acrylamide functionality = 100% * (260.8/194.4) / (4/3) = 100%. Finally, also of note is that no sharp t-butyl signal is present around 1.4 ppm, which shows that the deprotection of the Boc groups was quantitative.

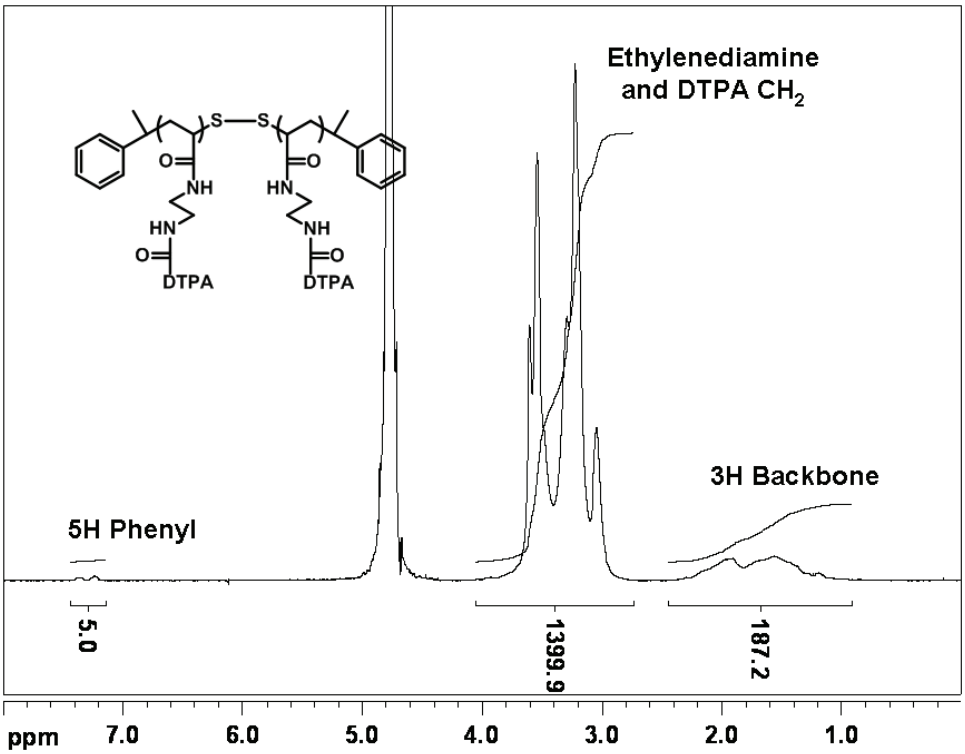

Figure S5. ^{1}H NMR Spectra (D_2O) of P(DTPA)-Disulfide (DP_n = 67). In this spectrum, DP_n is calculated by comparing the 5H Phenyl end-group to the *a* and *b* backbone signals, yielding DP_n = (187.2/3) = 62. Note, however, that this analysis is less reliable due to difficulty in assigning a baseline to the broadened polymer backbone peaks. Within NMR error, the polymer is fully functionalized with DTPA groups. This is calculated by comparing the backbone signals to the ethylenediamine and DTPA signals, where DTPA functionality = 100% * (1399.9/187.2) / (22/3) = 100%.

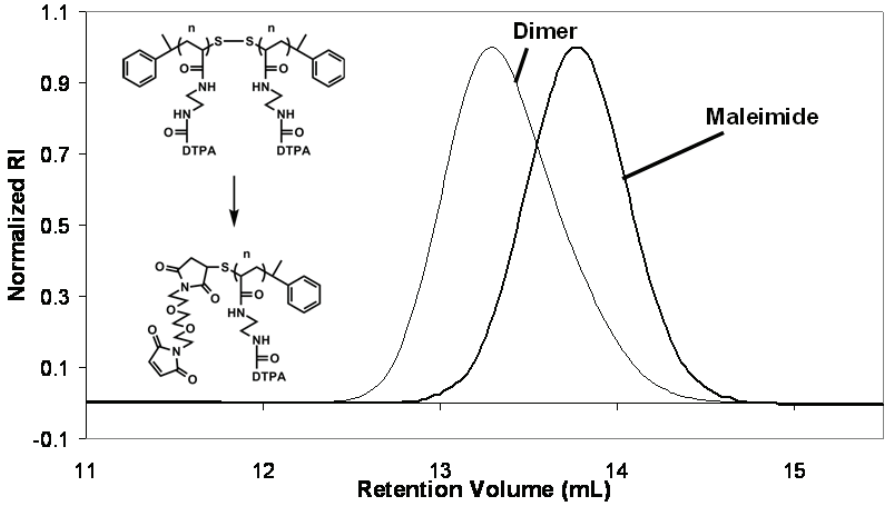

Figure S6. Aqueous GPC Chromatographs of P(DTPA)-disulfide and P(DTPA)-maleimide (sample 67). The initial DTPA polymer is in the form of a polymeric disulfide. After reduction with DTT and reaction with the bismaleimide linker, the polymers are in their final unimeric form with a maleimide end group. Before reaction M_n = 36,800 Da (PDI = 1.18), and after reaction M_n = 22,500 Da (PDI = 1.15).

Thermal gravimetric analysis of disodium EDTA·2H$_2$O

Analysis of the mass loss for $EDTA^{2-}2Na^{+}\cdot 2H_2O$ shown in Figure 2 of the main text corresponds closely with the expected results based on the previous studies of Gonzales-Vilchez and coworkers.[3] These results are presented in Table S2.

Table S3. Expected and Observed Mass Losses & Ceramic Yields for TGA Analysis of $EDTA^{2-}2Na^{+}\cdot 2H_2O$.

	Expected Values	Experimental Values
$MassLoss_{H2O}$	9.7%	9.7%
$ApparentMassLoss_{organic}$	61.8%	62.9%
Ceramic Yield	28.5%	27.4%

Isothermal titration calorimetry (ITC)

Figure S7 (Top) shows the ITC thermogram of Gd^{3+} (5.0 mM) in citrate buffer at pH 5.5 titrated into citrate buffer (blank) and into a solution of DTPA (0.5 mM) in the same buffer at 25 ºC. The enthalpy of dilution for Gd-Citrate is represented by the small exothermic signals observed for the titration of blank sample. Endothermic signals correspond to the exchange of Gd^{3+} between citrate and DTPA complex. After saturation of DTPA ligands, the thermogram shows small exothermic peaks similar to those in the blank sample.

The binding isotherms for DTPA and for the blank solution were obtained by integrating the signals from the ITC thermograms and are presented in the bottom part of Figure S7. The data were analyzed with a one site binding model provided by the Origin software. This model, which ignores the competitive interaction between citrate and Gd, gave an apparent binding constant of $4.70 \pm 0.13 \times 10^5$ M^{-1} and an enthalpy of binding of 4.0 ± 0.1 kcal/mol. A stoichiometry of 1:1 was obtained for the DTPA-Gd pair (n = 1.04 ± 0.03). The isotherm for the titration of Gd in citrate buffer shows a constant value that corresponds to the enthalpy of dilution (ΔH_{dil}). This constant value is used to shift the binding curve for the titration of DTPA before nonlinear least-squares regression with Origin as recommended by the manufacturer.

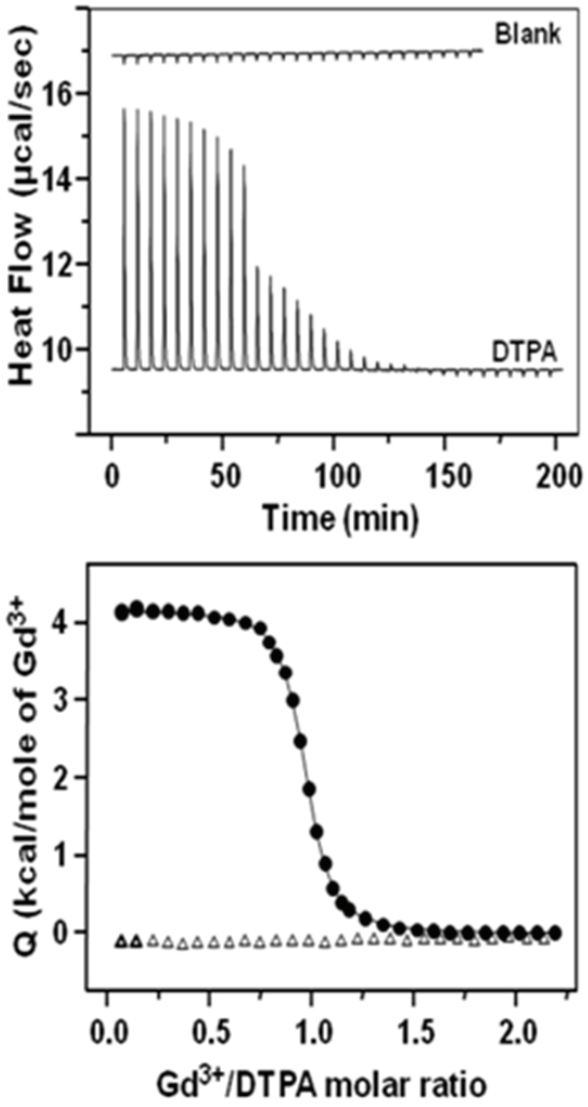

Figure S7. Top: Isothermal titration calorimetric thermogram of Gd^{3+} (5.0 mM) in citrate buffer at pH 5.5 into citrate buffer (blank) and into a solution of DTPA (0.5 mM) in the same buffer at 25 ºC. The enthalpy of dilution for Gd-Citrate is represented by the small exothermic signals observed for the titration of blank sample. Endothermic signals correspond to the exchange of Gd^{3+} between citrate and DTPA complex. Bottom: Binding isotherms calculated from the titration of citrate buffer (blank) and DTPA (0.5 mM) with Gd^{3+} (5.0 mM) at 25 ºC. The equivalence point (n) for the fitted curve shows that one DTPA ligand binds one Gd^{3+} ion ($n = 1.04 \pm 0.03$).

Antibody Dilution Series

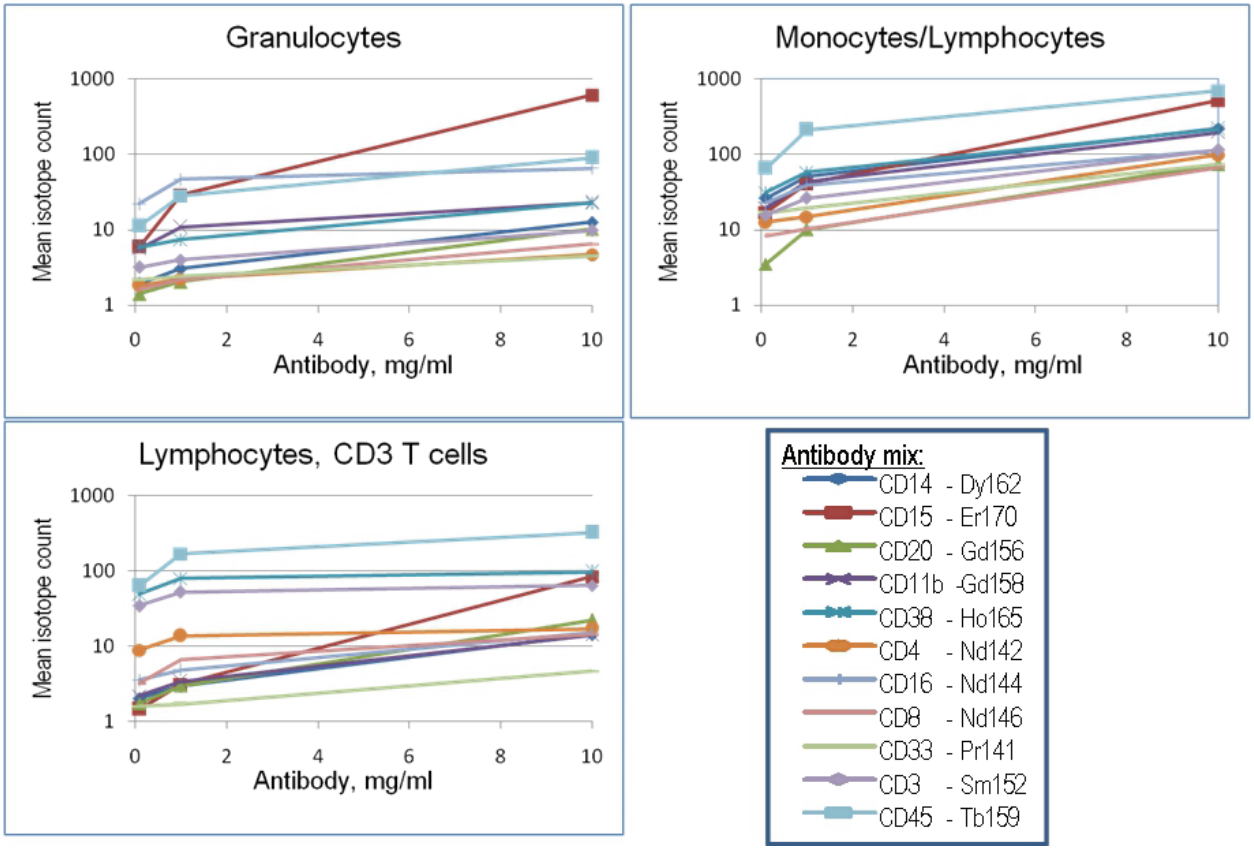

Figure S8. Antibody titration of a mixture of 11 metal-tagged antibodies on whole umbilical cord blood. Titration curves are shown for Granulocytes, Monocytes and CD3 T lymphocytes. The gating strategy is presented in Figure 5 of the main text.

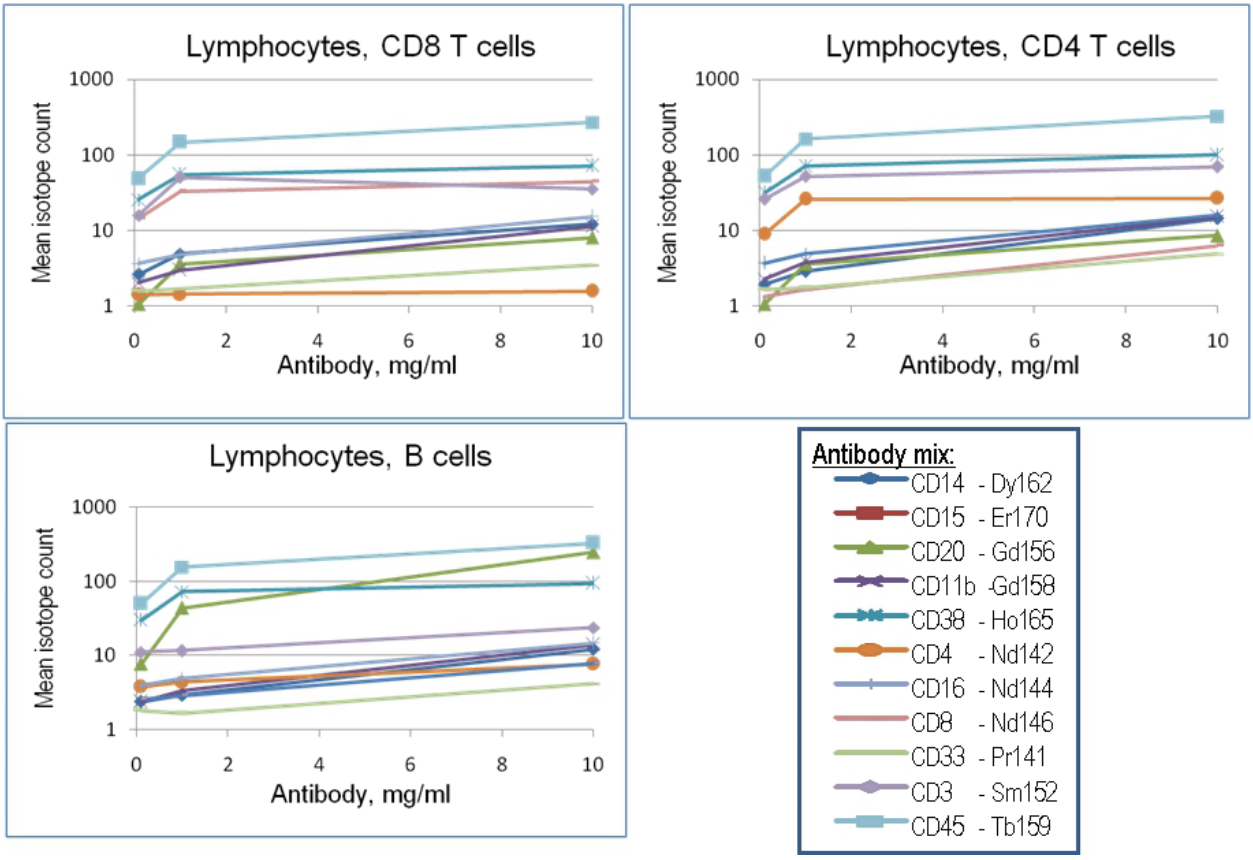

Figure S9. Antibody titration of a mixture of 11 metal-tagged antibodies on whole umbilical cord blood. Titration curves are shown for CD4 T and CD8 T lymphocytes, and CD20 B-cells. The gating strategy is presented in Figure 5 of the main text.

Clusters of Differentiation (CD) and Gating Strategy in Figure 4 (Main Text)

CD is an abbreviation of the cluster of differentiation nomenclature used for the identification and analysis of cell surface biomarkers found on blood cells. We selected 11 antibodies to the most commonly used CD molecules such as CD45, CD4, CD8, CD38, CD11, etc from the list of more than 300 known CD markers now available to the research community.[4]

Gating Strategy in Figure 4. Single nucleated cells that bind the Ir-intercalator (with ^{191}Ir and ^{193}Ir stable isotopes) are distinguished from debris in the (Ir191)D vs (Ir193)D dot plot by the high expression of both Ir isotopes. Within this gate cells that express different levels of CD15-Er170 and CD45-Tb159 in the (Er170)D vs (Tb159)D plot are grouped into lymphocytes (Lymphs, low Er170, high Tb159), monocytes (Mono, high Er170, high Tb159) and granulocytes (Gran, high Er170, low Tb159). Each of these cell populations is subdivided according to the defining biomarker expression for the respective subtype. For example, the Lymphoytes from the (Er170)D vs (Tb159)D plot are further

analyzed for T cell markers ((Nd142)D vs (Nd146)D plot within the (Sm153)D vs (Nd144)D plot and the (Ho165)D vs (Gd156)D plot).

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