

Supporting Information

Beyond Global Charge: Role of Amine Bulkiness and Protein Fingerprint on Nanoparticle-Cell Interaction

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1. Experimental Section

Inorganic synthesis

All chemicals were purchased from Sigma-Aldrich (Switzerland) and used without additional purification unless otherwise specified.

The procedure from Bastus et al.^[1] was adapted. Synthesis of Au Seeds. A solution of 2.0 mM sodium citrate in Milli-Q water (150 mL) was heated with an oil bath in a 250 mL three-necked round-bottomed flask for 15 min under magnetic stirring. A condenser was utilized to prevent the solvent evaporation. After boiling had started, 1 mL of HAuCl₄ (25 mM) was added. The color of the mixture changed from yellow to bluish gray and then to soft pink in 10 min. The resulting particles (around 10 nm at a concentration of 3E+12 NPs/mL) are coated with negatively charged citrate ions and hence are well suspended in H₂O.

Seeded Growth of Au NPs of up to 30 nm in Diameter

Immediately after the Au seeds in the same vessel, the reaction was cooled down to 90 °C. Then, 1 mL of sodium citrate (60 mM) and 1 mL of HAuCl₄ solution (25 mM) were sequentially injected (time delay 2 min). After 30 min, aliquots were extracted for further characterization by UV-Vis spectroscopy.

Characterization of Au NPs

TEM images were taken using a Phillips CM20 Biotwin operating at 80 kV. The suspension was washed twice via centrifugation (1 ml centrifuged twice $8,000 \times g$ for 5 minutes) and re-dispersed in water. This suspension was drop cast onto a TEM grid and dried in air. A minimum of 200 particles were analyzed to obtain the size distribution, summarized in Table S1.

Table S1. Au NPs characteristics

Property [units]	Value
Core diameter (TEM), $n = 250$ [nm]	26 ± 3
Mean surface area per Au NP [nm ²]	2124 ± 28
Mean volume per Au NPs [nm ³]	9202 ± 14
Extinction coefficient at 405 nm [M ⁻¹ cm ⁻¹]	$1.76E +09$
Zeta potential [mV]	-43 ± 5

Functionalized Au NPs Colloidal Stability Characterization

UV-Visible spectroscopy was carried out on a Jasco V-670 spectrophotometer; Depolarized Dynamic Light Scattering (DDLS) on a 3D LS spectrometer (LS Instruments); Zeta-potential measurements on a 90Plus PALS (Brookhaven Instruments Cooperation). Zeta-potential measurements were made on a Brookhaven ZetaPALS (Table S1).

Organic Synthesis

All chemicals were purchased from Sigma-Aldrich (Switzerland) and used without additional purification unless otherwise specified. *N*-vinylacetamide was purchased from TCI. Vinyl acetate and *N*-methyl-vinylacetamide were freshly distilled to remove polymerization inhibitors. Carbon disulfide were purchased from Acros and ethanol was from VWR. Deuterated solvents were obtained from Cambridge Isotope Laboratory. Thin layer chromatography (TLC) plates were purchased from Merck.

All reactions were carried out under inert atmosphere using Argon flux and all reactants were added via syringe. Solvent was removed by rotary evaporation at 40 °C and compounds were dried under high vacuum on a Schlenk-line.

Chain Transfer Agent Synthesis

i. Synthesis of O-ethyl S-2-phthalimidomethoxylxanthate CTA (I)

The procedure of Huang and coworkers was adapted. A mixture of phthalamide (41.1 g, 0.3 mol, 1.0 eq.) and deionized water (100 mL) was stirred for 10 min at room temperature. Formaldehyde solution (40 %, 27 mL, 0.4 mol, 1.3 eq.) was added. The solution was refluxed for 1.5 hours. After cooling to 0-5 °C, the resulting precipitate was collected by filtration, washed with cold water (0-5 °C, 200 mL) and dried in-vacuo to give the corresponding hydroxymethylphthalimide. Yield: 47.7 g, 0.3 mol, 96.0 %.

A solution of thionyl chloride (16.1 mL, 26.4 g, 0.2 mol, 1.1 eq.) in dichloromethane (35 mL) was slowly added to a mixture of the resulting *N*-hydroxymethylphthalimide (35.1g, 0.2 mol, 1 eq.) in dichloromethane (380 mL) and *N,N*-dimethylformamide (241 mL) during 30 min at room temperature. The reaction was stirred overnight at room temperature under mechanical agitation. water (200 mL) was slowly added. The solution was neutralized to pH 6.7-7.0 using saturated aqueous NaHCO₃ solution. The organic layer was separated, dried over magnesium sulfate and concentrated in-vacuo. The residue was dissolved in chloroform, precipitated in ten-fold excess of hexane affording the *N*-chloromethylphthalamide. Yield: 9.832 g, 50.3 mmol, 26.4 %.

N-chloromethylphthalimide (9.832 g, 50.3 mmol, 1 eq.) was dissolved in acetone (150 mL). Potassium O-ethyl xanthate (8.5 g, 52.9 mmol, 1.1 eq.) was added portionwise in an ice water bath under stirring. The solution was let stirred overnight at room temperature. Solvent was removed under reduced pressure. The crude residue was redissolved in dichloromethane. The resulting solution was washed 3 times with water, dried over magnesium sulfate and

concentrated in-vacuo. Pale yellow solid was obtained and recrystallized from ethyl acetate affording the desired xanthate as colorless crystals. Yield: 12.0 g, 42.7 mmol, 85.0%

^1H NMR (CDCl_3 , 300 MHz) δ [ppm]: 7.89 (dd, $J=5.5$ Hz, 2H, ArH), 7.77 (dd, $J=5.5$ Hz, 2H, ArH), 5.36 (s, 2H, CH_2), 4.7 (q, $J=7.1$ Hz, 2H, CH_2), 1.48(t, $J=7.1$ Hz, 3H, CH_3).

(ESI) m/z : $[\text{M} + \text{Na}]^+$ calculated for $\text{C}_{12}\text{H}_{11}\text{NNaO}_3\text{S}_2$, 304.01; found, 304.0.

ii. Synthesis of Ethyl 2-(ethoxythiocarbonylthio) propanoate CTA (IV)

To a stirred solution of potassium hydroxide (13 g, 232.0 mmol, 1.0 eq.) in ethanol (100 g), carbon disulfide (18 g, 236.8 mmol, 1.0 eq.) was added dropwise. After stirring for 2 h, the solution was cooled to 5 °C, filtered and the crude product recrystallized twice from warm ethanol, giving a yellow powder . Yield: 24.2 g, 150.9 mmol, 65.0%.

Ethyl-2-bromo propionate (10.6 g, 58.6 mmol, 1.0 eq.) in dry acetonitrile (2 mL) was cooled to 0 °C and Potassium O-ethyl xanthate (10 g, 62.4 mmol, 1.1 eq.) was added portionwise. The suspension was stirred at room temperature for 2 h before the solvent was removed under reduced pressure. After redissolving in dichloromethane, the organic phase was washed with water and brine, dried over magnesium sulfate and concentrated. The crude product was purified via column chromatography with hexane: diethylether (19:1 to 9:1) as eluent giving a colorless liquid (IV). Yield: 8.1 g, 36.4 mmol, 62.1%.

^1H NMR (CDCl_3 , 300 MHz) δ [ppm]: 4.63 (q, $J=7.18$ Hz, 2 H), 4.37 (q, $J=7.37$ Hz, 1 H), 4.20 (q, $J=7.18$ Hz, 2 H), 1.56 (d, $J=7.55$ Hz, 3 H), 1.41 (t, $J=7.18$ Hz, 3 H), 1.28 (t, $J=7.08$ Hz, 3 H)

(ESI) m/z : $[\text{M} + \text{Na}]^+$ calculated for $\text{C}_8\text{H}_{14}\text{NaO}_3\text{S}_2$, 245.05; found, 245.0.

i. RAFT Polymerization of Vinyl Acetate (II)

The procedure described by Smith et al.^[2] was modified. A mixture of freshly distilled vinyl acetate (17.025 g, 197.8 mmol), AIBN (29.0 mg, 0.2 mmol) and *O*-ethyl S-2 phthalamidomethylxanthate (**I**, 479.0 mg, 1.7 mmol) was degassed and fully immersed in a pre-heated oil bath (70 °C).

The polymerization was thermally quenched using an ice bath after 16 hours. The polymer was subsequently dissolved in dichloromethane and precipitated in a 10-fold excess of ice-cold hexane.

¹H-NMR (400 MHz, DMSO-d₆, δ): 7.90-7.70 (m, Ar H phthalamide), 4.90 (s, CH backbone), 4.70-4.55 (m, 2H, S=COCH₂CH₃ RAFT agent moiety), 4.20-4.10 (m, 2H, NCH₂ RAFT agent moiety), 2.10-1.85 (m, CH₃ backbone), 1.85-1.60 (m, CH₂ backbone).

ii. Phthalamide deprotection and subsequent hydrolysis of poly(vinyl acetate) (III)

A mixture of phthalamide end functionalized poly(vinyl acetate) (**II**, 1.0 g, 0.1 mmol) in methanol (6 mL) was heated to 60 °C and treated with hydrazine hydrate aqueous solution (1.029 g/mL) 55% w/v (2.4 mL, 27.8 mmol) during 1.5 hours under stirring. A mixture of sodium hydroxide (980 mg, 24.5 mmol) in methanol (6 mL) was added dropwise. The reaction was allowed to stir at 65 °C overnight. After cooling down to room temperature, the crude polymer was filtrated under gravity and washed with ice cold methanol. Final mass: 234 mg, 4.5×10^{-2} mmol, 45.2% yield.

¹H-NMR (400 MHz, D₂O, δ): 4.10-3.95, 1.80-1.30 (m, CH₂ backbone).

iii. RAFT copolymerization of vinyl acetate and N-vinylacetamide (V)

A mixture of freshly distilled vinyl acetate (3.528 g, 41.0 mmol), *N*-vinylacetamide (387 mg, 4.6 mmol), AIBN (1.9 mg, 1.0×10^{-2} mmol), ethyl 2-(ethoxythiocarbonylthio) propanoate

(IV, 26.1 mg, 1.2×10^{-1} mmol) and dry dioxane (2 mL) was degassed and fully immersed in a pre-heated oil bath (70 °C). The polymerization was thermally quenched using an ice bath after 19 hours. The polymer was subsequently dissolved in dichloromethane and precipitated in a 10-fold excess of ice-cold hexane.

$^1\text{H-NMR}$ (400 MHz, $\text{CHCl}_3\text{-d}$, δ): 4.90 (s, CH backbone), 4.70-4.55 (m, 2H, $\text{S=COCH}_2\text{CH}_3$ RAFT agent moiety), 2.15-1.95 (m, CH_3 backbone), 1.95-1.60 (m, CH_2 backbone).

iv. Basic hydrolysis of poly(vinyl acetate-co-N-vinylacetamide) (VI)

A mixture of poly(vinyl acetate-co-*N*-vinylacetamide) (V, 1.0 g, 15.4×10^{-2} mmol) in methanol (10 mL) was added dropwise to a preheated solution (65 °C) of sodium hydroxide (4.0 g, 100 mmol) in methanol (10 mL). The reaction was allowed to stir overnight at 65 °C. After cooling down to room temperature, solvent was removed under reduced pressure. The crude polymer was re-dissolved in Milli-Q water (30 mL) and neutralized using HCl 37%. The crude polymer was purified by dialysis in Milli-Q water.

Final mass: 420 mg, 11.3×10^{-2} mmol, 73.4% yield.

$^1\text{H-NMR}$ (400 MHz, D_2O , δ): 4.05-3.85 (s, CH backbone), 2.05-1.92 (m, CH_3 acetamide backbone), 1.80-1.40 (m, CH_2 backbone).

v. Acidic hydrolysis of poly(vinyl alcohol-co-N-vinylacetamide) (VII)

The procedure describe by Dréan et al.^[3] was adapted. A mixture of poly(vinyl alcohol-co-*N*-vinylacetamide) (VI, 420 mg, 11.3×10^{-2} mmol) in Milli-Q water (6 mL) was heated to 75 °C. HCl 37% (6 mL) was added dropwise. The reaction was allowed to stir for 64 hours at 75 °C. The mixture was neutralized using NaOH 0.1 M. The crude polymer was purified by dialysis in Milli-Q water.

Final mass: 242.3 mg, 7.2×10^{-2} mmol, 63.7% yield. $^1\text{H-NMR}$ (400 MHz, D_2O , δ): 4.05-3.85 (s, CHOH backbone), 3.70-3.50 (s, CHNH_2 backbone), 2.0-1.80 (m, CH_2CHNH_2 backbone), 1.80-1.40 (m, CH_2CHOH backbone).

vi. RAFT copolymerization of vinyl acetate and *N*-methyl-*N*-vinylacetamide (VIII)

A mixture of freshly distilled vinyl acetate (3.528 g, 41.0 mmol), *N*-methyl-*N*-vinylacetamide (451 mg, 4.6 mmol), AIBN (1.9 mg, 1.0×10^{-2} mmol), ethyl 2-(ethoxythiocarbonylthio) propanoate (**IV**, 26.1 mg, 12.0×10^{-2} mmol) and dry dioxane (4 mL) was degassed and fully immersed in a pre-heated oil bath (70 °C). The polymerization was thermally quenched using an ice bath after 19 hours. The polymer was subsequently dissolved in dichloromethane and precipitated in a 10-fold excess of ice-cold hexane.

$^1\text{H-NMR}$ (400 MHz, $\text{CHCl}_3\text{-d}$, δ): 5.10-4.50 (s, CH backbone), 4.20-4.05 (m, 2H, $\text{O}=\text{COCH}_2\text{CH}_3$ RAFT agent moiety), 2.90-2.65 (m, NCH_3 backbone), 2.15-1.95 (m, CH_3 backbone), 1.95-1.60 (m, CH_2 backbone), 1.45-1.35 (m, 3H, $\text{O}=\text{COCH}_2\text{CH}_3$ RAFT agent moiety), 1.30-1.20 (m, 3H, $\text{S}=\text{COCH}_2\text{CH}_3$ RAFT agent moiety), 1.20-1.10 (m, 3H, $\text{O}=\text{CCHCH}_3$ RAFT agent moiety).

vii. Basic hydrolysis of poly(vinyl acetate-co-*N*-methyl-*N*-vinylacetamide)

A mixture of poly(vinyl acetate-co-*N*-methyl-*N*-vinylacetamide) (**VIII**, 188 mg, 3.8×10^{-2} mmol) in methanol (4 mL) was added dropwise to a preheated solution (65 °C) of sodium hydroxide (490 mg, 12.3 mmol) in methanol (8 mL). The reaction was allowed to stir overnight at 65 °C. After cooling down to room temperature, the solvent was removed under reduced pressure. The crude polymer was re-dissolved in Milli-Q water (12 mL) and neutralized using HCl 1M. The crude polymer was purified by dialysis in Milli-Q water.

viii. Acidic hydrolysis of poly(vinyl alcohol-co-*N*-methyl-*N*-vinylacetamide) (IX)

A mixture of poly(vinyl alcohol-co-*N*-methyl-*N*-vinylacetamide) in Milli-Q water (6 mL) was heated to 75 °C. HCl 37% (6 mL) was added dropwise, the reaction was allowed to stir at 75 °C for 64 hours. The crude polymer was neutralized using NaOH 1 M and dialyzed against Milli-Q water.

Final mass: 43.8 mg, 0.016 mmol, 42.1% yield. ¹H-NMR (400 MHz, D₂O, δ): 4.20-3.85 (s, CH backbone), 2.80-2.55 (m, NCH₃ backbone), 1.85-1.30 (m, CH₂ backbone).

ix. Polymer methylation (X)

Poly (vinyl alcohol-co-*N*-Methyl-*N*-vinyl amine) (IX, 21.9 mg, 87.6×10^{-4} mmol) and para-formaldehyde (122 mg, 30.3 mmol) were dissolved in Milli-Q water (2 mL) and stirred at 65 °C for 2.5 hours. Dimethylamine complex 97% (31 mg, 52.6×10^{-2} mmol) was added at 5 °C.

The reaction was allowed to stir overnight at 5 °C. The water was removed under reduced pressure (10^{-2} mbar). The crude polymer was purified by size exclusion chromatography using (SEPHADEX cartridge). The collected fractions were freeze dried.

Final mass: 10.4 mg, 4.2×10^{-4} mmol, 47.5% yield.

¹H-NMR (400 MHz, D₂O + TFA, δ): 4.05-3.80 (s, CH backbone), 2.90-2.70 (m, N(CH₃)₂ backbone), 2.70-2.60 (s, NCH₃ backbone), 2.00-1.30 (m, CH₂ backbone).

Instrumentation

Visualization after TLC was performed with a 254 nm or 365 nm UV lamp. Flash column chromatography was performed on an Isolera One from Biotage. ¹H NMR spectra were recorded on a Bruker Avance DPX 400 or DPX 300 MHz spectrometers at frequencies of 400.19 MHz or 300.23 MHz, respectively, for ¹H nuclei and 100.63 MHz or 75.49 MHz, respectively, for ¹³C nuclei. Chemical shifts were expressed in parts per million (ppm; δ) and referenced internally to the residual solvent signal. Coupling constants (J) are reported in Hz.

Multiplets are abbreviated as follows: s (singlet), d (doublet), dd (doublet of doublet), t (triplet), m (multiplet).

Flash column chromatography was performed on a Biotage Isolera One chromatography system.

Size exclusion chromatography (SEC) experiments were performed on an Agilent 1200 series HPLC system equipped with an Agilent PLgel mixed guard column (particle size = 5 μm) and two Agilent PLgel mixed-D columns (ID = 7.5 mm, L = 300 mm, particle size = 5 μm). Signals were recorded by a UV detector (Agilent 1200 series), an Optilab REX interferometric refractometer, and a miniDawn TREOS light scattering detector (Wyatt Technology Corp.). Samples were run using THF as the eluent at 30 °C and a flow rate of 1.0 mL/min. Data analyses were carried out on Astra software (Wyatt Technology Corp.) and molecular weights were determined based on narrow molecular weight polystyrene standards calibration (from 2,340 to 364,000 g/mol).

Table S2. Calculated molecular weights from GPC traces and ¹H-NMR

Polymer	Calculated M_n	M_n [Da] GPC	M_w [Da] GPC	\bar{D}	M_n [Da] ¹ H-NMR	M_n [Da] after hydrolysis GPC
Phtalmide Poly(vinyl acetate) (II)	10,000	10,000	15,000	1.5	10,000	5,200
Poly(vinylacetate-co-N-vinyl acetamide) (V)	34,000	6,600	13,700	2.1	6,500	3,300
Poly(vinyl acetate-co-N-Vinyl-N- methyl acetamide) (VIII)	34,000	5,000	6,700	1.3	4,000	2,100-2,200

Table S3. Poly (vinyl alcohol) determination by Iodine KI blue complexometry

Nanoparticle type	Total gold mass [mg]	Total polymer mass [mg]	Polymer mass in supernatant [mg]	Final packing density [units/nm ²]	Amine density [units/nm ²]
Primary Pendant	7.5	5.3	0.5	10.9	99
Secondary Pendant	7.5	3.3	1.8	5.4	38
Tertiary Pendant	7.5	3.3	2.4	3.1	19
Primary Chain End	7.5	6.8	4.4	3.6	4

Au NPs Functionalization and Characterization

Citrate Au NPs at a concentration of 376 µg Au/mL was obtained and used as a stock solution for subsequent polymer functionalization. All citrate Au NPs were functionalized by ligand exchange with the corresponding polymer at an initial packing density of 12 polymer units/nm². All reactions were allowed to stir overnight at room temperature. The resulting functionalized Au NPs were centrifuged at 10,000 g for one hour. Supernatant was removed and kept to analyze the uncoated polymer via iodine- PVA boric acid-complexometry (Figure S17). The NPs were re-dissolved in water Milli-Q and passed through a cotton filters to remove large aggregates formed during centrifugation.

The final packing density was assessed via iodine- PVA boric acid-complexometry (Table S3). The final Au concentration was estimated via UV-Vis spectroscopy following reports by Haiss et al.^[4]

Colloidal stability measurements were made using dynamic light scattering (DLS). First, NPs were buffered in a 0.1 M NaOH solution. Particles were then diluted into RPMI-1640 cell culture media supplemented with 10% fetal bovine serum to a final concentration of

40 $\mu\text{g Au/mL}$. Depolarized DLS measurements were made on a 3D LS spectrometer using a linearly polarized and collimated Cobolt 05-01 diode pumped solid state laser ($\lambda = 660 \text{ nm}$, $P_{\text{max}} = 500 \text{ mW}$) over 24 hours at 37°C .

The previous formulations prepared for colloidal stability were centrifuged at $10,000 \text{ g}$ during one hour and re-suspended in PBS. This procedure was performed three times. Zeta-potential measurements were performed on a 90Plus PALS (Brookhaven Instruments Cooperation), equipped with a platinum electrode. The ζ -potential values were calculated from electrophoretic mobility using the Smoluchowski method for data processing (10 cycles). The viscosity, refractive index and dielectric constant of pure water were used to characterize the solvent.

Cellular Uptake by Inductively Coupled Plasma-Optical Emission Spectroscopy

A549 human lung epithelial cells and J774A.1 mouse monocyte/macrophages were acquired from ATCC/LGC Standards. For cell uptake studies, cells were used between passage 5 to 25. Into a 6-well plate, A549 human lung epithelial cells or J774A.1 murine monocyte/macrophages were seeded at $570,000 \text{ cells/well}$ ($60,000 \text{ cells/cm}^2$). Cells were subcultured in RPMI-1640 supplemented with 10% fetal bovine serum (FBS) and 1% penicillin-streptomycin. For each uptake time point, cells were seeded in triplicate (i.e. three wells per time point per particle type/treatment). There were two main reasons for using serum supplemented cell-culture media, as opposed to human plasma or even whole blood, in this study. Firstly, the A549 and J774A.1 cell lines were optimised for culture in FBS-supplemented media, which also means the vast majority of comparative studies to date have used similar expose conditions. Secondly, recent findings indicate that even more complex environments, e.g. plasma or whole blood with anti-clotting agents, do not necessarily correlate any closer with the protein corona formed in vivo. Therefore, further efforts should be made to understand correlations between protein coronas formed in different exposure

scenarios to that formed in vivo – something currently very challenging, technically, to accomplish accurately.

Cells attached overnight, and the next day the different NP formulations were added. Each NP formulation was buffered 1:1 in a 0.1 M NaOH solution, before diluting to a final concentration of 40 µg Au/mL in supplemented RPMI-1640 cell culture media. Cells were incubated with NPs overnight in an incubator at 37 °C and 5% CO₂. After incubation, cells were washed twice with sterile phosphate buffered saline (PBS), excess PBS was removed, and cells were sealed and stored at -80 °C until further preparation for ICP-OES analysis.

Samples were brought to room temperature, and then 200 µL of nitric acid 60% and 100 µL of hydrogen peroxide 35% were added sequentially to the cells. The cells were predigested for 3 hours and sonicated at 50 °C for 15 minutes. Following, 400 µL of hydrochloric acid 37% solution were added to cells and digested overnight. The residual digestion mixture was transferred into a 15 mL Falcon tube and diluted to 5 mL with Milli-Q water. The cellular-uptake was measured using ICP-OES (Optima 7000DV, Perkin Elmer). Measurements were performed at a wavelength of 242.8 nm, at an axial plasma view. The plasma flow was 15 L/min and the sample flow rate 1.5 mL/min. A washing step was performed by the instrument between each measurement and each sample was measured three times on each repetition. The cellular uptake was calculated by dividing the measured Au mass by the mass of 1 NP divided by the seeded cells number/well.

Toxicity Experiments

For cytotoxicity studies, A549 human lung epithelial or J774A.1 murine monocyte/macrophages were seeded in a 96-well plate at 10,000 cells/well (31,250 cells/cm²) or 20,000 cells/well (62,500 cells/cm²), respectively. Each NP formulation was buffered in 0.1 M NaOH as described above, and added to cells at a final concentration of 40 µg Au/mL in

supplemented RPMI-1640 cell culture media. Supplemented media only was used as a negative control, and 0.2% Triton X-100 was used as a positive control.

After overnight incubation of NPs at 37 °C and 5% CO₂, an aliquot of cell culture media (100 µL) was removed and tested for lactate dehydrogenase activity using a standard cytotoxicity kit (Sigma-Aldrich). Remaining CCM was removed from wells, and cells were washed 2 × with sterile PBS. Next, 100 µL of 88 µM resazurin HCl dissolved in CCM was added to each well. Cells were incubated protected from light for 3 hr at 37 °C and 5% CO₂. Fluorescent intensity was measured at an excitation wavelength of 560 nm and emission wavelength of 590 nm, and cell viability was determined by comparing fluorescence values of cells exposed to NPs to cells treated with only media.

1D SDS-PAGE and Silver Staining Analysis

Sample solutions prepared for the zeta potential measurement were used for protein analysis. Each formulation was mixed 4:1 with a 5 × concentrated Laemmli reducing sample buffer. Samples were denatured/desorbed for 48 hr at 75 °C. Next, 40 µL of each sample was loaded into a 4-15% gradient Mini-Protean® TGXTM precast gel (Bio-Rad, Hercules, California, USA) besides a Precision Plus Protein™ Dual Color (Bio-Rad) molecular weight standard. Samples were run using a Bio-Rad electrophoresis system, first at 80 V until they entered the gel and then at 120 V. Proteins were stained using a previously described silver staining protocol.^[5]

Mass Spectrometry Sample Preparation and Analysis

For MS analysis, NPs incubated with CCM were concentrated by ultrafiltration using vivaspin columns (30 kDa MWCO). Samples were heated in SDS-PAGE loading buffer, reduced with 1 mM DTT for 10 min at 75 °C and alkylated using 5.5 mM iodoacetamide for 10 min at room temperature. Protein mixtures were separated on 4-12% gradient gels (Nupage, Thermo

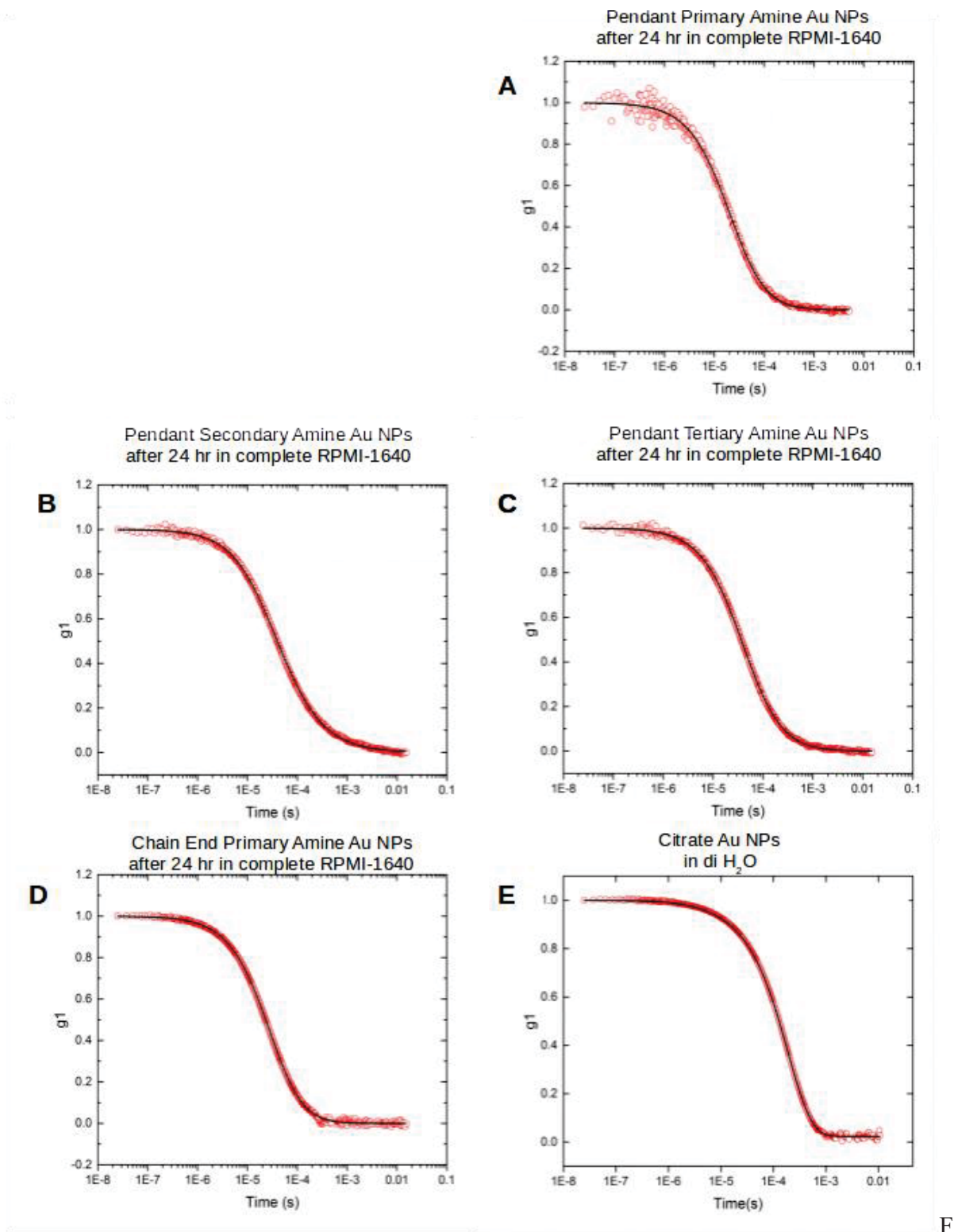
Fisher). The gel lanes were cut into two equal slices, the proteins were in-gel digested with trypsin (Promega) and the resulting peptide mixtures were processed on STAGE tips and analyzed by LC-MS/MS.^[6,7]

Mass spectrometric (MS) measurements were performed on a QExactive Plus mass spectrometer coupled to an EasyLC 1000 nanoflow-HPLC. HPLC-column tips (fused silica) with 75 μm inner diameter were self-packed with Reprosil-Pur 120 C18-AQ, 1.9 μm (Dr. Maisch) to a length of 20 cm. Samples were applied directly onto the column without a pre-column. A gradient of A (0.1% formic acid in water) and B (0.1% formic acid in 80% acetonitrile in water) with increasing organic proportion was used for peptide separation (loading of sample with 0% B; separation ramp: from 5-30% B within 85 min). The flow rate was 250 nL/min and for sample application 600 nL/min. The mass spectrometer was operated in the data-dependent mode and switched automatically between MS (max. of 1×10^6 ions) and MS/MS. Each MS scan was followed by a maximum of ten MS/MS scans using normalized collision energy of 25% and a target value of 1000. Parent ions with a charge state from $z = 1$ and unassigned charge states were excluded for fragmentation. The mass range for MS was $m/z = 370-1750$. The resolution for MS was set to 70,000 and for MS/MS for 17,500. MS parameters were as follows: spray voltage 2.3 kV; no sheath and auxiliary gas flow; ion-transfer tube temperature 250 °C.

The MS raw data files were uploaded into the MaxQuant software version 1.4.1.2 for peak detection, generation of peak lists of mass error corrected peptides, and for database searches.^[8] A full-length UniProt bovine database additionally containing common contaminants such as keratins and enzymes used for in-gel digestion (based on UniProt bovine FASTA version December 2016) was used as reference. Carbamidomethylcysteine was set as fixed modification and protein amino-terminal acetylation {deamidation (NQ), pyro-Glu, pyro-Gln} and oxidation of methionine were set as variable modifications. iBAQ was chosen as quantitation mode. Three missed cleavages were allowed, enzyme specificity

was trypsin/P, and the MS/MS tolerance was set to 20 ppm. The average mass precision of identified peptides was in general less than 1 ppm after recalibration. Peptide lists were further used by MaxQuant to identify and relatively quantify proteins using the following parameters: peptide and protein false discovery rates, based on a forward-reverse database, were set to 0.01, minimum peptide length was set to 7, minimum number of peptides for identification and quantitation of proteins was set to one which must be unique, minimum ratio count was set to two, and identified proteins were re-quantified. The “match-between-run” option (2 min) was used.

2. Supporting Information Results



F

Figure S1: (A-D) DDLs field auto-correlation functions of NPs in supplemented RPMI after 24 hours exposure. (E) DLS field auto-correlation function of citrate Au NPs in Milli-Q water. Solid lines correspond to the best fit curve and red circles represent experimental data.

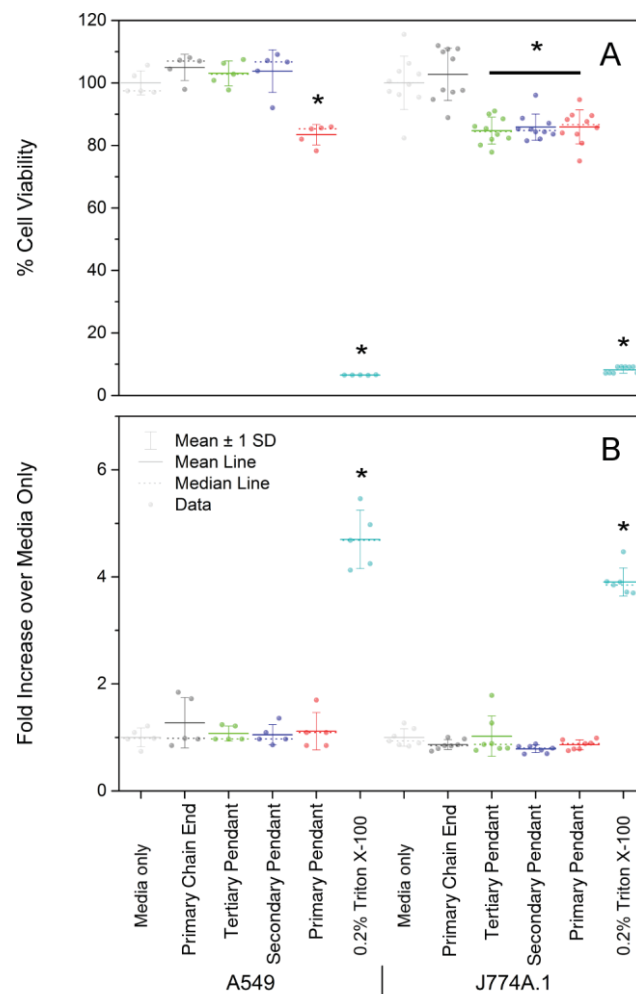


Figure S2. (A) Resazurin and (B) LDH cell viability assays in A549 human lung epithelial cells and J774A.1 mouse monocyte/macrophages. Cells treated with only cell culture media acted as the negative control, and cells treated with 0.2% Triton X-100 were the positive control. Statistical significance was determined using ANOVA and Tukey's HSD test ($p < 0.05$).

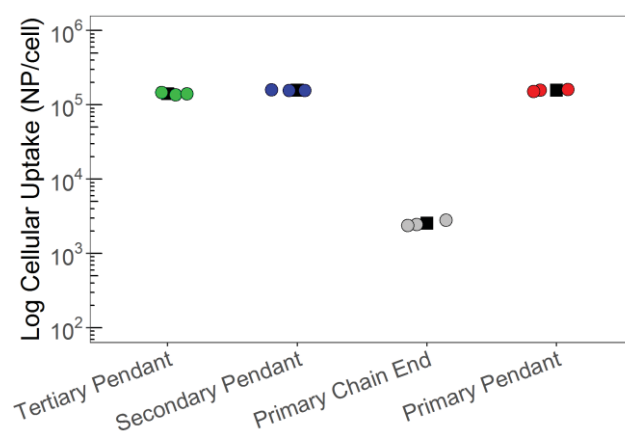


Figure S3. Cellular uptake of particles by J774A.1 mouse monocyte/macrophages.

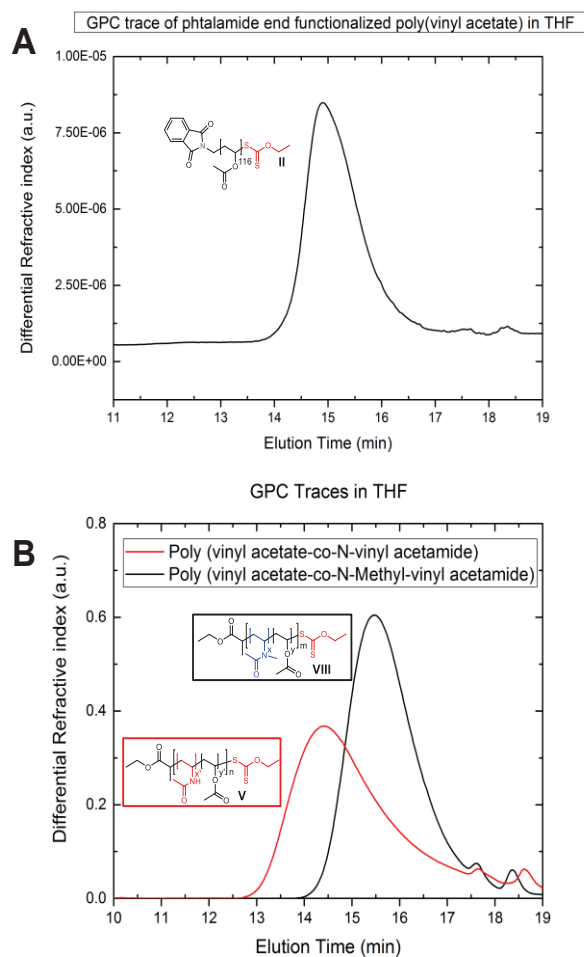


Figure S4. GPC traces of (A) phtalamide end functionalized poly(vinyl acetate) (II). (B) poly(vinyl acetate-*co*-*N*-vinyl acetamide) (V) and poly(vinyl acetate-*co*-*N*-Methyl-*N*-vinyl acetamide) (VIII) in THF.

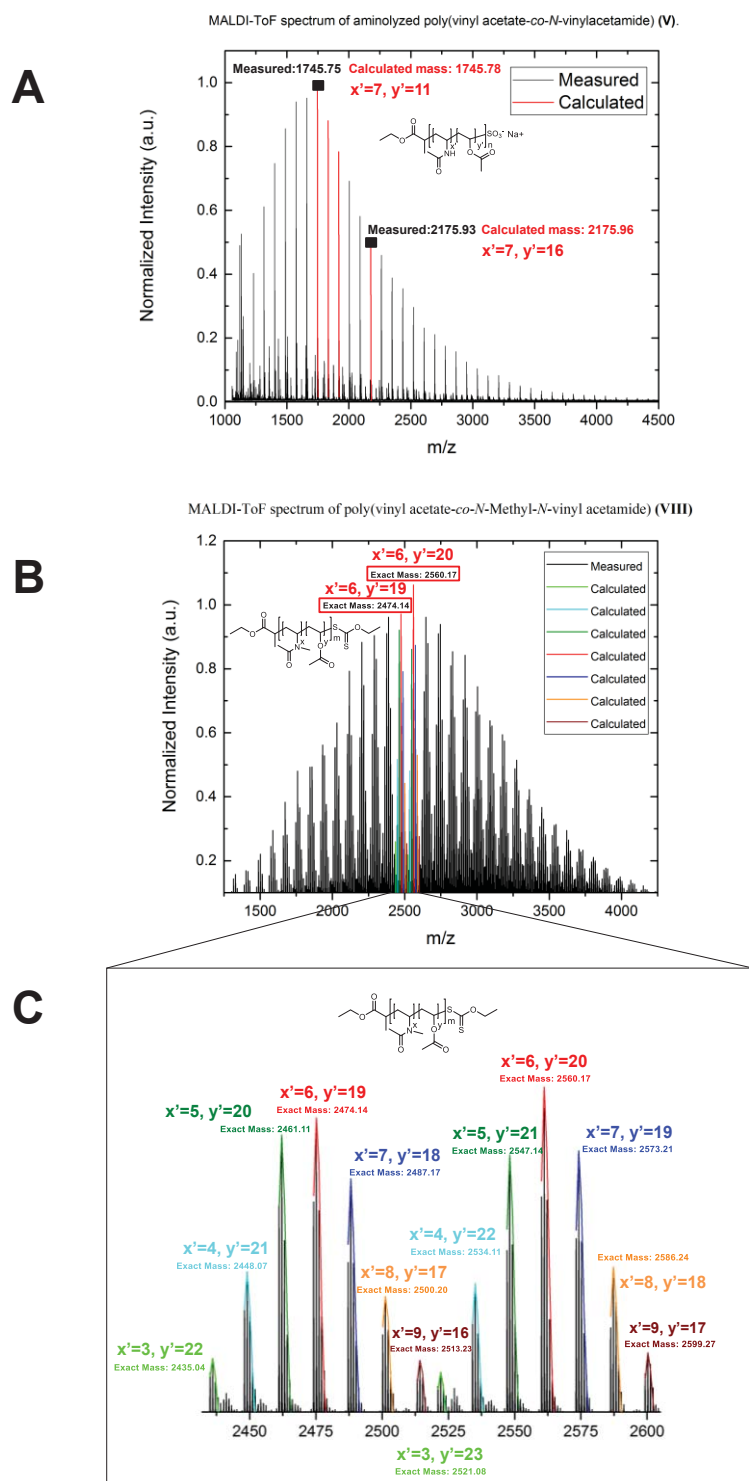


Figure S5. (A) MALDI-ToF mass spectrum of aminolyzed poly(vinyl acetate-*co*-*N*-vinyl acetamide) (V), red lines depict the calculated position of the ^{12}C peaks with increasing numbers of repeat units. (B) MALDI-ToF mass spectrum of poly(vinyl acetate-*co*-*N*-Methyl-*N*-vinyl acetamide) (VIII). (C) Zoom on the maximum peaks in mono-isotopic resolution, colored lines depict calculated values and isotope patterns.

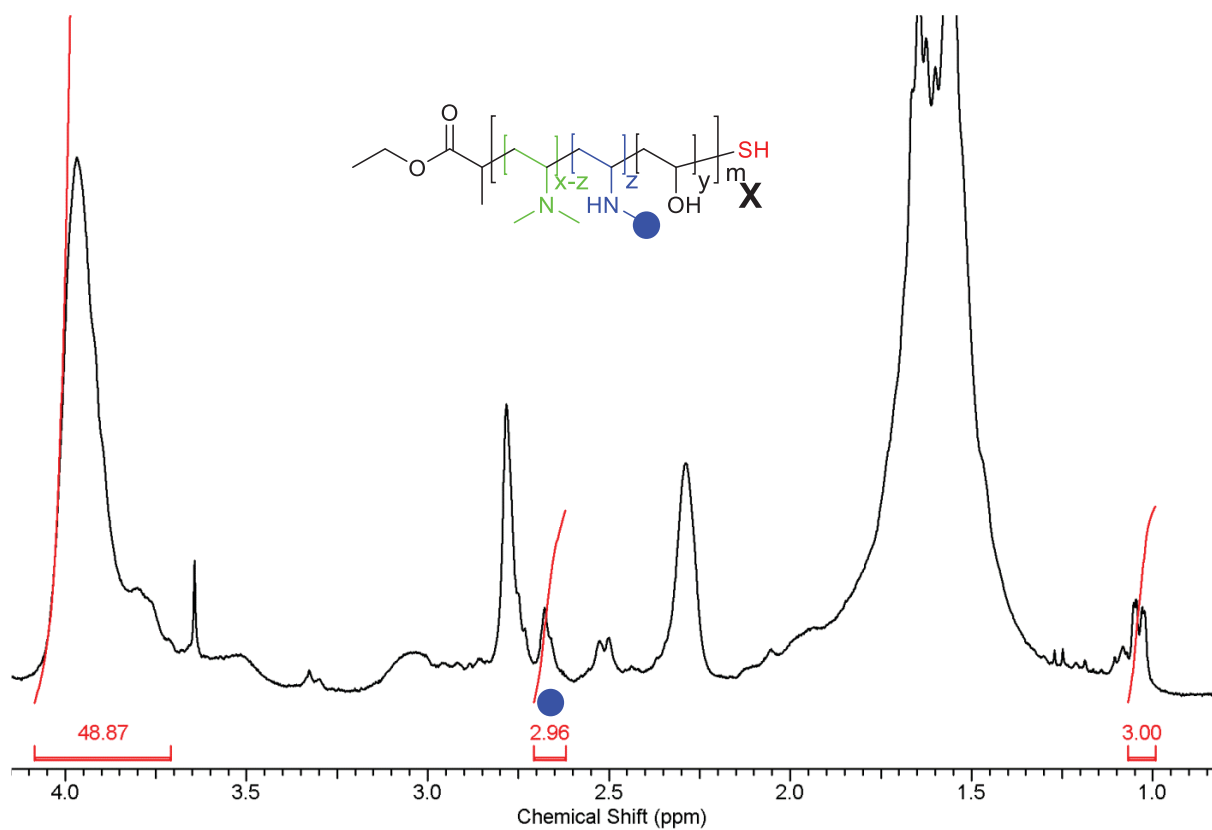


Figure S6. ^1H -NMR spectrum of thiolated poly(vinyl alcohol-*co*-*N*-di-Methyl-*N*-vinyl amine) (X) recorded in D_2O .

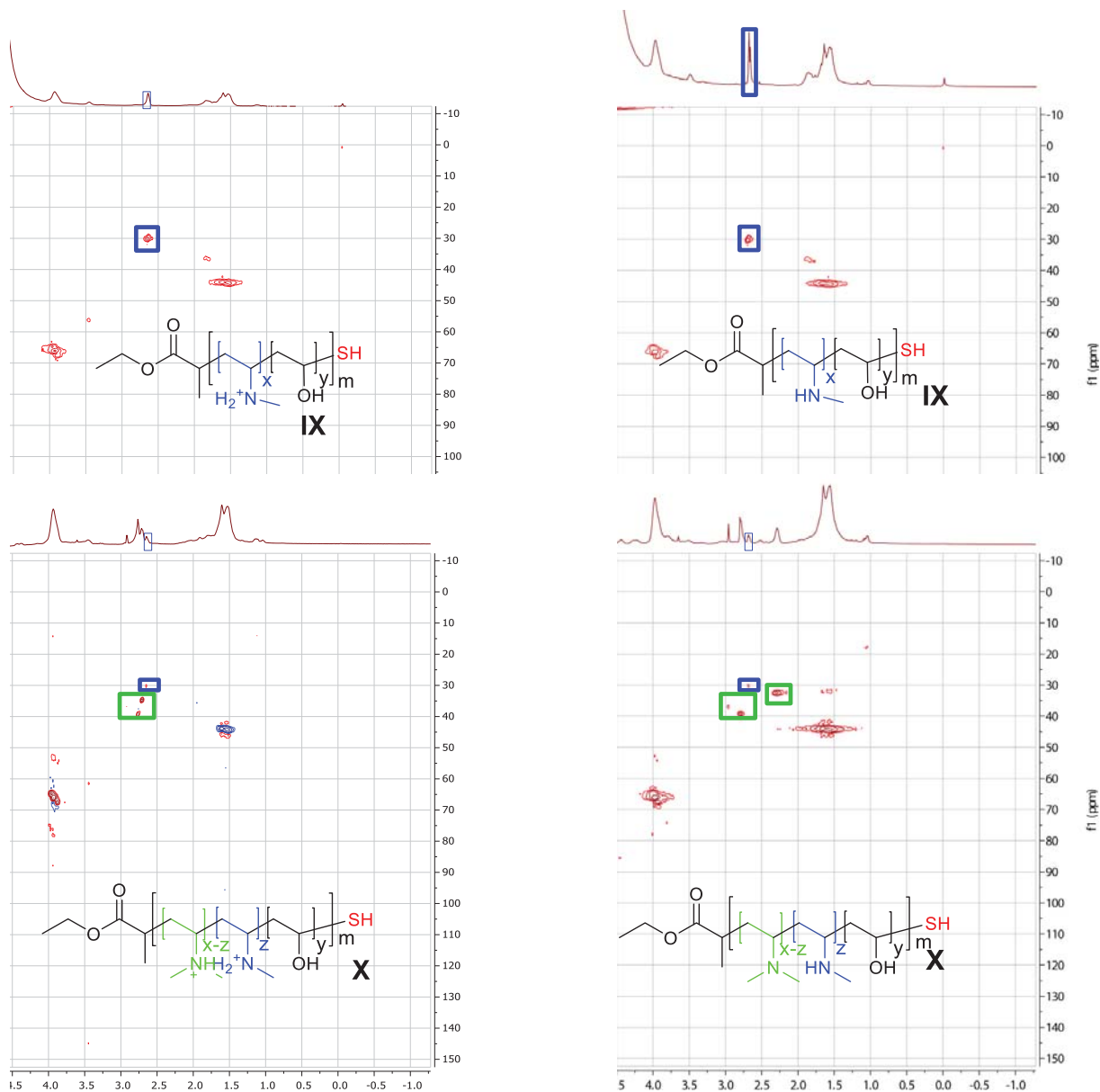


Figure S7. HSQC 2D ^{13}C - ^1H -NMR spectra of thiolated poly(vinyl alcohol-co-N-Methyl-N-vinyl amine) and thiolated poly(vinyl alcohol-co-N-di-Methyl-N-vinyl amine) with or without TFA recorded in D_2O (IX/X).

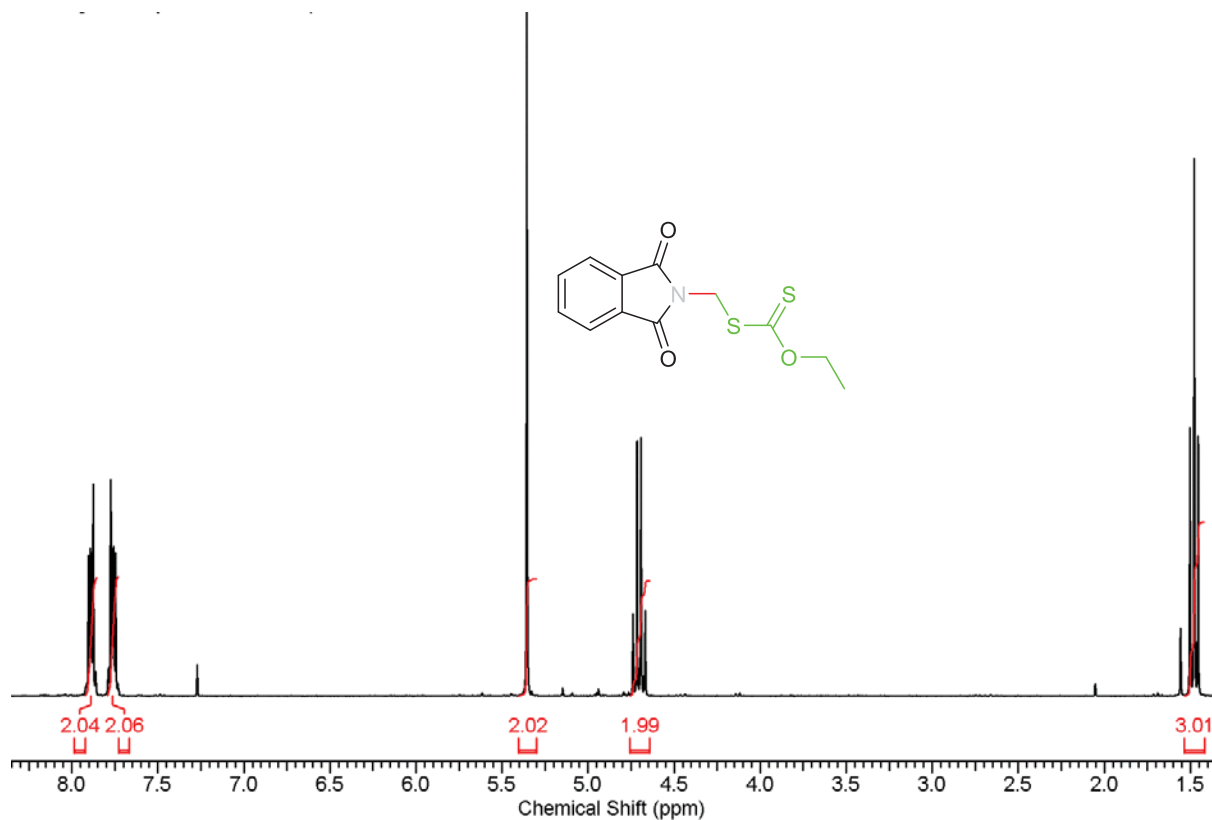


Figure S8. ¹H-NMR spectrum of O-ethyl S-2-phthalimidomethylxanthate CTA (**I**) recorded in CDCl₃.

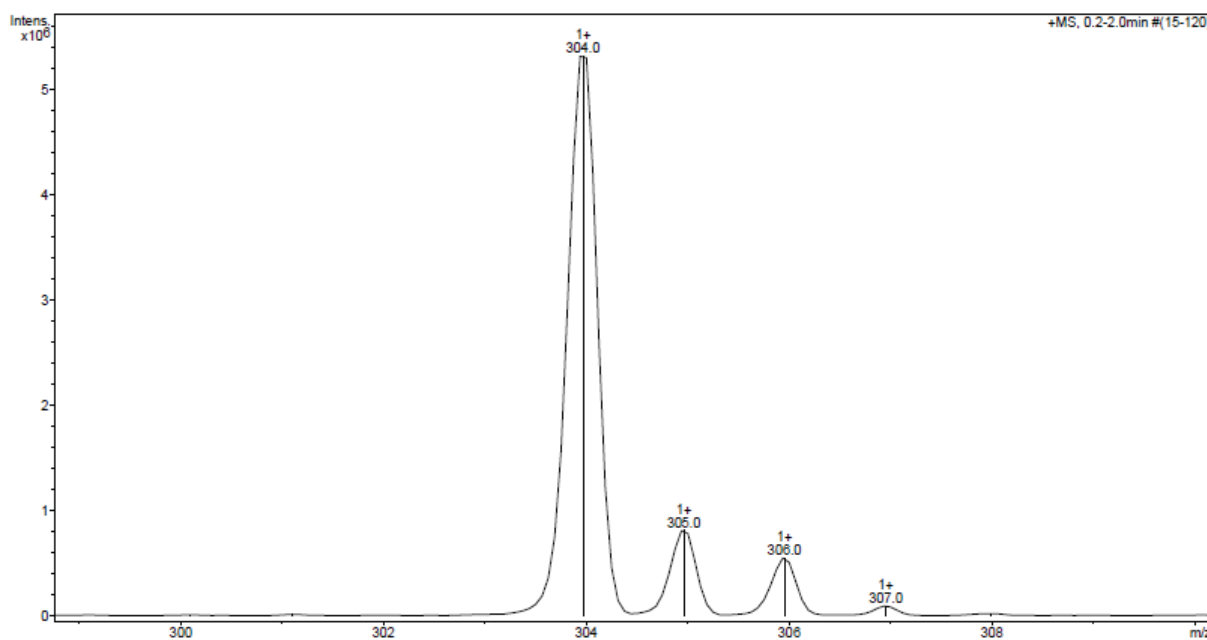


Figure S9. ESI spectrum of O-ethyl S-2-phthalimidomethylxanthate CTA (**I**).

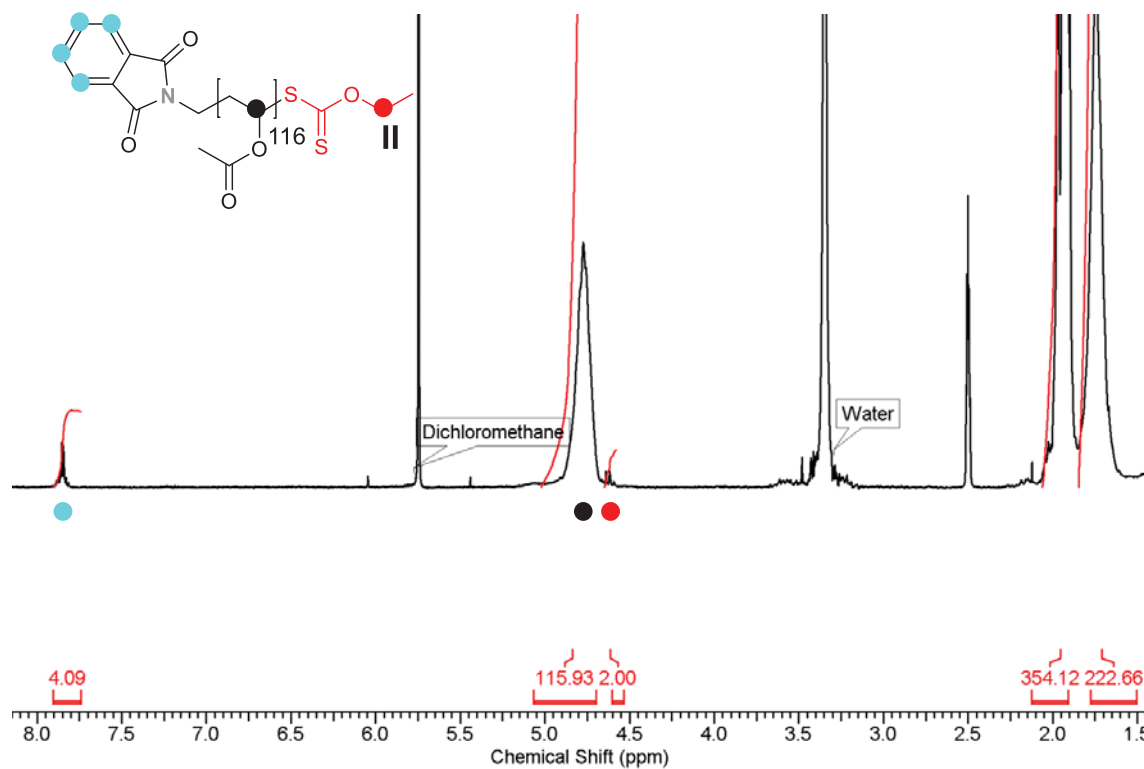


Figure S10. ^1H -NMR spectrum of poly(vinyl acetate) using O-ethyl S-2-phthalimidomethylxanthate CTA (II) recorded in DMSO-d_6 .

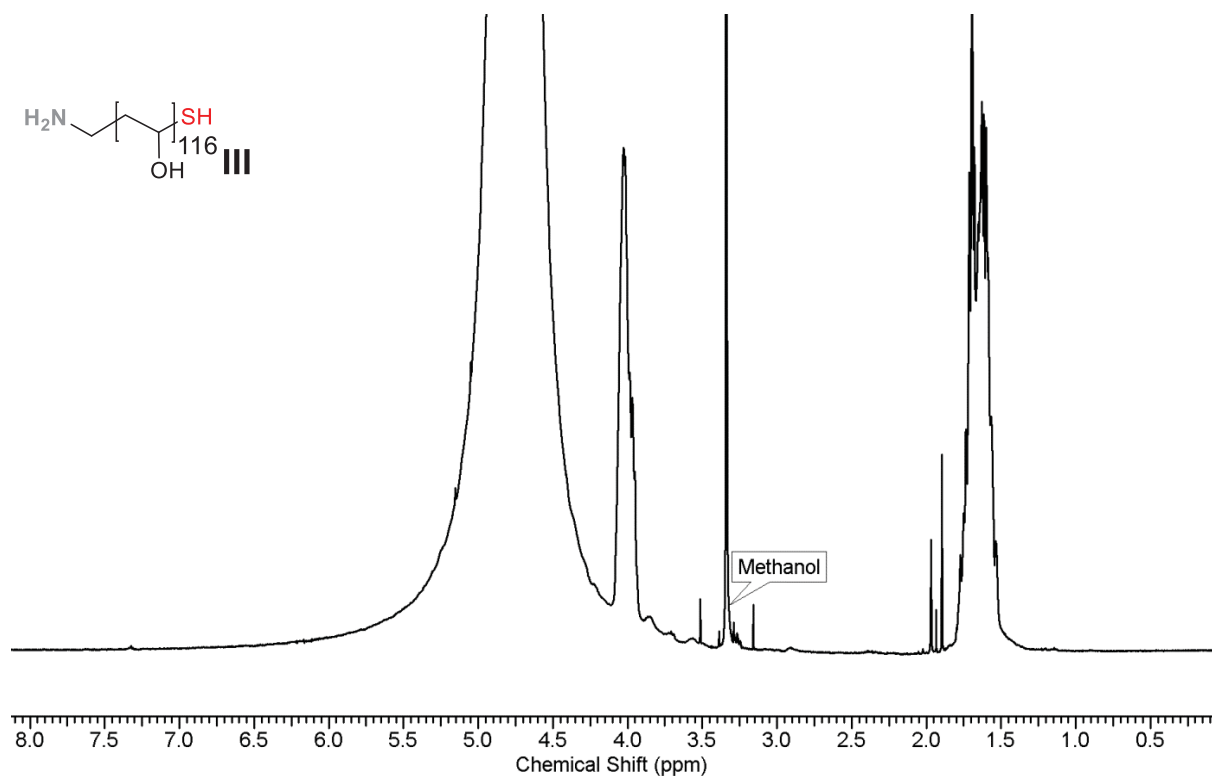


Figure S11. ^1H -NMR spectrum of poly(vinyl alcohol) amino end functionalized (III) recorded in D_2O .

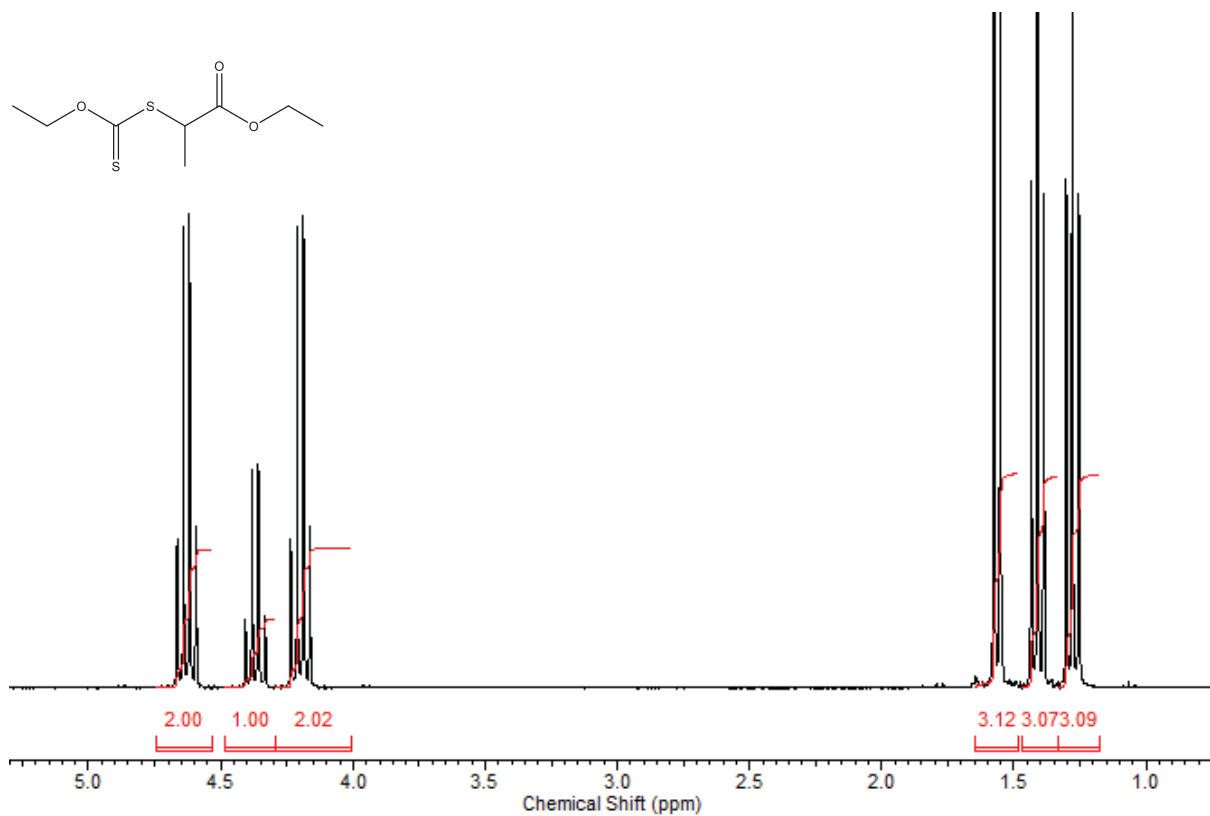


Figure S12. ¹H-NMR spectrum of ethyl 2-(ethoxythiocarbonylthio) propanoate (IV) recorded in CDCl₃.

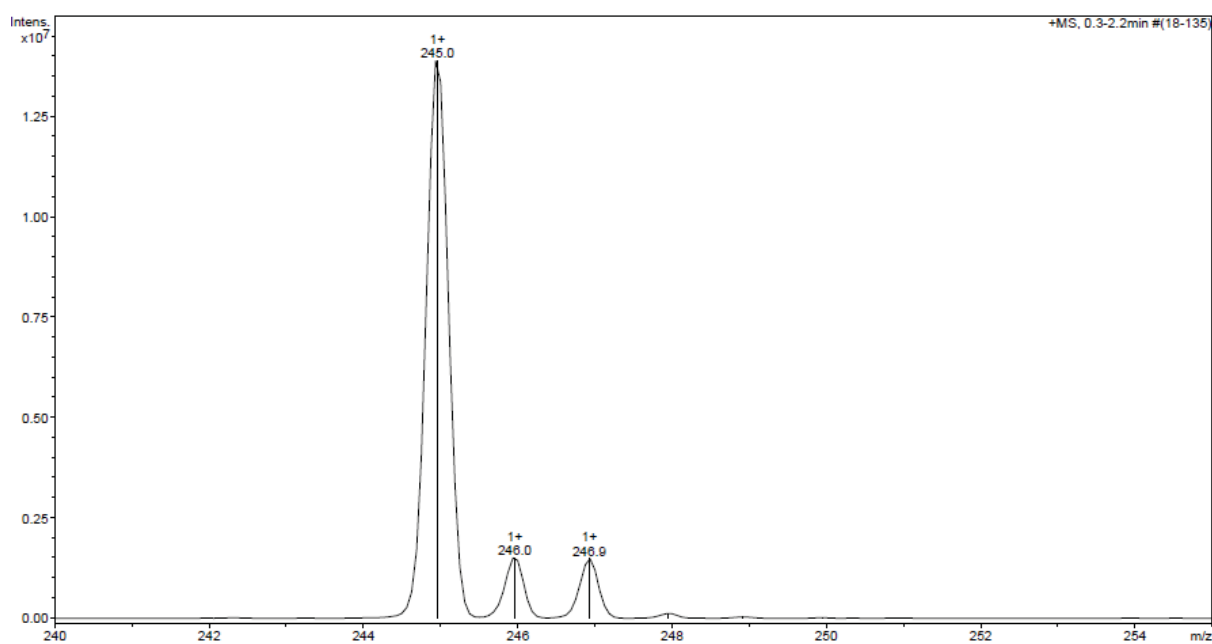


Figure S13. ESI spectrum of Ethyl 2-(ethoxythiocarbonylthio) propanoate CTA (IV).

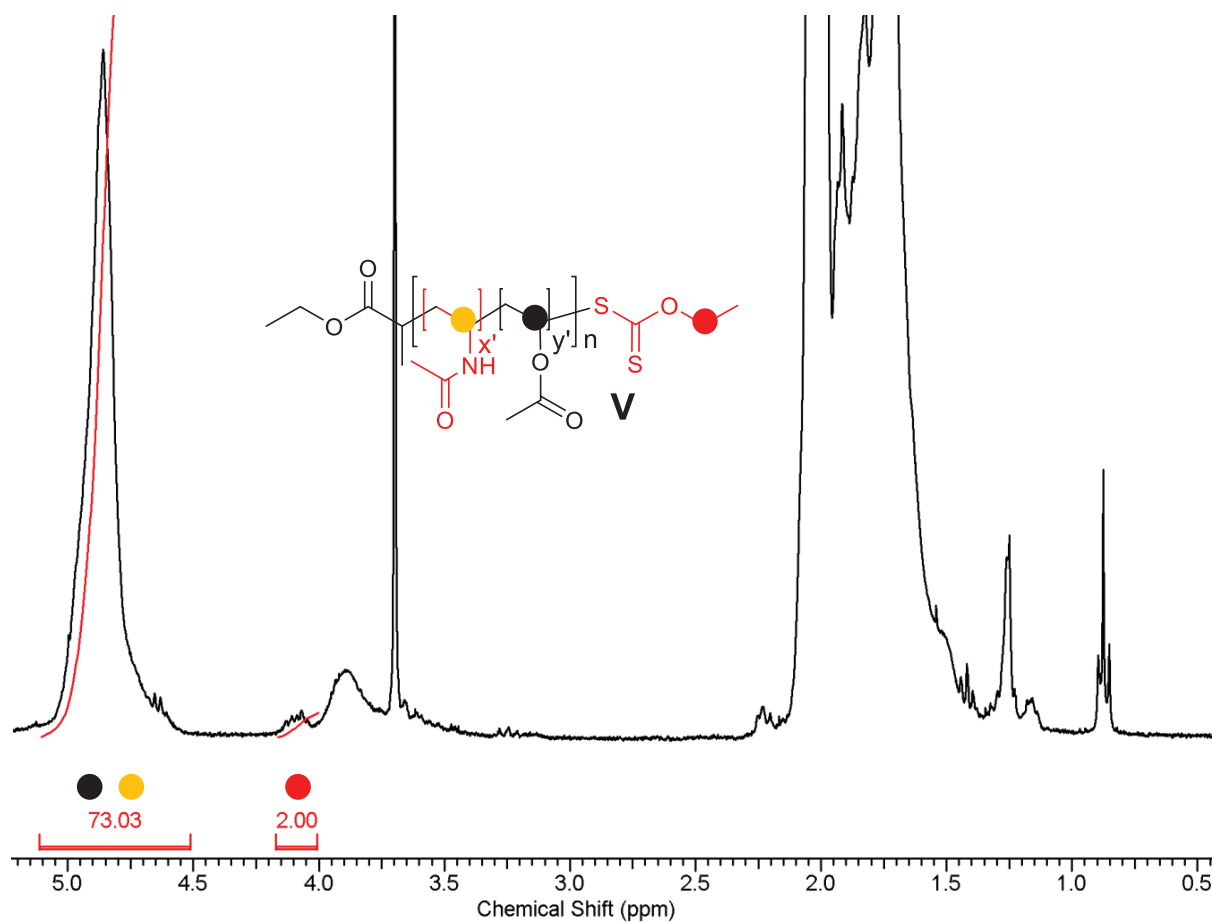


Figure S14. ^1H -NMR spectrum of poly(vinyl acetate-*co*-*N*-vinylacetamide) (V) recorded in CDCl_3 .

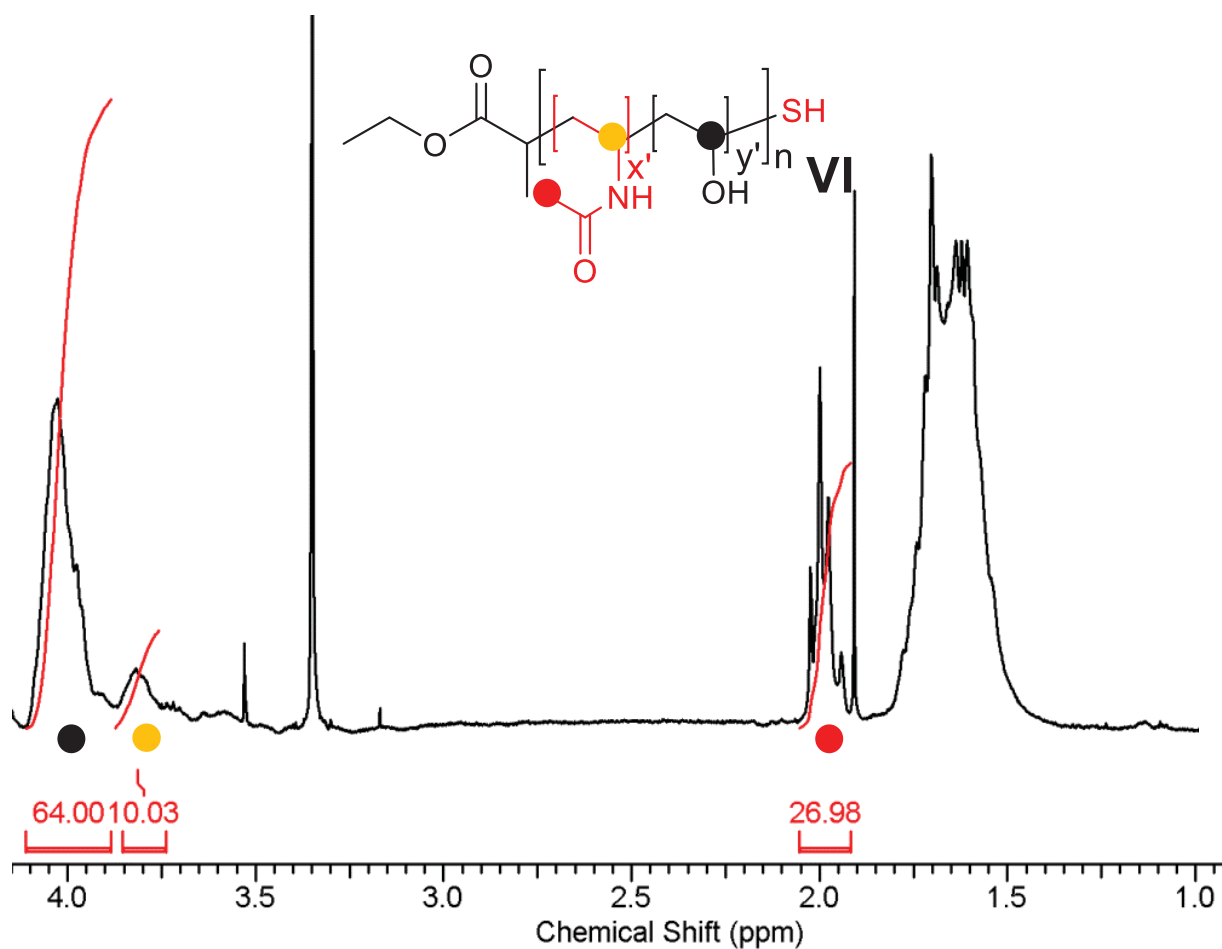


Figure S15. ¹H-NMR spectrum of thiolated poly(vinyl alcohol-*co*-*N*-vinylacetamide) (VI) recorded in D₂O.

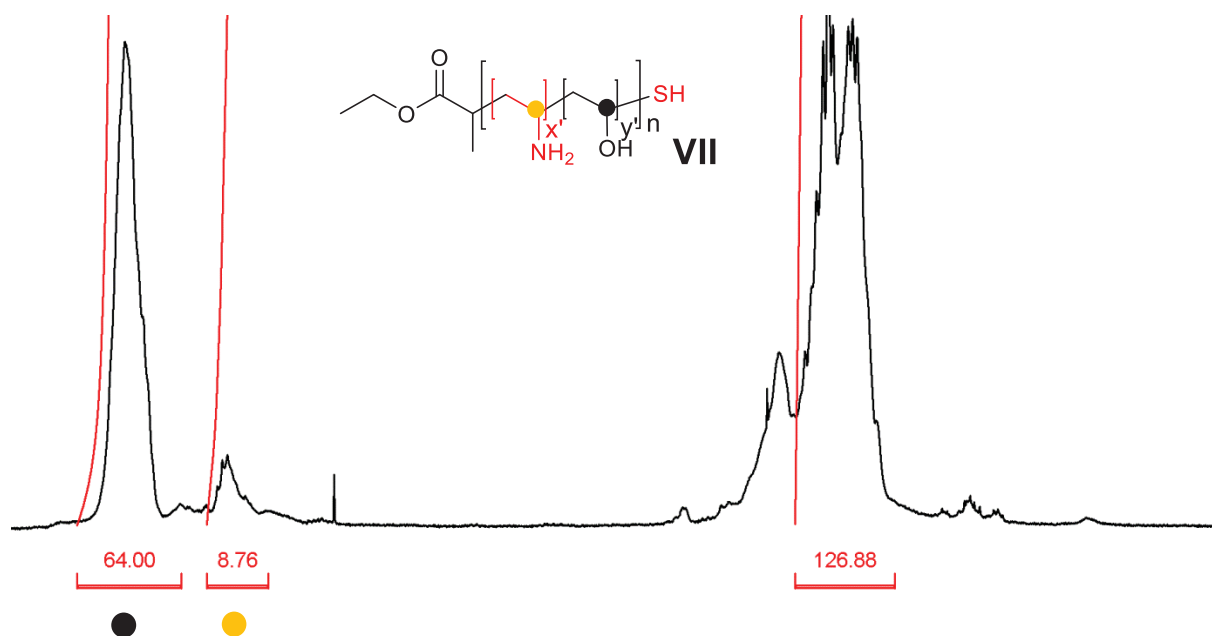


Figure S16. ¹H-NMR spectrum of thiolated poly(vinyl alcohol-*co*-*N*-vinylamine) (VII) recorded in D₂O.

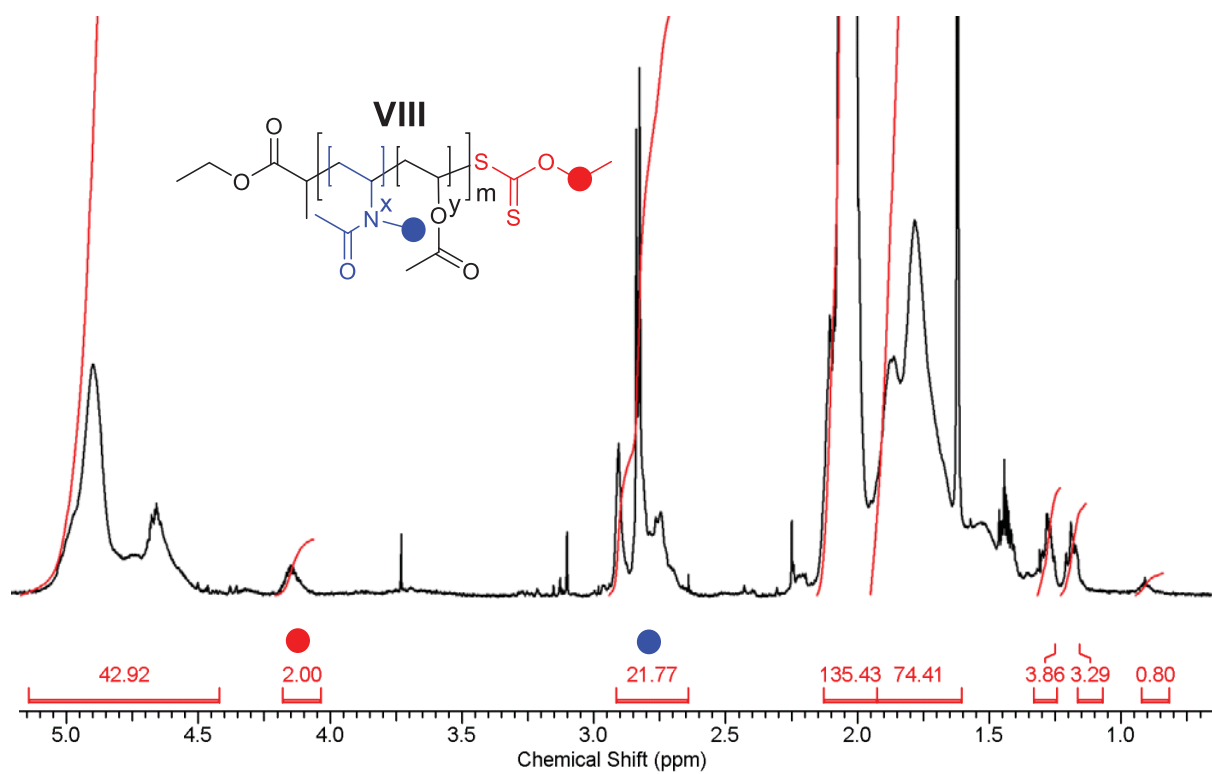
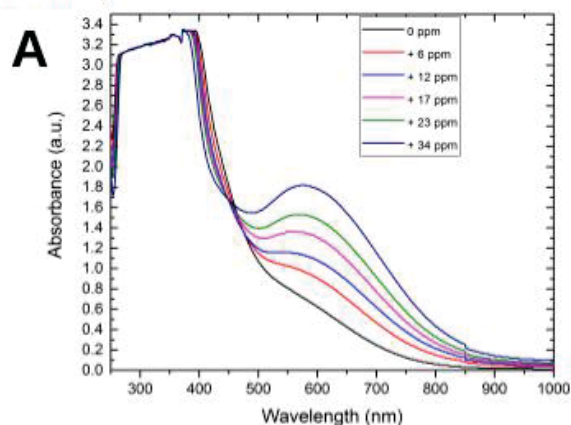
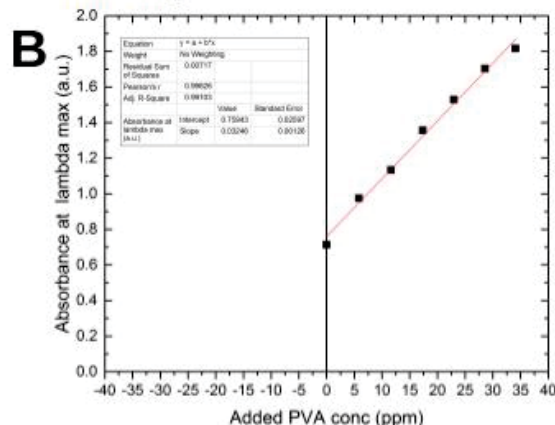


Figure S17. ^1H -NMR spectrum of Poly(vinyl acetate-*co*-N-Vinyl-N-methyl acetamide) (VIII) recorded in CDCl_3 .

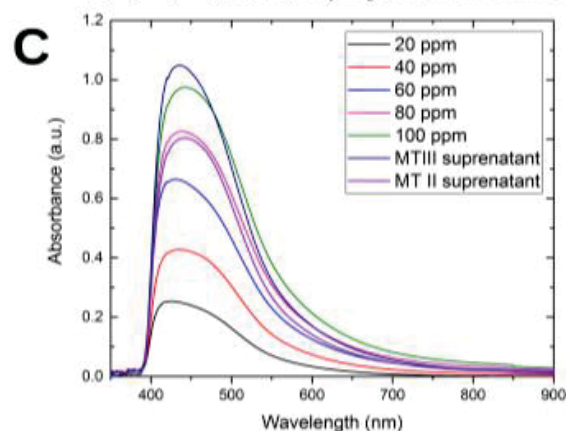
Pendant -NH₂ : PVA B(OH)₃ KI/I₂ Complex UV-Vis spectra standard addition



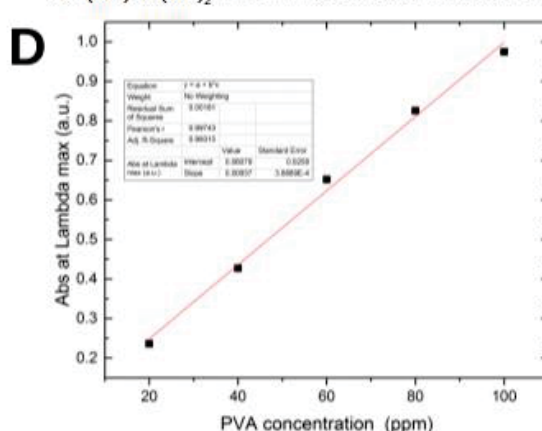
Pendant -NH₂ : Free PVA standard addition calibration curve



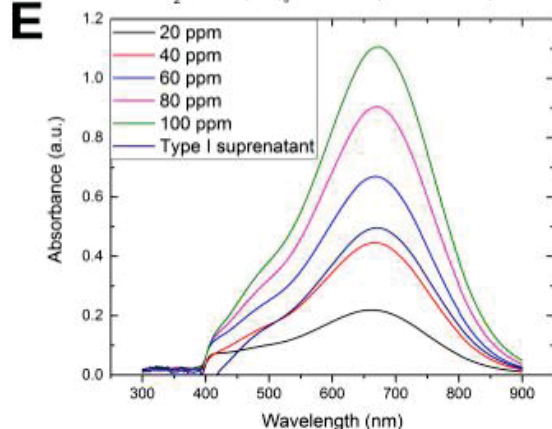
Pendant -NH(Me)N(Me)₂ : PVA B(OH)₃ KI/I₂ complex UV-Vis spectra



Pendant -NH(Me)N(Me)₂ : Free PVA quantification calibration curve



Chain End -NH₂ : PVA B(OH)₃ KI/I₂ Complex UV-Vis spectra



Chain End -NH₂ : Free PVA quantification calibration curve

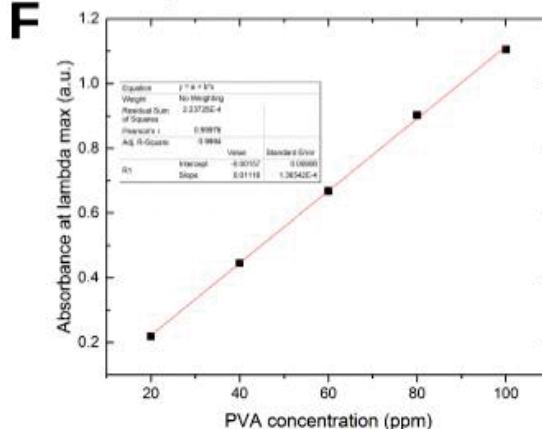


Figure S18. (A) UV-Vis extinction spectra of primary pendant amino polymer supernatant complexed with a mixture of boric acid and KI/I₂ using standard addition method and (B) the associated calibration curve. (C) UV-Vis extinction spectra of secondary and tertiary amino polymers using a standard method and (D) the associated calibration curve. (E) UV-Vis extinction spectra of primary chain end polymers and (F) the associated calibration curve.

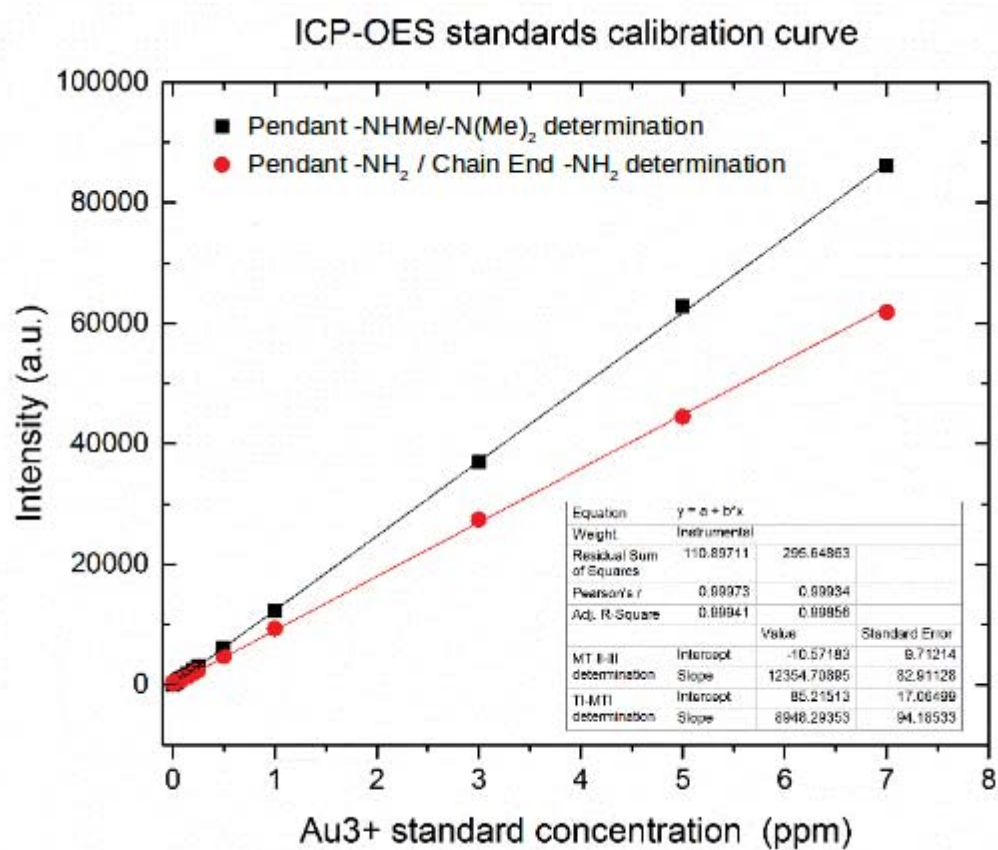


Figure S19. ICP-OES calibration curve for the cellular uptake determination.

Table S4. Cellular uptake quantification. Au³⁺ calibration standards determined by ICP-OES

Au ³⁺ standards [ppm]	Net intensity [a.u.]
0.000	47 ± 30
0.025	279 ± 16
0.050	584 ± 5
0.075	932 ± 3
0.100	1220 ± 8
0.125	1818 ± 9
0.150	1818 ± 9
0.200	2384 ± 23
0.250	3099 ± 27
0.500	6157 ± 144
1.000	12196 ± 109
3.000	36989 ± 109
5.000	62843 ± 254
7.000	86054 ± 333

Table S5. Quantification of cellular uptake for Secondary and Tertiary NPs. Au³⁺ determined by ICP- OES

Sample	Cells	Au ³⁺ [ppm]	Number of NPs/cell	Average
Pendant Secondary x1	A549	0.7	10,925	10,296 ± 622
Pendant Secondary x2	A549	0.6	10,281	
Pendant Secondary x3	A549	0.6	9,681	
Pendant Tertiary x1	A549	0.7	11,822	11,327 ± 433
Pendant Tertiary x2	A549	0.7	11,022	
Pendant Tertiary x3	A549	0.7	11,136	
Pendant Secondary x1	J774A.1	9.9	156,203	157,542 ± 1,632
Pendant Secondary x2	J774A.1	10.1	159,360	
Pendant Secondary x3	J774A.1	9.9	157,062	
Pendant Tertiary x1	J774A.1	8.7	136,907	141,244 ± 4,781
Pendant Tertiary x2	J774A.1	8.9	140,456	
Pendant Tertiary x3	J774A.1	9.3	146,370	

Table S6. Cellular uptake quantification. Au³⁺ calibration standards determined by ICP-OES

Au ³⁺ standards [ppm]	Net intensity [a.u.]
0.000	295 ± 27
0.025	351 ± 16
0.050	581 ± 9
0.075	796 ± 9
0.100	987 ± 5
0.125	1207 ± 32
0.150	1404 ± 3
0.200	1863 ± 5
0.250	2347 ± 5
0.500	4685 ± 37
1.000	9299 ± 63
3.000	27396 ± 110
5.000	44462 ± 223
7.000	61847 ± 423

Table S7. Cellular uptake quantification for Primary Pendant and Chain End NPs. Au³⁺ determined by ICP- OES

Sample	Cells	Au ³⁺ [ppm]	Number of NPs/cell	Average
Primary Chain End x1	A549	0.029	459	448 ± 174
Primary Chain End x2	A549	0.039	617	
Primary Chain End x3	A549	0.017	269	
Primary Pendant x1	A549	11.88	187,906	179,207 ± 9,787
Primary Pendant x2	A549	11.45	181,105	
Primary Pendant x3	A549	10.66	168,610	
Primary Chain End x1	J774A.1	0.179	2,831	2,562 ± 236
Primary Chain End x2	J774A.1	0.156	2,467	
Primary Chain End x3	J774A.1	0.151	2,388	
Primary Pendant x1	J774A.1	10.03	158,645	157,221 ± 4,979
Primary Pendant x2	J774A.1	10.20	161,334	
Primary Pendant x3	J774A.1	9.59	151,685	

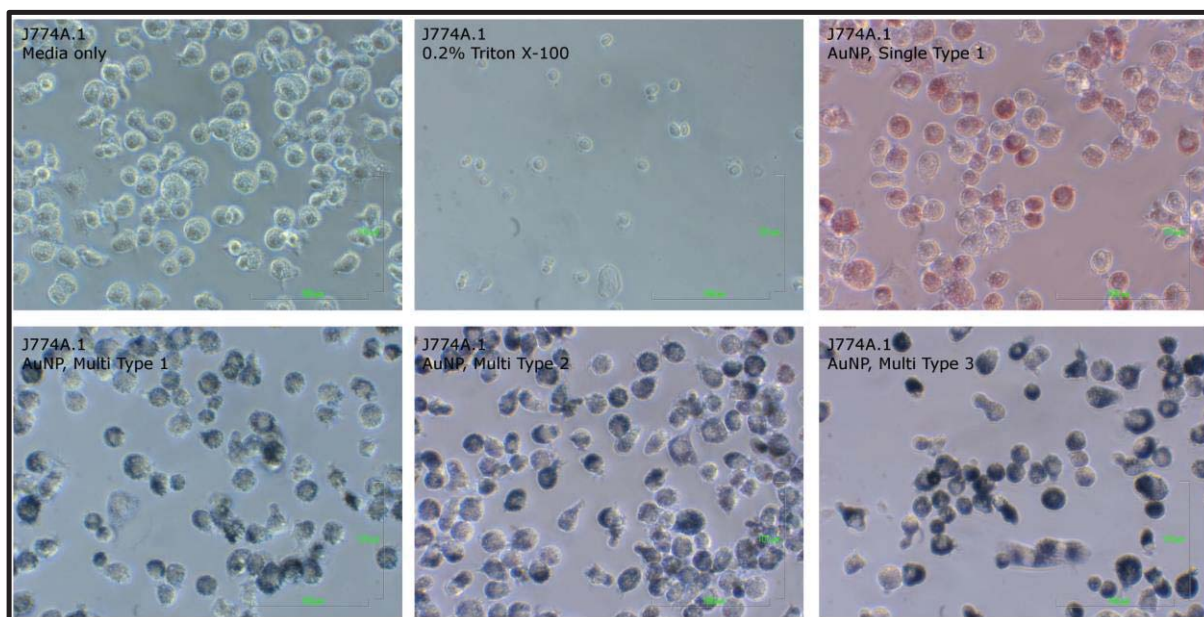


Figure S20. Brightfield microscopy images of J774A.1 mouse monocyte/macrophages after 24 hours incubation with Au NPs at 40 $\mu\text{g Au/mL}$.

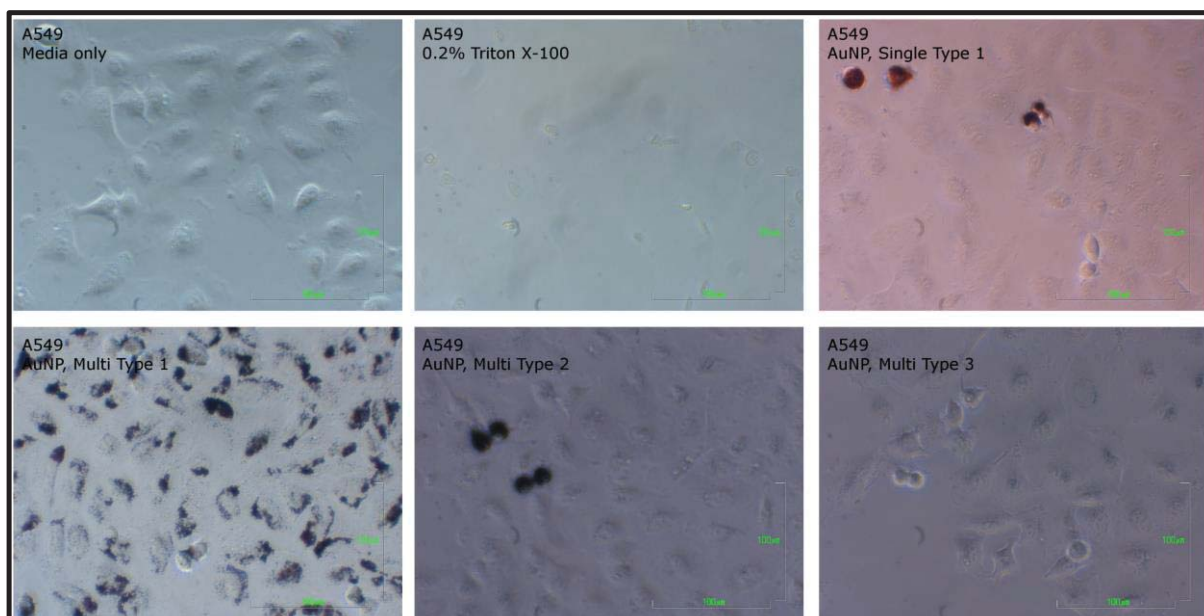


Figure S21. Brightfield microscopy images of A549 human lung epithelial cells after 24 hours incubation with Au NPs at 40 $\mu\text{g Au/mL}$.

3. Supporting Information References

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