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Supporting Information

Siderophore-Inspired Nanoparticle-based Biosensor for the Selective Detection of Fe^{3+}

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Materials and Methods

Materials:

All chemicals were used as supplied. Diethyl ether, ethyl acetate, hexane, methanol, triethylamine and toluene were all purchased from Fisher Scientific at laboratory reagent grade. Deuterated chloroform (99.8 atom % D), deuterated methanol (99.8 atom % D), *N*-hydroxysuccinimide (98.0 %), *N,N*-diisopropylcarbodiimide (99.0 %), *N*-hydroxyethyl acrylamide (97.0 %), 4,4'-azobis(4-cyanovaleric acid) (≥ 98.0 %), mesitylene (97.0 %), calcium chloride dehydrate (≥ 99.0 %), cobalt(II) chloride hexahydrate (98.0 %), copper(II) chloride (≥ 99.0 %), iron(III) chloride hexahydrate (97.0 %), iron(II) tetrahydrate (98.0 %), gadolinium(III) chloride hexahydrate (99.0 %), manganese(II) chloride tetrahydrate (≥ 98.0 %) and bovine plasma were all purchased from Sigma-Aldrich. Dopamine hydrochloride (99.0 %) was purchased from VWR International Ltd. Gold nanoparticle suspension (40 nm) was purchased from BBI Solutions. Benzyl 2-[(*tert*-butoxycarbonyl)amino]ethyl trithiocarbonate[1] and 2-(dodecylthiocarbonothioylthio)-2-methylpropanoic acid[2, 3] were synthesized as described in previous reports.

Analytical Methods:

NMR spectroscopy (^{1}H, ^{13}C) was conducted on a Bruker AV-250, Bruker DRX-500 or Bruker AV III-600 spectrometer using deuterated chloroform or methanol as solvent. All chemical shifts are reported in ppm (δ) relative to the solvent used. High resolution mass spectra were recorded on a Bruker Electrospray Ultra-High Resolution tandem TOF mass spectrometer using electrospray ionization (ESI) in positive mode on samples prepared in methanol. FTIR spectra were acquired using a Bruker Vector 22 FTIR spectrometer with a Golden Gate diamond attenuated total reflection cell. A total of 64 scans were collected on samples in their native (dry)

state. UV-visible spectra were obtained using a Biotech Synergy HT and processed using the Gen5 software package. "Normalized absorbance" refers to data that has been normalized between 0 and1. All other data referred to as "absorbance" has been normalized such that the absorbance at 450 nm equals 1. SEC analysis was performed on a Varian 390-LC MDS system equipped with a PL-AS RT/MT autosampler, a PL-gel 3 μm (50 × 7.5 mm) guard column, two PL-gel 5 μm (300 × 7.5 mm) mixed-D columns using DMF with 5 mM NH_4BF_4 at 50 °C as the eluent at a flow rate of 1.0 mL.min^{-1}. The GPC system was equipped with ultraviolet (UV) (set at 280 nm) and differential refractive index (DRI) detectors. Narrow molecular weight PMMA standards (200 - 1.0 × 10^6 g.mol^{-1}) were used for calibration using a second order polynomial fit. Particle size analysis was determined by Dynamic Light Scattering using a Malvern Zetasizer Nano ZS instrument. A 4 mW He-Ne 633 nm laser module was used and scattered light was measured at 173° (back scattering). The attenuator and position was selected automatically by the instrument and particle sizes reported as the average of 4 measurements. TEM images were obtained on a JEOL 2000fx microscope, 200 kV, LaB_6 instrument operated with a beam current of ~115 mA; images were captured using a Gatan Orius 11 megapixel camera. Samples were prepared by deposition and drying of nanoparticle samples (10 μL of stock solutions) onto lacey carbon 300-mesh copper TEM grids (Agar Scientific). Diameters were measured using ImageJ version 1.48a; average values were calculated by counting a minimum of 60 particles.

***Synthetic Procedures*:**

Synthesis of 2,5-Dioxopyrrolidin-1-yl 2-(((dodecylthio)carbonothioyl)thio)-2-methylpropanoate (2): *N*-hydroxysuccinimide (0.47 g, 4.11 mmol) and 2-(dodecylthiocarbonothioylthio)-2-methylpropanoic acid (1.00 g, 2.74 mmol) were dissolved in

anhydrous tetrahydrofuran (20 mL) in a 100 mL 3-necked flask purged with nitrogen. The flask was cooled to 0 °C and *N,N*'-diisopropylcarbodiimide (0.52 g, 0.64 mL, 4.11 mmol) was added dropwise over 10 minutes. The reaction mixture was stirred at room temperature overnight. After this time, solvent was removed *in vacuo* and the crude solid was re-dissolved in ethyl acetate. The organic layer was washed with saturated sodium hydrogencarbonate solution, twice with water, once with brine and dried over magnesium sulfate. Removal of the solvent left a yellow solid which was recrystallized from ethyl acetate/hexane and washed with water to yield yellow crystals (1.10 g, 86.6 %).

^{1}H NMR (250 MHz, $CDCl_3$) δ_{ppm}: 3.31 (2H, t, $J_{12\text{-}11}$ = 7.57 Hz, H^{12}); 2.81 (4H, s, H^{18}); 1.88 (6H, s, H^{15}); 1.69 (2H, p, $J_{11\text{-}12,\ 11\text{-}10}$ = 7.57 Hz, H^{11}); 1.20 – 1.45 (18H, m, $H^{2\text{-}10}$); 0.89 (3H, t, $J_{1\text{-}2}$ = 6.93 Hz, H^{1})**; ^{13}C NMR** (250 MHz, $CDCl_3$) δ_{ppm}: 218.7 (C^{13}); 169.1 (C^{16}); 168.7 (C^{17}); 54.3 (C^{14}); 37.2 (C^{12}); 30.9 (C^{15}); 27.8 (C^{11}); 24.5(C^{18}); 31.9, 29.6, 29.5, 29.4, 29.3, 29.1, 29.0, 25.6, 22.7 ($C^{2\text{-}10}$); 14.1 (C^{1}); **FTIR** cm^{-1}: 2951, 2916, 2847 (alkyl-H stretch); 1776, 1735 (C=O stretch); 1073 (C=S) stretch; **HRMS** (ESI +) m/z: 484.1619 $[M+Na]^{+}$; expected 484.1620 ($C_{21}H_{35}NO_4S_3Na$).

Synthesis of 1-((3,4-dihydroxyphenethyl)amino)2-methyl-1-oxopropan-2-yl dodecyl carbonotrithioate: Compound **2** (0.9 g, 1.95 mmol) and dopamine hydrochloride (0.41 g, 2.14 mmol) were added to a 100 mL round bottomed flask. Anhydrous methanol (40 mL) was added and stirred to dissolution under nitrogen. Triethylamine (0.24 g, 0.30 mL, 2.34 mmol) was added and the yellow solution stirred at room temperature for 2 days. After this time, solvent was removed *in vacuo* and the yellow residue was re-dissolved in diethyl ether and extracted three times with 1M HCl, twice with water, once with brine and dried over magnesium sulfate. The

solvent was removed to give an orange oil (0.9 g) to which hexane (100 mL) was added. The mixture was stirred for 30 minutes, after which time a yellow precipitate was observed. This was collected by filtration (gravity) (0.65 g, 67.0 %).

^{1}H NMR (500 MHz, $CDCl_3$) δ_{ppm}: 6.78 (1H, d, $J_{24\text{-}25}$ = 8.04 Hz, H^{22}); 6.67 (1H, d, $J_{21\text{-}25}$ = 2.00 Hz, H^{27}); 6.57 (2H, m, $H^{17,\ 21}$); 6.18, 5.42 (2H, s br., $H^{24,\ 26}$); 3.44 (2H,q, $J_{18\text{-}19}$ = 7.04 Hz, H^{18}); 3.26 (2H, t, $J_{12\text{-}11}$ = 7.56 Hz, H^{12}); 2.66 (2H, t, $J_{19\text{-}18}$ = 7.04 Hz, H^{19}); 1.63-1.69 (8H, m, $H^{11,\ 15}$); 1.38 (2H, p, $J_{10\text{-}9,\ 10\text{-}11}$ = 7.28 Hz, H^{10}); 1.20-1.37 (16H, m, $H^{2\text{-}9}$); 0.88 (3H, t, $J_{1\text{-}2}$ = 7.04 Hz, H^{1}); **^{13}C NMR** (500 MHz, $CDCl_3$) δ_{ppm}: 220.1 (C^{13}); 172.8 (C^{16}); 143.8 (C^{23}); 142.7 (C^{22}); 131.2 (C^{20}); 121.0 (C^{21}); 115.5 (C^{27}); 115.4 (C^{22}); 57.1 (C^{14}); 41.6 (C^{18}); 37.2 (C^{12}); 34.6 (C^{19}); 31.9, 29.7, 29.6, 29.5, 29.3, 29.1, 29.0, 28.9, 27.7, 22.7 ($C^{2\text{-}11}$); 25.9 (C^{15}); 14.3 (C^{1}); **FTIR** cm^{-1}: 3342 (N-H stretch); 3196 (O-H stretch); 3042 (aryl-H stretch); 2920, 2850 (alkyl-H stretch); 1623, 1532 (C=O stretch); 1605 (aromatic ring); 1361 (O-H bend); 1071 (S-(C=S)-S) stretch; **HRMS** (ESI +) m/z: 522.2138$[M+Na]^+$; expected 522.5141 ($C_{25}H_{41}NO_3S_3Na$).

Example polymerisation of N-hydroxyethyl acrylamide using 1-((3,4-dihydroxyphenethyl)amino)2-methyl-1-oxopropan-2-yl dodecyl carbonotrithioate: *N*-hydroxyethyl acrylamide (1.00 g, 8.69 mmol), compound **3** (75.90 mg, 115.91 μmol) and 4,4'-azobis(4-cyanovaleric acid) (6.50 mg, 23.18 μmol) were added to a vial fitted with stir bar and rubber septum and dissolved in methanol:toluene (50:50) (2 mL). Mesitylene (0.20 mL) was added as internal reference and the mixture stirred (5 mins). An aliquot of this starting mixture was removed for ^{1}H NMR analysis. The mixture was degassed by bubbling through nitrogen gas for 30 mins and placed in an oil bath thermostated at 70 °C for 35 minutes. The reaction was

quenched in liquid nitrogen, an aliquot removed and conversion determined by ^{1}H NMR. The product was purified three times by precipitation from methanol into cold diethyl ether, the solid isolated by centrifugation and dried to yield a pale yellow solid. Conversion (NMR): 78.5% M_n (theoretical): 6800 g.mol^{-1}; M_n (SEC): 9700 g.mol^{-1}; M_w/M_n (SEC): 1.17.

Polymerisation of N-hydroxyethyl acrylamide using benzyl 2-[(tert-butoxycarbonyl)amino]ethyl trithiocarbonate: *N*-hydroxyethyl acrylamide (1.00 g, 8.69 mmol), benzyl 2-[(*tert*-butoxycarbonyl)amino]ethyl trithiocarbonate (39.76 mg, 115.91 μmol) and 4,4'-azobis(4-cyanovaleric acid) (6.50 mg, 23.18 μmol) were added to a vial fitted with stir bar and rubber septum and dissolved in methanol:toluene (50:50) (2 mL). Mesitylene (0.20 mL) was added as internal reference and the mixture stirred (5 mins). An aliquot of this starting mixture was removed for ^{1}H NMR analysis. The mixture was degassed by bubbling through nitrogen gas for 30 mins and placed in an oil bath thermostated at 70 °C for 45 minutes. The reaction was quenched in liquid nitrogen, an aliquot removed and conversion determined by ^{1}H NMR. The product was purified three times by precipitation from methanol into cold diethyl ether, the solid isolated by centrifugation and dried to yield a pale yellow solid. Conversion (NMR): 74.1% M_n (theoretical): 6400 g.mol^{-1}; M_n (SEC): 10200 g.mol^{-1}; M_w/M_n (SEC): 1.16

Functionalisation of gold nanoparticles with polymer (pHEA-1/2/3/4@AuNP$_{40}$): Polymer was dissolved in citrated coated gold nanoparticle suspension at a concentration of 2 mg.mL^{-1} and left at 5 °C overnight. The solution was centrifuged (15 mins, 10000 rpm), supernatant removed and

the pellet re-dispersed in distilled water. This washing procedure was repeated a total of 4 times, the pellet re-dispersed in equal volume of distilled water and stored in the fridge until required.

UV/visible spectrophotometry assay: In triplicate in a 96-well plate, polymer-coated gold nanoparticle suspension (95 μL), concentrated sodium chloride solution (5 μL) and freshly-prepared, concentrated iron(III) chloride hexahydrate or iron(II) chloride tetrahydrate solution were added to give the desired final concentrations. After incubation for 30 minutes at room temperature, the UV-visible spectrum between 450 and 700 nm was recorded. Data presented is the mean average of three replicates.

Additional small molecule characterisation data

^{1}H NMR, ^{13}C NMR and FTIR characterisation of compounds **2** and **3** is shown in Figures S1-3.

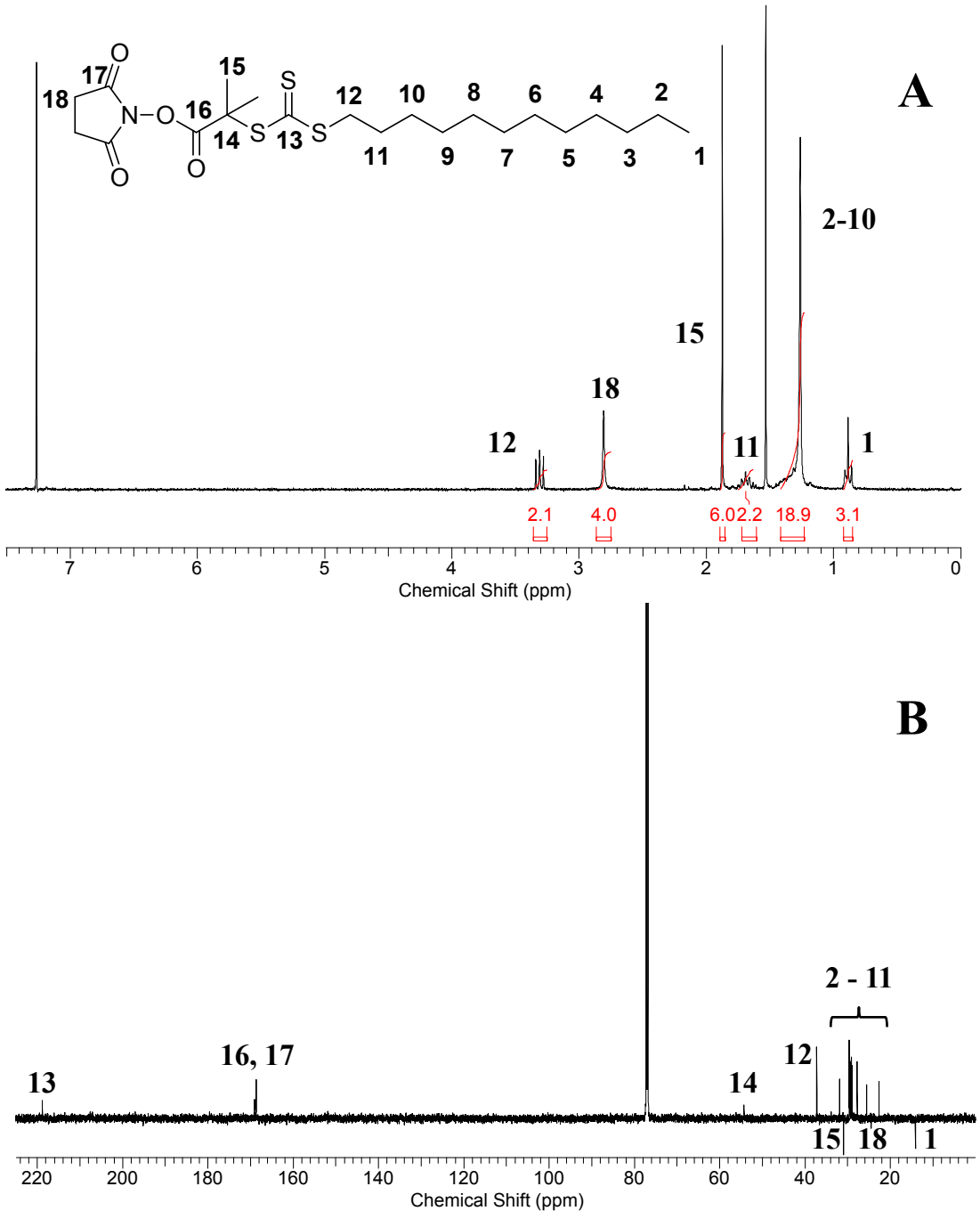

Figure S1. A) ^{1}H NMR and B) ^{13}C NMR spectra of 2,5-Dioxopyrrolidin-1-yl 2-(((dodecylthio)carbonothioyl)thio)-2-methylpropanoate in $CDCl_3$.

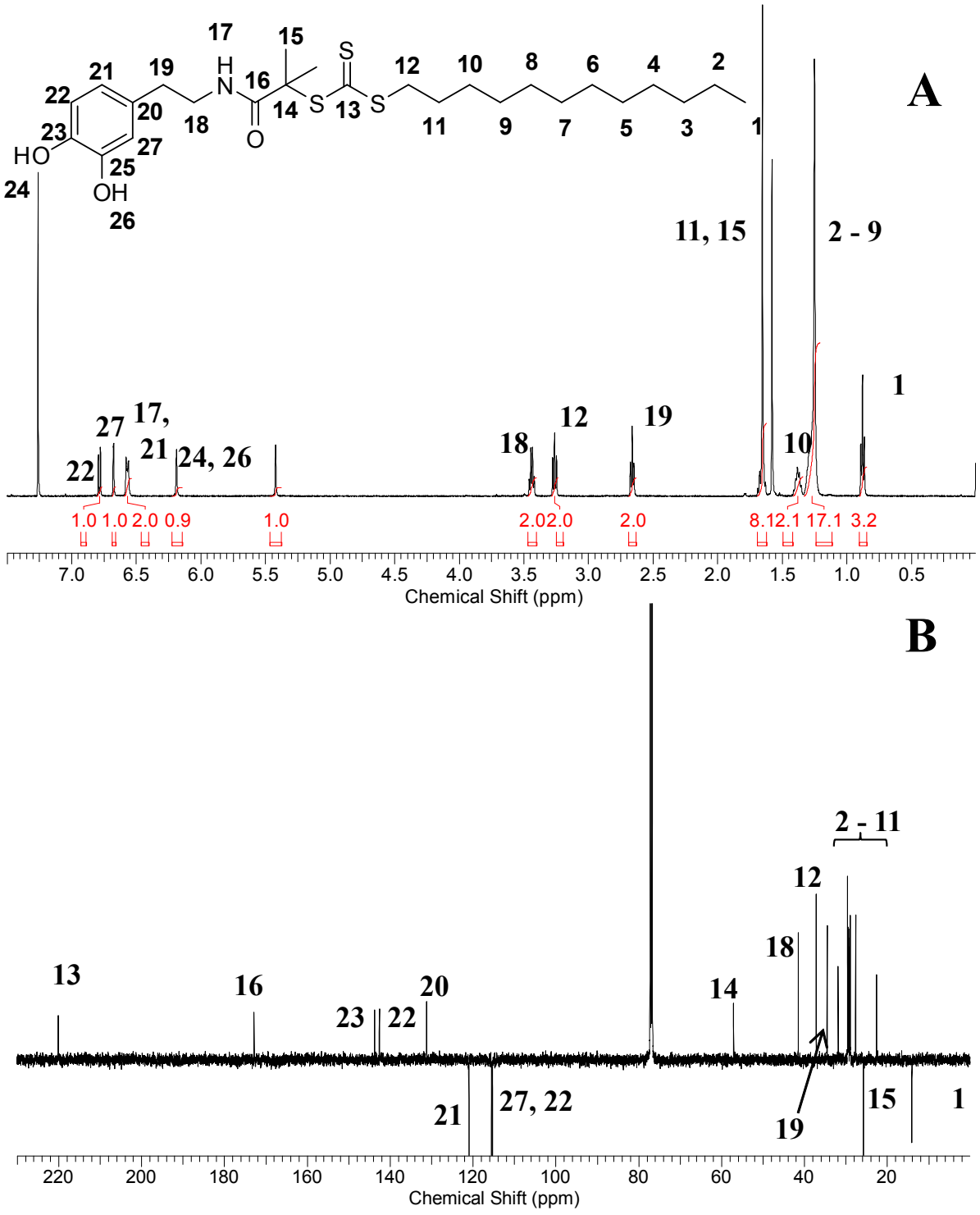

Figure S2. A) ^{1}H NMR and B) ^{13}C NMR spectra of 1-((3,4-dihydroxyphenethyl)amino)2-methyl-1-oxopropan-2-yl dodecyl carbonotrithioate in $CDCl_3$.

Infrared spectroscopy can be used to follow the successful preparation of chain transfer agent **3** (Figure S3). Activation of the carboxylic acid terminus of **1** with an NHS ester was confirmed by the loss of the C=O stretch at 1700 cm^{-1} and replacement with two C=O stretches at 1735 and 1776 cm^{-1}. Elaboration to catechol-functional chain transfer agent **3** is reflected in the gain of an N-H stretch at 3342 cm^{-1} and an O-H stretch at 3196 cm^{-1}. Furthermore, the two C=O stretches at 1735 and 1776 cm^{-1} have been replaced by two characteristic amide stretches at 1623 and 1523 cm^{-1}.

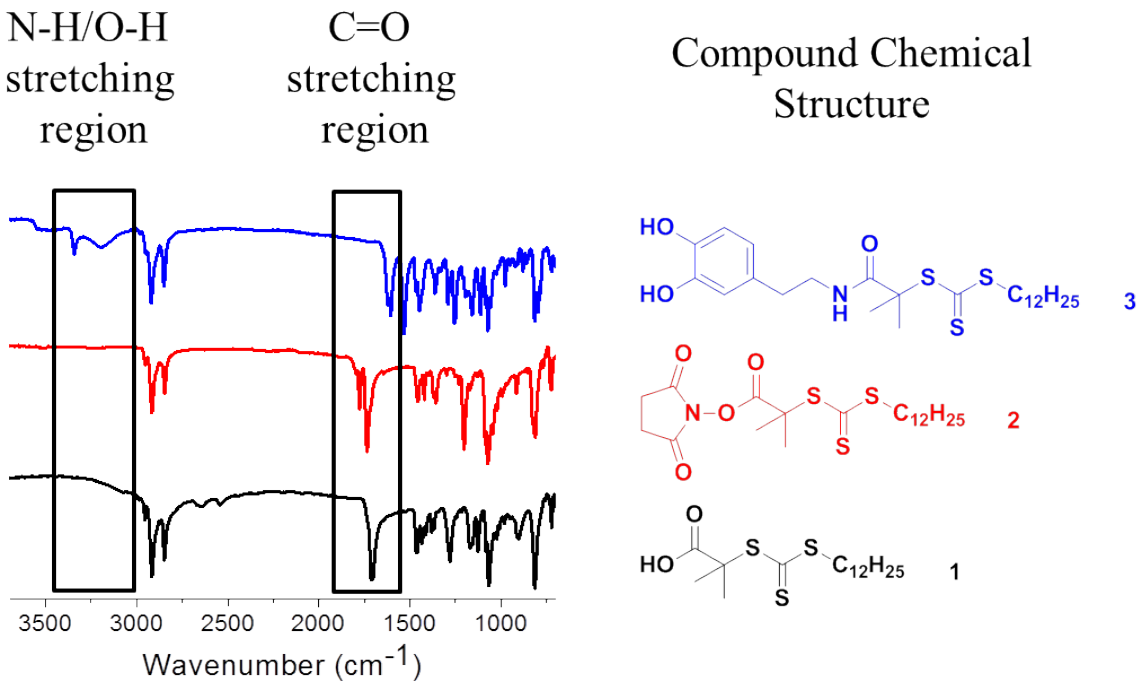

Figure S3. Infrared spectra showing sequential modification of the carboxylic acid terminus of chain transfer agent **1** to the activated ester **2** and subsequent modification to produce a catechol terminus **3**.

Additional polymer characterisation data

SEC characterisation of the polymers prepared in this study is shown in Figure S4.

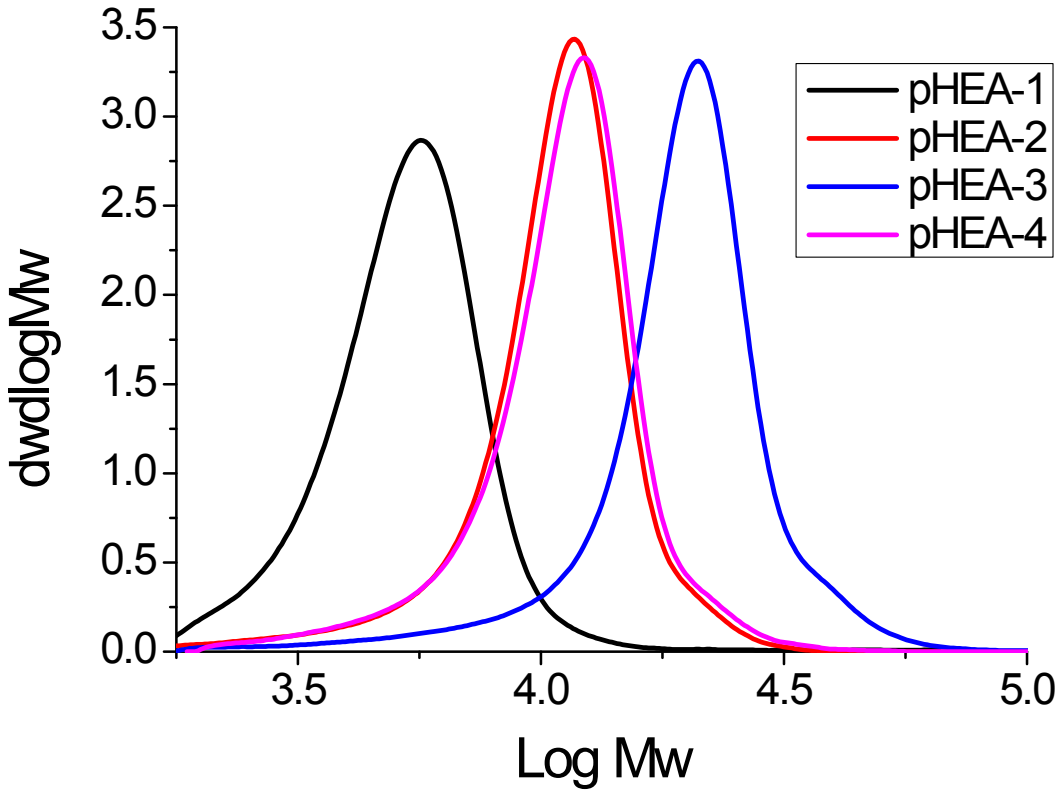

Figure S4. SEC Characterisation of polymers synthesised in this study.

Additional gold nanoparticle characterisation

Gold nanoparticle characterisation of **pHEA-1/3/4@AuNP$_{40}$** is shown in Figures S6-7.

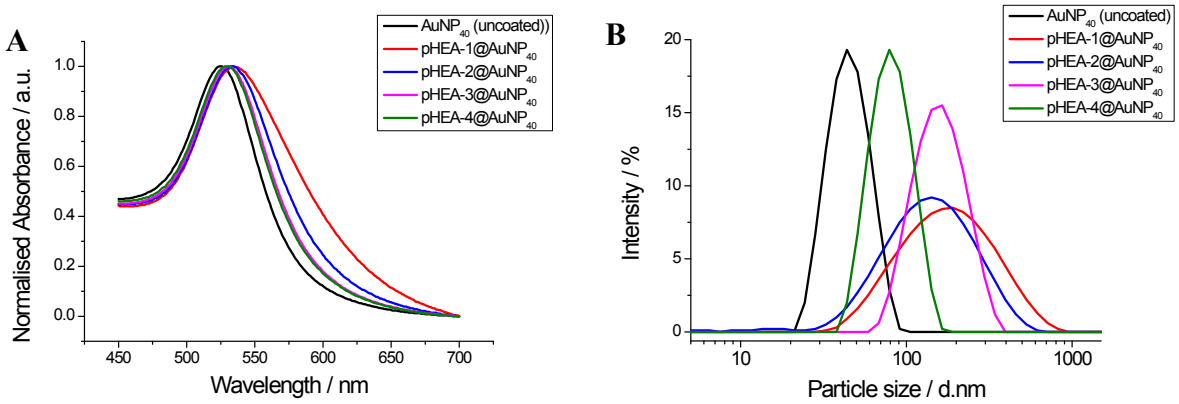

Figure S5. (A) UV/visible spectra and (B) DLS analysis of **AuNP$_{40}$ (uncoated)** and **pHEA-1/2/3/4@AuNP$_{40}$**.

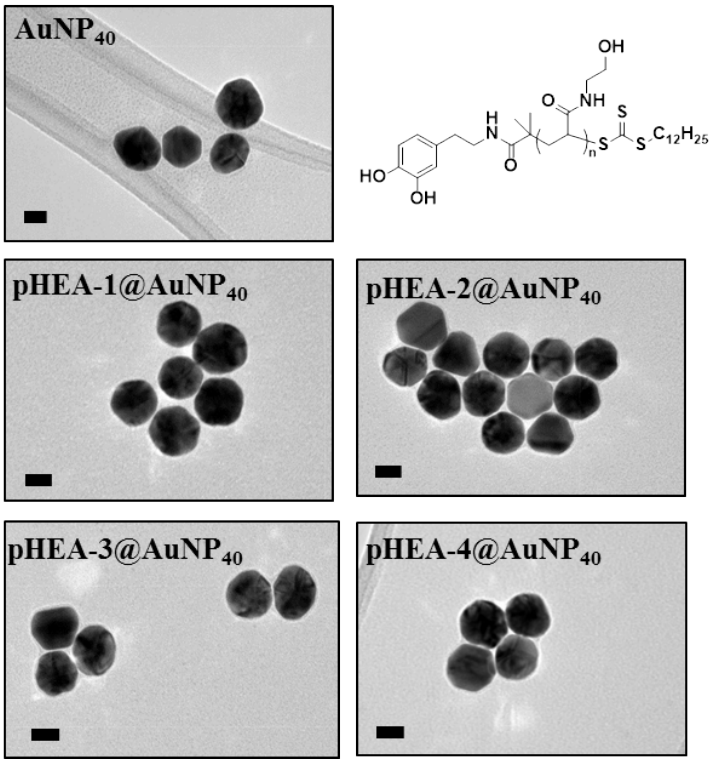

Figure S6. Transmission electron microscope (TEM) images of uncoated and polymer coated nanoparticles, as labelled, and the chemical structure of the coating polymer.

Assessing AuNP stability in the presence of 150 mM NaCl

DLS data showing the stability of **pHEA-2@AuNP$_{40}$** in the presence of 150 mM NaCl over time is shown in Figure S7. No significant changes in particle size were observed over an 8 hour period. A slight increase in particle size was observed between 8 and 24 hours. All the assays presented in this study utilised a 30 minute incubation time.

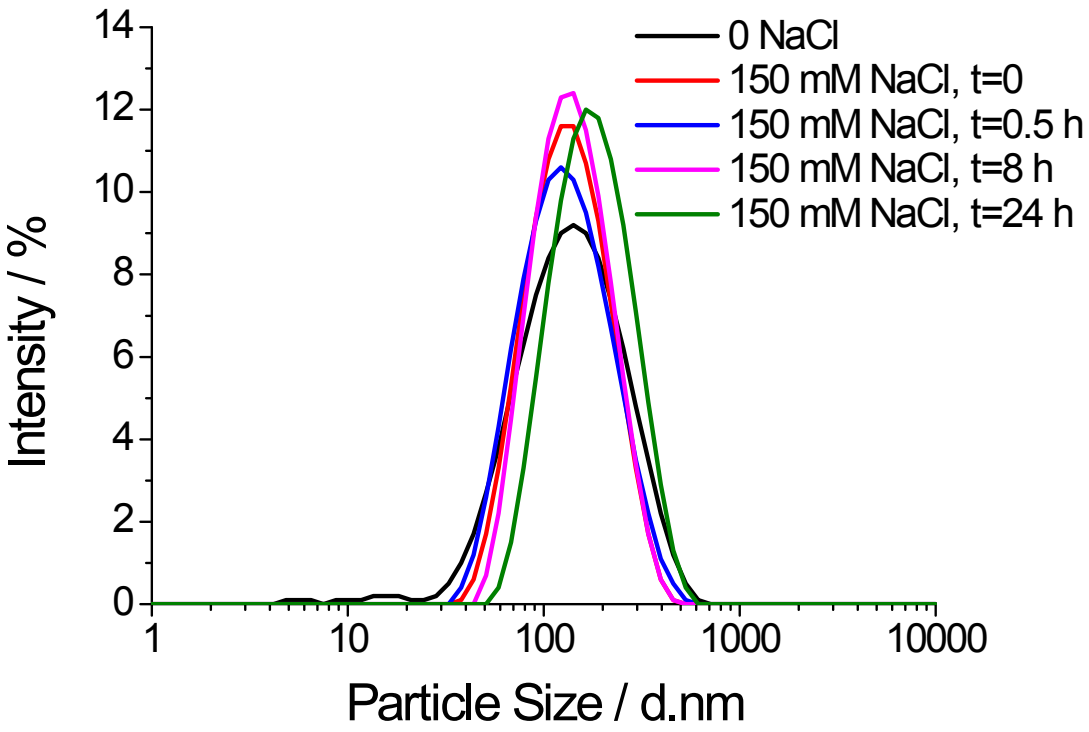

Figure S7. DLS of **pHEA-2@AuNP$_{40}$** in the presence of 150 mM NaCl at various time-points.

Control Data

As described in the main text, two additional controls were performed: (A) the interaction of Fe^{3+} with non-catechol functionalised AuNPs (**pHEA-4@AuNP$_{40}$**) and (B) catechol-functionalised AuNPs (**pHEA-2@AuNP$_{40}$**) in the presence of Fe^{2+} (Figure S8). No significant change in either SPR_{max} or Abs@700 nm was observed in either case.

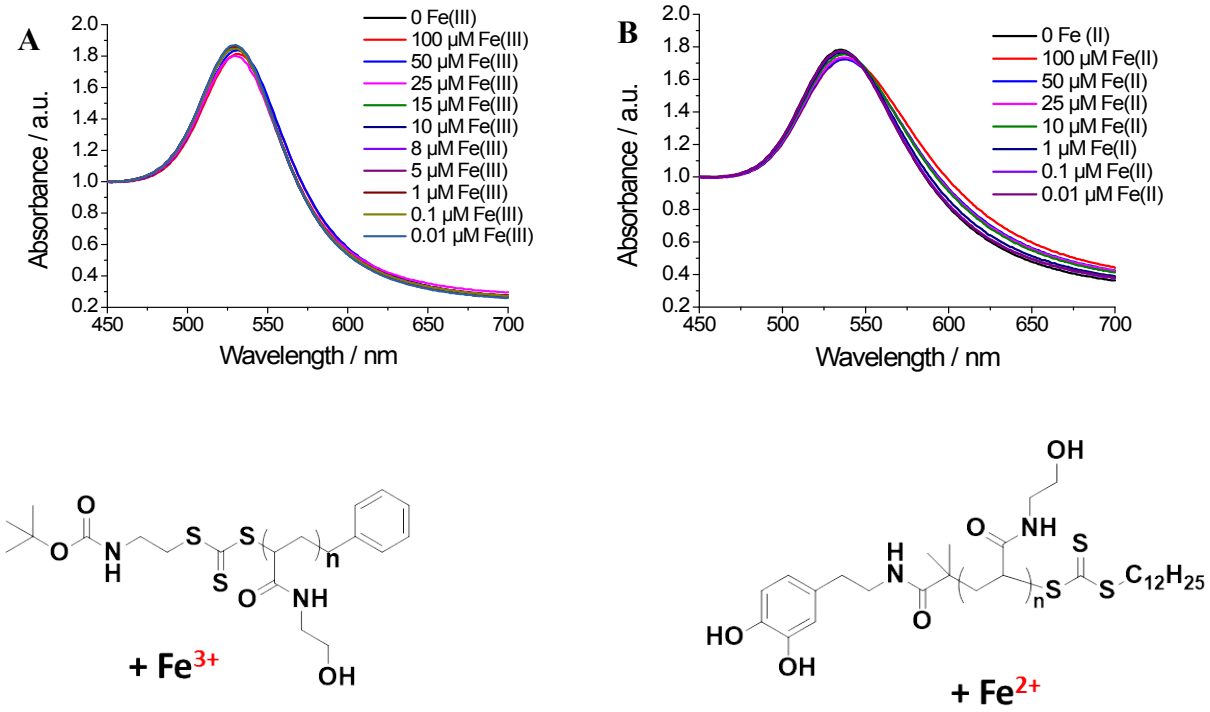

Figure S8. Response of (A) **pHEA-4@AuNP$_{40}$** to various concentrations of Fe^{3+} and (B) **pHEA-2@AuNP$_{40}$** to various concentrations of Fe^{2+}.

1. D. J. Phillips, J. P. Patterson, R. K. O'Reilly and M. I. Gibson, *Polym. Chem.*, 2014, 5, 126.
2. D. J. Phillips and M. I. Gibson, *Biomacromolecules*, 2012, 13, 3200.
3. J. Skey and R. K. O'Reilly, *Chem. Commun.*, 2008, 4183.