Electronic Supplementary Information

Tunable Functionalization of Silica Coated Iron Oxide Nanoparticles Achieved Through a Silanol-Alcohol Condensation Reaction

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Experimental Section

Reagents and Chemicals. All of the chemicals were of analytical grade and were used as received without further purification. Iron (III) chloride hexahydrate (97%), gold (III) chloride trihydrate (≥99.9%), sodium citrate tribasic dihydrate (ACS reagent, ≥99.0%,), sodium dodecyl sulfate (≥98.5%), propylene carbonate (99%), polyethylene glycol 5,000 monomethyl ether (PEG, 5,000 MW), and tetraethyl orthosilicate (98%) were all obtained from Sigma-Aldrich (USA). Chemicals such as 11-mercapto-1-undecanol (97%), 11-undecanoic acid (96%), 1,10-decandiol (98%), and polyvinylpyrrolidone (≤100%, 10,000 MW) were all obtained from Aldrich. An aqueous solution of ammonium hydroxide (28% to 30%) was obtained from BDH (USA). Ellman's reagent (95 to 100%, lot # TC262582) was obtained from Thermo Scientific (USA). Urea (99%) and hydrogen peroxide (a 30% aqueous solution) were obtained from Fisher Scientific (USA). Polyethylene glycol (PEG, 100%, 200 MW) was obtained from Alfa Aesar (Belgium). Methoxy terminated PEG thiol (mPEG$_{5000}$-SH, 5,000 MW) was obtained from NanoCS (USA). Hydrochloric acid (36 to 38.5% v/v in water) was obtained from VWR (USA). Nitric acid (68 to 70% v/v in water) was obtained from ACP (USA). Sulfuric acid (95 to 98% v/v in water) was obtained from Caledon (USA). Ultrapure, deionized (DI) water (18.2 MΩ·cm) was obtained using a NANOpure DIamond^{TM} system from Barnstead (USA).

Synthesis of Iron Oxide Nanoparticles. These nanoparticles (NPs) were synthesized via a modified solvothermal method.[1] Iron (III) chloride hexahydrate (0.12 M), sodium citrate tribasic dihydrate (0.06 M), and urea (1.0 M) were dissolved in ethylene glycol (10 mL) with the aid of vigorous mechanical stirring for 60 min. This mixture was transferred to a Teflon lined stainless-steel autoclave (Model No. 4749, Parr Instruments Co., Moline, IL, USA), sealed, and heated at 200 °C for 8 h. After cooling the solution to room temperature, black particles were

isolated from the solution using an external magnet. The supernatant was decanted and these isolated particles were suspended in about 20 mL of ethanol. These steps for washing and isolation of the magnetic NPs were repeated a total of three times. After this process of washing the NPs, the isolated solids were dried in an oven at 70 °C for 2 h.

Silica Coating of the Iron Oxide Nanoparticles. The magnetic NPs were coated with a layer of silica using a Stöber sol-gel process.[2] A portion of the dried iron oxide NPs (10 mg) were re-suspended in a mixture of 16 mL DI water and 64 mL ethanol with the aid of sonication (Bransonic, Ultrasonic cleaner, 50-60 Hz) for 1 h. This solution of NPs was stirred using an overhead mechanical stirrer (Fisher Scientific, Compact Overhead Stirrer, 50 Watt output) rotating at 500 rpm during the addition of ammonium hydroxide and tetraethyl orthosilicate (TEOS). The ammonium hydroxide (1 mL) was added all at once, and subsequently the TEOS (1 mL) was added dropwise over a period of 5 min. This mixture was stirred continuously for 6 h. After this process, the particles were magnetically isolated from the solution, and the particles were washed by suspending these solids in 20 mL of ethanol with agitation of the solution for 3 min. This purification process was repeated at least two more times.

Functionalization of the Silica Coated Iron Oxide Nanoparticles. Functionalization of the silica coated iron oxide NPs was achieved using a modified silanol-alcohol condensation reaction.[3] The silica coated iron oxide NPs were washed again, but propylene carbonate was used in place of ethanol. The supernatant was removed after isolating the NPs from the solution, and these pelleted solids were suspended into a fresh solution of propylene carbonate. This purification process was repeated one more time to remove traces of ethanol from the solution. The desired alcohol reagent, such as 11-mercapto-1-undecanol, was prepared as a 500 mM solution in 2 mL of propylene carbonate. This solution of alcohol reagent was mixed with the

pelleted NPs. The vial containing this solution was sealed with a plastic cap and wrapped in parafilm. A suspension of these NPs was obtained by vortexing the solution for 5 min and sonicating for another 5 min. The sealed container was subsequently heated at 80 °C over 24 h by immersion in an oil bath under magnetic stirring. This temperature can also be easily achieved using oil, water, or sand bath assisted heating. The set-up was covered with aluminum foil to reduce exposure to ambient light. After 24 h, the NPs were isolated from the unreacted, excess reagent by use of an external magnet. The isolated NPs were magnetically washed three times with isopropanol as the rinse solution. Following each step of this procedure, the washed NPs were suspended in 10 mL of isopropanol by agitation using a vortexer for 3 min followed by sonication for another 3 min.

Synthesis of the Gold Nanoparticles. The gold NPs (Au NPs) were prepared via a modified citrate reduction method.[4] A 250 mL glass round bottom flask and a stir bar (Fisherbrand stir bar, 7 mm x 2.5 mm) were cleaned by immersion in aqua regia (3:1, v/v, HCl:HNO$_3$) for 15 min. These acid cleaned supplies were rinsed several times with DI water. This cleaning process was repeated using a piranha solution (7:2, v/v, H$_2$SO$_4$:H$_2$O$_2$). These supplies were further rinsed with DI water. *CAUTION: Aqua regia and piranha solutions are extremely corrosive. These solutions should be handled with extreme care.* A solution of gold salt (5.11 mM HAuCl$_4$) was prepared in DI water one day prior to the synthesis of the Au NPs to maximize the dissolution of the gold salt. A portion of this solution was diluted from 1.45 mL to 50 mL using DI water, and transferred to the precleaned 250 mL round bottom flask. A water cooled condenser was attached to this flask prior to heating the solution. The solution was subsequently stirred with a magnetic stir bar while heating to 100 °C using a heating mantle connected to a variable current controller (GC-15005-01, Chemglass, USA). While heating the

gold salt solution, 5 mL of a 4.46 mM solution of sodium citrate tribasic dihydrate was separately prepared and heated for 10 min at 60 °C using a water bath. Upon boiling the solution of gold, the hot solution of sodium citrate tribasic dihydrate was added all at once to the stirred solution in the round bottom flask. The mixture was heated for an additional 10 min before removing the heat and allowing the stirred flask to cool to room temperature. The as-synthesized Au NPs were isolated from the unreacted excess gold salts through centrifugation (Thermo Electron Corporation, IEC microlite microcentrifuge) at 9,500 rpm (8,400 g) for 15 min, decanting of the supernatants, and re-dispersion of the isolated solids in DI water with the assistance of a vortexer for 3 min.

Assembly of Gold Nanoparticles onto the Silica Coated Iron Oxide Nanoparticles. A solution of Au NPs (10 nM in 0.1 mL water), a solution of silica coated iron oxide NPs (1 mg in 0.1 mL isopropanol) and a solution of mPEG$_{5000}$-SH (10 µL, 3% w/v in water) were mixed in a 1.5 mL Eppendorf tube (VWR, 89000-028). This mixture was agitated on an orbital shaker at 250 rpm (VWR orbital shaker, VWR 57018-754) for 1 h at room temperature while covered with aluminum foil to prevent exposure to ambient light. After this process, the magnetic NPs were isolated from the excess reagents, including freely suspended Au NPs, through the use of external magnets. The isolated solids were washed three times with isopropanol. During this process, the magnetic particles were suspended in 1 mL of isopropanol with the assistance of vortexing for 3 min followed by sonicating for another 3 min. This procedure for assembling the Au NPs onto the magnetic core-shell particles was used, as outlined above, regardless of the surface chemistry on the silica coated iron oxide NPs. When evaluating the influences on the self-assembly process of adding non-covalently interacting surfactants with the particles, the amount of each surfactant added to the suspension was maintained in place of the mPEG$_{5000}$-SH.

Characterization of the Nanoparticles and Their Assemblies. The concentration of Au

NPs in solution was estimated using extinction spectroscopy.[5] The extinction spectra of the

suspensions of Au NPs were obtained using an 8453 UV-Vis spectrometer from Agilent (USA).

Cuvettes used for these measurements were Fisherbrand™ disposable cuvettes (Fisher, Catalog

#14-955-127) with a fixed path length of 1 cm. The peak maxima at ~521 nm associated with the

plasmon resonance of the Au NPs was used when determining the particle concentration. The

extinction coefficient for these particles ($8.78 \times 10^8\,\mathrm{M^{-1}cm^{-1}}$) was obtained from prior art.[5]

Analyses of the particles by transmission electron microscopy (TEM) and energy

dispersive X-ray spectroscopy (EDX) were obtained using an FEI Tecnai Osiris operating at 200

kV (Thermo Fisher, USA). Dimensions of the NPs were estimated through TEM analyses by

measuring between 20 and 50 particles per sample set. Tomographic analyses by TEM were

obtained by tilting the sample from $+60°$ to $-60°$ using a Fischione advanced TEM tomography

holder (Model 2020, USA). The TEM images used for the tomography reconstruction were

obtained at a tilt of every 2°. The reconstruction was performed by Inspect 3D (Thermo Fisher,

USA), and the data visualized by Amira 6.5 (Thermo Fisher, USA).

The number of Au NPs attached to the surfaces of the silica coated iron oxide NPs were

calculated through TEM analyses. For these measurements, the average number of Au NPs was

determined by assessing at least 5 different core-shell particles observed in the TEM samples.

The results of these analyses are summarized in Table S1 and Table S2. As the projected bright

field TEM images only clearly depict the NPs on one side of the assemblies, it was assumed that

the number of Au NPs would be equal on the bottom of the iron oxide particles. The number of

Au NPs coated on the core-shell particles in the projected images was, therefore, doubled for the

reported values. The TEM tomographic analyses indicated that this was a valid assumption given the uniformity of the coatings observed for the thiol functionalized magnetic NPs.

The Ellman's Test was used to quantify the amount of thiol functional groups present on the surfaces of the core-shell particles after reacting with 11-mercapto-1-undecanol. The purified particles were suspended in isopropanol. A calibration curve for the Ellman's Test was established using a series of standards containing 11-mercapto-1-undecanol (i.e., 0 mM, 0.08 mM, 0.17 mM, 0.25 mM, 0.33 mM, 0.41 mM, and 0.50 mM 11-mercapto-1-undecanol) each prepared by dissolving 11-mercapto-1-undecanol in isopropanol. A portion of each sample (250 µL of the particles in isopropanol) or the standard solutions of 11-mercapto-1-undecanol (250 µL, prepared in isopropanol) were each separately mixed with 2.5 mL aliquots of 0.1 M sodium phosphate buffer and 50 µL of the Ellman's reagent (4 mg/mL, suspended in 0.1 M sodium phosphate buffer). These solutions were agitated with the assistance of a vortexer (Vortex-Genie 2, Scientific Industries, USA) for 15 min before obtaining an absorbance spectrum for each mixture with an Agilent 8453 UV-Vis spectrometer. These assessments were performed at room temperature. The concentration of thiol functional groups present on the surfaces of the NPs was estimated from the standard curve.

The density of thiols on the surfaces of the magnetic particles was estimated using the quantitative results from the Ellman's Test and the total surface area estimated for the silica coated iron oxide NPs. The number of thiol groups in a sample was determined using the standardized results from the Ellman's Test. The total available surface area on each core-shell nanoparticle was calculated using the following steps: (i) the total number of NPs in a sample was calculated using the mass of silica coated iron oxide NPs used for the Ellman's Test in combination with the density of these materials and the average volume per NP; (ii) the density

of the iron oxide NPs was estimated to be 5.19 g/cm^3, and the density of the silica shell was estimated to be 1.90 g/cm^3;[6] (iii) the average volume of the silica shell and the iron oxide core were each determined from TEM based measurements; and (iv) the total collective surface area of particles in the sample was calculated by multiplying the estimated total number of NPs by the nominal surface area per nanoparticle, which was also estimated using particle diameters obtained from the TEM measurements and assuming each particle had a spherical shape.

The powder X-ray diffraction (XRD) pattern of the sample were acquired with a Rigaku R-Axis Rapid diffractometer equipped with a 3 kW sealed tube copper source (Kα radiation, λ = 0.15418 nm) collimated to 0.5 mm. The sample were packed into a cylindrical recess drilled into glass microscope slides (Leica 1 mm Surgipath Snowcoat X-tra Micro Slides) for acquiring XRD patterns for the products.
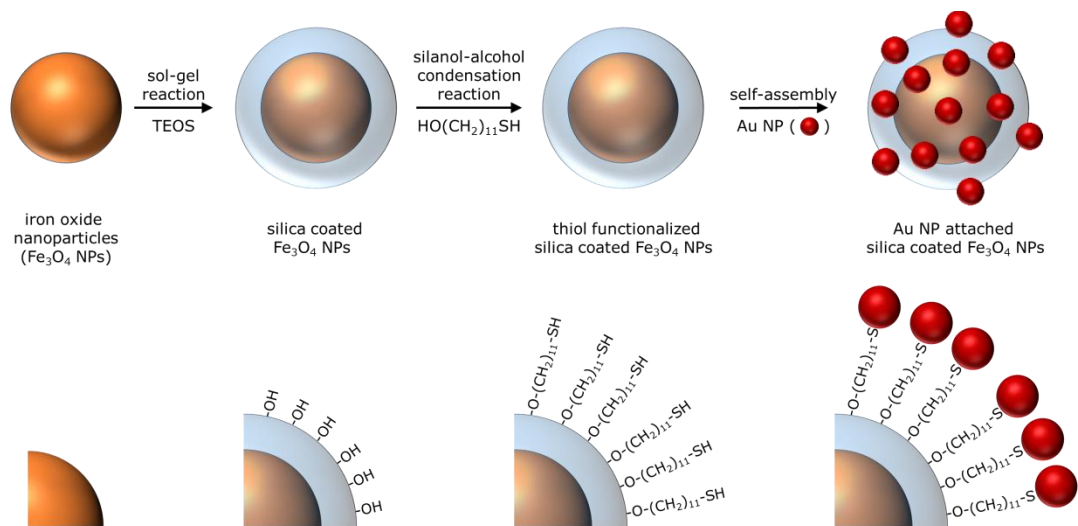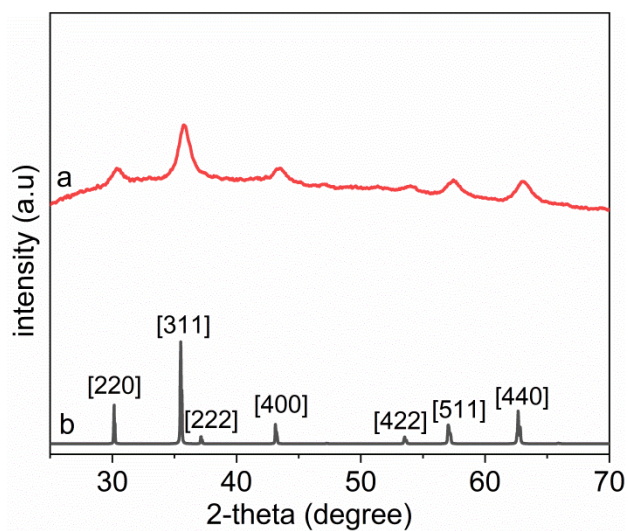
Fig. S1 Representative schematic diagram of thiol functionalization of silica coated iron oxide nanoparticles (Fe_3O_4 NPs) achieved using the silanol-alcohol condensation reaction. This diagram is not drawn to scale.
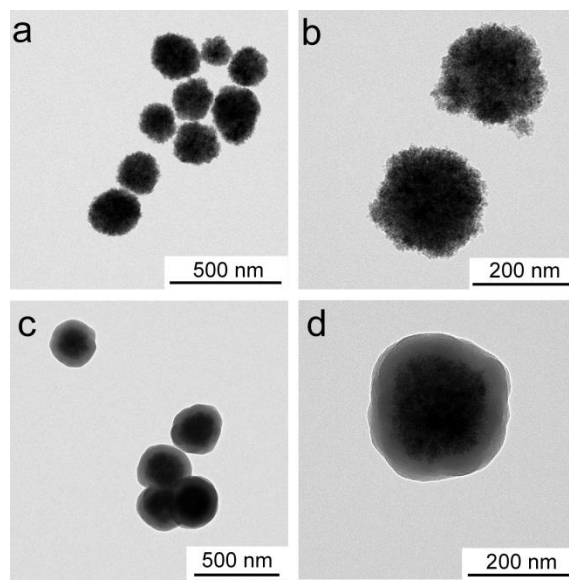
Fig. S2 Transmission electron microscopy (TEM) analyses of (a,b) the iron oxide nanoparticles (NPs), and (c,d) the silica coated iron oxide NPs.

Fig. S3 Powder X-ray diffraction patterns for: (a) Fe_3O_4 nanoparticles prepared by the described solvothermal synthesis; and (b) a reported Fe_3O_4 reference (ICSD no. 35000)

a

200 nm

b

50 nm

c

50 nm

d

50 nm
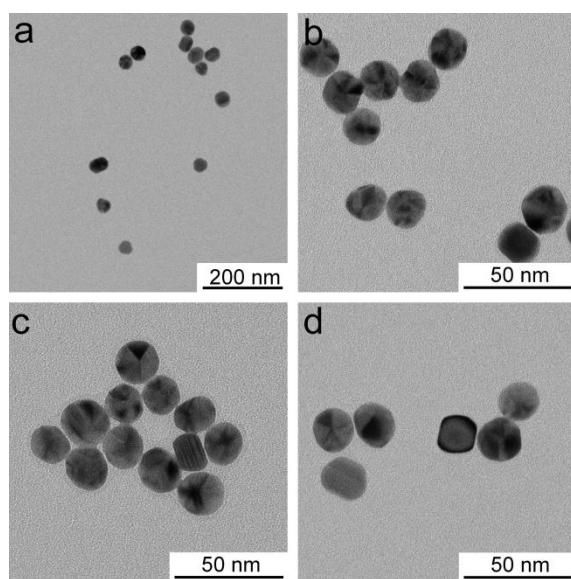
Fig. S4 Representative TEM analyses of the gold nanoparticles (Au NPs).
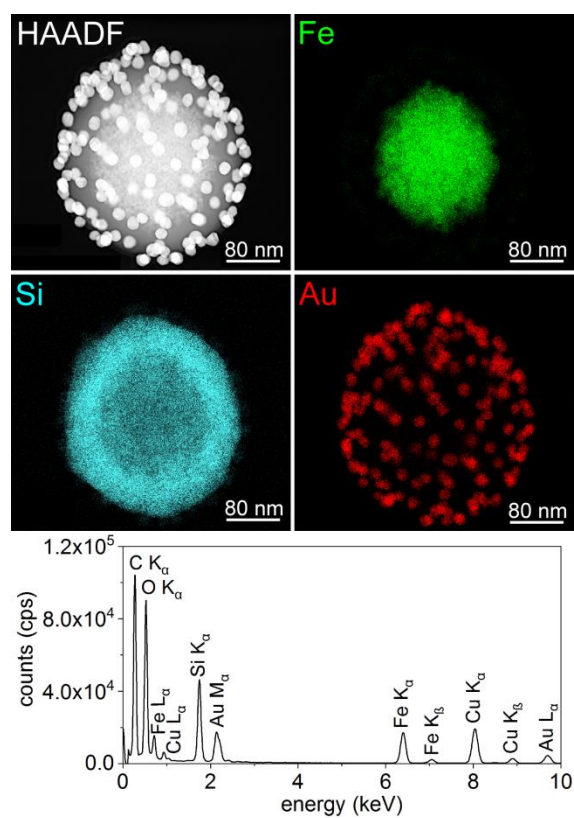
Fig. S5 Energy dispersive X-ray spectroscopy (EDX) analysis of the Au NPs assembled onto the surfaces of a thiol functionalized, silica coated iron oxide nanoparticle. These images show the high-angle annular dark field (HAADF) image obtained by scanning TEM techniques, and EDX maps of Fe, Si, and Au within these assemblies. A representative EDX spectrum is also included, depicting the composition of the assembly along with contributions from the copper TEM grid.
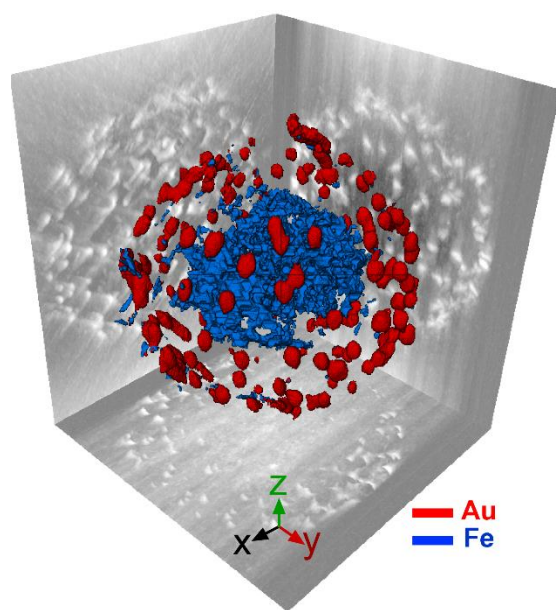
Fig. S6 A representative three-dimensional rendering produced from the tomographic data associated with the assemblies displayed in **Fig. 2**. This rendering was prepared from the reconstruction of a series of aligned images obtained at every 2° between +60° and -60°.
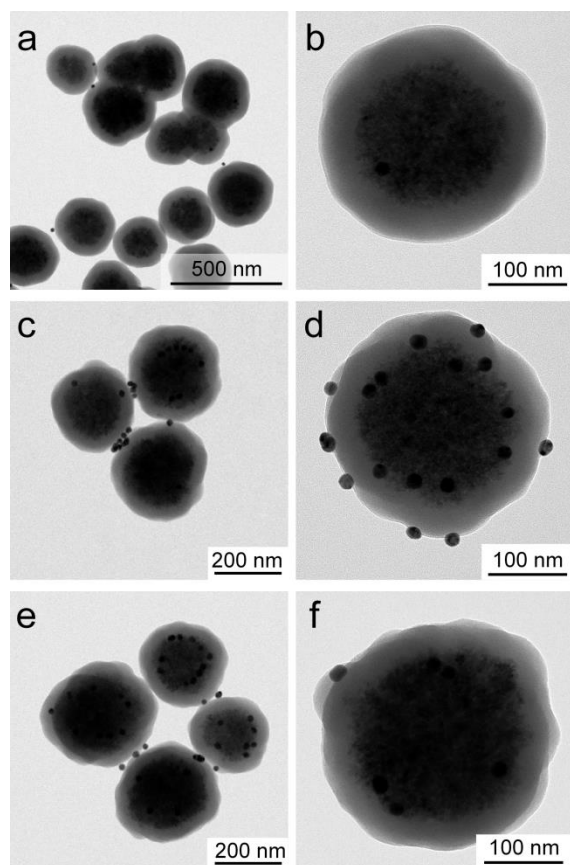
Fig. S7 The TEM analyses of particle assemblies prepared by mixing Au NPs with (a,b) silica coated iron oxide NPs, (c,d) carboxylic acid (i.e., 11-hydroxyundecanoic acid or HUDA) functionalized core-shell NPs, and (e,f) alcohol (i.e., 1,10-decandiol) functionalized core-shell NPs.
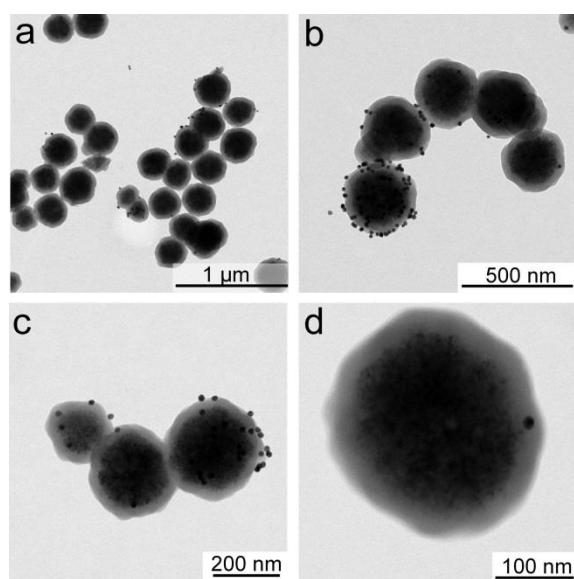
Fig. S8 Representative images from the TEM analyses of samples prepared by mixing Au NPs with the iron oxide nanoparticles that were incubated with thiol alcohol reagent at room temperature (i.e., without using elevated heating to trigger the silanol-alcohol condensation reaction).
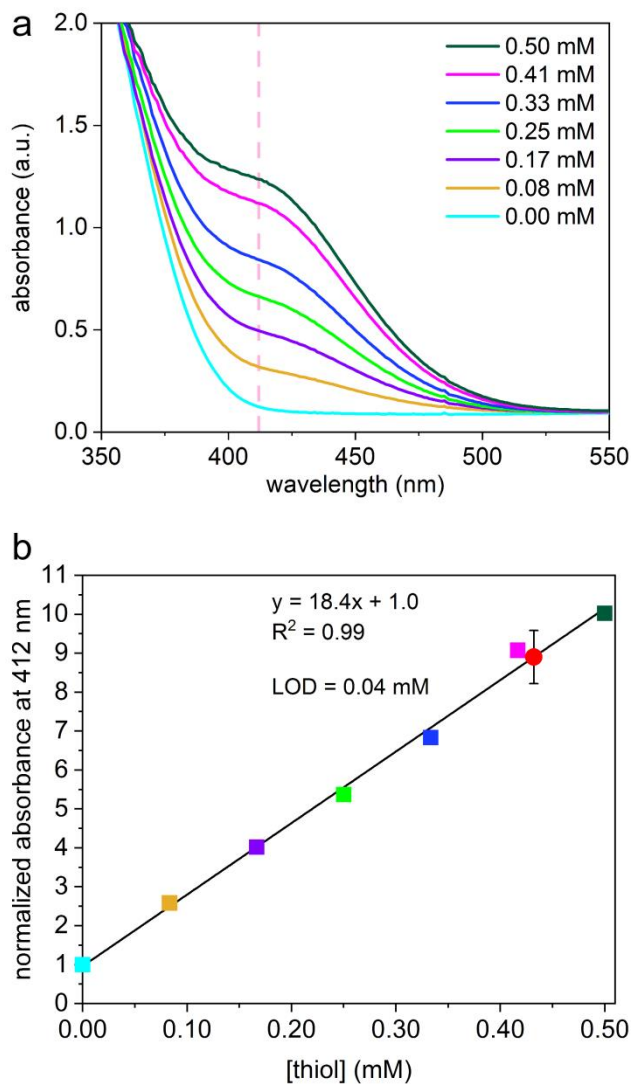
Fig. S9 (a) Absorbance spectra from the Ellman's Test for a series of standards prepared from 11-mercapto-1-undecanol at the concentrations noted in the legend. The response of each standard to the Ellman's Test was recorded as a function of its absorbance at 412 nm, as indicated by the dashed vertical pink line. (b) A calibration curve was prepared from these standard solutions containing increasing concentrations of 11-mercapto-1-undecanol. The red circle on this plot indicates the concentration of thiols associated with the core-shell particles functionalized with 11-mercapto-1-undecanol via a silanol-alcohol condensation reaction.
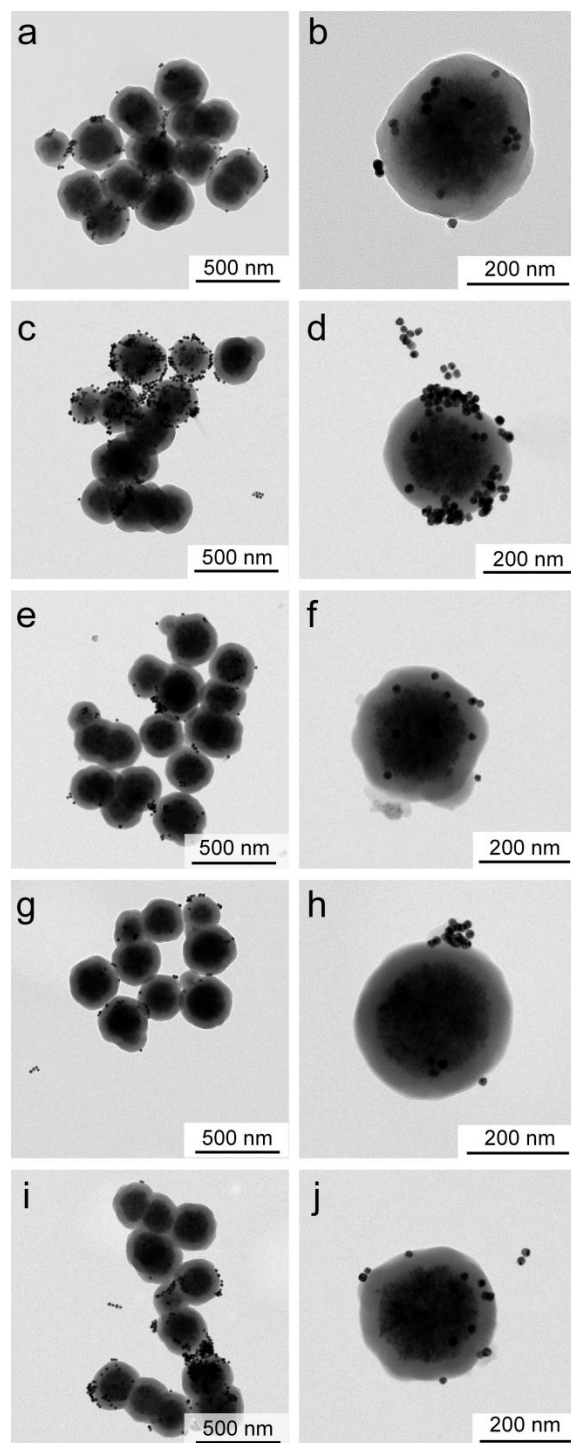
Fig. S10 The TEM analyses of samples prepared by mixing Au NPs with the thiol functionalized core-shell NPs and with the addition of: (a,b) no other surfactants; (c,d) sodium dodecyl sulfate (SDS); (e,f) poly(ethylene glycol) (PEG$_{5000}$) (MW 5,000); (g,h) polyvinylpyrrolidone (PVP); or (i,j) PEG$_{200}$ (MW 200).
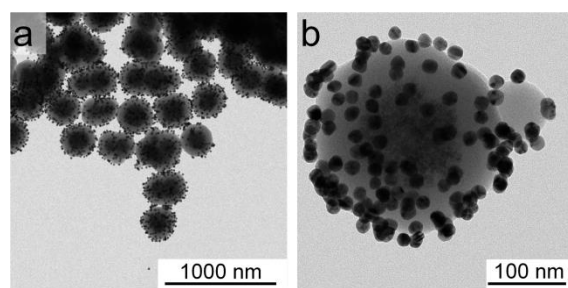
Fig. S11 The representative TEM analyses of the sample prepared by mixing 10 nM of Au NPs with 0.94 nM of the thiol functionalized core-shell magnetic NPs with addition of 0.06% (w/v) mPEG$_{5000}$-SH. The amount of mPEG$_{5000}$-SH in this sample was adjusted to maintain the same ratio of Fe$_3$O$_4$ NPs to PEG-SH that was used to prepare the majority of the assemblies (e.g., Fig. 3d, S5, S6, S7, S8, and S10).

Table S1. Quantification of the number of Au NPs per thiol functionalized core-shell nanoparticle as a function of varying the concentration of both the magnetic NPs and the Au NPs.

| [Fe$_3$O$_4$ NPs]	[Au NPs]	[PEG-SH]	# of Au NPs per magnetic particle [a]
0.94 nM	1.0 nM	0.3 % (w/v)	12 ± 3
4.7 nM	1.0 nM	0.3 % (w/v)	43 ± 12
0.94 nM	10 nM	0.3 % (w/v)	18 ± 6
4.7 nM	10 nM	0.3 % (w/v)	320 ± 90
0.94 nM	10 nM	0.06% (w/v)	190 ± 40

[a] Mean values calculated for each sample along with the distribution from these means reported as one times the standard deviation. These values were each calculated from a sample of at least 5 separate core-shell particles.

Table S2. Quantification of the number of Au NPs on the surfaces of the magnetic particles with different functionalities [+]

type of functionalization	# of Au NPs per magnetic particle [a]
native silica surfaces	6 ± 3
room temperature incubation with 11-mercapto-1-undecanol	16 ± 16
carboxylic acid functionalization (11-hydroxyundecanoic acid)	38 ± 7
alcohol functionalization (1,10-decandiol)	21 ± 9
thiol functionalization (11-mercapto-1-undecanol)	320 ± 90

[+] All particles were prepared from a shell of silica on an iron oxide core.

[a] Mean values calculated for each sample along with the sample variance reported as one times the standard deviation from the mean. These values were each calculated from a sample of at least 5 separate core-shell particles.
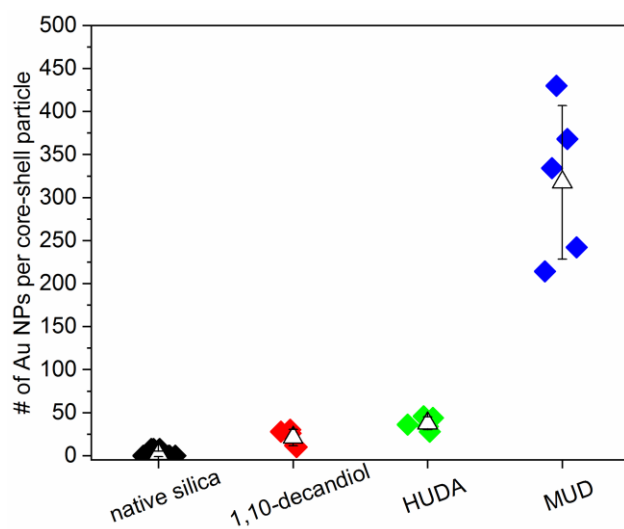
Fig. S12 Scatter plot of the number of Au NPs on the surfaces of the silica coated iron oxide NPs functionalized using the Stöber method or further tuned through an alcohol-condensation reaction (i.e., reacting with 1,10-decandiol, 11-hydroxyundecanoic acid, or 11-mercapto-1-undecanol). The white triangles represent the calculated mean values, and the error bars represent one times the standard deviation from these mean values.

References

1   C. Cheng, Y. Wen, X. Xu and H. Gu, *J. Mater. Chem.*, 2009, **19**, 8782.
2   W. Stöber, A. Fink and E. Bohn, *J. Colloid Interface Sci.*, 1968, **26**, 62–69.
3   A. W. H. Lee and B. D. Gates, *Langmuir*, 2017, **33**, 8707–8715.
4   J. Kimling, M. Maier, B. Okenve, V. Kotaidis, H. Ballot and A. Plech, *J. Phys. Chem. B*, 2006, **110**, 15700–15707.
5   X. Liu, M. Atwater, J. Wang and Q. Huo, *Colloids Surf B Biointerfaces*, 2007, **58**, 3–7.
6   A. van Blaaderen and A. Vrij, *J. Colloid Interface Sci.*, 1993, **156**, 1–18.