Supporting Information

Ligand-Mediated Self-Assembly of Hybrid Plasmonic and Superparamagnetic Nanostructures

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Materials

All reagents used in this study were used without further purification. Cetyltrimethylammonium bromide (CTAB) was purchased from AMRESCO, LLC (Solon, OH, USA). Gold (III) chloride trihydrate ($HAuCl_4$), silver nitrate ($AgNO_3$), iron (III) acetylacetonate ($Fe(acac)_3$), tri(ethylene glycol), trisodium citrate, hydrochloric acid (HCl), and tetraethyl orthosilicate (TEOS, stored in a glovebox) were bought from Sigma-Aldrich (St. Louis, MO, USA). Sodium borohydride ($NaBH_4$) came from Acros (Morris Plains, NJ, USA). L-(+)-ascorbic acid (ascorbic acid) was purchased from Alfa Aesar (Ward Hill, MA, USA). Thiol-poly(ethylene glycol)-acid and methoxy-poly(ethylene glycol)-thiol both had molecular weights of 5000 Da and were ordered from Creative PEG Works (Winston Salem, NC, USA) and Laysan Bio (Arab, AL, USA), respectively. Sodium hydroxide (NaOH) was purchased from JT Baker (Phillipsburg, NJ, USA). Ethanol, ethyl acetate, isopropyl alcohol (IPA), potassium iodide (KI) were all products from Fisher Scientific (Hampton, NH, USA). Deionized and ultrafiltered, or DIUF,, water (18.2 MΩ-cm, deionized and filtered with a Thermo Scientific Barnstead Diamond Water Purification System) was used. Any glassware used was cleaned prior with aqua regia and dried in a 90°C oven before use.

Gold Nanorod Synthesis

CTAB-coated gold nanorods (CTAB-AuNRs) were synthesized with an approach similar to the seed-mediated protocol reported by Nikoobakht and El-Sayed.[1] A nanorod growth solution was prepared in a 250 mL glass beaker by mixing 80 mL of 0.2 M CTAB, 12 mL of 0.01 M $HAuCl_4$, 4.8 mL of 0.004 M $AgNO_3$, and 68 mL of DIUF water. The growth solution was magnetically stirred at 500 rpm and heated to 30°C. Meanwhile, a gold nanoseed solution was prepared by mixing 2.5 mL of 0.2 M CTAB, 1.5 mL of 0.001 M $HAuCl_4$, and 2.5 mL of DIUF water in a 20 mL glass scintillation vial while magnetically stirring at 1100 rpm. Ice cold $NaBH_4$ solution (0.60 mL of 0.01 M) was added to reduce the gold ions in the nanoseed solution and form small gold nanospheres, as indicated by an immediate pale yellow-to-brown color change. While the gold nanoseed solution was allowed to stir for 2 min, 2.0 mL of 0.0788 M ascorbic acid was added dropwise to the growth solution, reducing the gold ions present and inducing a yellow-to-colorless color change. After stirring the gold nanoseed solution for 2 min, the stir bar in the growth solution was removed, and the reaction was allowed to sit for at least 5 min before adding 1.6 mL of the gold nanoseed solution to the growth solution. The growth solution with nanoseeds was allowed to continue stirring for 5 min at 500 rpm before the stir bar was removed. Upon removal of the stir bar, the nanorod solution already exhibited a pale red-purple color, becoming a deep purple-red color within 20 min. The CTAB-AuNR solution was covered with Parafilm and allowed to sit overnight at 35°C. After approximately 24 hr, the CTAB-AuNR solution had an OD of approximately 6 at wavelengths between 750 and 800 nm. The CTAB-AuNR solution was centrifuged once at 36,000 g for 30 min and resuspended with DIUF water to a volume that yielded a final CTAB-AuNR solution with an OD between approximately 13 and 15.

Gold Nanorod PEGylation

CTAB-AuNRs were functionalized with COOH-PEG-SH and mPEG-SH in 5 mL batches. To make a batch of COOH-AuNRs/mPEG-AuNRs, 5 mL of CTAB-AuNR solution was added to a 20-mL glass scintillation vial and mixed with 5 mL of a 1 mg/mL COOH-PEG-SH-mPEG-SH in DIUF water solution. The CTAB-AuNR and COOH-PEG-SH/mPEG-SH mixture was sonicated at room temperature for 5 min before being allowed to sit overnight at 30°C. After approximately 20 to 24 hr, the COOH-AuNRs/mPEG-AuNRs were collected by centrifugal filtration in 100 kDa MWCO Millipore Centrifugal Filters: the COOH-AuNR/mPEG-AuNR solutions were centrifuged twice at 3000 g for 20 min and resuspended after each centrifugation with DIUF water. After the second centrifuge cycle, the collected PEGylated AuNRs were resuspended to their original volume of 5 mL. In this step, it was common for some of the COOH-AuNRs to adhere to the centrifugal filter, while negligible adherence of mPEG-AuNRs to the filter was observed.

Extinction maxima approximately blue-shifted by no more than about 20 nm after PEGylation of the AuNRs with COOH-PEG-SH/mPEG-SH. Any decreases in OD of the COOH-AuNRs/mPEG-AuNRs inevitably resulted from the centrifugal filtration step.

"Bare" Superparamagnetic Iron Oxide Nanoparticle (SPION) Synthesis

"Bare", TREG-coated, superparamagnetic iron oxide nanoparticles (TREG-SPIONs) were synthesized through a thermal decomposition reaction of Fe(acac)$_3$ in TREG without a surfactant present.[2] Fe(acac)$_3$ (1 g) was added to 20 mL of TREG in a 50 mL 3-neck flask. Under reflux in an inert argon atmosphere, the reaction mixture was magnetically stirred at 1000 rpm and heated to a temperature between 250-260°C, at which point the reaction was allowed to react for 4 hr. The black TREG-SPION solution produced was allowed to cool to room temperature before it was transferred to a 20 mL glass scintillation vial for long-term storage.

Self-Assembly Method for Synthesizing Superparamagnetic Iron Oxide Nanoparticle-Studded Gold Nanorods (SPION-AuNRs)

Before they could be mixed with the COOH-AuNRs, the TREG-SPIONs were first cleaned using a series of centrifugation steps. 100 μL of TREG-SPION in TREG solution was mixed with 0.75 mL ethanol and 0.75 mL of ethyl acetate. The TREG-SPIONs dispersed easily in ethanol, while the ethyl acetate caused them to flocculate and become easier to collect by centrifugation. The TREG-SPION in ethanol and ethyl acetate mixture was centrifuged for 5 min at 14100 g in a 2 mL microcentrifuge tube, and the TREG-SPION pellet was collected by decanting the ethanol, ethyl acetate, and any remnant reaction byproducts from the TREG-SPION synthesis. The pellet was redispersed by sonification in 0.75 mL of ethanol and 0.75 mL ethyl acetate and centrifuged again. This process was repeated so that the TREG-SPIONs were collected in a pellet three times. On the final centrifugation, the pellet of TREG-SPIONs was decanted, dried by flow of nitrogen gas for about 1 min, then resuspended in 2 mL of pH = 3-4 HCl solution and transferred to a 20 mL glass scintillation vial. Brief sonication was sometimes required to redisperse small flocculated aggregates. To this TREG-SPION in HCl solution, 2 mL of COOH-AuNR solution was quickly added. The mixture of TREG-SPIONs and COOH-AuNRs was sonicated for 5 min and shaken with a vortex mixer at 500 rpm for 60 min.

After 60 min of shaking, a UV-Vis spectrum was obtained for the reaction mixture containing SPION-AuNRs and excess TREG-SPIONs. Across multiple experiments, the reaction mixture's extinction maxima repeatedly red-shifted by about 50 nm compared with that of the COOH-AuNRs. Microcentrifugation of the reaction mixture for 30 min at 1,500 g provided a means for removing most of the unbound TREG-SPIONs from the SPION-AuNRs, however this also resulted in some loss of SPION-AuNRs, as confirmed by UV-Vis spectra of the resuspended SPION-AuNR pellet and the supernatant decanted from the pellet (see Figure S2 below and Figure 2 in main manuscript) Centrifuged pellets of hybrid nanostructures were resuspended to a volume of 2 mL

For the mPEG-AuNR control study, the steps above for performed identically, except the 2 mL of COOH-AuNR solution was substituted by 2 mL of mPEG-AuNR solution.

Gold Nanoplate Synthesis

CTAB-coated gold nanoplates (CTAB-AuNPs) were prepared following a seed-mediated protocol adopted from previous reports.[3,4] First, a gold nanoplate seed solution was prepared by mixing 5 mL of 1 mM $HAuCl_4$, 5 mL of 1 mM sodium citrate, and 10 mL of DIUF water in a 20 mL scintillation vial at 750 rpm. Ice cold 0.6 mL of 0.1 M $NaBH_4$ was added to the gold nanoplates seed solution, causing the solution to become orange in color. The seed solution was stirred for 2 min before the stir bar was removed and the solution was allowed to sit for 2 hr.

Meanwhile, two gold nanoplate growth solutions were made by mixing 396 mL of 0.101 M CTAB, 4 mL of 25 mM $HAuCl_4$, 0.4 mL of 10 mM KI (resulting in a slow orange-to-yellow color change), 0.24 mL of 1 M NaOH, and 8 mL of 0.1 M ascorbic acid (resulted in a yellow-to-colorless color change) in two 500 mL Erlenmeyer flasks. The gold nanoplate growth solutions were prepared at 30ºC and stirred at 400 rpm. After the gold nanoplate seed solution had sat for 2 hr, 1 mL of the seed solution was added to both of the gold nanoplate growth solutions. The stir bars in each growth solution were removed after 15 s had elapsed. Within 1 min, the growth solutions were purple in color, and the gold nanoplates were allowed to grow for approximately 48 hr. After 1 hr had elapsed from the addition of the seed solution, 7.3 g of CTAB was added to each growth solution, their temperatures were increased to 60˚C, and they were allowed to sit overnight.

During this time, the gold nanoplates adhered to the bottoms of the Erlenmeyer flasks, leaving the gold nanospheres produced in the growth solutions.[iv] A simple aspiration system was used to aspirate the solutions of gold nanospheres from the flasks, and 30 mL of DIUF water was added to each flask. The flasks were sonicated for 1 min to collect the gold nanoplates, and the resulting pale green solutions were centrifuged at 20,000 g for 12 min. The supernatants were discarded, and the pellets were combined and resuspended with DIUF water to an OD of approximately 30.

Gold Nanoplate PEGylation

A 6 mL solution of 0.4 mM COOH-PEG-SH was prepared and added to 6 mL of the OD=30 CTAB-AuNP solution. The reaction mixture was sonicated for 30 s and was allowed to sit for 24 hr. It was then centrifuged in 100 kDa MWCO Millipore Centrifugal Filter at 2500 g for 10 min, the collected PEGylated AuNPs (COOH-AuNPs) were resuspended in DIUF water, and the centrifugation step was repeated. The collected COOH-AuNPs were resuspended to a total volume of 3 mL with DIUF water. As observed with the COOH-AuNRs, some of the COOH-AuNPs adhered to the centrifugal filter, causing a loss of COOH-AuNPs.

Self-Assembly Method for Synthesizing Superparamagnetic Iron Oxide Nanoparticle-Studded Gold Nanoplates

TREG-SPIONs (10 μL) were first cleaned and suspended in 2 mL of pH = 3.5 HCl solution following the same steps outlined for the SPION-AuNR synthesis. This TREG-SPION in HCl solution was added to a 20 mL scintillation vial along with 0.75 mL of the COOH-AuNP solution and 1.25 mL DIUF water. The reaction mixture was sonicated for 5 min and shaken with a vortex mixer at 500 rpm for 60 min.

Silica-Coating SPION-AuNRs

To create silica-coated SPION-AuNRs, a concentrated batch of SPION-AuNRs was first prepared from a 4 mL reaction mixture of TREG-SPIONs and COOH-AuNRs with twice the concentration of TREG-SPIONs and COOH-AuNRs. This reaction mixture was prepared by first centrifuging 6 mL of the COOH-AuNR solution for 20 min at 26,000 g and resuspending the collected COOH-AuNR pellet to a total volume of 3 mL with DIUF water. 100 µL of TREG-SPIONs were cleaned as previously described and suspended in 2 mL of pH = 3.5 HCl solution. Lastly, this 2x concentrated TREG-SPION in HCl solution was mixed with 2 mL of the 2x concentrated COOH-AuNR solution, and the reaction mixture was shaken with a vortex mixer at 500 rpm for 60 min. Meanwhile, a 3% TEOS in IPA solution was prepared by adding 150 µL of TEOS to 4.85 mL of IPA.

After 60 minutes of shaking, the concentrated reaction mixture of TREG-SPIONs and COOH-AuNRs containing SPION-AuNRs was removed from the vortex mixer. A small cross-shaped stir bar was added to the reaction mixture, and it was magnetically stirred at 400 rpm. Next, 5 mL of IPA was added to and allowed to thoroughly mix with the reaction mixture. Lastly, 1.2 mL of the 3% TEOS in IPA solution was added to begin the silica-coating reaction.

UV-Vis spectroscopy was used to track the progression of the silica-coating procedure.[5] UV-Vis extinction spectra were obtained from dilutions of the silica-coating reaction solution prepared at various times by adding 150 µL of the reaction solution to 2.5 mL of DIUF water (see Figure S4). At 16 hr, 2 mL of the reaction mixture and ~10 mL DIUF water were added to a 100 kDa MWCO Millipore Centrifugal Filter and centrifuged at 2500 g for 5 minutes. The collected pellet was resuspended in 2 mL DIUF water and filtered with a 0.2 µm nylon Whatman syringe filter to remove large, silica-coated aggregates.

Supplemental UV-Vis Spectrophotometry Data and TEM Images of Control Studies

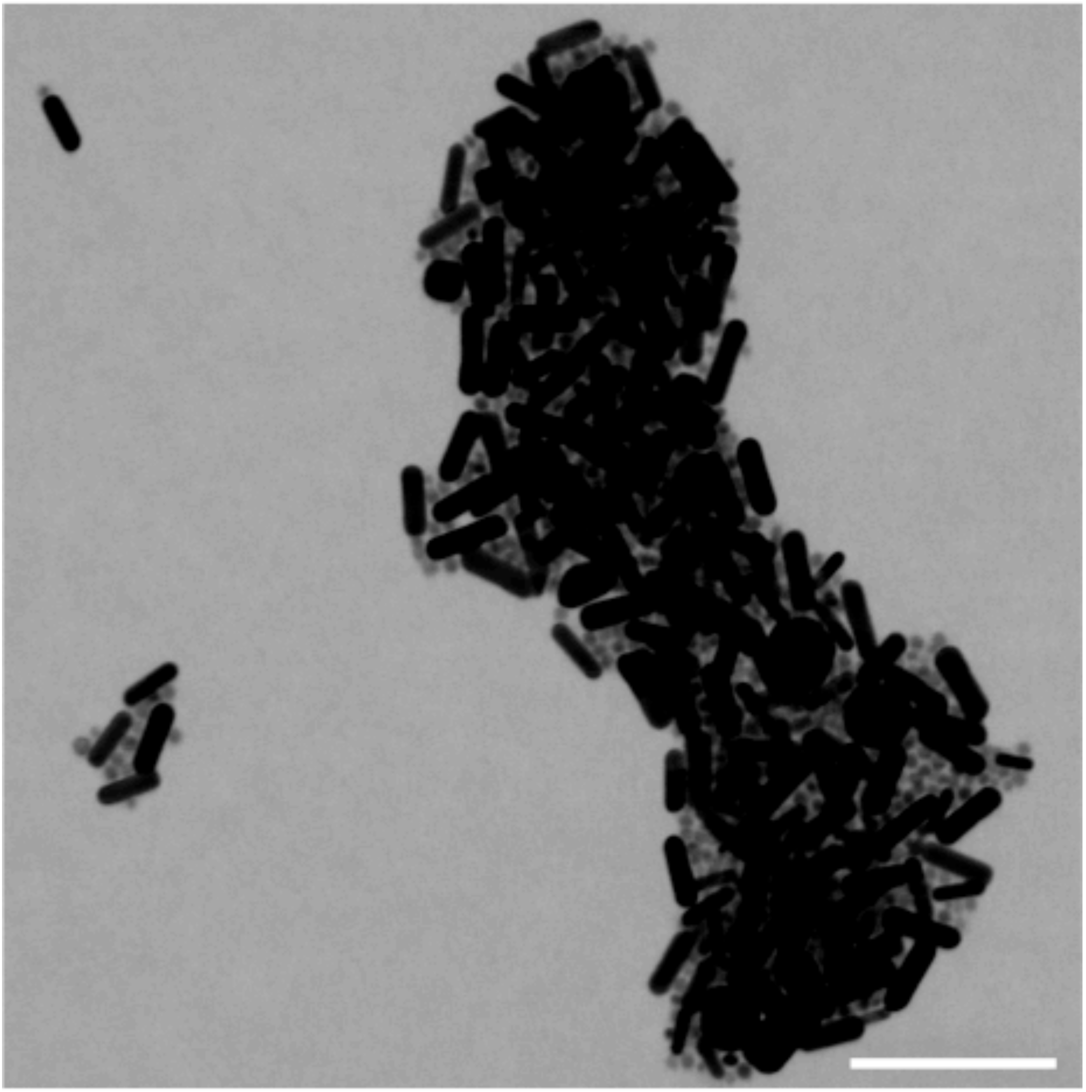


Figure S1. Visible aggregation between COOH-AuNRs and TREG-SPIONs occurs when the ratio of COOH-AuNRs to TREG-SPIONs reacted is too high. In this reaction, 2 mL of COOH-AuNRs was reacted with 1.7 μL of cleaned TREG-SPIONs that had been resuspended in 2 mL of pH = 3-4 HCl solution. The scale bar represents 100 nm.

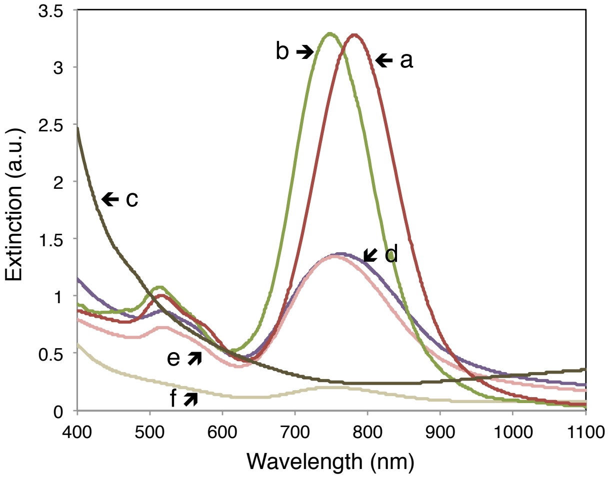


Figure S2. The UV-Vis extinction spectra for (a) 4x diluted CTAB-AuNRs, (b) 2x diluted mPEG-AuNRs, (c) 2x diluted cleaned TREG-SPIONs in pH = 3.5 solution, (d) a 2x dilution of the reaction solution of TREG-SPIONs mixed with mPEG-AuNRs, (e) a 2x dilution of the SPION-AuNRs collected by centrifugation and resuspended in DIUF water, and (f) a 1.3x dilution of the supernatant collected from the centrifuged pellet of mPEG-AuNRs and TREG-SPIONs.

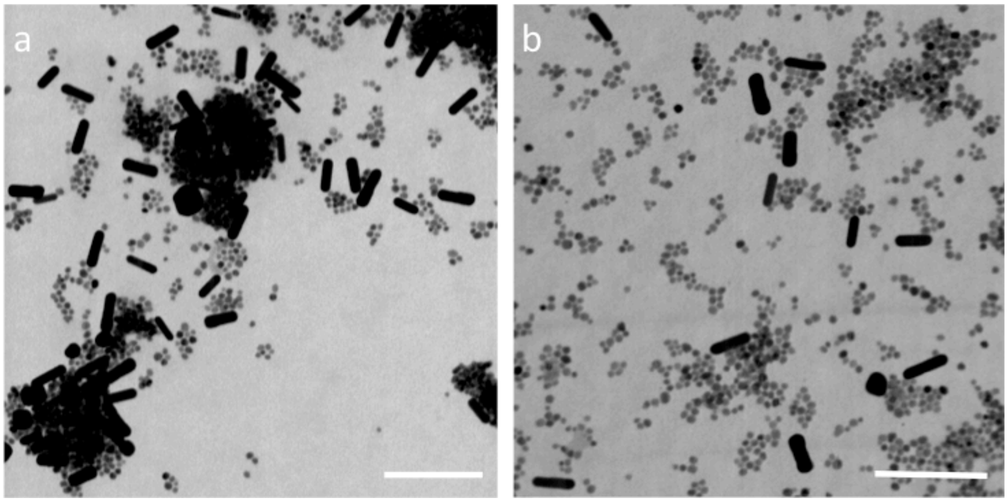


Figure S3. When COOH-AuNRs are reacted with cleaned TREG-SPIONs suspended in (a) DIUF water or (b) pH = 10.5 NaOH solution, no TREG-AuNRs are formed. In DIUF water, the TREG-SPIONs have poor colloidal stability and clump together. COOH-AuNRs will assemble at the edges of these clusters of clumped TREG-SPIONs in order to improve their colloidal stability. Scale bars represent 100 nm.

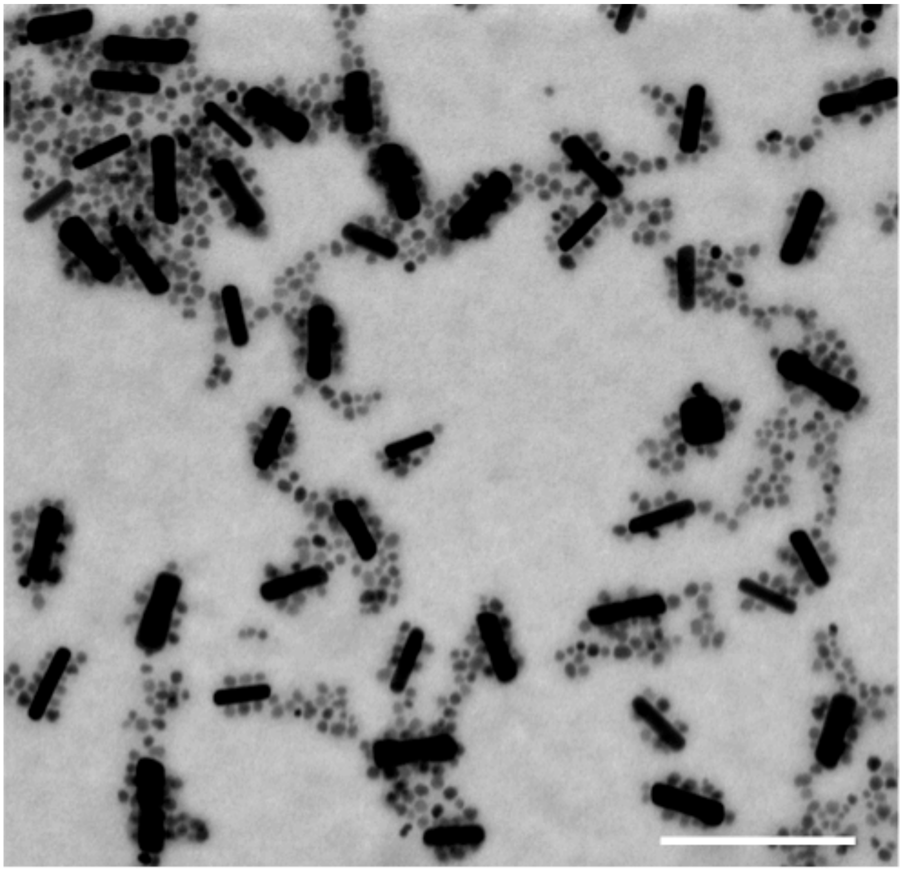
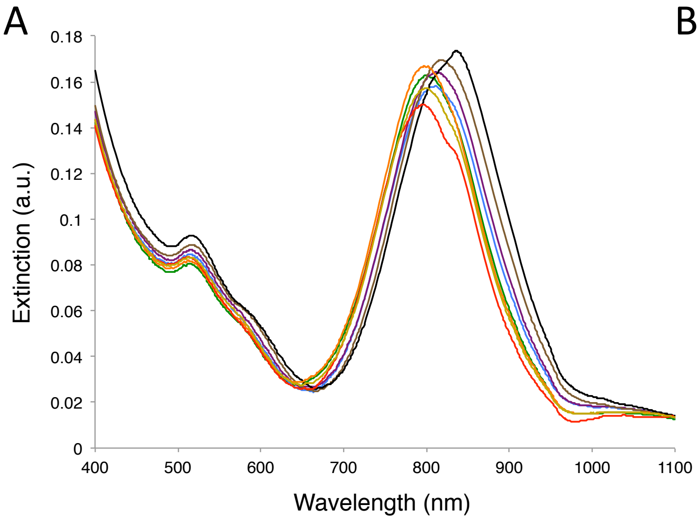


Figure S4. TEM image of TREG-AuNRs collected by microcentrifugation and resuspended in DIUF water. The currently employed microcentrifugation step does not remove all unbound TREG-SPIONs from the SPION-AuNRs, nor does it collect all SPION-AuNRs formed. Upon microcentrifugation and resuspension of the collected SPION-AuNRs to neutral pH with DIUF water, any unbound TREG-SPIONs are removed from the proximity of the AuNRs, resulting in the observation of SPION-AuNRs with some AuNR surface area free of chemisorbed SPIONs. Some nanoscale aggregation between TREG-SPIONs is also evident. The scale bar represents 100 nm.



B

Time of UV-Vis Reading	Wavelength at Extinction Maximum (nm)
40 sec	797 nm
0.5 hr	797 nm
1 hr	800 nm
2 hr	801 nm
4.5 hr	811 nm
5.5 hr	812 nm
16 hr	818 nm
24.5 hr	837 nm

Figure S5. (A) UV-Vis spectra of samples from the silica-coating reaction mixture at 40 s (red), 0.5 hr (orange), 1 hr (yellow), 2 hr (green), 4.5 hr (blue), 5.5 hr (purple), 16 hr (brown), and 24.5 hr (black). (B) The wavelengths of the red-shifting extinction maxima are listed for each time point and correspond to the UV-Vis spectra to the left.

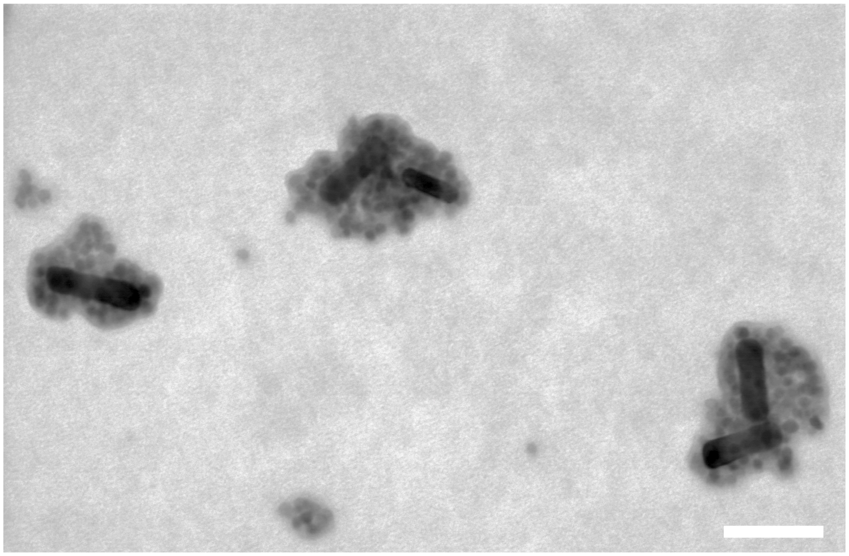


Figure S6. TEM view of multiple silica-coated SPION-AuNRs. Silica-coated TREG-SPIONs are visible at the bottom and left-hand sides of the field of view as well. The scale bar represents 50 nm.

References

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