Supporting Information

Glutathione coated luminescent gold nanoparticles: A surface ligand for minimizing serum protein adsorption

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Experimental Section

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Figure S2: TEM images of HBNPs in H_2O containing 15 ± 3 AuNPs per SPION (A) and in PBS with 10% FBS containing 14 ± 3 AuNPs per SPION (B) after 48 h of incubation period, which shows that the AuNPs and SPIONs are still intact.

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Table 1: Average hydrodynamic diameter of NPs in different solutions, including their polydispersity indices (PDIs).

Experimental Section

Materials and Equipment

$FeCl_3$ and L-glutathione (reduced) were purchased from Sigma-Aldrich, $FeCl_2(4H_2O)$ from Fluka, NH_4OH and sodium citrate dihydrate from Fischer Scientific, $HAuCl_4(3H_2O)$ from Acros Organics, and the NAP5 or NAP10 columns from GE Healthcare Life Sciences. All of other chemicals were purchased from Sigma-Aldrich and used as received. The hydrodynamic diameter (HD) and zeta potential of the nanoparticles in aqueous solution were analyzed using a Brookhaven 90Plus Dynamic Light Scattering Particle Size Analyzer (DLS) equipped with ZetaPALS Zeta Potential Analyzer. Transmission electron microscopy (TEM) images were obtained with a 200 kV JEOL 2100 transmission electron microscope. Fluorescence measurements were conducted using a PTI QuantaMaster^{TM} 30 Fluorescence Spectrophotometer. Absorption spectra were collected using a Varian 50 Bio UV-Vis spectrophotometer. Fourier Transform Infrared (FTIR) spectra were obtained using Nicolet 380 ThermoScientific. The elemental analysis for Fe and Au were conducted using an Agilent 7700x ICP Mass Spectrometer. A Vibrating Sample Magnetometer (VSM) was used to measure the magnetic properties of the nanoparticles at 25 ^oC (NPs were first freeze-dried for 24h and the powdered samples were collected for measurements). T_2 relaxation time measurements were obtained also at room temperature (25 ^oC) using Maran Ultra relaxometer (23 MHz, 0.5T) and Fe concentrations from 0.7 mM to 24 mM.

Synthesis of superparamagnetic γ-Fe_2O_3 nanoparticles (SPIONs). First, 50 ml of deionized H_2O in a round bottom (RB) flask was N_2 bubbled for 30 minutes, with mild stirring to deoxygenate the H_2O. Meanwhile, 0.1M (100 ml) and 0.01M (50 ml) HNO_3 were prepared. After 30 min of bubbling, 5 ml of the deoxygenated H_2O (d- H_2O) was taken out of the RB flask with a syringe and placed into a 20 ml scintillation vial, and the remaining volume was kept under N_2 with stirring. Then, 0.17 ml of 12 M HCl was added into the vial and swirled to mix. 1.04 g $FeCl_3$ and 0.64 g $FeCl_2(H_2O)$ was were dissolved in this acid solution. The addition of $FeCl_3$ resulted in a clear brown solution, whereas the addition of $FeCl_2(H_2O)$ resulted in a slightly cloudy yellowish brown

solution. The vial was mixed using a vortex mixer for 2 min, sonicated for 10 min, and vortex mixed again for 1 min. 15 ml of d- H_2O was placed in a 3-neck RB flask containing a stir bar, and the solution of $FeCl_3$ and $FeCl_2(H_2O)$ was added. An addition funnel was placed into the middle neck and a needle (attached to a hose that connects to the N_2 source) was inserted into one of the other two necks of the flask covered with rubber stoppers. Three syringe needles were placed through the rubber stopper as a means for air to escape. The N_2 source was switched on and the stir bar was set to the max. Afterwards, 25 ml of NH_4OH was transferred into the addition funnel, which was immediately capped in order to prevent the loss of NH_4OH in gaseous form. The stopcock was turned so that 1 to 2 drops of the base were added per second. Black precipitates immediately formed. The mixture was left stirring for 2 hours. Then, the stir bar was removed and the flask was set on a magnet for a few minutes until all of the black precipitates aggregated at the bottom. The supernatant was discarded and the precipitates were dispersed in 25 ml deionized (d.i.) H_2O for washing. This dispersion was transferred into a large capped vial and shaken manually. The precipitates were washed 2 more times with d.i. water, but after decanting the 3rd supernatant, the precipitates were re-dispersed in 25 ml 0.01 HNO_3. Again, the precipitates were collected using a magnet and dispersed into 35ml 0.1M HNO_3. A vortex mixer was used to mix the solution thoroughly for 1 m. This solution was centrifuged @ 3000g for 1 min and the supernatant was transferred into a RB flask with a condenser for a 30 min reflux (T = 120 ºC). During reflux, the brown solution turned more red indicating that the Fe_3O_4 was oxidized to γ-Fe_2O_3 (maghemite). Afterwards, the maghemite solution was completely removed from heat and cooled to room temperature (with continued stirring and water flowing through the condenser for 1 h). When the solution was cool enough, it was centrifuged @ 6000g for 3 min, then @ 21000g for 2 min, and the supernatant was stored.

Citrate modification of SPIONs (Cit-SPIONs). To the SPION solution as described above, trisodium citrate powder was added to make a 1 M citrate solution and mixed well until all the citrates was dissolved. The solution was transferred into a RB flask with a stir bar, placed in a 37

ºC oil bath, and left to mildly stir for 2 h. Then, the solution was centrifuged at 3000g for 3 min, and the precipitates were redispersed in d.i. H_2O.

Glutathione modification of SPIONs (GSH-SPIONs). The GSH powder was directly added into Cit-SPION solution to make 0.14 M solution and vigorously stirred at room temperature for 24 h. Then, the precipitates were collected using a magnet and dispersed in d.i. H_2O. EtOH was added for the purification process in a 1:2 ratio by volume with the solution. The pH was adjusted to 2 (isoelectric point of GSH) by the addition of 1M HCl. The mixture was shaken and the precipitates were collected using a magnet, and dispersed in d.i. H_2O. The pH was adjusted back to neutral using 1 M NaOH. Finally, the GSH-SPION solution was passed through a NAP10 column for further purification.

Preparation of GS-AuNPs. First, aqueous solutions of 25mM GSH and 25mM $HAuCl_4$ were prepared. Second, 1 ml of the GSH solution was pipetted into a screw capped vial containing 1 ml gold acid solution (without shaking). Third, the sample was incubated for 30 days at room temperature, the time necessary for the fluorescent gold nanoparticles to form. And finally, the sample was centrifuged at 3000 g for 5 min, 6000 g for 3 min, and 21000 g for 2 min.

Integration of the GSH-SPIONs with GS-AuNPs. The newly synthesized GSH-SPION solution was first diluted with d.i. H_2O to half the original concentration and placed in a RB flask with a stir bar. GS-AuNP solution (with double the volume) was added into the GSH-SPION solution and vigorously stirred for 2 days. The sample was purified by adding EtOH in a 1:2 ratio directly into the Au-SG-SPION solution and by adjusting the pH to 2 using 1M HCl. The precipitates were collected using a magnet and dispersed in d.i. H_2O, followed by pH re-adjustment to neutral by adding 1M NaOH. This was repeated 7 more times. The seven supernatant solutions were saved and exposed to a UV lamp. After purification using a magnet, the hybrid sample was purified further by diluting it in PBS at 37 ºC for 1 h, followed by purification using the EtOH and magnet method 8 to 10 times. The sample was centrifuged (3000g for 3 min, 6000g for 2 min, and 21000g for 1 min) and purified even further by passing it through NAP10 column once.

Nanoparticles' Stability Study. The hybrid nanostructures were diluted ¼ of its original concentration in PBS and PBS with 10% FBS and incubated at RT and 37 ºC for 48 h. Within this incubation period, the fluorescence intensity of the particles was measured and monitored. Afterwards, these hybrid nanostructures were purified similar to the procedure above and imaged using TEM. The particles incubated in PBS containing 10% FBS were purified differently by adding 1M HCl until the solution became cloudy (pH ~6), centrifuged at 21000 g for 1 min, and dispersed in H_2O. In a separate study, GSH-SPIONs and hybrid nanostructures were also incubated in PBS and PBS with 10% FBS at 37 ºC for 1 h. Then, the particles were purified by adding EtOH (same volume), magnetically separated, and dispersed in PBS. This was repeated two more times. A Dynamic Light Scattering Particle Size Analyzer was used to measure the hydrodynamic diameter of the nanoparticles.

Determination of Quantum Yield. First, the absorbances of the NPs (GS-AuNPs and HBNPs), as well as the R6G dye, were measured using a Varian UV-Vis spectrophotometer. Their absorptions were kept below 0.05 A.U. for linearity and accuracy purposes. Then, the fluorescence spectra of the NPs and R6G were obtained using PTI fluorescence spectrophotometer. The emission spectra were plotted and the area under the curve was analyzed using the software OriginPro version 8. The calculation of quantum yield is shown in Fig. S5.

Supplementary Figures

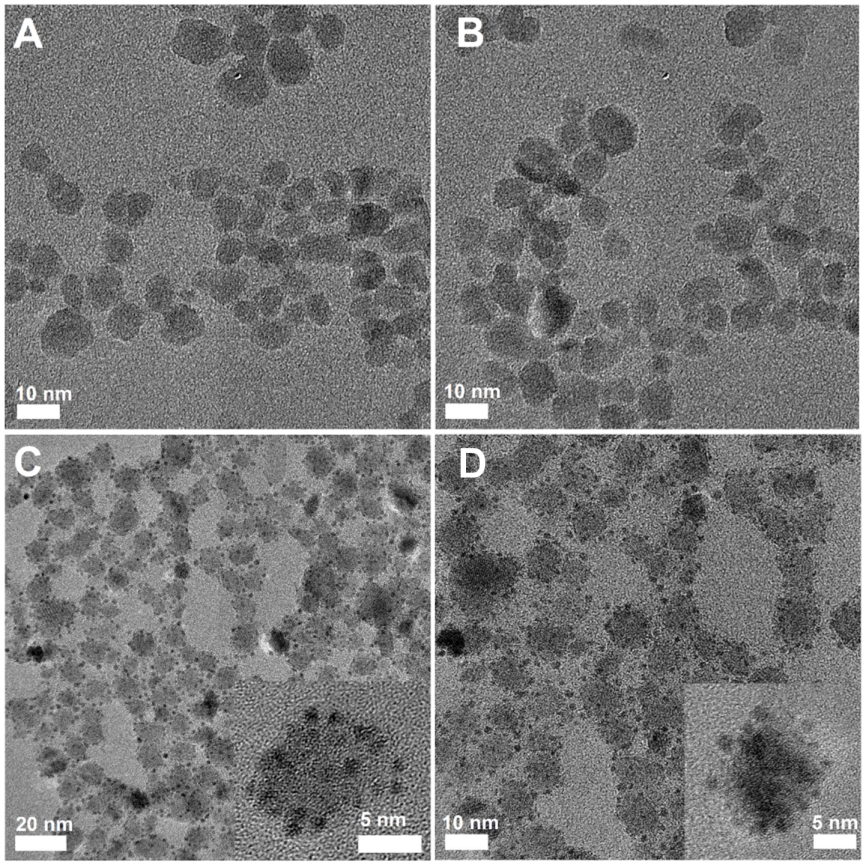


Figure S1. Additional TEM images of the GSH-SPIONs (A & B) and hybrid nanostructures (C & D).

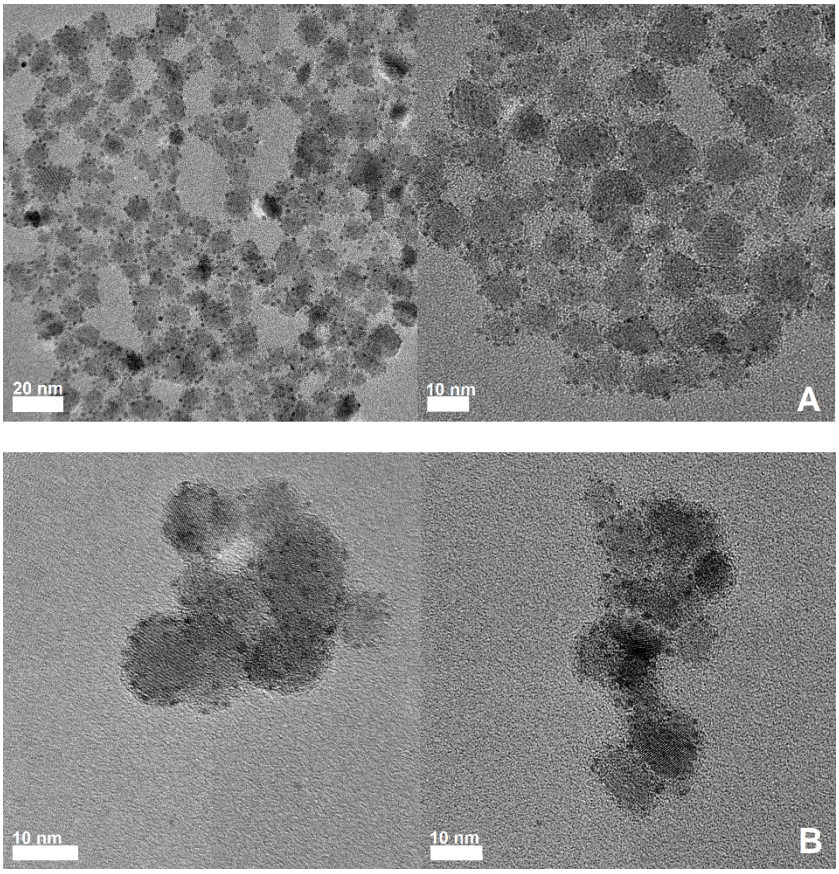


Figure S2. TEM images of HBNPs in H_2O containing 15 ± 3 AuNPs per SPION (A) and in PBS with 10% FBS containing 14 ± 3 AuNPs per SPION (B) after 48 h of incubation period, which shows that the AuNPs and SPIONs are still intact.

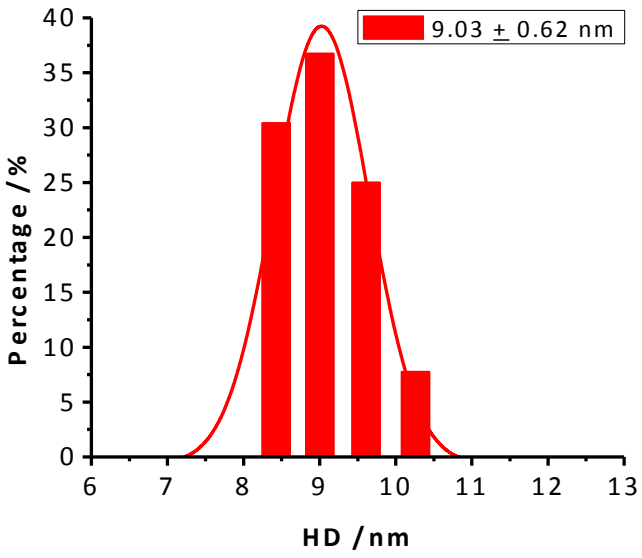
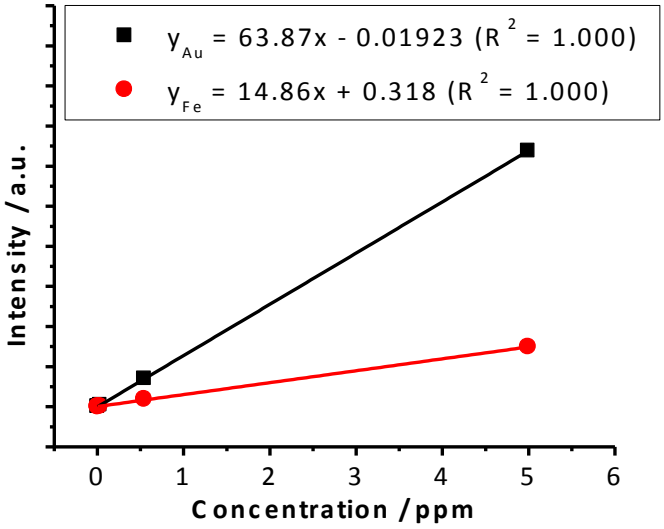


Figure S3. Particle distribution of citrate-SPIONs in aqueous solution.



Sample Name	Fe [ppm]	$Fe_{HBNPstock}$ [ppm]	Au [ppm]	$Au_{HBNPstock}$ [ppm]
HBNP 10kx	0.004438282	4438.28	-	-
HBNP 1kx	0.036790488	3679.05	0.005797161	579.72
HBNP 100x	0.597129206	5971.29	0.082860772	828.61
HBNP 10x	-	-	1.042630086	1042.63
	Average	**4696.21**		**816.98**

Figure S4. ICP-MS data used to determine the Fe and Au content in the nanoparticles.

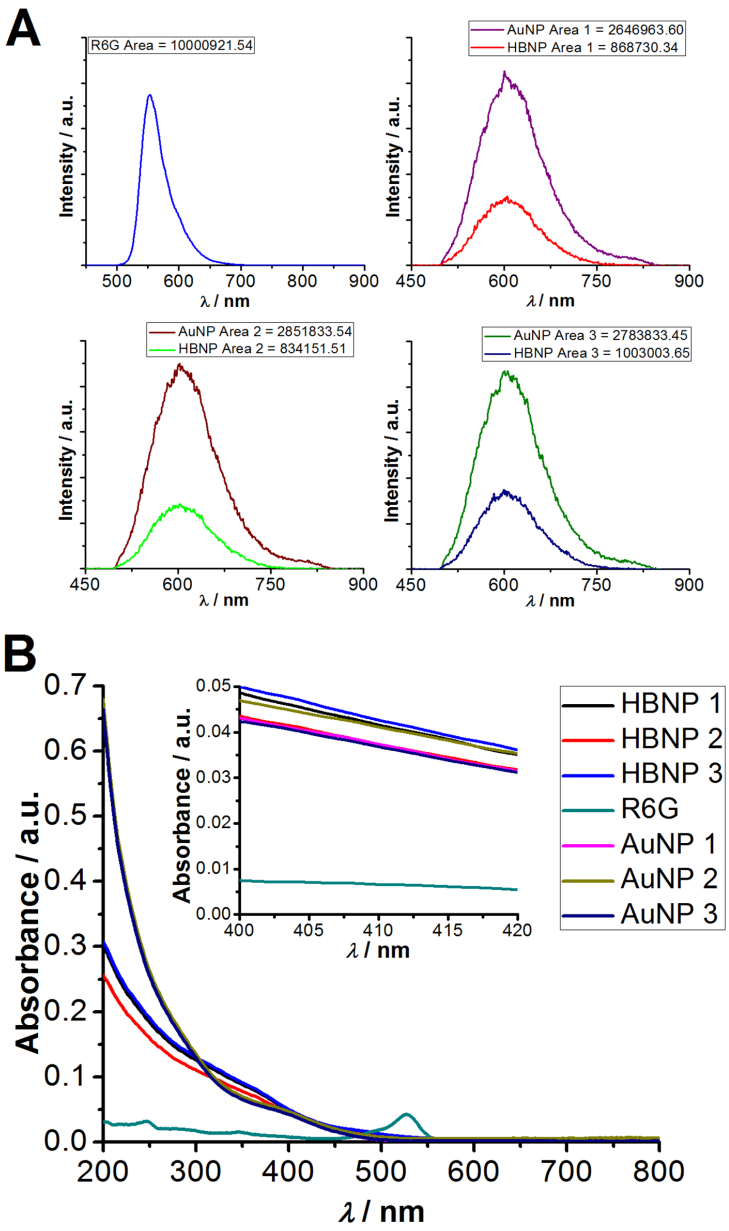


Figure S5. The Quantum Yield of the HBNPs was determined by comparing it with R6G, as the reference. This illustrates the fluorescence (A) and absorption (B) spectra of R6G and HBNPs.

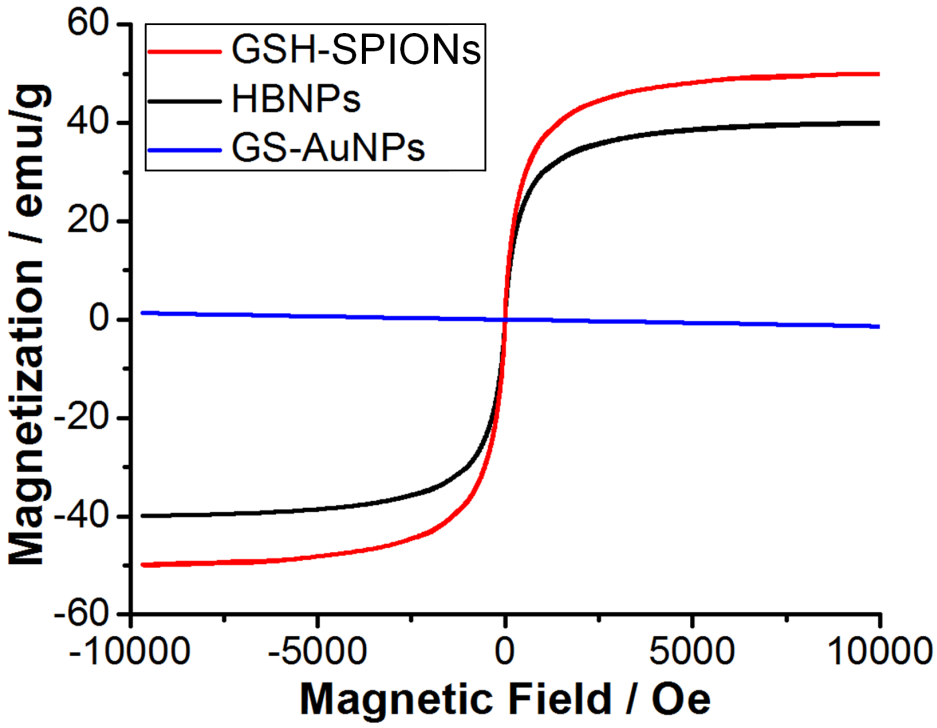


Figure S6. HBNPs and GSH-SPIONs showing their magnetization saturation at the same iron content.

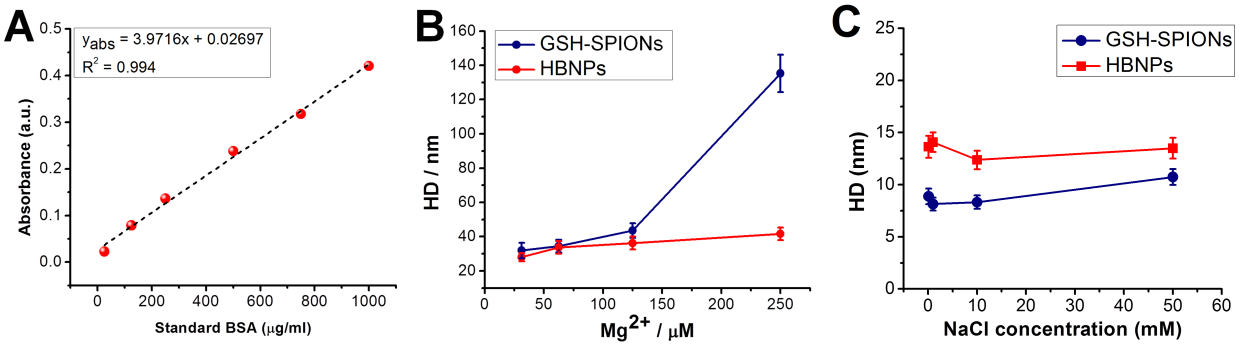


Figure S7. BSA calibration standard for Bradford Protein Assay (quantitative analysis shown below) (A). Each sample (GSH-SPIONs and HBNPs) was diluted in Mg^{2+} (B) and NaCl (C) solutions of different concentrations, and HDs of the NPs were measured after 1h incubation.

	BSA in S* (µg/ml)	**BSA in S* (µg)**	**BSA adsorbed on NP (µg)**	**BSA adsorbed (µg/mg NP)**	**Std. Error (±µg/mg NP)**
GSH-SPIONs	276.43	82.93	63.42	123.45	4.17
HBNPs	433.84	130.15	16.19	12.63	0.43
Control (no NPs)	487.83	146.35	- -	- -	- -

S = supernatant*

BSA in S (µg) = **[**BSA in S (µg /ml)][$V_{solution}$(ml)]

BSA adsorbed on NP (µg) = $(BSA\ in\ S)_{control} - (BSA\ in\ S)_{NP}$

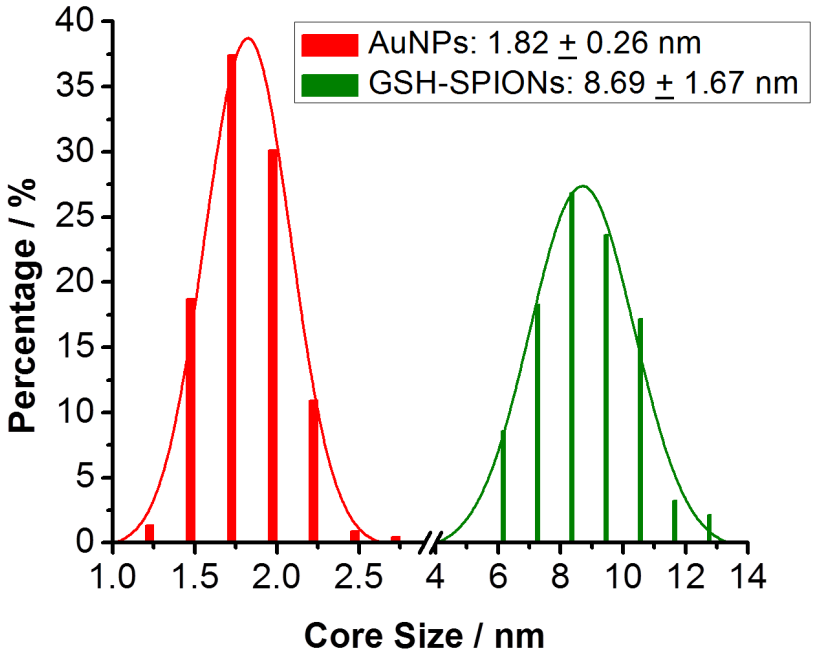


Figure S8. TEM core size analysis of individual AuNPs and SPIONs present in the hybrid nanostructures.

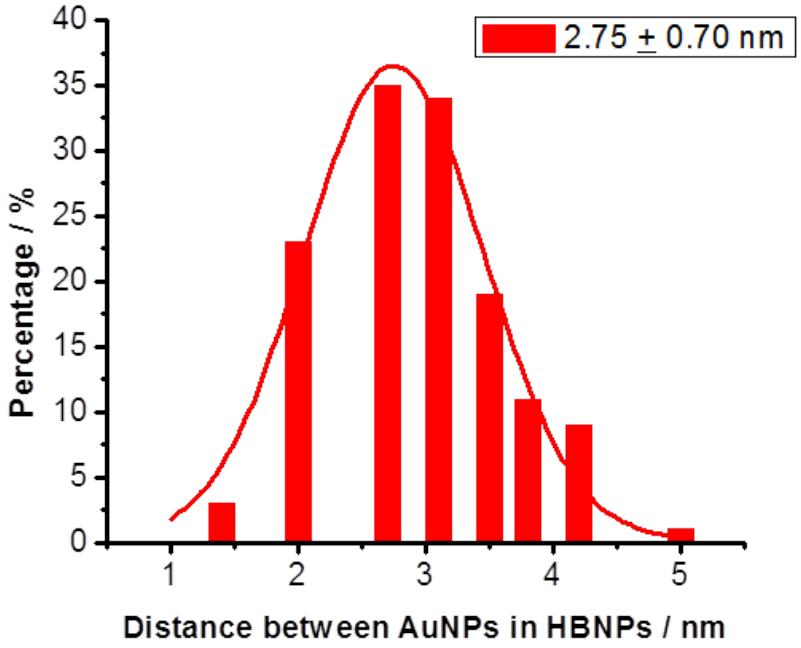


Figure S9. Average distance between the GS-AuNPs attached on the SPION's surface.

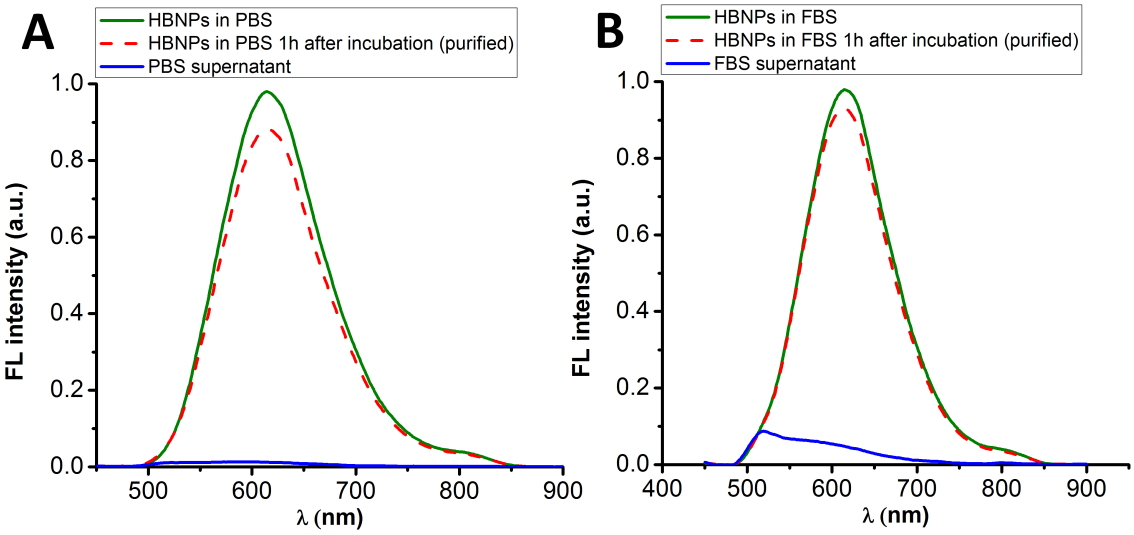


Figure S10. The fluorescence spectra of HBNPs in PBS (0 h), HBNPs in PBS after 1h incubation (purified), and PBS supernatant (A). The fluorescence spectra of HBNPs in FBS (0 h), HBNPs in PBS with 10% FBS after 1h incubation (purified), and FBS supernatant (B).

Sample	Solution	Mean ± Std. Dev. (nm)	Polydispersity Index (PDI)
HBNPs	H_2O	13.63 ± 0.96	0.260
HBNPs	PBS	12.82 ± 0.99	0.277
HBNPs	PBS with 10% FBS	18.02 ± 2.12	0.283
GSH-SPIONs	H_2O	9.18 ± 0.68	0.227
GSH-SPIONs	PBS	50.79 ± 4.86	0.246
GSH-SPIONs	PBS with 10% FBS	110.54 ± 12.26	0.249

Table 1. Average hydrodynamic diameter of NPs in different solutions, including their polydispersity indices (PDIs).