Electronic Supplementary Material (ESI) for Analytical Methods.

Glutathione detection in human serum by gold nanoparticle decorated, monodisperse-porous silica microspheres in magnetic form

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Supporting Information

1. Materials

Tetraethoxysilane (TEOS), tetrabutylammonium iodide (TBAI), 3-aminopropyltriethoxysilane (APTES), concentrated ammonia solution (25 % w/w), isopropyl alcohol (Iso-PrOH), triethylamine (TEA), chloroauric acid tetrahydrate ($HAuCl_4.4H_2O$), trisodium citrate, sodium borohydride phosphate buffer saline (PBS), hydrogen peroxide (50 % w/w) were obtained from Sigma-Aldrich Chem. Co., U.S.A. and used without further purification. *O*-phenylenediamine (OPDA), L-glutathione and human serum (H4522, Human Serum, from human male AB plasma, USA origin, sterile-filtered) were also obtained from Sigma-Aldrich. Distilled deionized (DDI) water with a resistivity of 18 MΩcm obtained from Direct-Q3, Millipore, U.S.A. was used in all runs.

2. Synthesis of SiO_2@Fe_3O_4@SiO_2 microspheres

Fe_3O_4@SiO_2 microspheres were coated by a thin SiO_2 layer according to the following protocol. Fe_3O_4@SiO_2 microspheres (0.2 g) were dispersed in Iso-PrOH/water solution (Iso-

PrOH/water ration: 10/1 v/v, 27.5 mL) containing TBAI (0.125 g) and concentrated ammonia (0.125 mL). TEOS/IsoPrOH solution (2 mL) containing 50 % v/v TEOS was added dropwise in 1 min. The resulting dispersion was mechanically stirred at 300 rpm for 24 h at 40^{o}C. SiO_2 coated Fe_3O_4@SiO_2 microspheres (i.e.SiO_2@Fe_3O_4@SiO_2 microspheres) were isolated by centrifugation at 5000 rpm for 5 min and washed with ethanol and DDI water three times by successive centrifugation and decantation and finally redispersed in DDI water by ultrasonication for 1 min.

3. Attachment of APTES onto SiO_2@Fe_3O_4@SiO_2 microspheres

For hydroxyl enrichment, SiO_2@Fe_3O_4@SiO_2 microspheres were interacted with aqueous HCl solution (25 mL, 5 % w/w) at 80^{o}C, for 6 h with a shaking rate of 120 cpm. The microspheres were extensively washed with DDI water by a successive centrifugation and decantation protocol and dried at 60^{o}C for 24 h. SiO_2@Fe_3O_4@SiO_2 microspheres (0.10 g) were degassed at 250^{o}C in vacuo for 6 h.

The microspheres were dispersed in Iso-PrOH (20 mL) containing TEA (0.3 mL) by vortexing. APTES (3 mL) was added dropwise and the resulting dispersion was shaken at 120 cpm for 24 h, at 80^{o}C, in a sealed Pyrex® glass reactor. APTES attached form SiO_2@Fe_3O_4@SiO_2 microspheres were extensively washed with ethanol and water, by successive centrifugation and decantation.

4. Synthesis of Au nanoparticles (Au NPs)

The synthesis of Au NPs was performed according to Turkevich method.[2] $HAuCl_4$ solution (1 mM, 24 mL) was heated until boiling under magnetic stirring at 250 rpm. Aqueous trisodium citrate solution (2 mL, 1.75 % w/w) was added dropwise into the medium. The boiling was continued for 15 min. The resulting dispersion was cooled down to room temperature, under magnetic stirring at 250 rpm.

5. Characterization of nanozyme

The mean size, size distribution and morphological properties of Au@SiO_2@Fe_3O_4@SiO_2 microspheres were determined by Scanning Electron Microscope (FEI, Quanta 200, FEG, a

part of Thermo Fisher Scientific, Waltham, MA, U.S.A.). The crystal structure of $Au@SiO_2@Fe_3O_4@SiO_2$ microspheres was investigated using X-ray diffractometer with CuKa1 radiation (λ) 1.54060 nm) operating at 30 mA and 40 kV (Scintag, a part of Thermo Fisher Scientific, Waltham, MA, U.S.A.). A surface area and pore size analyzer (Nova 2200e, Quantachrome, a part of Thermo Fisher Scientific, Waltham, MA, U.S.A.) was used for the determination of porous properties (i.e. pore size distribution, median pore size, pore volume and specific surface area, SSA) of the same microspheres, according to nitrogen adsorption/desorption method.

6. Peroxidase-like activity of $Au@SiO_2@Fe_3O_4@SiO_2$ microspheres

Peroxidase-mimetic activity of $Au@SiO_2@Fe_3O_4@SiO_2$ microspheres were investigated in the pH range of 4-9 using OPDA as the synthetic substrate. Before the enzyme-mimetic activity run, the microspheres were washed with the selected buffer solution two times, by centrifugation and decantation. The microspheres (10 mg) were dispersed in the OPDA solution (400 μM, 4 mL) at a certain pH and H_2O_2 solution (50 % w/w, 2 μL) was added into the resulting dispersion. The reaction was continued with a prescribed time with a rotating rate of 120 cpm in dark at room temperature (22^{o}C). The magnetic separation was applied for the removal of $Au@SiO_2@Fe_3O_4@SiO_2$ microspheres from the reaction medium using an external magnet. The absorbance of sample was measured in a UV-Vis spectrophotometer (Thermo Scientific, U.S.A.) at 416 nm. Initial OPDA consumption rate ($-r_{OPDA}$, μM OPDA/min) was calculated as described elsewhere.[1] The Michaelis-Menten plot of nanozyme was obtained by repeating this protocol with different OPDA initial concentration ranging between 50-500 μM.

7. Equilibrium GSH adsorption onto the nanozyme.

The microspheres were washed with 50 mM pH 7 phosphate buffer by centrifugation and decantation. The microspheres (10 mg) were dispersed in the same buffer (3 mL) containing GSH at an initial concentration ranging between 810-19500 μM. The adsorption was continued for 2 h with a rotating rate of 120 cpm at room temperature (22^{o}C). At the end of the equilibrium period, magnetic separation was applied for $Au@SiO_2@Fe_3O_4@SiO_2$

microspheres. The supernatant was analyzed using HPLC (Shimadzu, SPD-10 AV, Japan) using a reversed phase column (Phenomenex, SphereClone, 5 μm silica, 250x4.6 mm) in a UV-Vis spectrophotometer (Thermo Scientific, U.S.A.) at 416 nm. The equilibrium GSH adsorption, Q_{GSH} (mg GSH/g nanozyme) was calculated using the initial (C_o) and final concentration (C_f) in the adsorption medium (V, mL) according to the following expression.Where, M_n (g) is the amount of nanozyme used in the adsorption run.

$$Q_{GSH} = (C_o - C_f)xV/M_n \quad (1)$$

8. Determination of GSH in human serum by Ellman's method

The total glutathione concentration of the human serum was determined by the glutathione reductase-DTNB (5,5′-Dithio-bis-[2-nitrobenzoic acid], Ellman's reagent) recycling method as described in previous studies.[3-5] This method is based on the reaction of GSH with DTNB that produces a yellow colored TNB (5-thio-2-nitrobenzoic acid), which has a maximal absorbance at 412 nm. The rate of change in absorbance is linearly proportional to the total GSH concentration. The concentration of an unknown sample is determined by using linear regression to calculate the values obtained from the standard curve. Briefly, human serum, which is low in protein (<1 mg/mL) due to dilutions, was used without the deproteination procedure. The assay buffer was 100 mM sodium phosphate with 5 mM EDTA disodium salt, pH 7.4. Standards (0-10 μM as GSH equivalent) were prepared by two fold serial dilutions using a GSSG stock solution. The microtiter plate was prepared by pipetting 20 μl of assay buffer (blank), standards and samples per well. At this time the following freshly prepared reagents are mixed: 60 μL of 1.68 mM DTNB and 60 μl of 3.3 U/ml glutathione reductase. Immediately, 120 μL of the mixture was pipetted into each well. Finally, 60 μL of β-NADPH were added to each well and the plate was read at 412 nm using the microplate reader (SpectraMax M2, Molecular Devices). Measurements was taken every 30 seconds for 2 min. A standard curve of GSH was generated using SoftMax Pro software (version 4.8; Molecular Devices, LLC, San Jose, CA, USA) and Microsoft Excel (Microsoft, Redmond, WA, USA). The samples and standards were assayed in triplicate.[6]

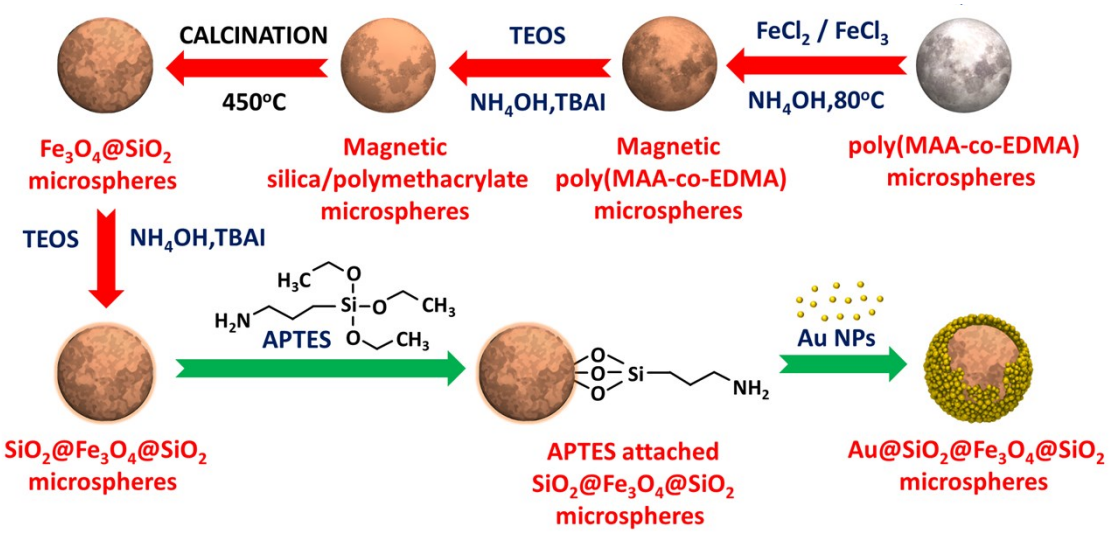

Figure S1. The schematical representation of the synthesis protocol for $Au@SiO_2@Fe_3O_4@SiO_2$ microspheres.

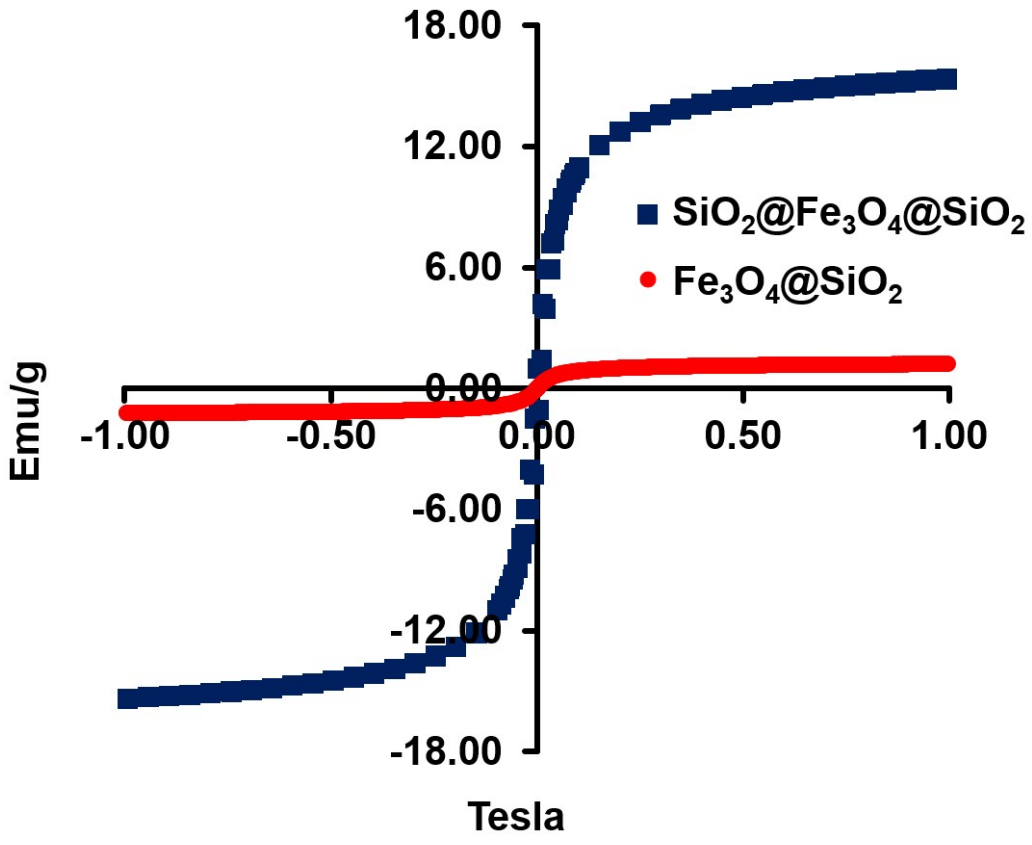

Figure S2. Magnetization curves of $Fe_3O_4@SiO_2$ and $SiO_2@Fe_3O_4@SiO_2$ microspheres obtained by vibrational sample magnetometry after acid leaching. Amount of microspheres: 100 mg, HCl solution (5 % w/w): 25 mL. Shaking rate: 120 cpm, Temperature: 80°C, Time: 6 h.

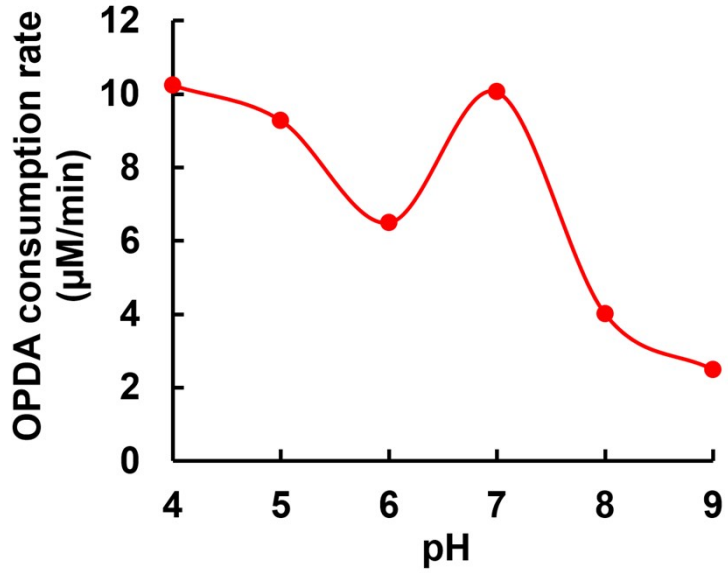

Figure S3. The variation of peroxidase like activity with pH for $Au@SiO_2@Fe_3O_4@SiO_2$ microspheres. Reaction volume: 4 mL, Microsphere amount: 10 mg, pH 7.0, H_2O_2 solution: 2 μL, OPDA concentration: 400 μM, Rotating rate: 120 cpm, Room temperature, 90 min.

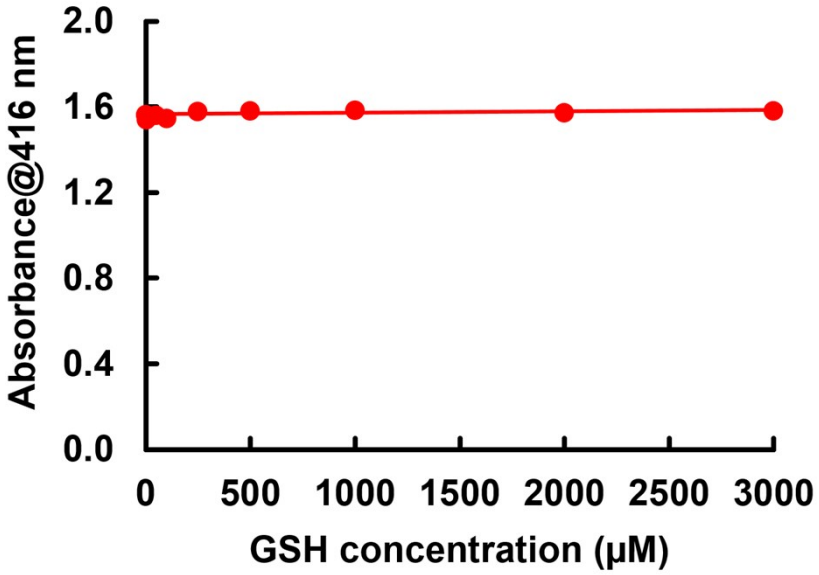

Figure S4. The effect of added GSH concentration on the resulting absorbance obtained by the colorimetric conversion in the absence of GSH. Concentration of $Au@SiO_2@Fe_3O_4@SiO_2$ microspheres: 2.5 mg/mL, H_2O_2: 2 μL, Initial OPDA concentration: 400 μM, Time: 30 min, Rotating rate: 100 cpm, Room temperature.

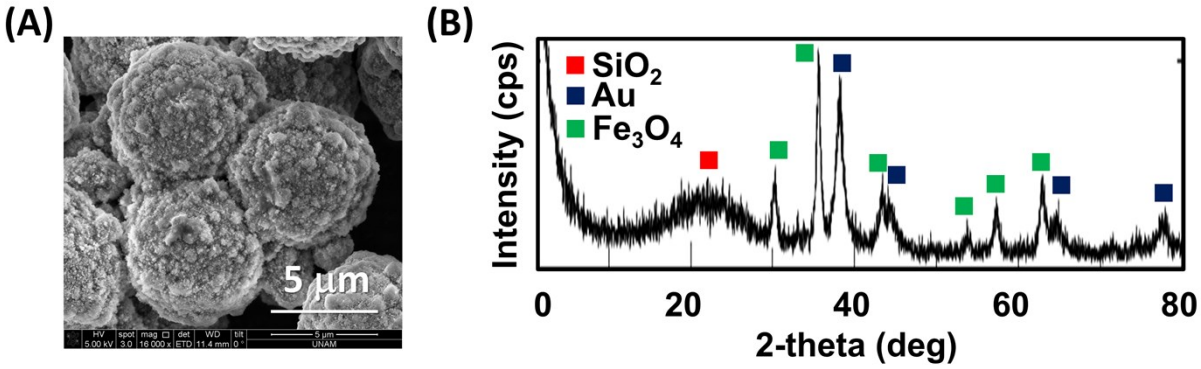

Figure S5. (A) SEM image, and (B) XRD spectrum of $Au@SiO_2@Fe_3O_4@SiO_2$ microspheres after colorimetric determination of GSH via peroxidase-like reaction.

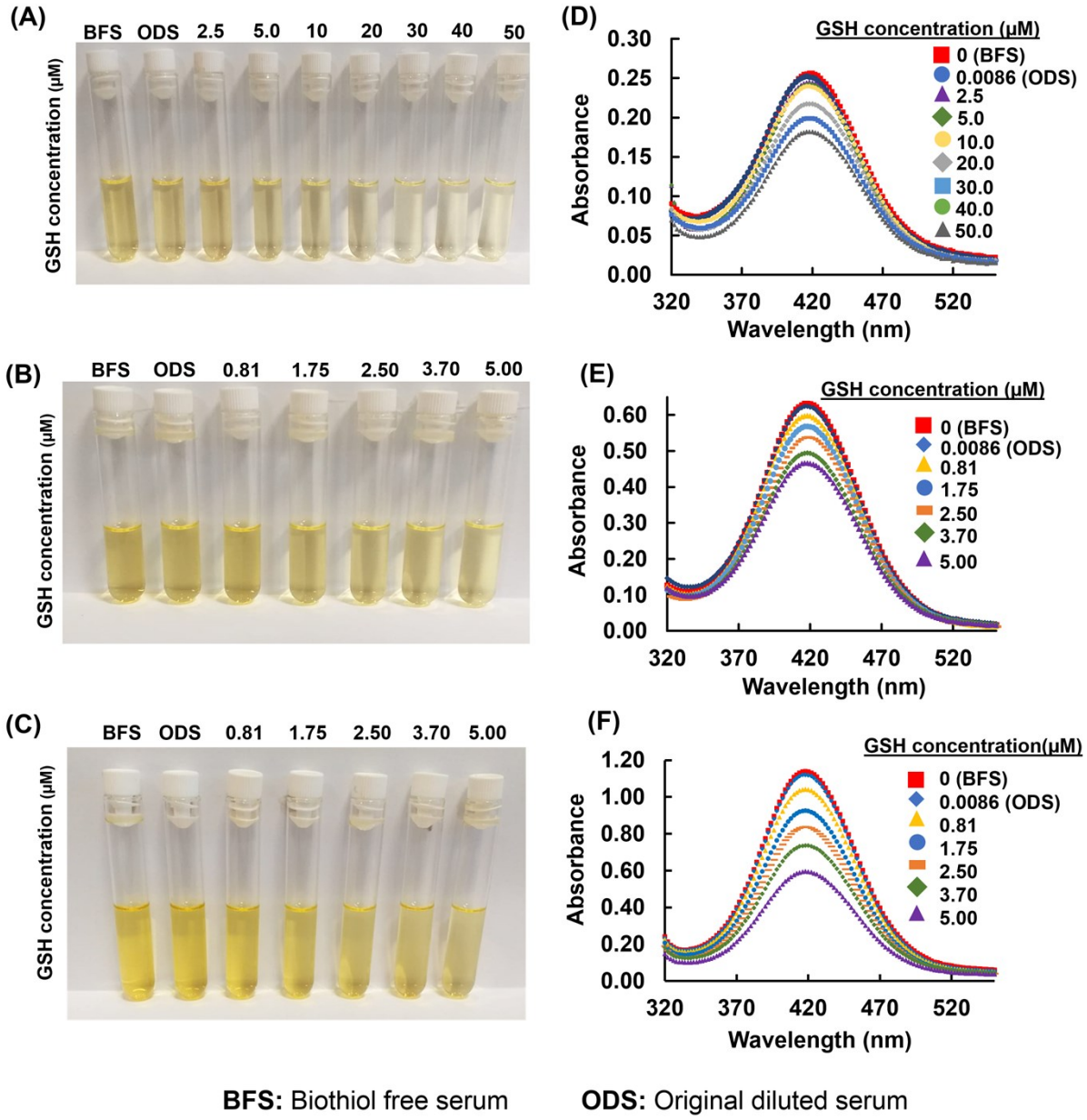

Figure S6. (A) The photographs of colorimetric solutions obtained by the completion of peroxidase-mimic reaction with $Au@SiO_2@Fe_3O_4@SiO_2$ microspheres in human serum samples spiked with GSH at different concentrations. Concentration of nanozyme (mg/mL): (A) 0.67, (B) 1.17, (C) 3.0, Corresponding UV-Vis spectra of colorimetric solutions in (A), (B), (C) are given in (D), (E), (F) respectively. Reaction volume: 4 mL, pH 7.0, Initial OPDA concentration: 400 μM for (A), (B) and 1000 μM for (C), H_2O_2 solution: 2 μL, room temperature, 100 rpm. These photographs and spectra were taken with the samples after the completion of colorimetric reaction before adding HCl.

References

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