Hierarchical Core/Shell Fe_3O_4@SiO_2@γ-AlOOH@Au Micro/Nanoflowers for Protein Immobilization

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Supplementary Information

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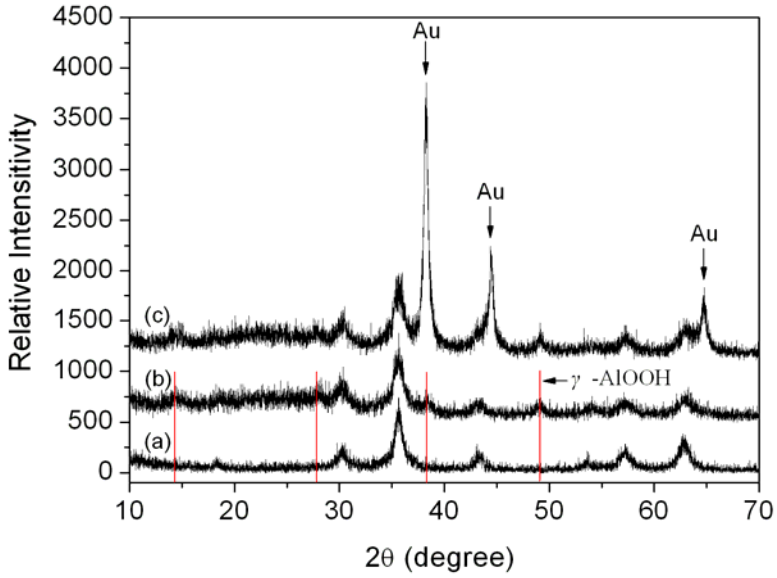

Figure S1. XRD patterns of the Fe_3O_4@SiO_2 (a), Fe_3O_4@SiO_2@γ-AlOOH (b), and Fe_3O_4@SiO_2@γ-AlOOH@Au composites. In comparison to the XRD of the Fe_3O_4@SiO_2 core/shell particles (Figure S1a), four additional peaks at 14.2º, 27.8º, 38.3º, and 49.1º, which represent the Bragg reflections from (020), (120), (031), and (200) planes of orthorhombic γ-AlOOH (JCPDS card 21-1307), were observed (Figure S1b), showing clearly the immobilization of γ-AlOOH onto the surface of the Fe_3O_4@SiO_2 core/shell particles.

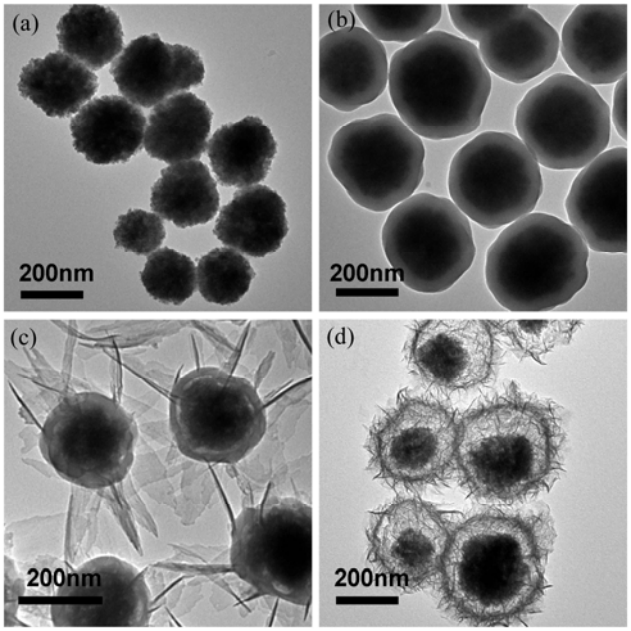

Figure S2. TEM images of the Fe_3O_4 particles (a), Fe_3O_4@SiO_2 core/shell microspheres (b), Fe_3O_4@SiO_2@γ-AlOOH flower-like structures (c), and yolk-like Fe_3O_4@$NiSiO_3$ microparticles (d).

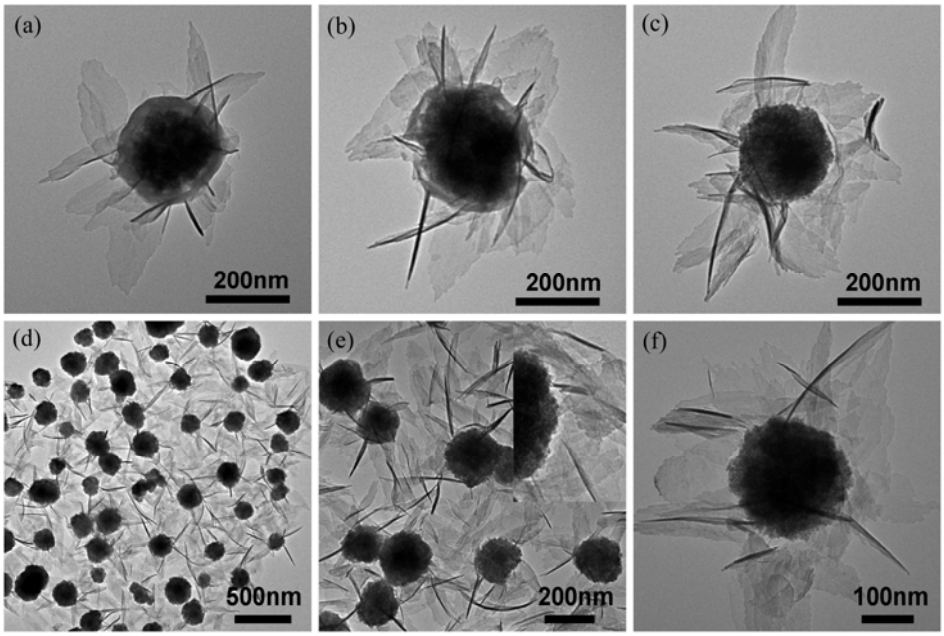

Figure S3. TEM images of the microflowers synthesized at different hydrothermal reaction time: 36 h (a), 45 h (b), and 72 h (c–f).

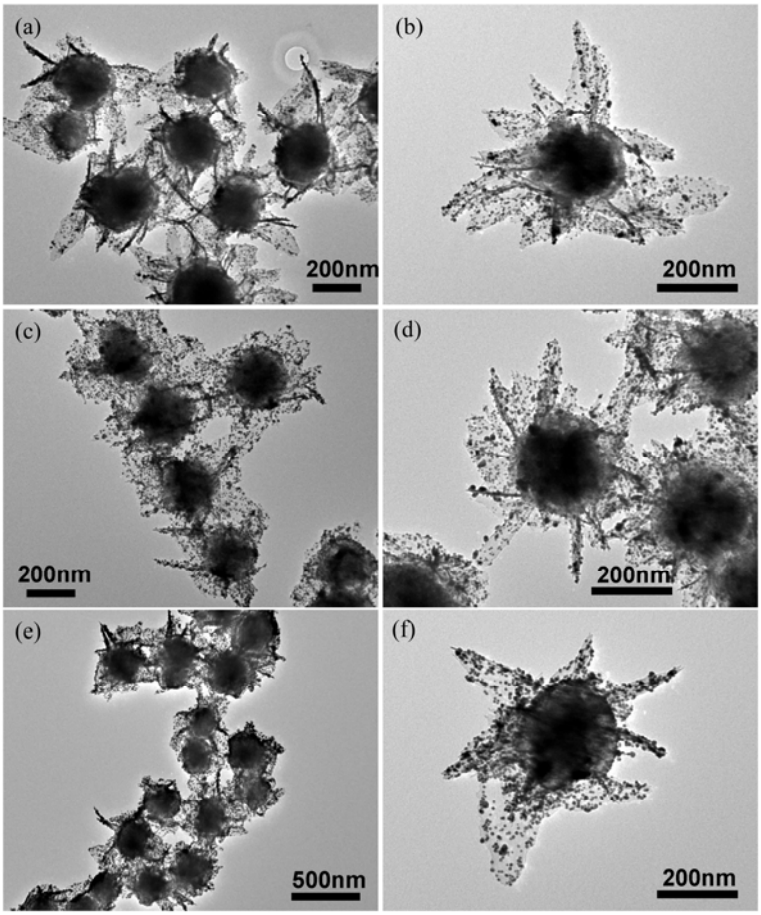

Figure S4. TEM images revealing the evolution of immobilized Au nanoparticles after 1 (a and b), 3 (c and d), and 6 (e and f) cycles of seeded growth.

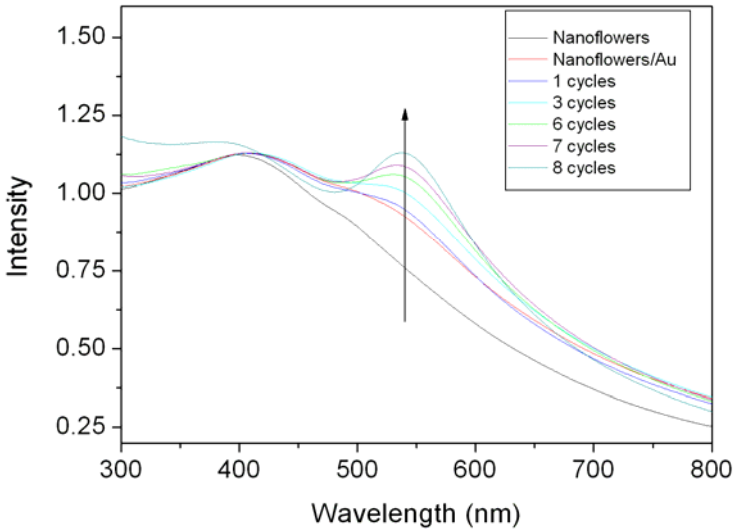

Figure S5. UV/visible absorption spectra of the Fe_3O_4@SiO_2@γ-AlOOH microflowers and Fe_3O_4@SiO_2@γ-AlOOH@Au microflowers, which present the evolution of optical properties of the immobilized Au nanoparticles after 0, 1, 3, 6, 7, and 8 cycles of seeded growth.

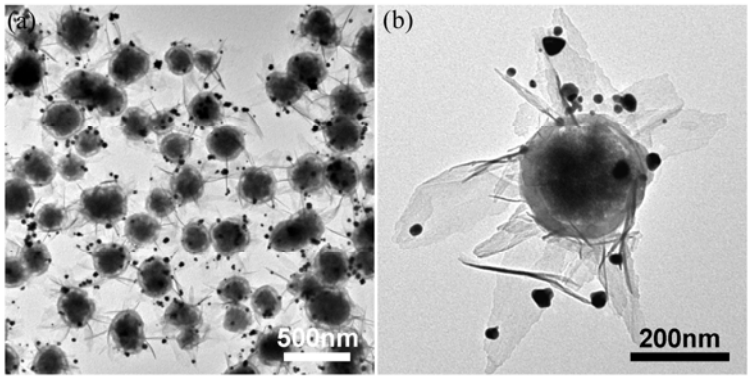

Figure S6. TEM images of the Fe_3O_4@SiO_2@γ-AlOOH@Au flower-like structures with larger Au nanoparticles. Sulfhydryl group was used to modify the γ-AlOOH nanosheets, however, the Au nanoparticles cannot be well dispersed on the surface of the nanosheets and many large aggregates were formed.

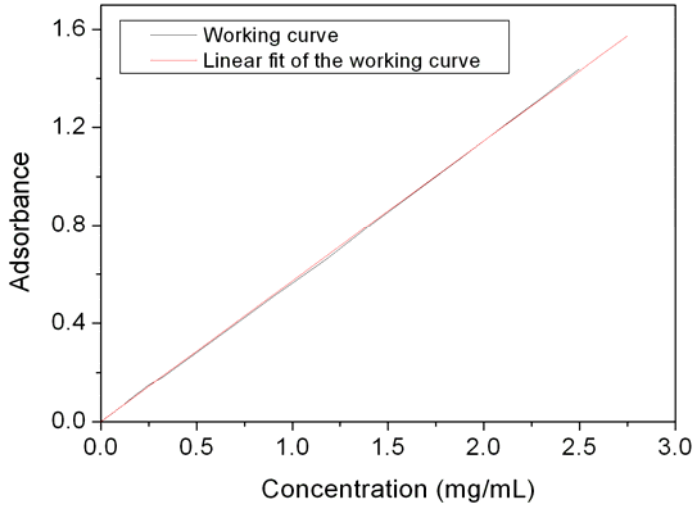

Figure S7. Standard curve based on UV/visible absorbance at 280 nm *vs* various standard concentrations of the BSA protein solution.

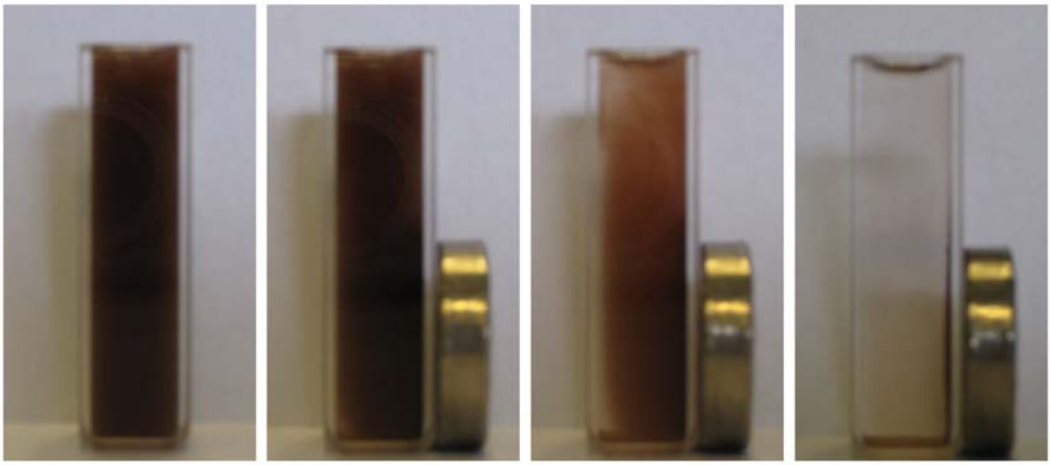

Figure S8. Separation of Fe_3O_4@SiO_2@γ-AlOOH@Au nano/micro-flowers from solution under an externally applied magnetic field.

Experimental section

General consideration: Ferric chloride hexahydrate ($FeCl_3 \cdot 6H_2O$), aluminium nitrate $Al(NO_3)_3 \cdot 9H_2O$, sodium acetate (NaOAc), sodium acrylate (Na acrylate), ethylene glycol (EG), diethylene glycol (DEG), tetraethyl orthosilicate (TEOS), 3-aminopropyltriethoxysilane (APTES), and urea were purchased from Aldrich. All chemicals were of analytical grade and used without further purification. Deionized water was obtained from Barnstead RO pure system and was bubbled with high-purity nitrogen for at least 30 min before use.

Characterization: X-ray powder diffraction patterns (XRD) of the products were obtained on a Bruker D8 Advance diffractometer equipped with graphite monochromatized Cu Kα radiation (λ = 1.5406 Å). Transmission electron microscopy (TEM) photographs were taken on a FEI CM120 microscope at an accelerating voltage of 120 kV and a high-resolution transmission electron microscope (HRTEM, Tecnai F20, FEI) at an accelerating voltage of 200 kV. UV/visible absorption spectra were obtained using Cary 5G UV/visible/NIR spectrophotometer with a scan rate of 600 nm/min. Measurements were performed twice.

Synthesis of Fe_3O_4 Nano/micro-spheres: Monodispersed superparamagnetic Fe_3O_4 nano/micro-spheres with tunable average sizes were synthesized according to a hydrothermal method. Typically, for the synthesis of 200 nm Fe_3O_4 microspheres, $FeCl_3 \cdot 6H_2O$ (2 mmol), NaOAc (1.5 g), and Na Acrylate (1.5 g) were dissolved in EG

(20 mL) in a beaker. After vigorous stirring for an hour, the homogeneous solution was transferred to a Teflon-lined stainless-steel autoclave (25 mL volume) and then sealed to heat at 200 °C. After a 12 h reaction period, the autoclave was cooled to room temperature. The obtained Fe_3O_4 spheres were washed with water and ethanol, and then dispersed in water (15 mL).

Synthesis of Fe_3O_4@SiO_2 Microspheres with Core/shell Nanostructure: The obtained Fe_3O_4 microspheres were coated by a layer of SiO_2 shell. In particular, an aqueous solution (2.5 mL) of Fe_3O_4 was diluted with water (0.5 mL) and ethanol (30 mL). The mixture was homogenized by ultrasonication for 30 min, prior to the addition of ammonia solution (1 mL). After 30 min, a solution of TEOS in ethanol was injected into the solution. The reaction was performed for 100 min and that the product was collected by the help of a magnet, then washed with ethanol and water for several times. Finally, the product was dried in vacuum for 12 h to obtain the Fe_3O_4@SiO_2 microspheres with core/shell nanostructure. The shell thicknesses of the Fe_3O_4@SiO_2 microspheres were easily controlled by varying the TEOS concentration.

Synthesis of Fe_3O_4@SiO_2@γ-AlOOH Nanoflowers: Fe_3O_4@SiO_2 core/shell particles were dispersed in 5 mL H_2O under sonication. 30 min later, 0.25 mmol $Al(NO_3)_3$ was added into the above solution and stirred vigorously. Then, a urea aqueous solution (2.25 M, 5 mL) was introduced. 20 min later, 5 mL ethanol was added under sonication. Subsequently, the obtained solution was transferred into a Teflon-lined

stainless-steel autoclave (25 mL volume) and then sealed to heat at 190 °C. After a 36 h reaction period, the autoclave was cooled to room temperature. The obtained flower-like particles were washed with water and ethanol, and then dried in vacuum overnight.

Synthesis of Flower-like Fe_3O_4@SiO_2@γ-AlOOH@Au Nanoflowers: The above Fe_3O_4@SiO_2@γ-AlOOH microparticles were firstly transferred to a mixture of isopropanol (20 mL) and APTES (0.4 mL) and heated to 80 °C for 2 h to functionalize the silicate surface with amino groups. The surface modified particles were washed with isopropanol and dispersed in deionized water. Then, the Fe_3O_4@SiO_2@γ-AlOOH@Au nanocomposites were synthesized by using an electrostatic attraction method. First, in order to induce positive charges at the surface of the Fe_3O_4@SiO_2@γ-AlOOH microparticles, acetic acid solution (0.1 mL) was added into the ethanolic dispersion of amine functionalized Fe_3O_4@SiO_2@γ-AlOOH (10 mg/mL, 1 mL) at room temperature and then sonicated at room temperature for 1 h. Subsequently, an aqueous solution of citrate-coated Au nanoparticles with an average diameter of 4 nm was prepared. In brief, an aqueous solution (20 mL) containing $HAuCl_4$ (2.5×10^{-4} M) and tri-sodium citrate (2.5×10^{-4} M) was prepared in a conical flask. Then, cold $NaBH_4$ solution (0.6 mL, 0.1 M) was added with vigorous stirring. The solution turned pink immediately after the addition of $NaBH_4$, indicating the Au nanoparticle formation. The freshly prepared Au nanoparticles solutions were used within 2-5 h after preparation. Finally, the solution of positively charged

Fe_3O_4@SiO_2@γ-AlOOH microparticles (1 mL) was added to the Au solution under sonication for 30 min. The Au nanoparticles were electrostatically attracted on the surface of the Fe_3O_4@SiO_2@γ-AlOOH nanocomposites, thereby the resultant Fe_3O_4@SiO_2@γ-AlOOH/Au product was removed from the solution by applying an external magnetic field, rinsed with water and ethanol several times. For increasing the Au loading, the Fe_3O_4@SiO_2@γ-AlOOH@Au product was dispersed in to an aqueous solution which contains PVP (0.1 g), sodium citrate (2.5×10^{-4} M) and $HAuCl_4$ (2.5×10^{-4} M). In each seeded growth, an ascorbic acid solution (2.5×10^{-4} M) was injected into the mixture and reacted for 1 h. The product was finally collected by magnetic separation. The Fe_3O_4@SiO_2@γ-AlOOH@Au product with desired Au weight percentage was prepared by varying the repeating reduction times.

The Adsorption and Magnetic Separation of BSA: A given amount of the Fe_3O_4@SiO_2@γ-AlOOH@Au nanoflowers were added into a phosphate buffer saline (PBS) solution with defined BSA and incubated with 12 h at room temperature. After the adsorption was completed, the Fe_3O_4@SiO_2@γ-AlOOH@Au nanoflowers were separated by magnetic field and the supernatant of the above protein solutions was measured using a UV/visible absorption spectrophotometer and monitored at 280 nm.