Supplementary Information for

A Colloidal Assembly Approach to Synthesize Magnetic Porous Composite Nanoclusters for Efficient Protein Adsorption

Qi Yang, Fang Lan*, Qiangying Yi, Yao Wu*, and Zhongwei Gu

1. Materials

Iron(III) chloride hexahydrate ($FeCl_3 \cdot 6H_2O$), Iron(II) chloride tetrahydrate ($FeCl_2 \cdot 4H_2O$), histidine (His), bovine serum albumin (BSA), apo-transferrin (TRT) and bovine hemoglobin (BHB) were purchased from Sigma Aldrich (St. Louis, MO, USA). Lysozyme (LYZ) was purchased from Solarbio Science and Technology Co. Ltd. (Beijing, China). Span 80 and liquid paraffin were purchased from Forest Science and Technology Development Co. Ltd. (Chengdu, China). Genipin was purchased from Com-pound Biotech Development Co. Ltd. (Chengdu, China). The commercial magnetic beads (Carboxylic functionalized MasterBeads with 500 nm in size) were purchased from SuperMed Trading Co. Ltd. (Shanghai, China). Pierce® BCA Protein Assay Kit was purchased from Wandao Biotech Development Co. Ltd. (Chengdu, China). Other chemicals were analytical pure reagents. Deionized water was used in this work.

2. Synthesis of Fe_3O_4/His composite nanoclusters

2.1 Synthesis of Fe_3O_4 nanoparticles

The Fe_3O_4 nanoparticles were synthesized by the co-precipitation of Fe^{2+} and Fe^{3+} salts using ammonia.[1] One hundred milliliters of deionized water was heated at 50 °C with the deaeration of O_2 by bubbling N_2 in a flask. Then 1000 mg of $FeCl_2 \cdot 4H_2O$, 2448 mg of $FeCl_3 \cdot 6H_2O$ and 35 mL of ammonia were added under nitrogen. After stirring for 1 h at 80 °C, the black suspension was precipitated with water using a magnet, and the sediment was collected, following by washing with deionized water until the pH = 7. Finally, Fe_3O_4 nanoparticles were re-dispersed in 100 mL of deionized water to form stable Fe_3O_4 suspension.

2.2 Synthesis of Fe_3O_4/His nanospheres

Sixty milliliters of above Fe_3O_4 suspension and 60 mL of histidine aqueous solution (5 mg mL^{-1}) were mixed by stirring at 80°C for 12h. The Fe_3O_4/His nanospheres were then collected using a magnet and re-dispersed in deionized water.

2.3 Synthesis of Fe_3O_4/His composite nanoclusters

Two hundred microliters of the prepared Fe_3O_4/His nanospheres suspension was added into 15 mL of liquid paraffin containing 5 mL of span 80 under vigorous stirring for 10 min. Then, 25 mg of genipin was added under vigorous stirring at 60 °C for 4 h. The sediment were collected by an external magnetic field, and then washed with petroleum ether, ethyl alcohol, and water. Finally, the Fe_3O_4/His nanoclusters were dispersed in deionized water for further use.

Fe_3O_4/His nanoclusters were further coated by polyethyleneimine (PEI) to obtain the Fe_3O_4/His-PEI nanoclusters. Briefly, 5 mL of PEI aqueous solution (5 mg mL^{-1}) were added into 5 mL of above Fe_3O_4/His

nanoclusters with vigorous stirring at 80 °C for 12 h. Then the brown product was collected under an external magnetic field. Finally, Fe_3O_4/His-PEI nanoclusters were stored in deionized water.

2.4 Synthesis of nonporous Fe_3O_4@His composite nanospheres

The nonporous Fe_3O_4@His composite nanospheres were prepared by inverse emulsion crosslinking method. Firstly, 50 mg of His was dissolved in 250 μL of water, and then 500 μL of Fe_3O_4 suspension was added, following the mixture was homogeneously blended by sonication at a power level of 50% for 5 min (Sonics & Materials Inc. VCX 130PB, 130W). The black mixture was added into 10 mL of liquid paraffin containing 3 mL of span 80 at 1000 rpm for 15 min. Then, 200 μL of genipin aqueous solution (50 mg mL^{-1}) was added to begin the cross-linking reaction by mechanical stirring at 1000 rpm. After allowing the reaction to proceed for 4 h at 60 °C, the product was collected by an external magnetic field, and then washed several times with petroleum ether, ethyl alcohol, and water. Finally, the obtained product was dried in a vacuum oven.

3. Protein experiments

Bovine hemoglobin (BHB) was dissolved in 10 mM PBS buffer. Three hundred micrograms of Fe_3O_4/His nanoclusters were added into BHB solution (300 μL, 500 μg) under shaking at 35 °C for 12 h. The magnetic nanoclusters were removed from the protein solution using a magnet within 10 s. The amount of free protein in the buffer was determined using a bicinchoninic acid assay (BCA). The absorbed amounts of BHB were estimated from the following equation:[2]

$$q = V_o (C_o - C)/W$$

Where q is the equilibrium adsorbed amount in the nanoclusters (mg g^{-1}); C_o and C are the protein concentrations at initial and equilibrium solution, respectively (mg mL^{-1}); V_o is the volume of the aqueous phase (mL) and W is the weight of the magnetic composite nanoclusters (g).

The protein adsorption experiments of the commercial magnetic beads, the Fe_3O_4@His nanospheres, and the Fe_3O_4/His-PEI nanoclusters were conducted similar to that of the Fe_3O_4/His nanoclusters described above. And the adsorption experiments of BSA, or TRT were similar to that of BHB.

Lysozyme (LYZ), BSA, BHB, and TRT, were dissolved in 10 mM PBS buffer, respectively, to obtain 500 μg mL^{-1} of protein solution. And the protein mixture solution was prepared with the mass ratio of each protein of 1. Then 300 μg of Fe_3O_4/His nanoclusters was added into 300 μL of protein mixture under shaking at 35 °C for 12 h. The magnetic nanoclusters were removed from the protein solution under the external magnetic field within 10 s. Finally, the supernate was characterized by sodium dodecyl sulfatepolyacrylamide gel electrophoresis analysis (SDS-PAGE).

4. Characterization

The morphology of the magnetic composite nanoclusters was observed by scanning electron microscopy (SEM, Hitachi S-4800, Japan) and transmission electron microscopy (TEM, JEM-2010, Japan electronic). The samples were deposited on silicon wafer for SEM and on copper grid coated carbon for TEM, and then dried at room temperature. The size and size distribution of the composite nanoclusters were calculated via dynamic light scattering (DLS, Zetasizer Nano ZS90, Malvern Company). Zeta potential measurements (Zetasizer Nano ZS90, Malvern Company) were carried out. Fourier transform infrared spectra (FTIR, PE spectrometer) were recorded with wave number range 500-4000 cm-1. Powder X-ray diffraction (XRD, X' Pert Pro MPD, Philips, Netherlands) was employed to study the crystal structure of samples with angles ranging from 10° to 80°. Thermogravimetric analysis (TGA) measurements were performed with simultaneous thermal analysis (STA 449 C Jupiter, NETZSCH). The mass loss of the dried sample was monitored under N_2 at temperatures from 35 to 800 °C with a

heating rate of 10 K min^{-1}. The magnetization of the dried sample was measured by a vibrating sample magnetometer (VSM, Model BHV-525, Riken Japanese Electronics Company) with field from 0 to 18,000 Oe at 300 K. Nitrogen adsorption and BJH pore size distribution were obtained at 77 K on a surface area and pore size analyzer (QuadraSorb SI, America).

5. **Fig. S1 SEM image (A) and N_2 adsorption–desorption isotherm (B) of the nonporous Fe_3O_4@His composite nanospheres.**

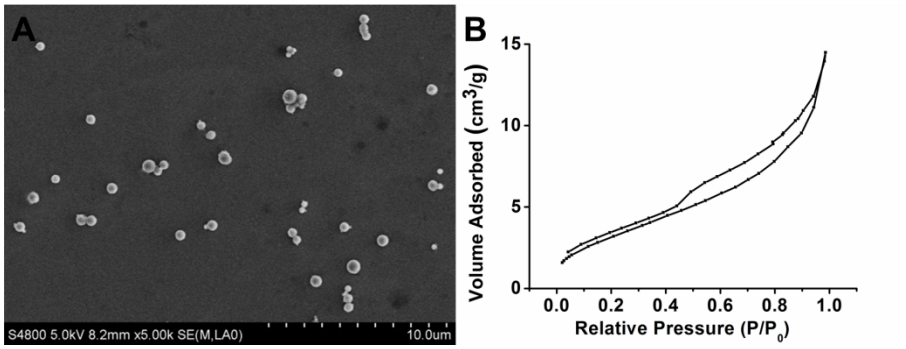

Fig. S1 SEM image (A) and N_2 adsorption–desorption isotherm (B) of the nonporous Fe_3O_4@His composite nanospheres.

6. **Fig. S2 The adsorption amounts and adsorption rates of Fe_3O_4/His MPCNs, nonporous Fe_3O_4@His nanospheres and electropositive Fe_3O_4/His-PEI MPCNs for bovine serum albumin (BSA), BHB and apo-transferrin (TRT).**

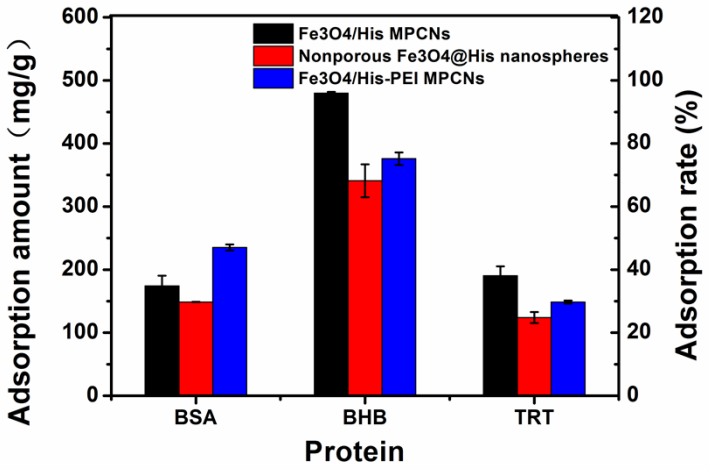

Fig. S2 The adsorption amounts and adsorption rates of Fe_3O_4/His MPCNs, nonporous Fe_3O_4@His nanospheres and electropositive Fe_3O_4/His-PEI MPCNs for bovine serum albumin (BSA), BHB and apo-transferrin (TRT).

References

1 Y. C. Chang and D. H. Chen, *J. Colloid Interface Sci.*, 2005, **283**, 446.
2 F. Lan, Y. Wu, H. Hu, L. Xie and Z. Gu, *RSC Adv.*, 2013, **3**, 1557.