Supporting Information

Superparamagnetic Nanocrystal Clusters for Enrichment of Low-Abundance Peptides and Proteins

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Materials

Oleic acid, iron pentacarbonyl ($Fe(CO)_5$), dioctyl ether, sodium dodecyl sulfate (SDS), trifluoroacetic acid (TFA), α-cyano-4-hydroxycinnamic acid (CHCA), cytochrome c, bovine serum albumin (BSA, fraction V) and trypsin were purchased from Aldrich Chemical Co. Angiotensin II was obtained from VWR. Ethanol, cyclohexane, acetonitrile and HyperSep^{TM} C18 Tip (TipC18) were obtained from Fisher Scientific. All solutions were prepared in deionized water (18 MΩ) from a Milli.Q^{TM} water purification system (Millipore, Billerica, MA, USA).

Synthesis of γ-Fe_2O_3 nanocrystals

Superparamagnetic γ-Fe_2O_3 nanocrystals were synthesized using a thermolysis process.[1] $Fe(CO)_5$ (0.2 mL, 1.52 mmol) was added to a mixture containing 10 mL of octyl ether and 1.28 g of oleic acid at 100 ^oC. Then the solution was heated to 290 °C under an argon atmosphere and maintained at this temperature for 1 h. After being cooled down to 200 ^oC, the solution was bubbled with air for 2 hours. After cooling down to room temperature, the solution was added ethanol to precipitate γ-Fe_2O_3 nanoparticles, which were then separated by centrifugation. Finally, the resulting black powder was redispersed in 10 mL cyclohexane.

Self-assembly of nanocrystals to colloidal clusters

In a typical experiment[2, 3], a cyclohexane solution of γ-Fe_2O_3 nanocrystals (1 mL) was mixed with an aqueous solution of SDS (56 mg in 10 mL H_2O) under sonication for 5~10 minutes. The mixture was then heated to 70 – 72 ^oC in a water bath for 4 hours. A clear nanoparticle "micelle" solution was obtained after evaporation of cyclohexane. The final products were washed with water three times and re-dispersed in 2 mL of water.

Tryptic digests of proteins

BSA (1 mg/mL, 1 mL) was mixed with trypsin (1 mg/mL, 20 μL) in aqueous ammonium bicarbonate (50 mM, pH = 8) and incubated at 37 ^oC for 24 hours. The resulting peptide mixtures were stored at -20 ^oC until further use.

Selective enrichment of peptides/proteins using the clusters

In a typical process, a suspension of nanocrystal clusters (10 μL, 10 mg/mL) was added into 500 μL of standard peptide, protein or protein digest at varied concentrations. (Angiotensin II (8 nM), cytochrome C (500 nM) and peptide mixture originating from tryptic digestion of BSA (6 nM)). Then the mixture was shaken at room temperature for 30 minutes. The peptide or protein-loaded clusters were collected by external magnet and washed with 100 μL of water three times. After that, 1 μL of CHCA aqueous solution (1 mg/mL, 50% acetonitrile and 0.1% TFA) was mixed with the obtained clusters. Finally, 0.5 μL of above slurry was deposited on the MALDI plate and applied for MALDI-TOF MS analysis.

Sample preparation using commercial TipC18

The sample preparation of peptides using commercial Hypersep^{TM} C18 pipette tips was performed according to the standard procedure provided by the technical note from Thermo Scientific corporation.

Structure characterization

The morphology of the nanostructures was investigated using a Philips Tecnai 12 transmission electron microscope (TEM). Dynamic light scattering (DLS) and zeta potential analysis were performed on a ZetaPALS system.

MALDI-TOF MS

Matrix assisted laser desorption/ionization mass spectra were obtained by using a Voyager-DE STR MALDI-TOF mass spectrometer (Applied Biosystems, Framingham, MA, USA) operating in positive reflector mode. The spectrometer is equipped with a pulsed nitrogen laser operated at 337 nm with 3 ns duration pulses. Mass spectra were acquired as an average of 100 laser shots. Peptide mass mapping was carried out by comparing the mass spectra with the protein sequence database in ExPASy.

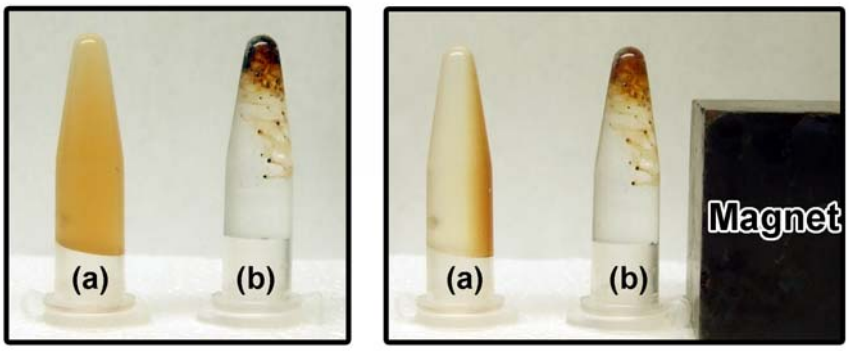

Figure S1. Digital photos of (a) as-synthesized clusters and (b) corresponding primary γ-Fe_2O_3 nanocrystals dispersed in water before and after exposure to external magnetic fields.

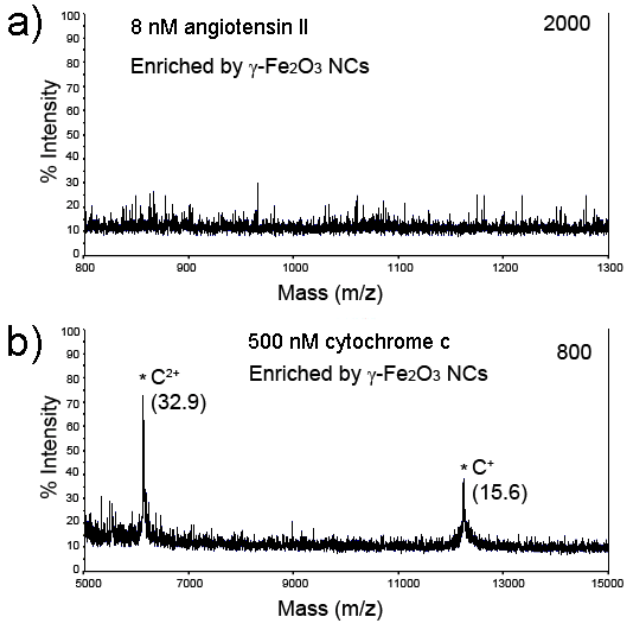

Figure S2. MALDI-TOF mass spectra of (a) angiotensin II (8 nM solution) and (b) cytochrome c (500) after enrichment with the non-clustered γ-Fe_2O_3 nanocrystals.

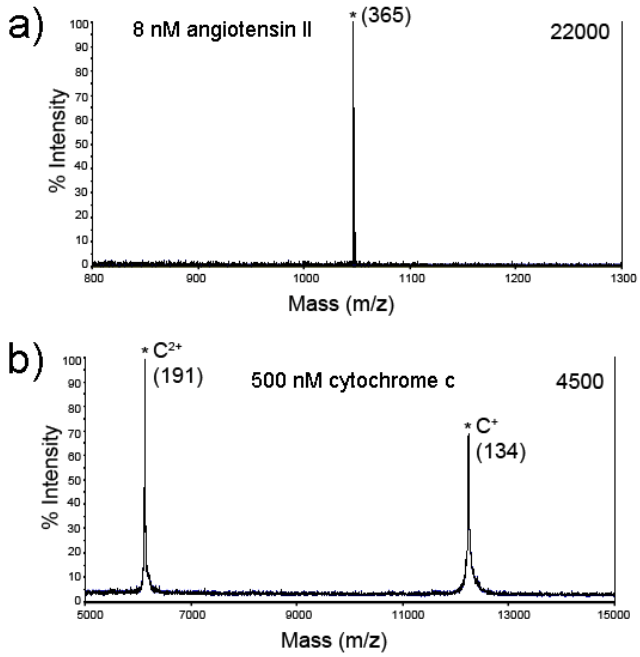

Figure S3. MALDI-TOF mass spectra of (a) angiotensin II (8 nM solution containing 100 mM of $CaCl_2$) and (b) cytochrome c (500 nM solution containing 100 mM of $CaCl_2$) after enrichment with the γ-Fe_2O_3 nanocrystal clusters.

Table S1. Peptide peaks of detected peptides from tryptic digests of BSA by MALDI-TOF mass analysis.

Peak No	Theroretical mono $[M+H]^+$	Theroretical av. $[M+H]^+$	Observed $[M+H]^+$	Sequence
b1	841.5	842.0	841.9	LCVLHEK
b2	927.5	928.1	927.8	YLYEIAR
b3	1015.5	1016.2	1018.0	SHCIAEVEK
b4	1052.5	1053.2	1052.9	CCTKPESER
b5	1163.6	1164.3	1164.0	LVNELTEFAK
b6	1069.5	1070.2	1171.0	EACFAVEGPK
b7	1249.6	1250.4	1250.0	FKDLGEEHFK
b8	1305.7	1306.5	1306.1	HLVDEPQNLIK
b9	1349.5	1350.5	1349.7	TCVADESHAGCEK
b10	1362.7	1363.6	1363.0	SLHTLFGDELCK
b11	1388.6	1389.5	1388.0	EYEATLEECCAK
b12	1479.8	1480.7	1482.2	LGEYGFQNALIVR
b13	1511.8	1512.7	1513.4	VPQVSTPTLVEVSR
b14	1578.6	1579.7	1577.1	ECCHGDLLECADDR
b15	1616.7	1617.8	1616.1	QEPERNECFLSHK
b16	1640.0	1640.9	1640.4	KVPQVSTPTLVEVSR
b17	1723.8	1724.9	1722.2	DAFLGSFLYEYSRR
b18	1756.7	1758.0	1756.3	CCAADDKEACFAVEGPK
b19	1823.9	1825.1	1825.4	RPCFSALTPDETYVPK
b20	1942.8	1944.2	1942.3	VHKECCHGDLLECADDR
b21	2003.8	2005.2	2003.3	ETYGDMADCCEKQEPER
b22	2045.0	2046.3	2044.3	RHPYFYAPELLYYANK
b23	2174.0	2175.5	2173.4	ECCDKPLLEKSHCIAEVEK
b24	2220.1	2221.6	2223.5	LFTFHADICTLPDTEKQIK
b25	2472.2	2473.8	2471.9	QNCDQFEKLGEYGFQNALIVR

Reference:

1 T. Hyeon, S. S. Lee, J. Park, Y. Chung, and H. Bin Na, *Journal of the American Chemical Society*, 2001, **123**, 12798.

2 F. Bai, D. S. Wang, Z. Y. Huo, W. Chen, L. P. Liu, X. Liang, C. Chen, X. Wang, Q. Peng, and Y. D. Li, *Angewandte Chemie-International Edition*, 2007, **46**, 6650.

3 Z. D. Lu, M. M. Ye, N. Li, W. W. Zhong, and Y. D. Yin, *Angewandte Chemie-International Edition*, **49**, 1862.