Electronic Supplementary Information

Room-temperature synthesis of core-shell structured magnetic covalent organic frameworks for efficient enrichment of peptides and simultaneous exclusion of proteins

Guo Lin,[a] Chaohong Gao,[a] Qiong Zheng,[a] Zhixian Lei,[a] Huijuan Geng,[a] Zian Lin,[a]* Huanghao Yang,[a] and Zongwei Cai [b]*

a) Ministry of Education Key Laboratory of Analysis and Detection for Food Safety, Fujian Provincial Key Laboratory of Analysis and Detection Technology for Food Safety, College of Chemistry, Fuzhou University, Fuzhou, Fujian, 350116, China

b) Partner State Key Laboratory of Environmental and Biological Analysis, Department of Chemistry, Hong Kong Baptist University, 224 Waterloo Road, Kowloon Tong, Hong Kong, SAR, P. R. China

- Corresponding author: Zian Lin; Zongwei Cai
- Postal address: College of Chemistry, Fuzhou University, Fuzhou, Fujian, 350116, China
- Fax: 86-591-22866135
- E-mail: zianlin@fzu.edu.cn (Z.A. Lin); zwcai@hkbu.edu.hk (Z.W. Cai)

EXPERIMENTAL SECTION

1. Chemicals and reagents

All reagents above were of analytical grade or better. Ferric chloride hexahydrate ($FeCl_3 \cdot 6H_2O$), sodium citrate dehydrate ($Na_3Cit \cdot 2H_2O$), dimethyl sulfoxide, tetrahydrofuran, ethanol and methanol were obtained from Sinopharm Chemical Reagent, Co., Ltd (Shanghai, China). 1,3,5-Tris(4-aminophenyl)benzene (TAPB) was purchased from J&K Chemical Ltd (Shanghai, China). Trifluoroacetic acid (TFA), formic acid (FA), sodium acetate, ethylene glycol, trypsin (DPCC treated, from bovine pancreas), insulin, Lysozyme (Lyz) and terephthaldicarboxaldehyde (TPA) were obtained from Aladdin Chemistry Co., Ltd (Shanghai, China). Bovine serum albumin (BSA) was purchased from Shanghai Lanji Co. Ltd. (Shanghai, China). Two peptides (Phe-Gly-Phe-Gly-Phe (Mw = 573.66, Sequence: FGFGF, Grand Average of Hydropathy (GRAVY) = 1.52), (Gly-Gly-Phe-Gly-Gly (Mw = 393.35, Sequence: GGFGG, Grand Average of Hydropathy (GRAVY) = 0.24) were purchased from Guotai Bio-technology Co. Ltd. (Hefei, China). Healthy human serum was kindly donated by Fujian Provincial Official Hospital (Fuzhou, China). Deionized water (18.2 $M\Omega\ cm^{-1}$) was prepared with a Milli-Q water purification system (Millipore, USA).

2. Preparation of Fe_3O_4 magnetic nanoparticles

The magnetic nanoparticles (MNPs) were synthesized according to the previous work [1,2] with some modification. Briefly, $FeCl_3 \cdot 6H_2O$ (8.1 g), $Na_3Cit \cdot 2H_2O$ (1.5 g) and sodium acetate (12.0 g) were dissolved in ethylene glycol (200 mL), followed by stirring intensively. Then, the obtained homogeneous yellow solution was transferred to autoclave, and then heated up to 200 °C for 12 h. After reaction, the product was separated with a magnet and washed with water and ethanol for several times. Finally, the product was dried under vacuum at room temperature for further use.

3. Preparation of core-shell structured Fe_3O_4@COFs

The synthesis of core-shell structured Fe_3O_4@COFs was carried out as follow. In brief, Fe_3O_4 (0.15 g), 1,3,5-Tris(4-aminophenyl)benzene (0.106 g) and terephthaldicarboxaldehyde (0.06 g) were dissolved in dimethyl sulfoxide (DMSO, 60 mL) and sonicated for 5 min. Subsequently, acetic acid (17.5 M, 2 mL) was slowly added with the assistance of sonication and the brown precipitates (Fe_3O_4@COFs) were formed during this process (~ 10 min). After incubation for 30 min, the yielded brown precipitate was collected with the help of magnet and then washed with anhydrous tetrahydrofuran, and anhydrous methanol for three times. Finally, the brown precipitate was dried under vacuum at room temperature prior to use.

4. Characterization

Scanning electron microscopy (SEM) images were recorded by a FEI Emission Scanning Electron Microscope (SU8020, Hitachi, Japan). Transmission electron microscopy (TEM) images were obtained on a FEI Tecnai G20 (USA) at 200 kV. Nitrogen adsorption-desorption isotherms were tested using an ASAP 2020 (Micromeritics, USA). The samples were degassed in a vacuum at 150 °C for 8 h before measurement. The surface area of the samples was reckoned using the Brunauer–Emmett–Teller (BET) equation. The total pore volume, V_T, was obtained using the desorption branch of the N_2 isotherm at $P/P_0 = 0.992$. By using the Barrett-Joyner-Halenda (BJH) model, the pore volume and pore size distribution were derived from the adsorption branches of the isotherms. Fourier-transform infrared spectroscopy (FTIR) spectra were obtained by Nicolet 6700 spectrometer (Thermo Fisher, USA) using KBr pellets. The crystal structure of the magnetic COFs was determined by X'Pert-Pro MPD (Philips, Holland). The magnetization curves were recorded by a superconducting quantum interference device magnetometer (SQUID) MPMS XL-7 (Quantum Design, USA) at 300 K. Thermogravimetric analysis (TGA) were performed using a Shimadzu

DTG-60AH in the temperature range 100 to 800 °C under flowing air (100 mL/min) with a heating rate of 10 °C/min.

HPLC analysis were measured out using a Shimadzu Prominence LC-20A HPLC (Kyoto, Japan) with an Agela Technologies Venusil XBP C8 (100 mm × 4.6 mm, 5 μm, 300 Å) column (Tianjin, China) and an GL Sciences Inc InertSustain C18 (150 mm × 4.6 mm, 5 μm) column (Japan). The separation of peptides, proteins and its mixture was performed by using mobile phase with various ratios under gradient mode: buffer A (0.1% TFA aqueous solution) and buffer B (0.08% TFA acetonitrile (ACN) solution); The flow rate was 1.0 mL/min; column temperature was 40 °C. The injected sample volume was 10 μL, and the samples were detected using a UV detector (214 nm). Quantitative analysis of the protein solutions were performed from a linear calibration curve of peak area versus concentration.

HPLC-Q-TOF MS was performed as follow. A 1260 series HPLC system with a binary SL pump was used. Detection was performed using an Agilent 6520 Q-TOF with dual electrospray ion source (ESI). The separation of all samples was performed on Agilent Poroshell 120 EC-C18 column (2.7 μm, 3.0 mm × 50 mm, Agilent). The flow rate was 0.3 mL/min. Solvent A was composed of water containing 0.1% formic acid. Solvent B was composed of ACN containing 0.1% formic acid. The following gradient program was used: 0–3 min, 3% B; 3–20 min, 3–40% B; 20–23 min, 40–80% B; 23–25 min, 80% B. A flush step was performed with 80 % B for 3 min and the column was equilibrated with 3 % B for 4 min. The column temperature was maintained at 40 °C. The sample injection volume was 10 μL. Nitrogen was used as drying gas at a temperature of 350 ℃ and a flow-rate of 10 L/min. Full scan MS data and MS/MS data were acquired at m/z 300–2000 and 100–3000, respectively. Scan rates for MS and MS/MS data were set to 3 spectra/s. The voltage set for the MS capillary was 4 kV and the fragmentor was set to 175 V. For MS2

experiments, the collision energy was set to according to formula, in which the top three highest intensity peaks in each MS were chosen for collision-induced dissociation. Isolation width for MS^2 was ± 4 amu.

All the LC-MS/MS raw data were searched with Spectrum Mill versionA.03.03 software (Agilent Technologies). Trypsin restriction was set with two missed cleavages. Cys carboxymethylation was set as the static modification. The mass tolerances were 100 ppm for parent ions and 200 amu for fragment ions.

5. In-vial adsorption and separation of peptides.

The adsorption experiments were carried out in a centrifuge tube at room temperature. Milli-Q water were the solvent of all sample solutions.

In the isothermal adsorption experiments, 0.3 mg of Fe_3O_4@COFs was vortex-mixed with 0.3 mL of FGFGF or GGFGG solutions at different concentrations for 20 min in the centrifuge tube. The nanocomposite-peptides conjugates were magnetically isolated by magnet. Then the supernatants were collected for HPLC analysis. The equilibrium adsorption capacity (Q_e, mg g^{-1}) was calculated according to

$$\mathbf{Q_e} = \frac{(\mathbf{C_0} - \mathbf{C_e})}{m}\mathbf{V} \quad (1)$$

The initial peptide concentration (μg mL^{-1}) denoted as C_0, the supernatant peptide concentration (μg mL^{-1}) denoted as C_e, the volume of protein solution (mL) denoted as *V* and the weight of the Fe_3O_4@COFs (g) denoted as *m*.

In the kinetic absorption experiment, 0.3 mg of Fe_3O_4@COF was incubated with 0.3 mL of FGFGF or GGFGG solution (each of peptide, 70 μg mL^{-1}) for different incubation times.

In the specific adsorption experiments, 5 mg of Fe_3O_4@COFs was incubated with 1 mL of peptide mixture containing FGFGF (50 μg mL^{-1}) and GGFGG (50 μg mL^{-1}), the mixture containing FGFGF (25 μg mL^{-1}), insulin (25 μg mL^{-1}) and Lyz (25 μg mL^{-1}), and human serum (diluted by 50-fold) spiked with FGFGF (50 μg mL^{-1}) for 5 min. After magnetic separation, the supernatants were collected. The captured peptides were eluted with eluent (50%water and 50%ACN). The supernatants and the eluates were analyzed by HPLC.

6. Digestion of BSA.

1.0 mg BSA in 1.0 mL of 50 mM NH_4HCO_3 buffer solution (pH 8.3) was denatured in a 100°C water bath for 5 min and reduced with dithiothreitol (DTT) (final concentration 5 mM) for 1h at 55 °C. Then, the reduced products were alkylated by iodoacetic acid (IAA) (final concentration 10 mM) in the dark for 1h at room temperature. Subsequently, digestion was performed by adding trypsin into the above mixture with a substrate-to-enzyme ratio of 30:1(w/w) at 37 °C for 24h. Finally, 1% formic acid was used to stop the reaction. The samples were stored at −20°C and further diluted to desired concentration prior to use. Similarly, the digestion of human serum was also performed by using 1.0 mL human serum (diluted by 60-fold with 50 mM NH_4HCO_3 buffer) as mentioned above.

References

[1] J.N. Zheng, Z.A. Lin, G. Lin, H.H. Yang, and L. Zhang, J. Mater. Chem. B, 2015, 3, 2185-2191.

[2] J. Liu, Z.K. Sun, Y.H. Deng, Y. Zou, C.Y. Li, X.H. Guo, L.Q. Xiong, Y. Gao, F.Y. Li, and D.Y. Zhao, Angew. Chem. Int. Ed. 2009, 48, 5875-5879.

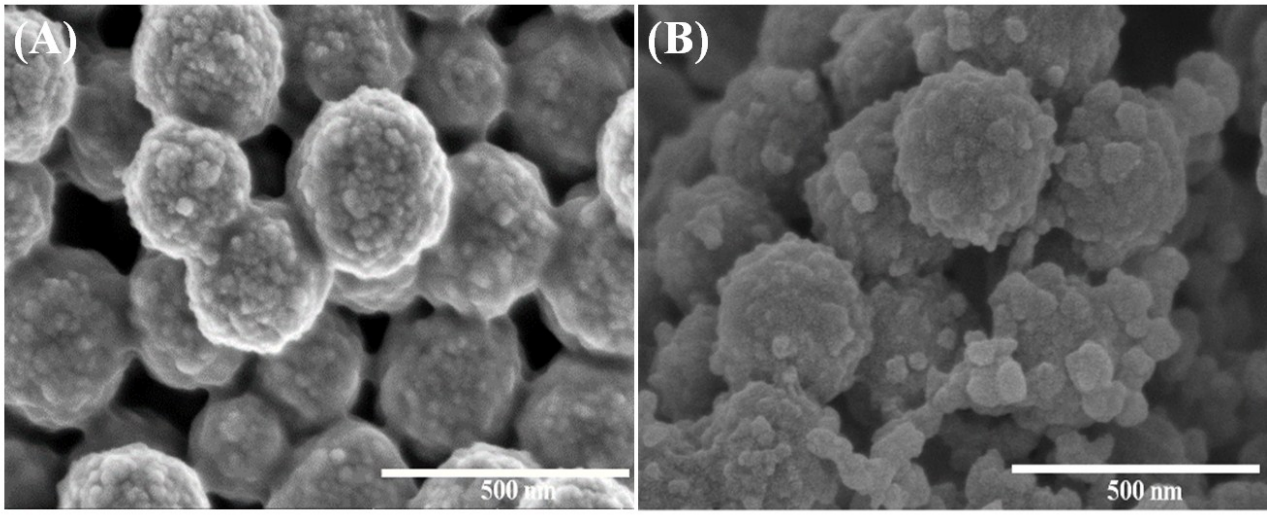

Fig. S1 SEM images of (A) Fe_3O_4 and (B) Fe_3O_4@COFs.

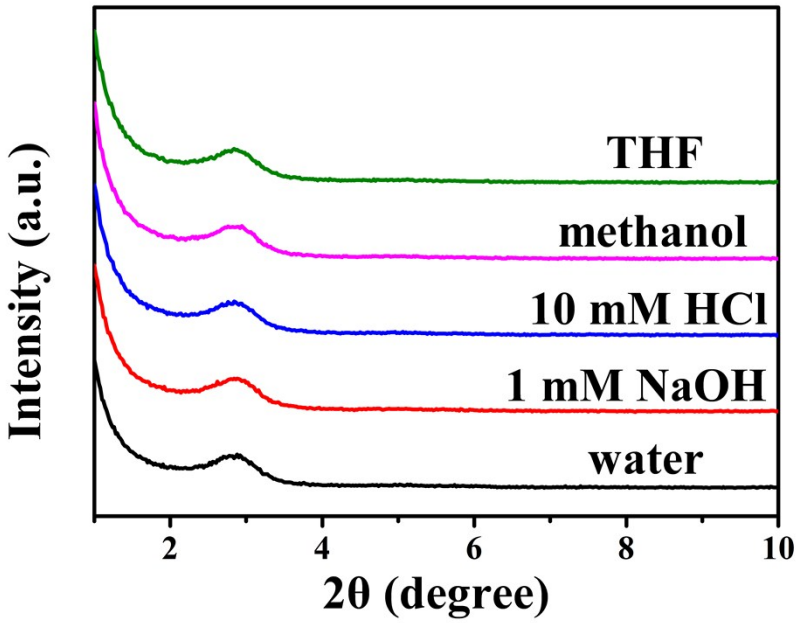

Fig.S2 XRD patterns of Fe_3O_4@COFs treated with different solvents for overnight

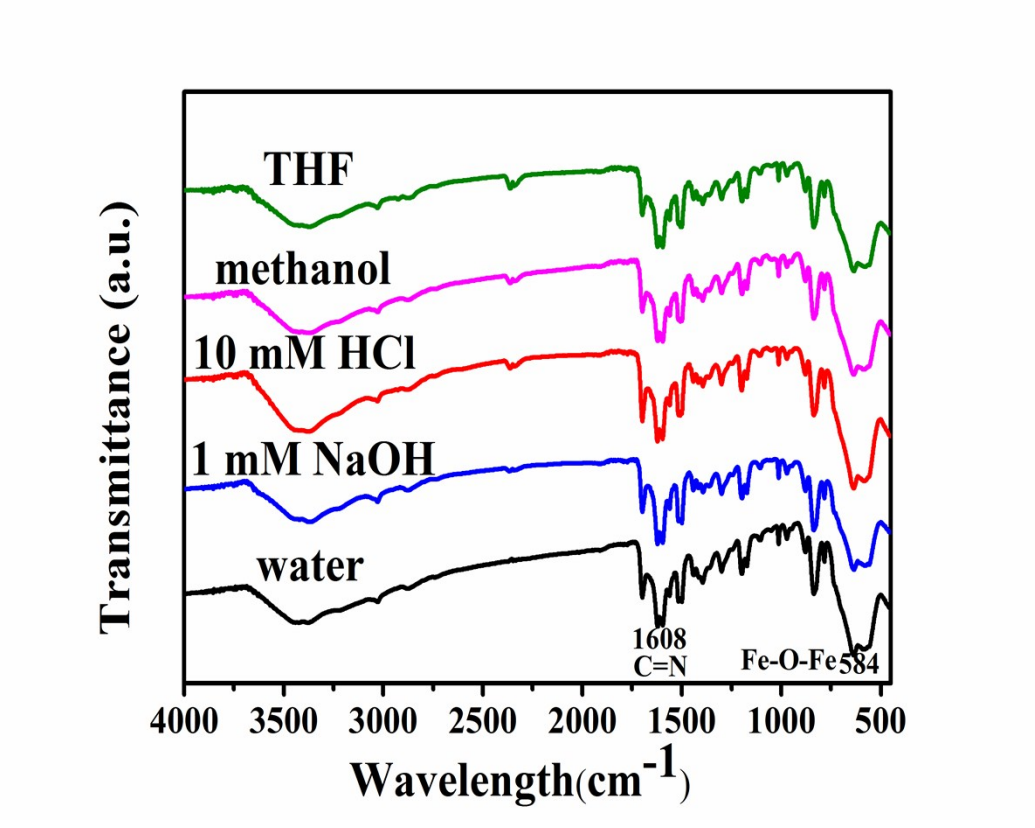

Fig.S3 FT-IR spectra of Fe_3O_4@COFs treated with different solvents for overnight

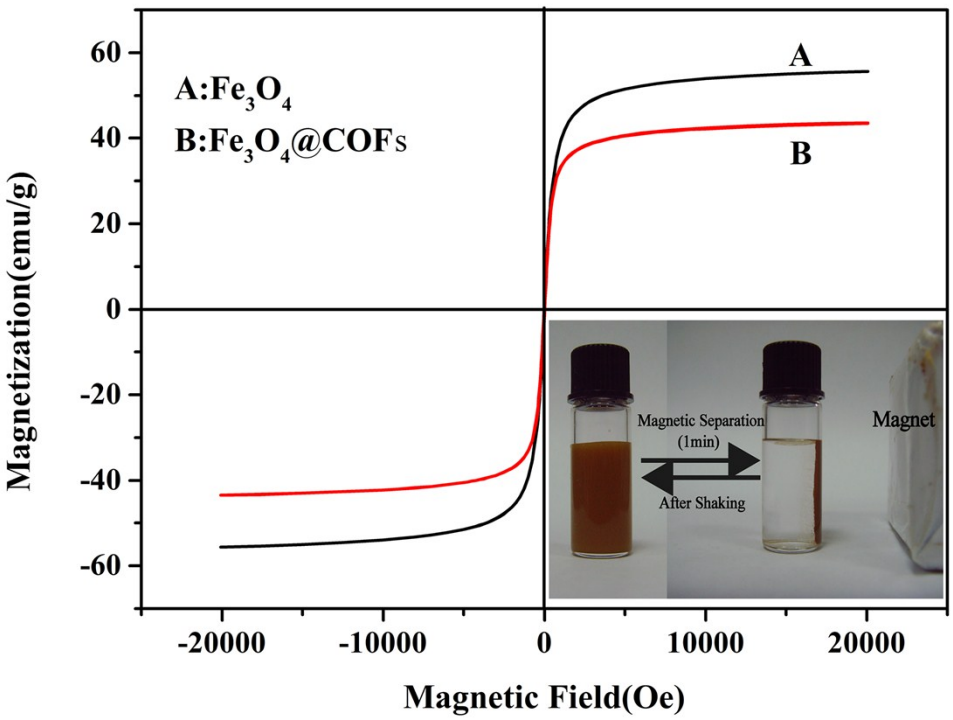
A:Fe3O4
B:Fe3O4@COFs
A
B
Magnetization(emu/g)
Magnetic Field(Oe)
60
40
20
0
-20
-40
-60
-20000
-10000
0
10000
20000
Magnetic Separation
(1min)
After Shaking
Magnet

Fig. S4 Hysteresis loops of Fe_3O_4 microspheres and Fe_3O_4@COFs at 300K. The inset shows the magnetic separation behavior of Fe_3O_4@COFs.

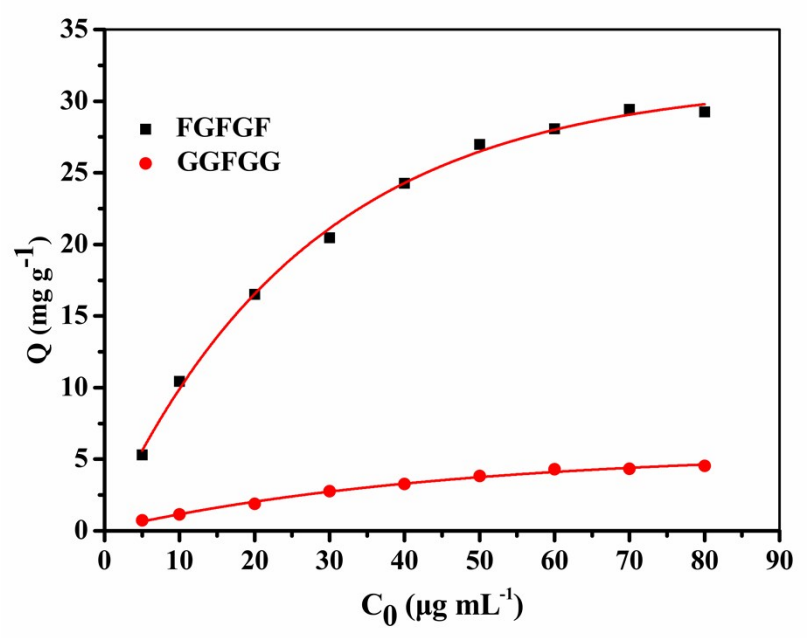

Fig.S5 Adsorption isotherms of the two peptides on the Fe_3O_4@COFs.

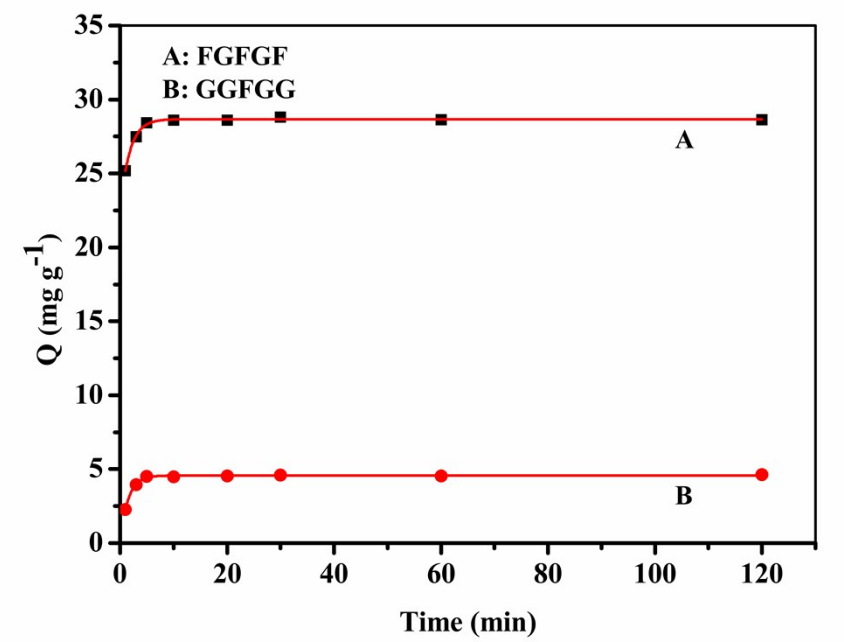

Fig.S6 Adsorption kinetics of the two peptides on the Fe_3O_4@COFs.

244

245

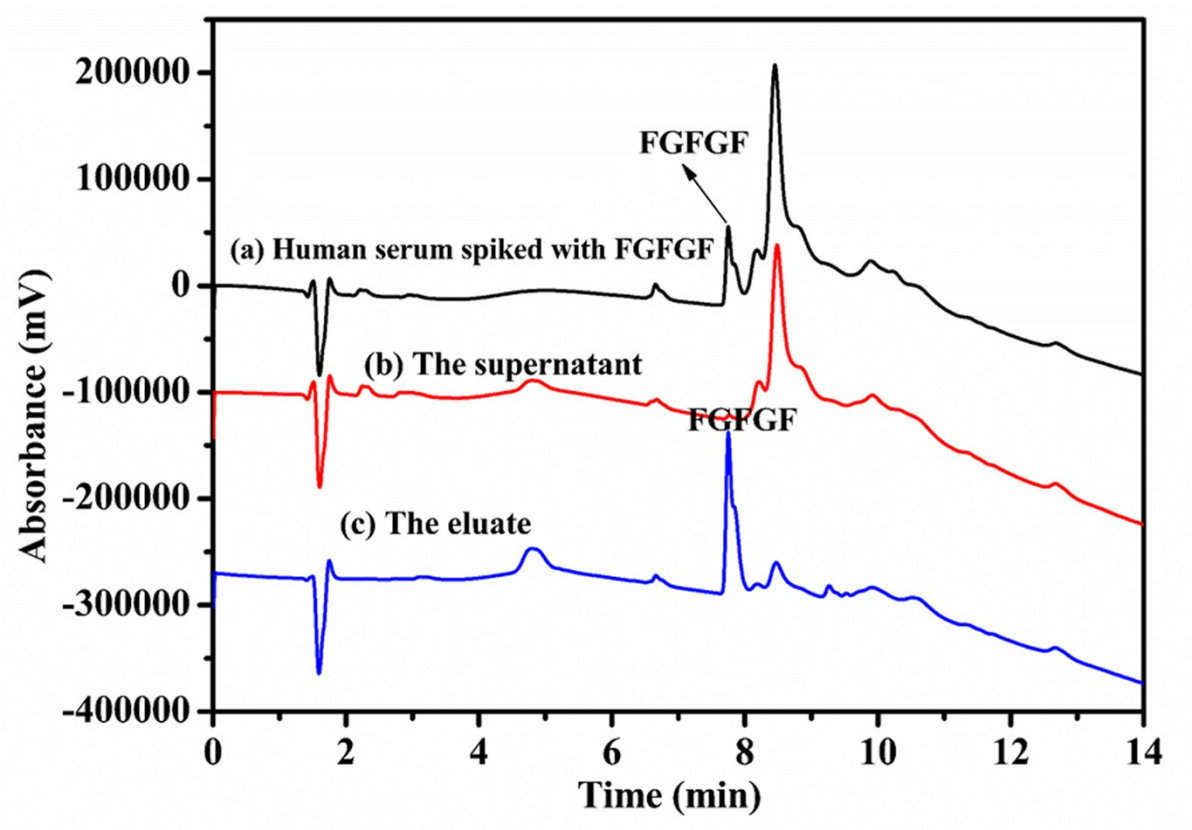

Fig.S7 Separation of 50-fold diluted human serum spiked with FGFGF before and after treatment with the Fe_3O_4@COFs.

Chromatographic conditions:

Buffer A (0.1% TFA aqueous solution) and buffer B (0.08% TFA acetonitrile solution); the flow rate was 1.0 mL/min; column temperature was 40 °C. Column: Venusil XBP C8 (100 mm×4.6 mm, 5 μm, 300 Å). The following gradient program was used: 0–12 min, 13%–90% B; 12–12.5 min, 90% B; 12.5–13 min, 90%–13% B; 14 min, stop. The flow rate of mobile phase: 1 mL/min, Wavelength: 214 nm.

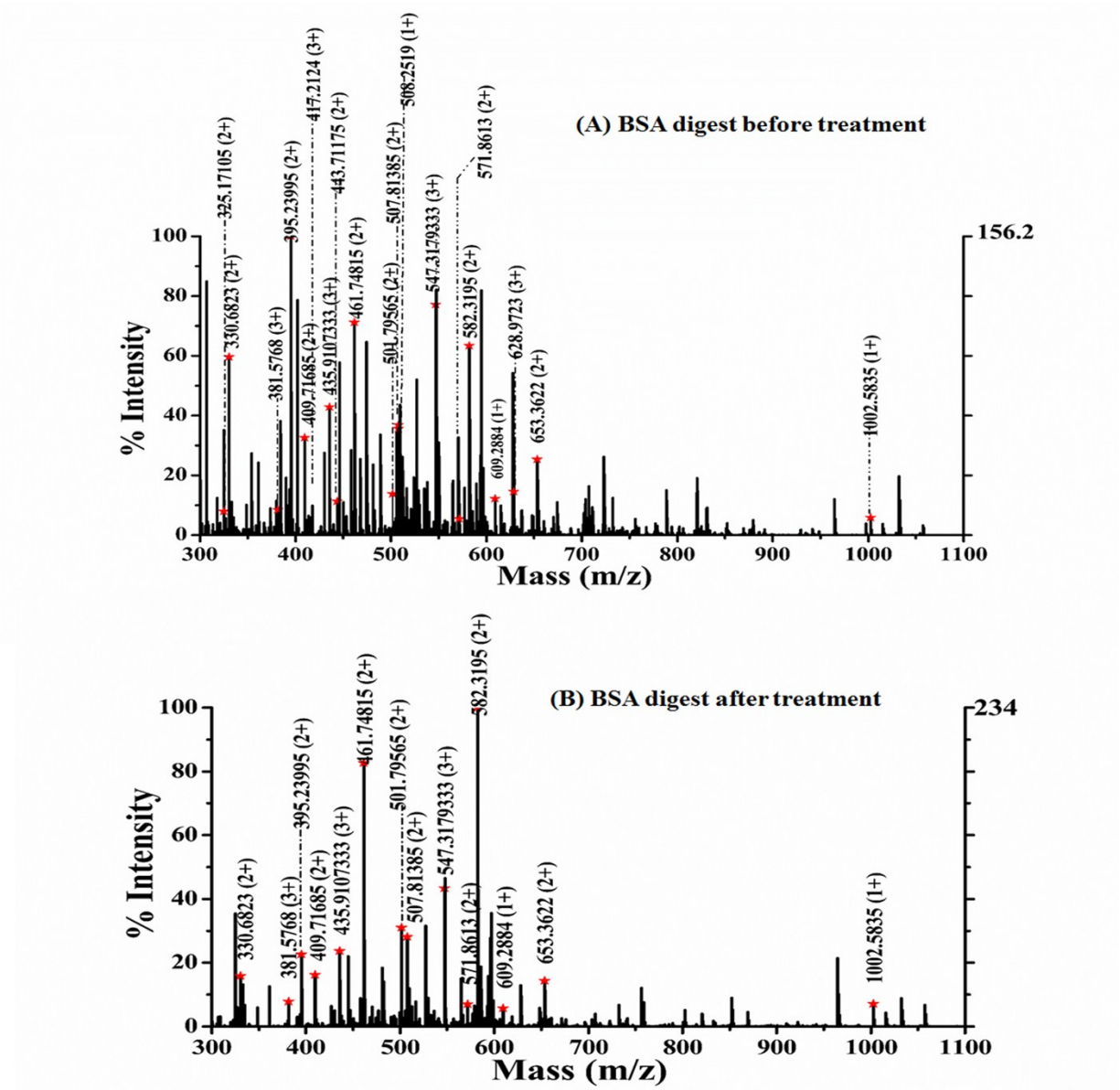

Fig.S8 HPLC-Q-TOF mass spectra of 5 μg mL^{-1} tryptic BSA digestion. (A) Direct analysis and (B) eluate from Fe_3O_4@COFs after peptide enrichment. Peptide fragments are marked with stars.

Table S1. Peptides of BSA digest (5 μg mL^{-1}) identified by HPLC-Q-TOF/MS before and after treatment with Fe_3O_4@COFs.

No	Position	Mw	Calculated m/z	Amino acid sequence[a]	GRAVY	Before enrichment[b]	After enrichment
1	229-232	507.2441	508.2519(1+)	FGER	-1.4	52.8	0
2	524-528	608.2806	609.2884(1+)	AFDEK	-1.26	18.3	13.3
3	205-209	648.3265	325.1711(2+)	IETMR	-0.46	15.6	0
4	490-495	659.349	330.6823(2+)	TPVSEK	-1.05	84.9	37.2
5	257-263	788.4643	395.2399(2+)	LVTDLTK	0.43	156.2	53.1
6	562-568	817.4181	409.7168(2+)	ATEEQLK	-1.36	46.9	38
7	131-138	885.4079	443.7117(2+)	DDSPDLPK	-1.825	16.4	0
8	249-256	921.4807	461.7481(2+)	AEFVEVTK	0.175	103.5	193.5
9	598-607	1001.5757	501.7956(2+)	LVVSTQTALA	1.39	25.4	72.5
10	598-607	1001.5757	1002.5835(1+)	LVVSTQTALA	1.39	9.5	16.7
11	549-557	1013.6121	507.8138(2+)	QTALVELLK	0.64	53.2	65.9
12	548-557	1141.707	381.5768(3+)	KQTALVELLK	1.09	12.3	18.3
13	548-557	1141.707	571.8613(2+)	KQTALVELLK	1.09	9	16.5
14	66-75	1162.6234	582.3195(2+)	LVNELTEFAK	0.13	93.1	234
15	35-44	1248.6138	417.2124(3+)	FKDLGEEHFK	-1.25	22.5	0
16	402-412	1304.7088	435.9107(3+)	HLVDEPQNLIK	-0.58	62.1	55.3
17	402-412	1304.7088	653.3622(2+)	HLVDEPQNLIK	-0.58	36.7	33.7
18	437-451	1638.9304	547.3179(3+)	KVPQVSTPTLVEVSR	-0.067	119.6	101.4
19	281-297	1883.8935	628.9723(3+)	ADLAKYICDNQDTISSK	-0.59	21.3	0

[a] The red mark in database sequence represents hydrophobic group-containing amino acids;
[b] The values refer to the abundant ratios of observed peptides.

Table S2. Peptides of BSA digest (0.5 μg mL^{-1}) identified by ESI-TOF/MS before and after treatment with Fe_3O_4@COFs.

No	Position	Mw	Calculated m/z	Amino acid sequence[a]	GRAVY	Before enrichment[b]	After enrichment
1	490-495	659.349	330.6823(2+)	TPVSEK	-1.05	8.5	9.9
2	257-263	788.4643	395.2399(2+)	LVTDLTK	0.43	12.4	18.2
3	257-263	788.4643	789.4721(1+)	LVTDLTK	0.43	0	1.5
4	562-568	817.4181	409.7168(2+)	ATEEQLK	-1.36	6.7	3.2
5	131-138	885.4079	443.7117(2+)	DDSPDLPK	-1.825	3.3	0
6	249-256	921.4807	461.7481(2+)	AEFVEVTK	0.175	0	17.8
7	598-607	1001.5757	501.7956(2+)	LVVSTQTALA	1.39	0	2.9
8	549-557	1013.6121	507.8138(2+)	QTALVELLK	0.64	4	19.3
9	548-557	1141.707	381.5768(3+)	KQTALVELLK	0.19	1.5	2.4
10	66-75	1162.6234	582.3195(2+)	LVNELTEFAK	0.13	0.4	10.6
11	402-412	1304.7088	435.9107(3+)	HLVDEPQNLIK	-0.58	2.5	0

[a] The red mark in database sequence represents hydrophobic group-containing amino acids;
[b] The values refer to the abundant ratios of observed peptides.

Table S3. Serum peptides of human serum digest (5 μg mL^{-1}) identified by ESI-TOF/MS before and after treatment with Fe_3O_4@COFs.

Database Accession	Protein Name	Before enrichment			After enrichment		
		Distinct Peptides	% AA Coverage	Mean Peptide Spectral Intensity	Distinct Peptides	% AA Coverage	Mean Peptide Spectral Intensity
P02768	Serum albumin precursor	34	58	1.09e+005	27	35	2.3e+005
P01871	Ig mu chain C region	/	/	/	8	18	1.66e+004
P04220	Ig mu heavy chain disease protein	/	/	/	5	12	1.34e+004
P02647	Apolipoprotein A-I precursor	/	/	/	6	25	3.21e+004
P01857	Ig gamma-1 chain C region	6	21	5.86e+004	5	15	1.24e+005
P01859	Ig gamma-2 chain C region	5	15	3.28e+004	5	15	6.62e+004
P01861	Ig gamma-4 chain C region	4	13	3.85e+004	4	13	7.52e+004
P01860	Ig gamma-3 chain C region	3	8	4.30e+004	3	8	9.92e+004
P02647	Ig lambda chain C regions	3	12	1.21e+004	6	18	2.61e+004
P01842	Ig kappa chain C region	2	23	2.20e+004	5	24	3.60e+004
P01876	Ig alpha-1 chain C region	/	/	/	3	7	2.43e+004
P01877	Ig alpha-2 chain C region	/	/	/	2	5	2.05e+004
P01023	Alpha-2-macroglobulin precursor	/	/	/	2	1	9.05e+003