Supporting Information

Experimental section

1. Materials and reagents

Dopamine hydrochloride and 1,3,5-benzenetricarboxylic acid were purchased from Aladdin Chemistry Co., Ltd. (USA). Copper (Ⅱ) acetate monohydrate was purchased from Sinopharm Chemical Reagent Co., Ltd (Shanghai, China). The NdFeB magnet was purchased from PCCW (Beijing, China), 2 cm long, 2 cm wide, 1 cm high, with surface magnetic field strength of 1000 Gauss.

Ammonium bicarbonate (NH_4HCO_3), sodium phosphate monobasic dihydrate ($NaH_2PO_4 \cdot 2H_2O$) and disodium hydrogen phosphate dodecahydrate ($Na_2HPO_4 \cdot 12H_2O$) were purchased from Chinasun Specialty Porducts Co., Ltd (Jiangsu, China). L-1-tosylamido-2-phenylethylchloromethyl ketone (TPCK) treated trypsin (from bovine pancreas), α-chymotrypsin (from bovine pancreas), Cytochrome c (Cytc), myoglobin (MYO), bovine serum albumin (BSA), α-Cyano-4-hydroxy-cinnamic acid (α-CHCA) and trifluoroacetic acid (TFA) were purchased from Sigma Chemical (St. Louis, MO, USA). Acetonitrile (ACN) was purchased from Merck (Darmstadt, Germany). Distilled water was purified by a Milli-Q system (Milford, MA, USA).

All other chemicals and reagents are of the highest grade commercially available and used as received.

2. Synthesis of Fe_3O_4@PDA@$[Cu_3(btc)_2]$

Fe_3O_4 particles were synthesized via a hydrothermal reaction according to our previous reports[1]. Briefly, 1.35 g of $Fe_3O_4 \cdot 6H_2O$ was dissolved in 75 mL of ethylene glycol under magnetic stirring. After complete dissolution, 3.60 g of sodium acetate (NaAc) was added to the solution. After being stirred for another 1 h, the resulting solution was transferred into a Teflon-lined stainless-steel autoclave. The autoclave was sealed and heated at 200 °C for 16 h. The obtained magnetic nanoparticles were thoroughly washed with deionized water and ethanol.

Fe_3O_4 particles were encapsulated with polydopamine through the polymerization of dopamine in alkaline buffer at room temperature. In brief, 40 mg of dopamine hydrochloride was dispersed in 40 mL of Tris buffer (10 mM) and sonicated for 5 min. Then, 10 mg of the Fe_3O_4 particles were added and mechanically stirred for 10-20 h. The as-synthesized Fe_3O_4@PDA particles were separated and collected

with a magnet and washed with deionized water three times. Fe_3O_4 and Fe_3O_4@PDA products were dried in vacuum at 50 °C.

To synthesize Fe_3O_4@PDA@[$Cu_3(btc)_2$] composites, 0.05 g of Fe_3O_4@PDA particles were dispersed in an ethanol solution (40 mL) containing 80 mg of $Cu(CH_3COO)_2 \cdot H_2O$ (0.01 M) and 84 mg of 1,3,5-benzenetricarboxylic acid (H_3btc, 0.01 M). The mixture was heated at 70 °C for 30 min. Finally, the product was collected by magnetic separation and washed with ethanol three times. The amount of solvent used for washing the Fe_3O_4@PDA@[$Cu_3(btc)_2$] nanocomposites and intermediate products was 75 mL every time in each step.

3. Characterizations and measurements

Transmission electron microscopy (TEM) images were taken on a JEOL 2011 microscope (Japan) operated at 200 kV. Samples were dispersed in ethanol beforehand and collected by using carbon-film-covered copper grids for analysis. Scanning electronic microscope (SEM) images and energy dispersive X-ray (EDX) spectra were recorded on a Philips XL30 electron microscope (Netherlands) operating at 20 kV. A thin gold film was sprayed on the sample before measurements. Fourier transform infrared (FT-IR) spectra were obtained with a Nicolet Fourier spectrophotometer (U.S.A) using KBr pellets. Raman spectra were collected by a LabRam-1B Raman spectrometer with a laser at an excitation wavelength of 632.8 nm at room temperature. Powder X-ray diffraction (XRD) patterns were recorded on a Bruker D4 X-ray diffractometer with Ni-filtered Cu K_α radiation (40 kV, 40 mA). Nitrogen adsorption-desorption isotherms were measured with a Micromeritcs Tristar 3000 analyzer (USA) at 77 K. The Brunauer-Emmett-Teller (BET) method was used to calculate the specific surface area.

4. Sample preparation

To prepare a phosphate buffer saline (PBS, pH = 6.5), we firstly prepared the aqueous solutions of Na_2HPO_4 (0.2 M) and NaH_2PO_4 (0.2 M). Afterwards, 31.5 mL of the NaH_2PO_4 solution was mixed with 68.5 mL of the Na_2HPO_4 solution to obtain the targeted PBS solution.

To prepare the α-CHCA matrix solution, we dissolved 8 mg of α-CHCA in 1 mL of 50%ACN/0.1%TFA buffer. Meanwhile 6 mg of ammonium hydrogen citrate was dissolved in 10 mL of 50%ACN/0.1%TFA buffer. The two solutions of equal volume were mixed to get the α-CHCA matrix.

To prepare the NH_4HCO_3 solution of Cytc or MYO, we dissolved 1 mg of the protein in 1 mL of

deionized water and denatured it in a boiling water bath for 10 min. The Cytc/MYO solution was exchanged into 25 mM NH_4HCO_3 buffer (with the final pH of 8.3 and the final concentration of 0.2 μg/μL). To investigate the feasibility for digesting low-concentration proteins, we adjusted the concentration of the Cytc solution to 2 ng/μL.

5. Immobilization of enzymes onto Fe_3O_4@PDA@$[Cu_3(btc)_2]$

About 1 mg of Fe_3O_4@PDA@$[Cu_3(btc)_2]$ composites were washed with the PBS (pH = 6.5) three times and then redispersed in a PBS solution of enzyme (2 μg/μL, 100 μL) with the help of a vortex. The immobilization reaction was performed at 37 °C for 16 h in a rotary shaker. The enzyme-immobilized magnetic MOFs were collected by magnetic separation and washed with the PBS for five times before the digestion of proteins. To test the stability of the enzyme-immobilized magnetic MOFs, we stored them at -20 °C for further use.

The UV absorption value of the supernatant was measured at λ = 595 nm and compared with the UV absorption value of the trypsin solution before immobilization to evaluate the amount of trypsin immobilized on the magnetic MOF composites. To achieve accurate calculation, a calibration curve was recorded by using a series of bovine serum albumin (BSA) solutions with different concentrations under the same condition.

6. Protein digestion by Fe_3O_4@PDA@$[Cu_3(btc)_2]$-enzyme nanocomposites

Fe_3O_4@PDA@$[Cu_3(btc)_2]$-enzyme nanocomposites were washed with 25 mM NH_4HCO_3 solution for three times in advance. Then, the composites were dispersed in 200 μL of Cytc solution and incubated in a rotary shaker at 37 °C for 10 min. After digestion, a magnet was used to retain the conjugates, and the supernatant was directly deposited on a MALDI plate.

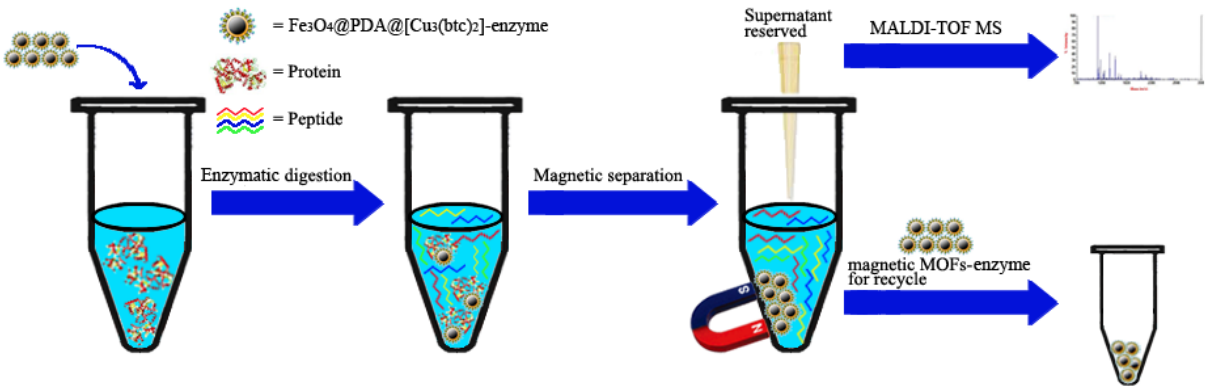
7. In-solution digestion of cytochrome c and MYO

In comparison, in-solution digestion was also carried out. The NH_4HCO_3 solution of Cytc (200 μL) was incubated with trypsin (40:1, w/w) at 37 °C for 16 h.

In order to calibrate the MALDI-TOF MS analyzer before the identification of the peptides, we also treated the 25 mM NH_4HCO_3 solution of MYO with the conventional in-solution digestion.

8. MALDI-TOF-MS analysis and database searching

The tryptic digests of Cytc (1μL) were deposited on a MALDI sample target (Applied Biosystems, MDS SCIEX, Foster City, CA, USA) and dried at room temperature. Then, 0.5 μL of CHCA matrix was dropped on it. Four replicate spots were taken for every sample. After the evaporation of the solvent, the substrates were submitted to MALDI-TOF MS for analysis. Mass spectra were acquired in positive reflective mode on a 5800 Proteomics Analyzer (Applied Biosystems, Framingham, MA, USA) with the Nd: YAG laser at 366 nm, the repetition rate of 200 Hz and the acceleration voltage of 20 kV. Before the identification of the peptides, the instrument was calibrated in an internal calibration mode by a tryptic digest of MYO. All the spectra were taken from signal-averaging of 800 laser shots with the laser intensity kept at a proper constant. GPS Explorer software (Applied Biosystems, Framingham, MA, USA) with MASCOT (Matrix Science, London, UK) searching engine and SwissProt (Version 54) database was used to interpret the MALDI-TOF MS data by searching the species of mammals. The searching parameters were set up as follows: enzyme was trypsin or α-chymotrypsin, the maximum of missed cleavages was allowed up to 1, the peptide mass tolerance was ± 0.3 Da.



Scheme S1 The procedure for the digestion of proteins by Fe_3O_4@PDA@$[Cu_3(btc)_2]$-enzyme nanocomposites.

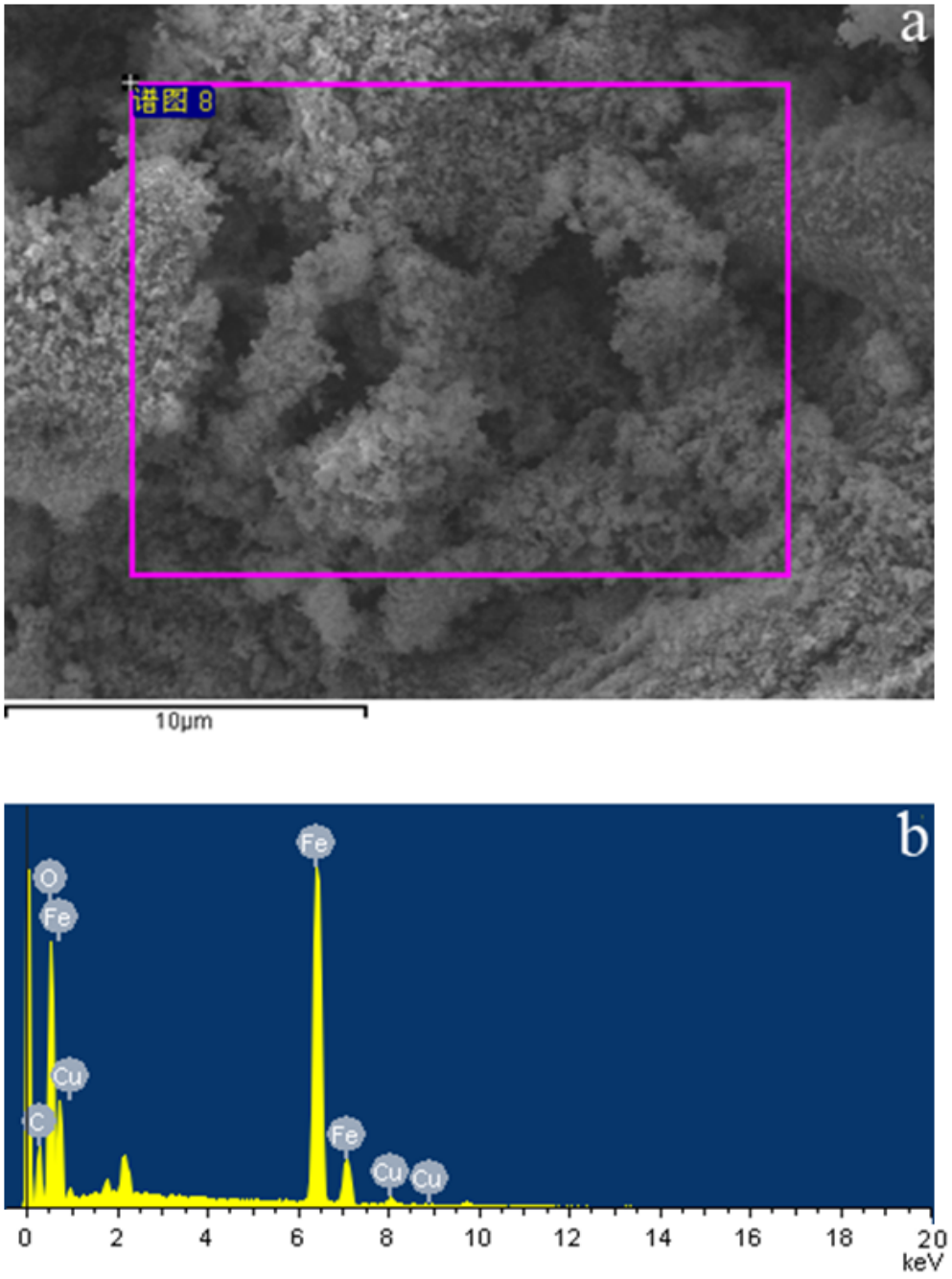


Fig. S1 a) The corresponding SEM image chosen for the energy dispersive X-ray (EDX) analysis and b)The EDX spectrum of Fe_3O_4@PDA@[$Cu_3(btc)_2$] composite microspheres.

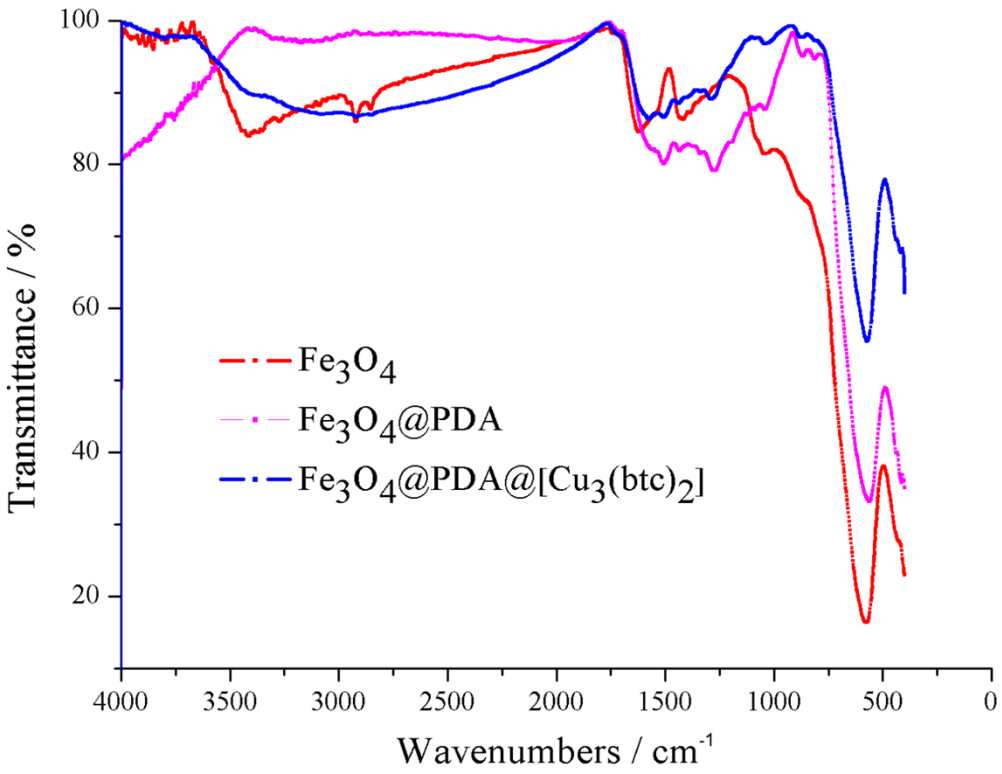


Fig. S2 The FT-IR spectra of Fe_3O_4, Fe_3O_4@PDA and Fe_3O_4@PDA@[$Cu_3(btc)_2$].

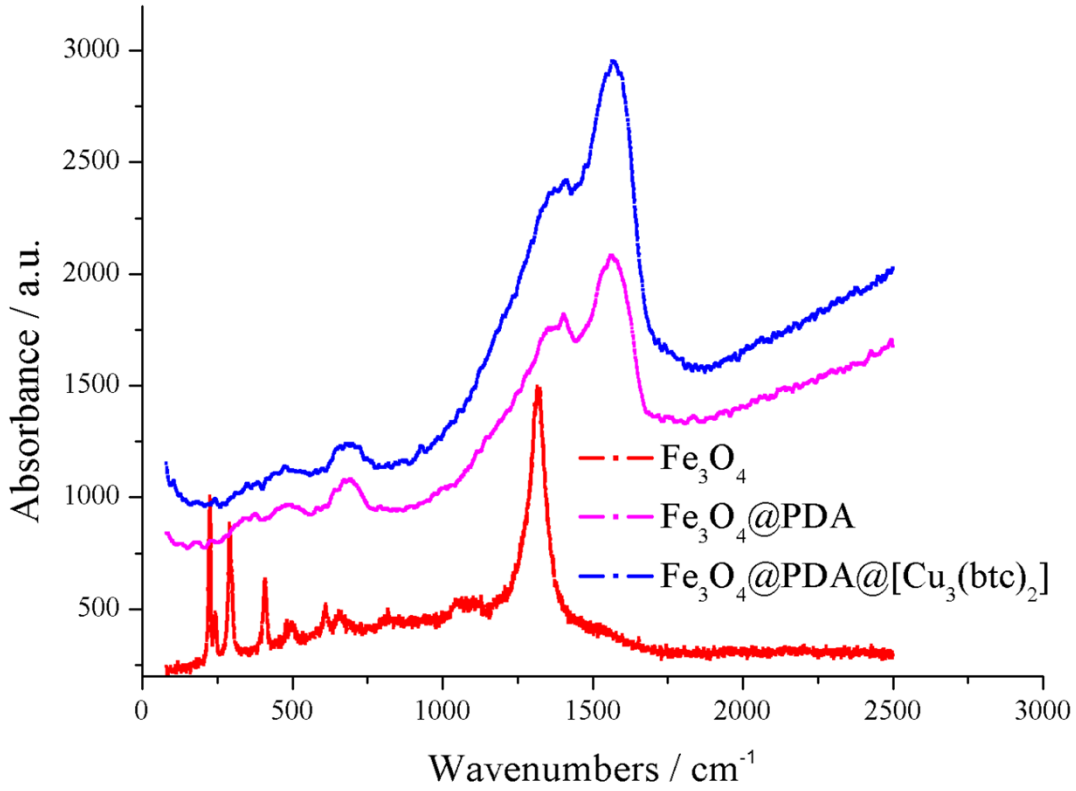


Fig. S3 The Raman spectra of Fe_3O_4 particles, Fe_3O_4@PDA and Fe_3O_4@PDA@[$Cu_3(btc)_2$] composite microspheres.

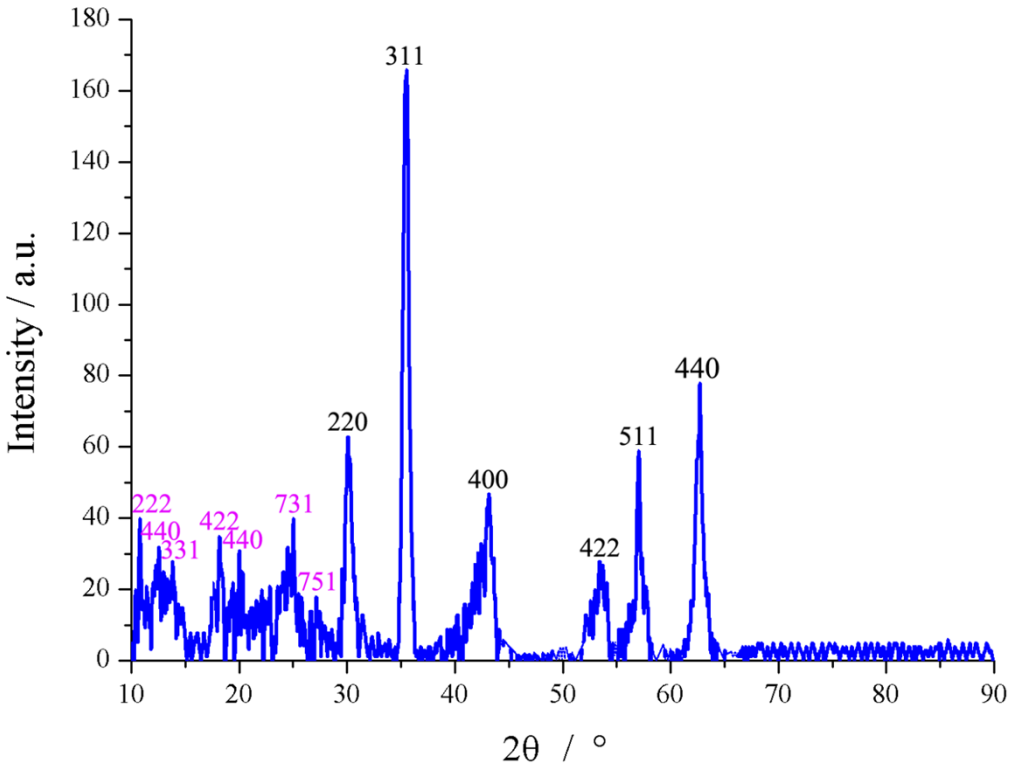


Fig. S4 The XRD patterns of Fe_3O_4@PDA@[$Cu_3(btc)_2$]. Peaks originated from [$Cu_3(btc)_2$] MOFs are marked with the miller indexes in pink and those originated from Fe_3O_4 are marked with the miller indexes in black.

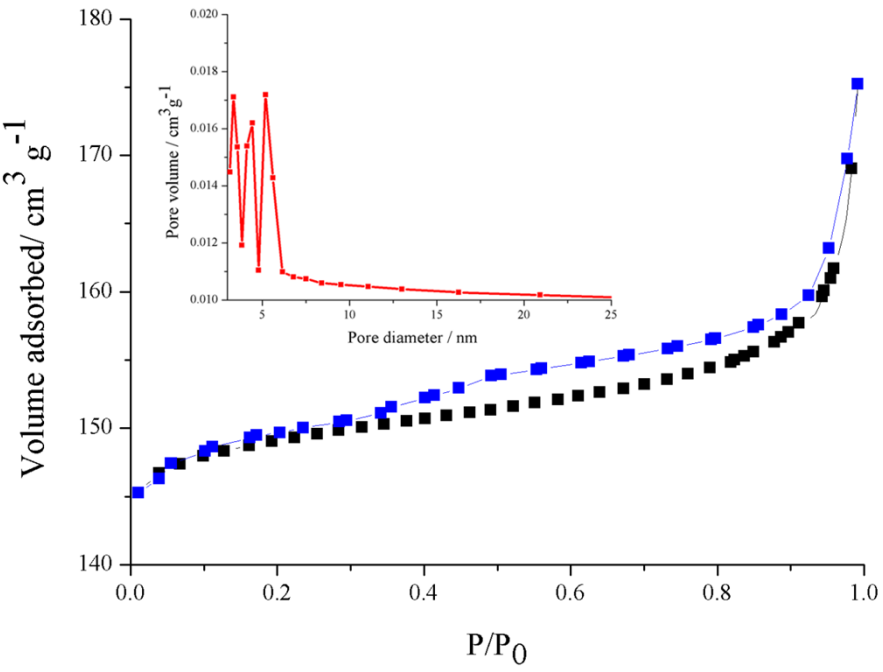


Fig. S5 The nitrogen adsorption-desorption isotherms of Fe_3O_4@PDA@[$Cu_3(btc)_2$] measured at 77 K. The inset shows corresponding pore size distribution analysis obtained using the Barrett-Joyner-Halenda (BJH) method.

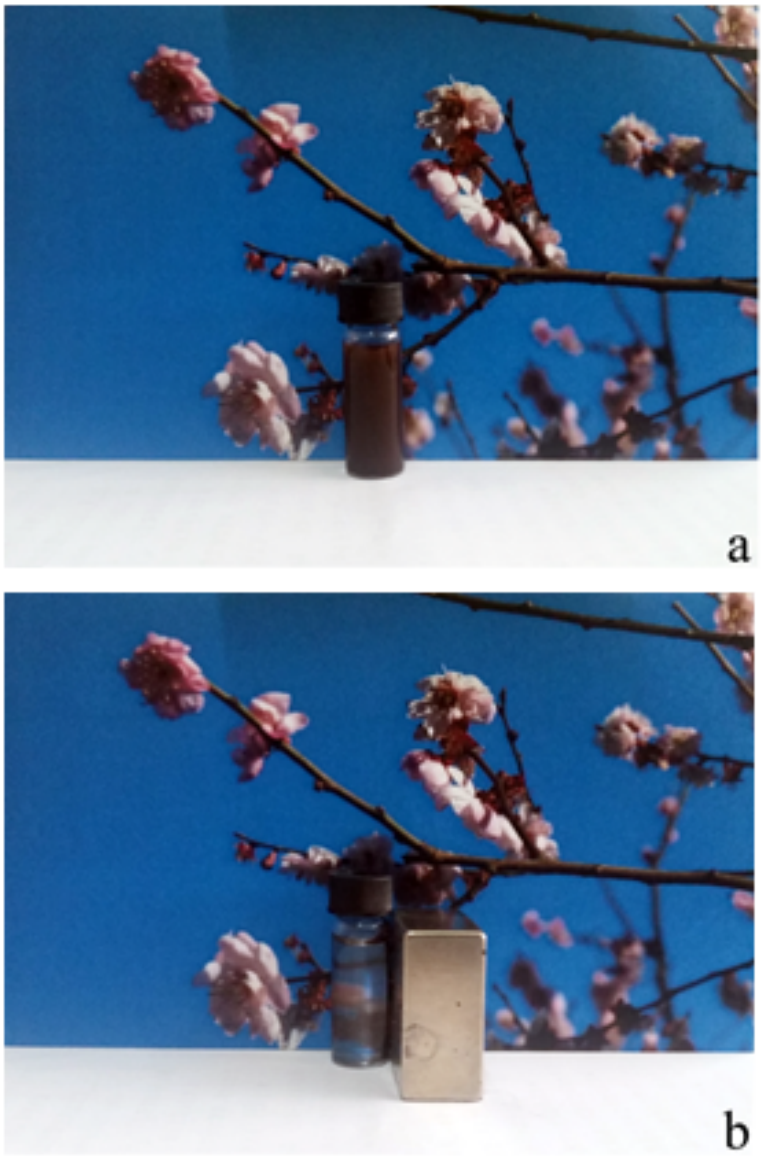


Fig. S6 The photos of the aqueous dispersion of Fe_3O_4@PDA@$[Cu_3(btc)_2]$: (a) before and (b) after separation with a magnet for 2 seconds.

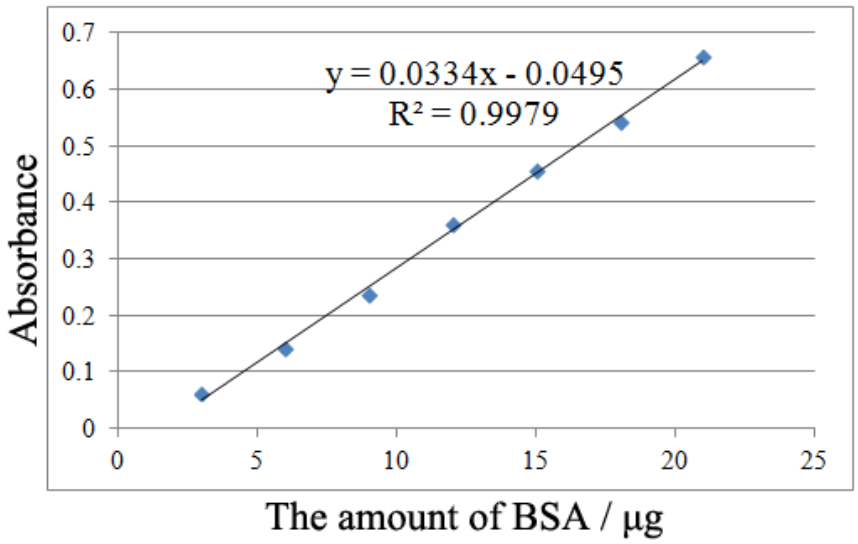


Fig. S7 The calibration curve obtained by using a series of BSA solutions with different concentrations.

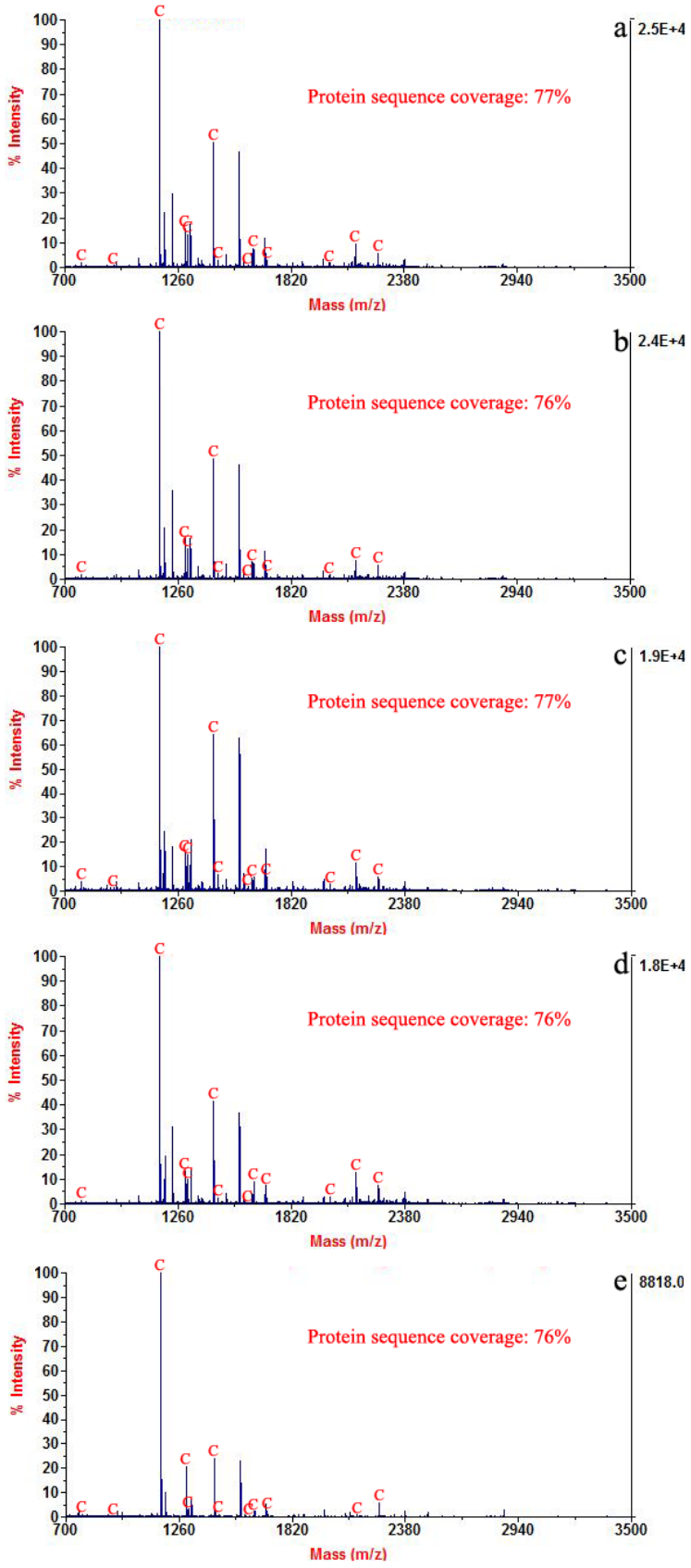


Fig. S8 MALDI-TOF mass spectra of a Cytc solution (0.2 μg/μL) after treatment with the Fe_3O_4@PDA@[$Cu_3(btc)_2$]-trypsin used: a) for the first time, b) the 2nd time, c) the 3rd time, d) the 4th time and e) the 5th time. The peaks marked with capital c represent peptides originated from Cytc.

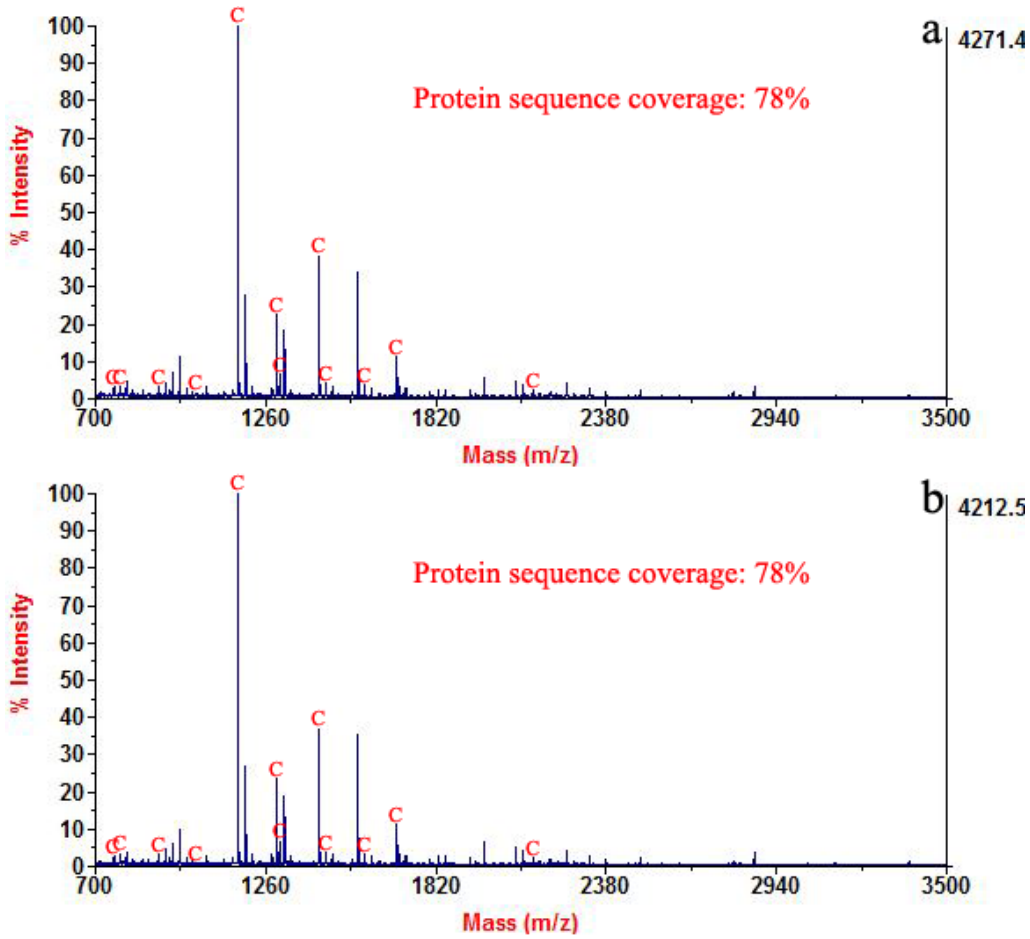


Fig. S9 MALDI-TOF mass spectra of a Cytc solution (0.2 μg/μL) after treatment with the Fe_3O_4@PDA@[$Cu_3(btc)_2$]-trypsin: a) freshly prepared and b) after being stored at -20 °C for one month. The peaks marked with capital c represent peptides originated from Cytc.

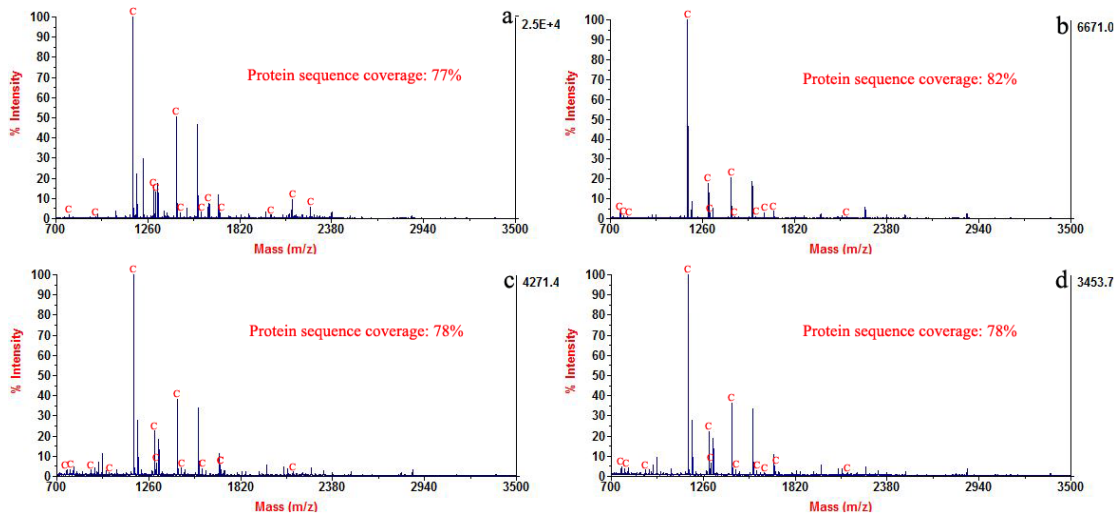


Fig. S10 MALDI-TOF mass spectra of Cytc solutions (0.2 μg/μL) after 10-min digestion with different batches of Fe_3O_4@PDA@[$Cu_3(btc)_2$]-trypsin nanocomposites. The peaks marked with capital c represent peptides originated from Cytc.

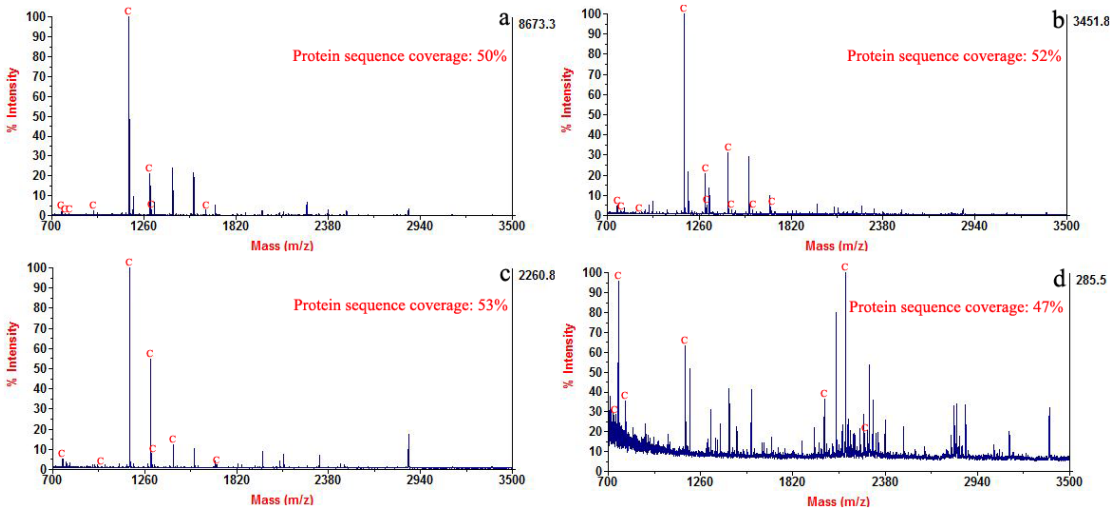


Fig. S11 MALDI-TOF mass spectra of Cytc solutions (2 ng/μL) after 10-min digestion with different batches of Fe_3O_4@PDA@[$Cu_3(btc)_2$]-trypsin nanocomposites. The peaks marked with capital c represent peptides originated from Cytc.

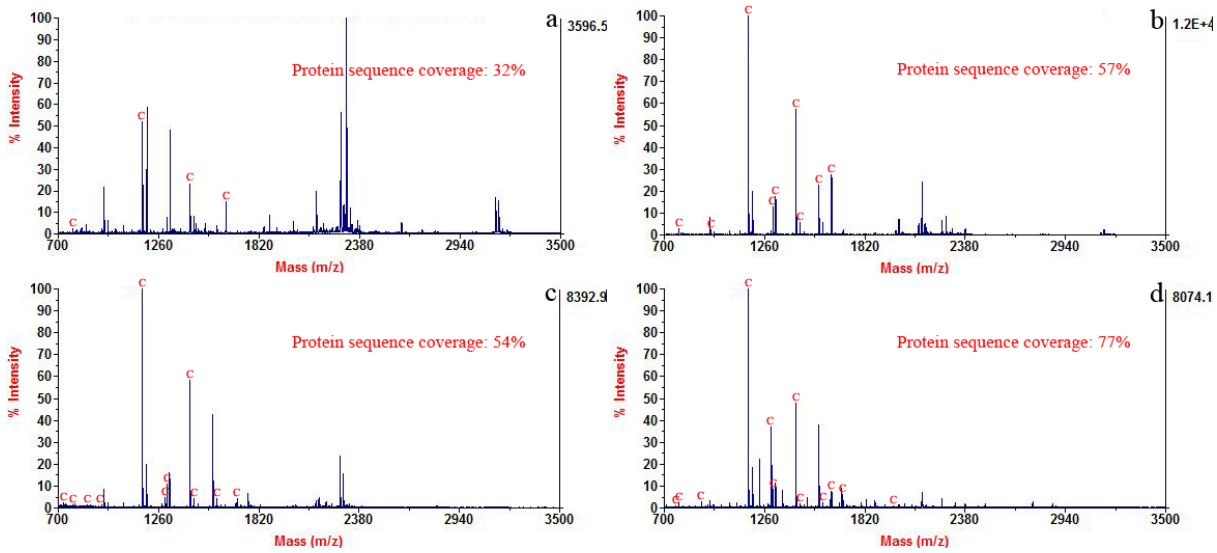


Fig. S12 MALDI-TOF mass spectra of a Cytc solution (0.2 μg/μL) after 10-min digestion with: a) Fe_3O_4-trypsin composites, b) PDA-trypsin composites, c) Fe_3O_4@PDA-trypsin composites and d) $Cu_3(btc)_2$-trypsin composites. The peaks marked with capital c represent peptides originated from Cytc.

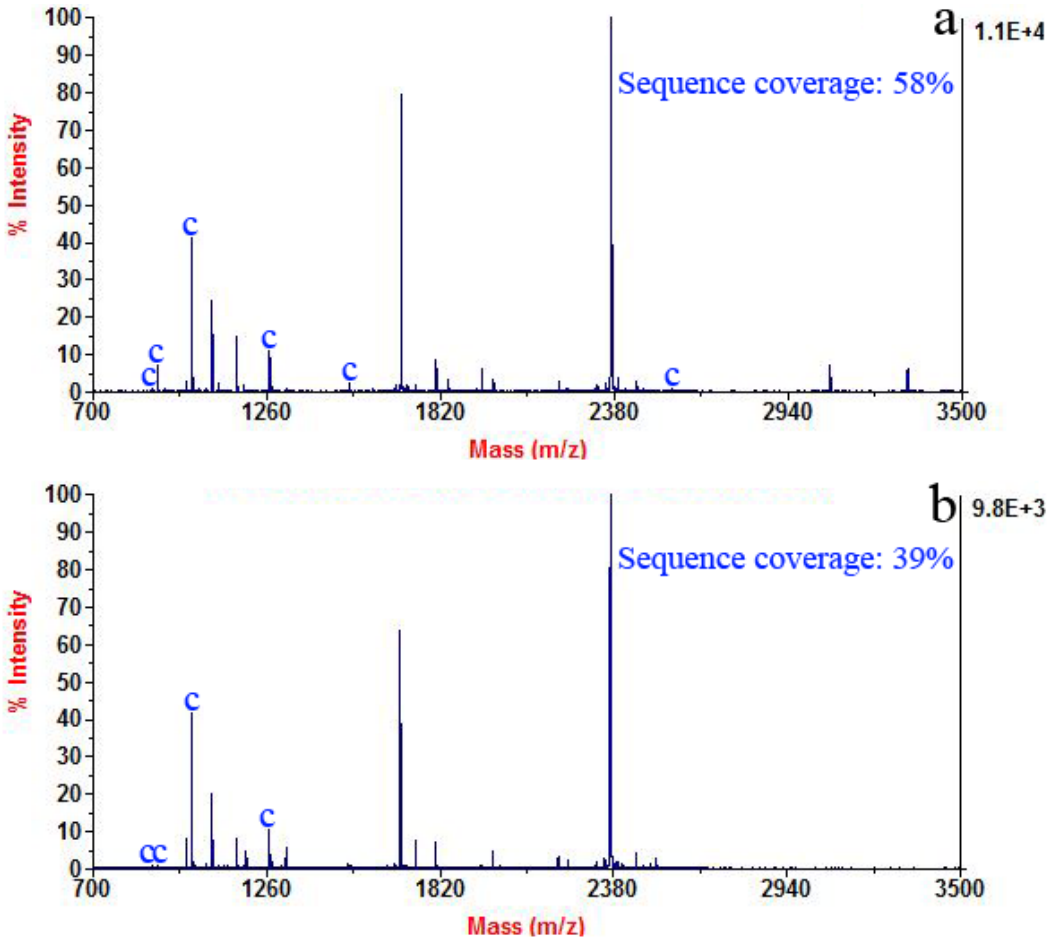


Fig. S13 MALDI-TOF mass spectra of a Cytc solution (0.2 μg/μL) after: a) in-solution digestion with α-chymotrypsin for 16 h and b) digestion with Fe_3O_4@PDA@[$Cu_3(btc)_2$]-chymotrypsin nanocomposites for 10 min. The peaks marked with capital c represent peptides originated from Cytc.

Table S1 The UV absorption values of the PBS solution of trypsin before and after the immobilization procedure and a series of BSA solutions with different concentrations.

Sample	3 μg of BSA	6 μg of BSA	9 μg of BSA	12 μg of BSA	15 μg of BSA	18 μg of BSA	21 μg of BSA	The trypsin solution before immobilization	The supernatant after immobilization
The UV absorption value	0.0619	0.1425	0.2374	0.3612	0.4553	0.5428	0.6573	0.2416	0.0527

Table S2 Peptides identified from the tryptic digests of Cytc after digestion with Fe_3O_4@PDA@[Cu_3(btc)$_2$]-trypsin nanocomposites and after treatment with in-solution digestion.

Start -End	Mr (expt)	Peptide	Digestion with Fe_3O_4@PDA@[Cu_3(btc)$_2$]-trypsin		Treatment with in-solution digestion
			0.2 μg/μL Cytc	2 ng/μL Cytc	0.2 μg/μL Cytc
9-14	761.3359	K.KIFVQK.C	√	√	
81-87	778.4189	K.MIFAGIK.K	√	√	√
74-80	805.3246	K.KYIPGTK.M	√		
81-88	906.5074	K.MIFAGIKK.K	√	√	
15-23	1017.5254	K.CAQCHTVEK.G	√	√	
29-39	1167.5786	K.TGPNLHGLFGR.K	√	√	√
29-40	1295.6861	K.TGPNLHGLFGRK.T	√	√	√
90-100	1305.6674	K.GEREDLIAYLK.K	√	√	√
27-39	1432.7426	K.HKTGPNLHGLFGR.K	√	√	√
41-54	1455.6238	K.TGQAPGFSYTDANK.N	√		√
40-54	1583.7107	R.KTGQAPGFSYTDANK.N	√		√
10-23	1632.5702	K.IFVQKCAQCHTVEK.G	√	√	√
41-56	1697.7285	K.TGQAPGFSYTDANKNK.G	√	√	
57-73	2008.8325	K.GITWGEETLMEYLENPK.K	√		√
57-74	2136.9380	K.GITWGEETLMEYLENPKK.Y	√		√
55-73	2250.9229	K.NKGITWGEETLMEYLENPK.K	√		

Table S3 Peptides identified from the chymotryptic digests of Cytc after digestion with Fe_3O_4@PDA@[Cu_3(btc)$_2$]-chymotrypsin nanocomposites and after treatment with in-solution digestion.

Start -End	Mr (expt)	Peptide	Digestion with Fe_3O_4@PDA@[Cu_3(btc)$_2$]-chymotrypsin	Treatment with in-solution digestion
			0.2 μg/μL Cytc	0.2 μg/μL Cytc
9-14	890.5632	K.KIFVQK.C	√	√
81-87	905.5790	K.MIFAGIK.K	√	√
74-80	1017.6383	K.KYIPGTK.M	√	√
81-88	1267.7611	K.MIFAGIKK.K	√	√
15-23	1522.9688	K.CAQCHTVEK.G	√	
29-39	2562.4522	K.TGPNLHGLFGR.K	√	

References

1 X. Xu, C. Deng, M. Gao, W. Yu, P. Yang and X. Zhang, *Adv. Mater.*, 2006, **18**, 3289.