Supporting Information

Electrospinning-based synthesis of highly ordered mesoporous silica fiber for lab-in-syringe enrichment of plasma peptides

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Experimental details

Chemicals and Materials. Polyvinyl alcohol (PVA, average MW 110,000), cetyltrimethylammonium bromide (CTAB), ethanol (EtOH), orthophosphoric acid (H_3PO_4), sodium hydroxide (NaOH), and other chemicals were supplied by Shanghai General Chemical Reagent Factory (Shanghai, China). Tetraethyl orthosilicate (TEOS) was obtained from the Chemical Plant of Wuhan University (Wuhan, China). HPLC grade acetonitrile (ACN) was obtained from Fisher Scientific (Pittsburgh, USA). Cotton wool was supplied by Xuzhou Hygiene of Material Factory (Xuzhou, China). α-Cyano-4-hydroxycinnamic acid (CHCA), trifluoroacetic acid (TFA), Angiotensin II, lysozyme, myoglobin, horseradish peroxidase (HRP) and bovine serum albumin (BSA) were purchased from Sigma-Aldrich (St Louis, USA). Sequencing grade trypsin was obtained from Promega (Madison, WI, USA). Human plasma sample was obtained from the Wuhan Zhongnan Hospital according to their standard clinical procedures and stored at -70^{o}C until use. Purified water was obtained with a Milli-Q apparatus (Millipore, Bedford, MA, USA).

Preparation of ordered mesoporous silica fiber (OMSF). The amorphous silica fiber (ASF) was fabricated by electrospinning according to previous report with some modifications.[1] The precursor sols for electrospinning were prepared by the following procedure. First, 2.5 g of CTAB was dissolved in 5 mL of CH_3CH_2OH and 15 mL of H_2O. Then after 16 g TEOS was added to the mixture, 200 μL of H_3PO_4 was added dropwise to the solution. After stirring for 5 h, 30 g of 8 wt % PVA solution was added into the silica sol to make the viscosity suitable for electrospinning. The

reactant mixture was further stirred for 8 h to get a spinnalbe sol. The electrospinning process was performed using a home-made device (Figure S1). Briefly, the sol of PVA/SiO_2 composites was added into a plastic syringe connecting with an iron needle with an inner diameter of 0.7 mm. The sol was driven by a four channels syringe pump with a flow rate of 0.4 mL/h. A grounded aluminum sheet covered with tinfoil served as collector and counter electrode and the distance of tip-to-collector was 15 cm. A high voltage of 15 kv was applied to the needle and a jet was moved and covered on the tinfoil. The fibers were collected and dried at 60°C for 12 h. The PVA and CTAB were removed after calcination at 550°C for 8 h.

The as-prepared ASF was transformed into OMSF via pseudomorphic synthesis.[2] The molar ratio of all components was 1 SiO_2: 0.3 NaOH: 0.1 CTAB: 80 H_2O. Typically, silica fiber (1.8 g) were added to a mixture of CTAB (1.092 g), water (43.2 mL) and NaOH (0.36 g) and then stirred at room temperature for 30 min. The hydrothermal reaction was carried out in a Teflon-lined autoclave at 130°C for 24 h. The products were washed with deionized water for several times. Finally, the fibers were heated at 550°C for 8 h to remove the template CTAB, and then OMSF was finally obtained.

Characterization of the prepared materials. Scanning electron microscopy images were taken using JSM-6700F field emission scanning electron microscope (FESEM, JEOL, Japan). Transmission electron microscopy images were obtained from JEM-2100 (HT) transmission electron microscope (TEM, JEOL, Japan). The powder X-ray diffraction (XRD) measurements were recorded on a D/MAX-RB X-ray

powder diffractometer (RIGAKU, Japan) using Cu Kα radiation (λ= 1.5406 Å) with scattering angles (2θ) of 0.5-5°. Nitrogen sorption measurement was performed at 77 K using a JW-BK surface area and pore size analyzer (JWGB Sci. & Tech., Beijing, China). The composites were activated by evacuating in vacuum and heating to 423 K for 6 h to remove any physically adsorbed substances before analysis. The specific surface area value was calculated according to the BET (Brunauer–Emmett–Teller) equation at P/P_0 between 0.05 and 0.3. The pore parameters (pore volume and pore diameter) were evaluated from the desorption branch of isotherm based on BJH (Barrett–Joyner–Halenda) model.

Preparation of OMSF packed syringe. As shown in Figure S4, the OMSF was packed in the hub of a 1 mL syringe. To ensure the hub was fully filled and avoid the OMSF moving during the extraction process, the OMSF was packed between two cotton layers. Additionally, the cotton layers could protect the OMSF and increase the reuse times of the lab-in-syringe system. Typically, a small piece of cotton wool (approximate 200 μg) was pushed into the hub. Then OMSF (1 mg) was added followed by covering with another piece of cotton wool (approximate 100 μg). The sandwich packing bed was compacted tightly when the barrel was connected to the hub.

Preparation of peptides. BSA (1 mg) was dissolved in 100 μL of denaturing buffer solution (8 M urea in 100 mM Tris-HCl, pH 8.5). The obtained protein solution was mixed with 5 μL of 100 mM tri(2-chloroethyl)phosphate (TCEP) and incubated for 20 min at room temperature to reduce protein disulfide bonding. 3 μL 500 mM

Iodoacetamide was added, and the resulting solution was incubated for an additional 30 min at room temperature in the dark. The reduced and alkylated protein mixture was diluted with 300 μL 100 mM Tris-HCl (pH 8.5). Subsequently, 9 μL of 100 mM $CaCl_2$ was added, and the mixture (~50 μL in volume) was digested with trypsin at an enzyme to substrate ratio of 1:50 (w/w) by incubating overnight at 37°C.

Peptides enrichment with OMSF using the lab-in-syringe SPE approach. The OMSF-packed syringe was directly used for peptides enrichment. BSA digests or standard protein aqueous solution (500 μL) was pipetted up and down 20 times to allow peptides to be adsorbed on the OMSF. After washing twice with 500 μL water, the trapped peptides were eluted with 50 μL 50% ACN containing 0.1% TFA. Finally, original solution (1 μL), solution after extraction (1 μL) and eluate (1 μL), were directly applied for MALDI-TOF MS analysis.

Human plasma (50 μL) was diluted with 450 μL water, and the solution was pipetted up and down 30 times. And then the packed bed was successively washed with water (500 μL) three times. The adsorbed peptides were eluted with 50 μL 50% ACN containing 0.1% TFA and 2 μL of the eluate was applied for MALDI-TOF MS analysis.

Enrichment of BSA digests with Fe_3O_4@mSiO$_2$. Fe_3O_4@mSiO$_2$ was prepared according to the method described in our previous work[3] and was used as a comparison for the enrichment of peptides. BSA digests (aqueous solution, 20 fmol/μL, 500 μL) was mixed with suspension of Fe_3O_4@mSiO$_2$ microspheres (5 μL, 5 mg/mL, in water), and shaken at room temperature for 3 min. After decanting the

supernatant, the residue was successively washed with 500 μL H_2O three times and the trapped peptides were eluted with 50 μL 50% ACN containing 0.1% TFA, and 1 μL of the eluate was applied for MALDI-TOF MS analysis.

Desalting with Zip-Tip C18 pipette tip. The sample preparation of human plasma for MALDI-TOF MS analysis with Zip-Tip C18 pipette tip was according to the manufacturer's recommended procedures as follow (Millipore Corporation, Billerica, MA, USA).

1. Prewet the tip twice by wetting solution (50% ACN containing 0.1% TFA).
2. Equilibrate the tip for binding by washing it twice with the equilibration solution (0.1% TFA in Milli-Q water).
3. Bind peptides or proteins to Zip-Tip C18 by aspirating and dispensing 10 μL human plasma (diluted 10 times by water) 10 cycles.
4. Wash the tip twice by washing solution (0.1% TFA in Milli-Q water).
5. Elute peptides or proteins by eluate solution (4 μL 80% ACN containing 0.1% TFA).

MALDI-TOF MS analysis. Sample solutions (peptides or protein) were deposited on the stainless steel target probe using the dried droplet method. An amount of 1 or 2 μL of sample solution was applied onto the target, and then another 1 μL of CHCA matrix solution (2 mg/mL, 0.1% TFA in 50% ACN/H_2O solution) was introduced. All MALDI-TOF mass spectra were collected with an Axima TOF^2 mass spectrometry (Shimadzu, Kyoto, Japan). The instrument was equipped with a 337 nm nitrogen laser with a 3 ns pulse width. All the mass spectra were performed in positive ion mode

with an accelerating voltage of 20 kV. Typically, 200 laser shots were averaged to generate each spectrum. Analysis of peptides and proteins was performed in the reflector and linear TOF detection modes, respectively.

Reference

1. C. Shao, H. Kim, J. Gong and D. Lee, *Nanotechnology*, 2002, **13**, 635-637.
2. T. Martin, a. Galarneau, F. Di Renzo, F. Fajula and D. Plee, *Angew. Chem. Int. Ed.*, 2002, **41**, 2590-2592.
3. G.-T. Zhu, X.-S. Li, Q. Gao, N.-W. Zhao, B.-F. Yuan and Y.-Q. Feng, *J. Chromatogr. A*, 2012, **1224**, 11-18.

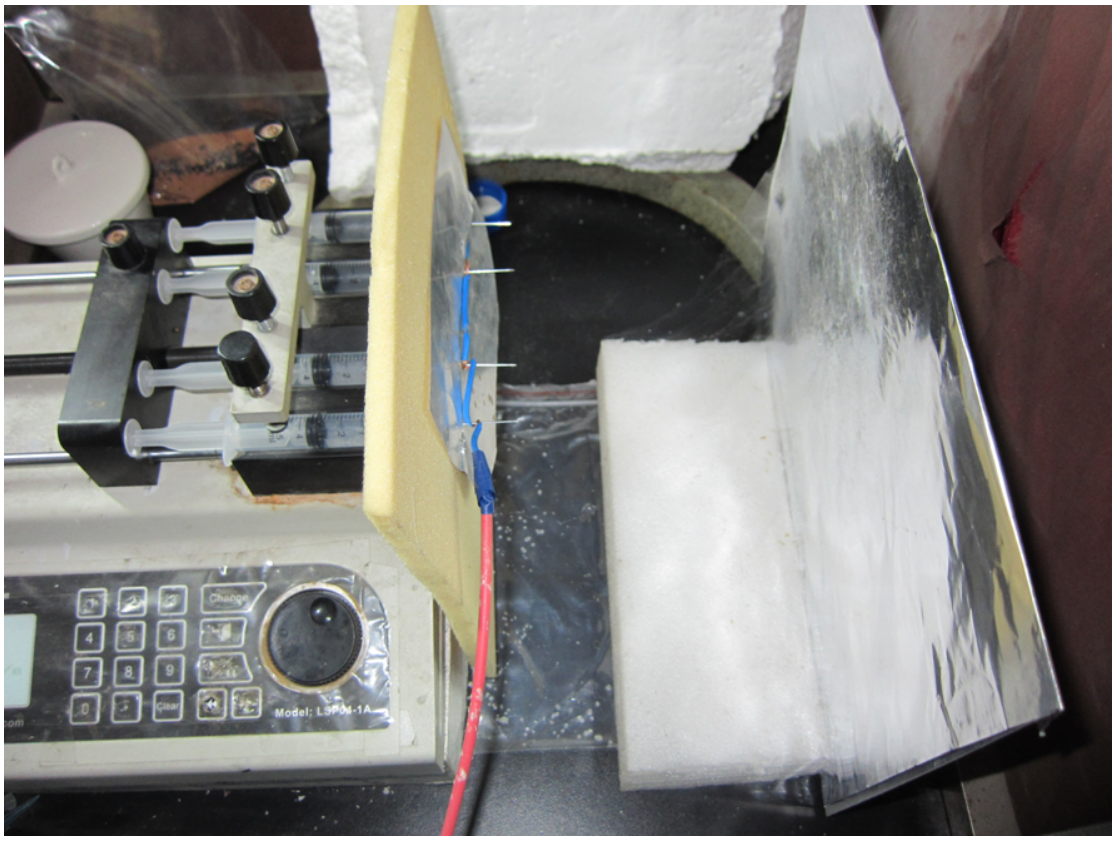

Figure S1. Photograph of the electrospinning setup in current study.

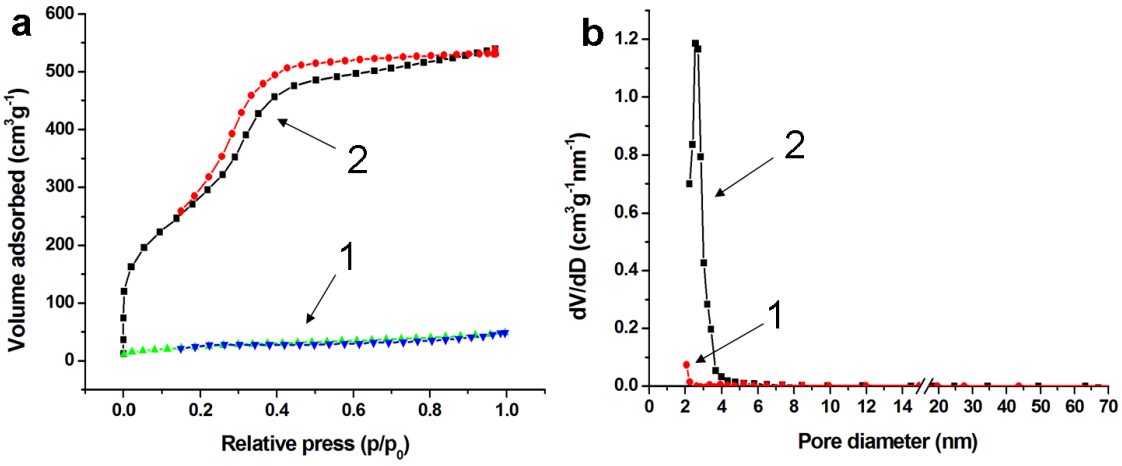

Figure S2. N_2 adsorption–desorption isotherms of ASF (a1) and OMSF (a2), and pore size distributions of ASF (b1) and OMSF (b2).

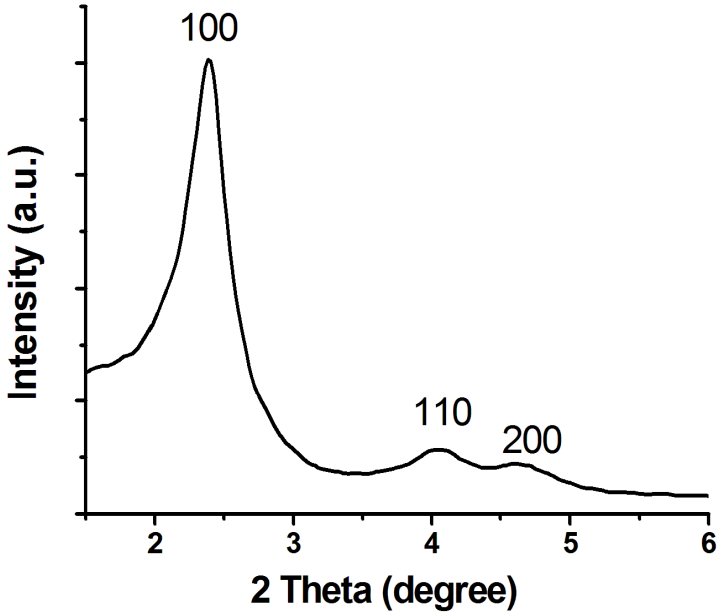

Figure S3. Low-angel XRD pattern of OMSF.

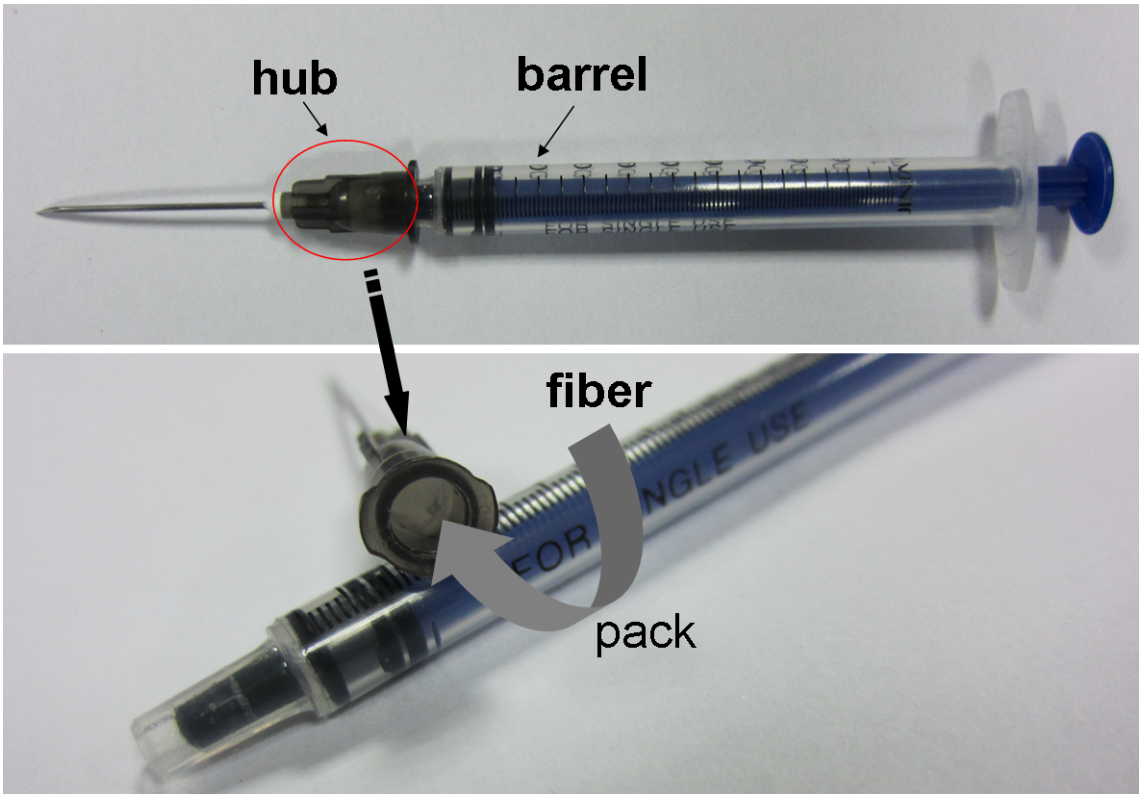

Figure S4. Photographs of the OMSF packed lab-in-syringe system for peptides enrichment in current study.

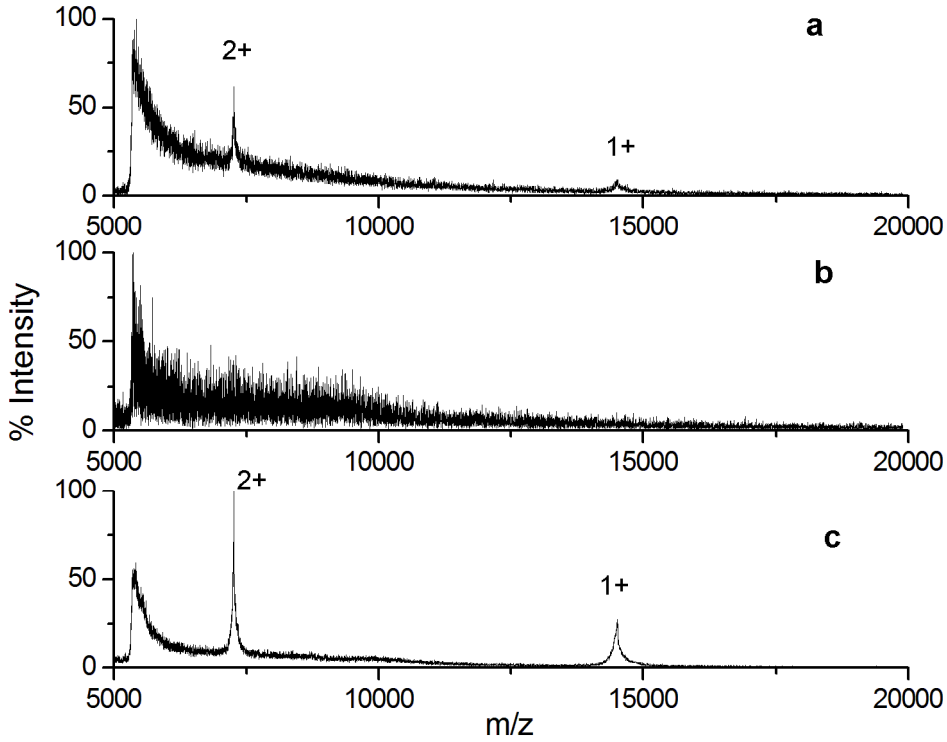

Figure S5. MALDI-TOF mass spectra of 50 nM lysozyme (MW 14.4 kDa) without any treatment (a), and solution after extraction (b) or eluate (c) after enrichment with OMSF using the lab-in-syringe approach.

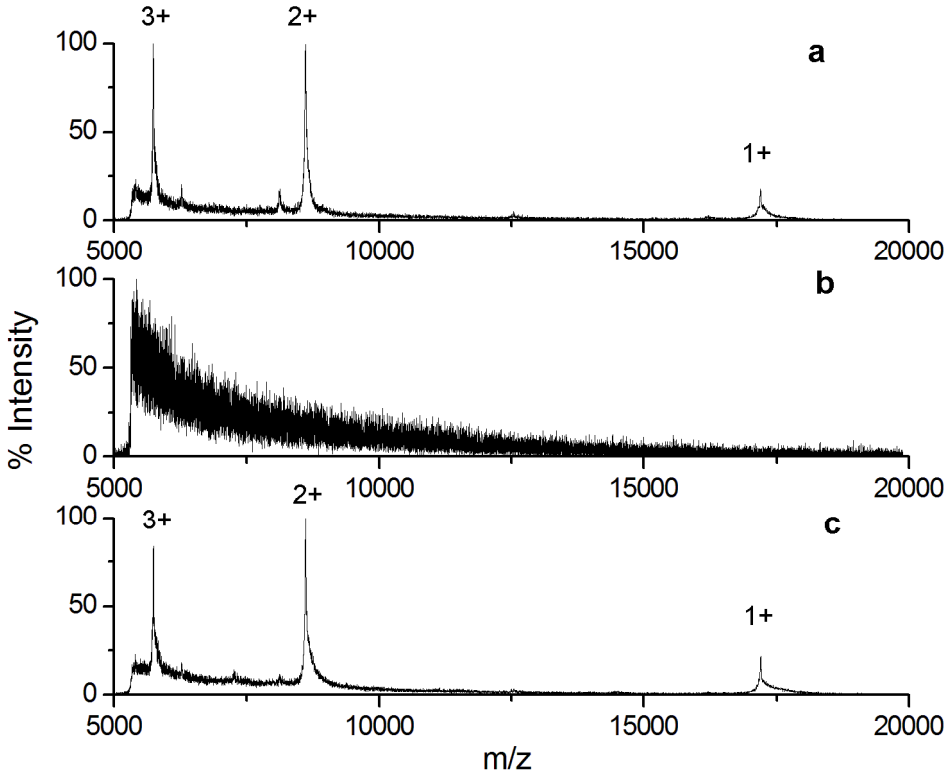

Figure S6. MALDI-TOF mass spectra of 250 nM myoglobin (MW 17.0 kDa) without any treatment (a), and solution after extraction (b) or eluate (c) after enrichment with OMSF using the lab-in-syringe approach.

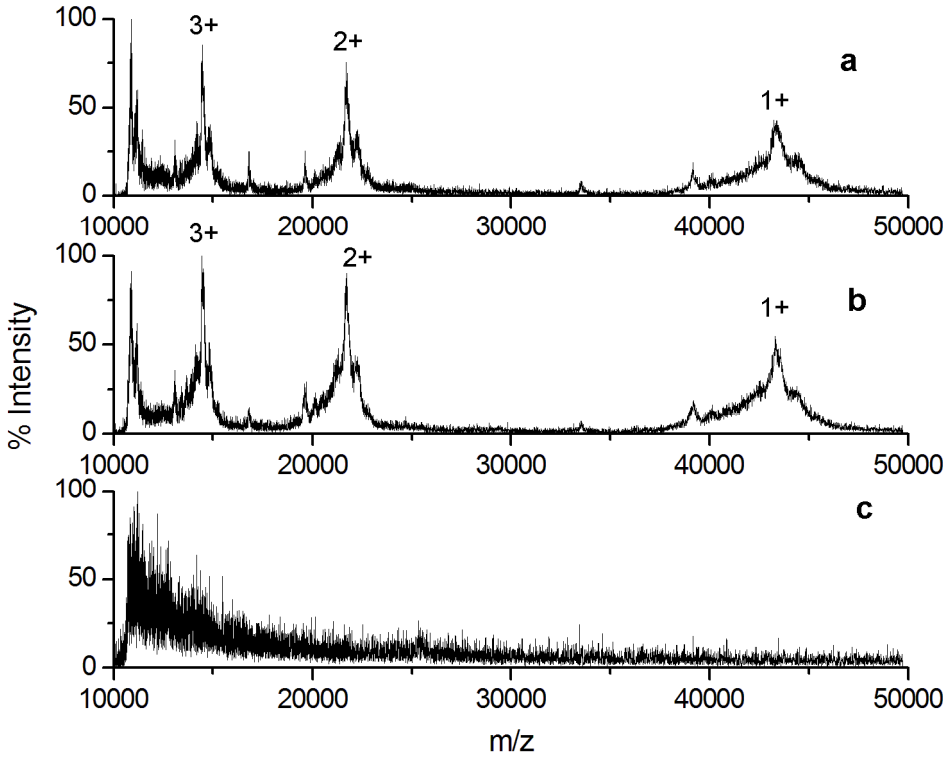

Figure S7. MALDI-TOF mass spectra of 500 nM HRP (MW 44.0 kDa) without any treatment (a), and solution after extraction (b) or eluate (c) after enrichment with OMSF using the lab-in-syringe approach.

Table S1. Detailed information of the observed peptides from BSA digests.

Calculated m/z	Database sequence	A	B	C	D
817.8841	K.ATEEQLK.T			√	√
838.9066	K.CCAADDK.E				√
927.0546	K.YLYEIAR.R		√	√	√
1163.3201	K.LVNELTEFAK.T	√	√	√	√
1283.4753	R.HPEYAVSVLLR.L		√	√	√
1305.4791	K.HLVDEPQNLIK.Q		√	√	√
1307.7197	K.HKPKATEEQLK.T		√	√	
1399.6106	K.TVMENFVAFVDK.C		√	√	√
1403.7265	K.GACLLPKIETMR.E		√	√	
1414.6803	K.TVMENFVAFVDK.C		√	√	√
1419.6016	K.SLHTLFGDELCK.V		√	√	√
1438.8045	R.RHPEYAVSVLLR.L	√	√	√	√
1443.5353	K.YICDNQDTISSK.L		√		
1456.8514	R.ALKAWSVARLSQK.F		√	√	
1463.5500	K.TCVADESHAGCEK.S		√		√
1467.7582	R.DTHKSEIAHRFK.D		√	√	
1478. 5813	R.ETYGDMADCCEK.Q	√	√	√	√
1493.9181	K.QIKKQTALVELLK.H			√	√
1502.6225	K.EYEATLEECCAK.D				√
1538.8127	R.LCVLHEKTPVSEK.V		√	√	√
1576.7689	K.LKPDPNTLCDEFK.A			√	√
1638.9305	R.KVPQVSTPTLVEVSR.S	√	√	√	√
1672.7627	K.QEPERNECFLSHK.D		√	√	√
1724.9953	R.MPCTEDYLSLILNR.L		√	√	√
1728.9463	K.KFWGKYLYEIARR.H		√		
1747.9264	R.QRLRCASIQKFGER.A	√	√	√	√
1881.1134	R.RPCFSALTPDETYVPK.A	√	√	√	√
1908.1352	K.LFTFHADICTLPDTEK.Q		√	√	√
2492.8454	K.GLVLIAFSQYLQQCPFDEHVK.L		√	√	√
Peptides identified		6	24	24	23

Mascot from Matrix Science Ltd. (London, U.K.) was used for the peptides search and identification. Search parameters: database, SwissProt; enzyme, trypsin; maximum of missed cleavages, 1; mass tolerance, 1 Da. 'A' represents the results of BSA digests (20 nM) without enrichment. 'B' represents the results of BSA digests (20 nM) after enriched by Fe_3O_4@mSiO_2 microspheres. 'C' represents the results of BSA digests (20 nM) after enriched by OMSF. 'D' represents the results of BSA digests (20 nM) after enriched by OMSF which has already been used for 50 times.

‘√’ represents the peptides identified by MALDI-TOF mass spectra from BSA digests.