Electronic Supplementary Material (ESI) for Nanoscale.

Supplementary Information

A Designed Combo-Pore Approach for Programmable Extraction of Peptides/Proteins

Kun Qian, Liang Zhou, Jun Zhang, Chang Lei, Chengzhong Yu*

Fax: (+) 61-7-33463973; E-mail: c.yu@uq.edu.au

Experimental:

Chemicals and Materials. Triblock copolymer $EO_{20}PO_{70}EO_{20}$ [denoted P123, where EO is poly(ethylene oxide) and PO is poly(propylene oxide)], potassium chloride (KCl, 99.5%, AnalaR, Australia), fuming hydrochloric acid (36%, Lab-Scan, Analytical Science, Thailand), ethanol (99.5%, Asia Pacific Specialty, Australia), ammonium bicarbonate, dry toluene, tetramethoxysilane (TMOS), 3-aminopropyltriethoxysilane (APTS) and 1,2-bis-(trimethoxysilyl)ethane (BTME, 96%) were purchased from Aldrich. E7 peptides (99%) and ammonium aqueous solution (25%) were obtained from Sigma. Acetonitrile (ACN, 99.9%), α-cyano-4-hydroxycinnamic acid (CHCA, 99%) and trifluoroacetic acid (TFA, 99.8%) were purchased from Merck. All reagents were used as received without further purification. The proteins (trypsin, cytochrome c, myoglobin, beta casein, horseradish peroxidase, ovalbumin and bovine serum albumin) were purchased from Sigma. The MW cut-off membranes (10 kDa and 3 kDa) were bought from the Pall Australia. Deionized water (18.2 MΩ·cm) used for all experiments was obtained from a Milli-Q system (Millipore, Bedford, MA).

Synthesis and characterization of materials. The MOSF and amino-functionalized MOSF were prepared according the previous reports.[1] MOSF was synthesized at 35 °C in buffer solutions (pH 5.0) with the presence of TMOS as a silica source and block copolymer (P123) as a template. The final MOSF products were obtained by calcination at 550 °C for 5 h. In a typical functionalization process, 0.2 g of dehydrated MOSF was dispersed and refluxed in 30 mL of APTS toluene solutions under stirring overnight, then filtrated and dried under vacuum. In the preparation of PMO, 0.33 g of P123 and 1 g KCl was dissolved in 20 g of 0.167 M HCl solution at 38 °C. Then 0.47 g of BTME was added and stirred for 10 min. The above reaction system was aged at 38 °C for 24 h and the resulting powder was filtered, washed and dried at room temperature.[2] The above as-made products were extracted by refluxing with ethanol/HCl (3.8 g of 36% HCl in 150 mL of 98% ethanol) for 6 h (2 times) to remove the templates. The final products were dried at room temperature under vacuum.

Nitrogen adsorption isotherms of samples were obtained by a Quantachrome's Quadrasorb SI analyzer at 77 K. Before the measurements, the samples were firstly degassed at 383 K for at least 8 h in vacuum. Scanning electron microscopy (SEM) images were recorded on a JEOL Philips XL30 microscope operating at 20 kV. The samples were coated with gold before observation. Transmission electron microscopy (TEM) images were directly taken with a JEOL 2011 microscope operated at 200 kV by dispersing the samples on a Cu grid covered with carbon films.

Adsorption of standard proteins and peptides. To test the immobilization ability of the materials towards proteins, 1 mg of MOSF or PMO material was dispersed in 1 mL of 10 μM cytochrome c proteins solution. After incubating for 15 min, the slurry was centrifugated to get the supernatant. The ultraviolet–visible spectrometry was employed to measure the absorbance at wavelength of 409 nm to determine the concentration of the proteins after material adsorption. To test the immobilization ability of the materials towards peptide, 1 mg of MOSF or PMO material was dispersed in 1 mL of 10 μM E7 peptides solution. After incubating for 15 min, the slurry was centrifugated to get the supernatant. The peptide quantification was performed based on the previous reported label-free MS method, where the signature peptide was used as the internal standard for E7 (RAHYNI**V**TF) with a sequence of RAHYNI**A**TF (molecular weight 1092.2 Da). 25 μL of signature peptides solution (3 μg/mL) was mixed with 25 μL of standard E7 peptides with different concentrations (0.89-8.9 μM) and supernatant obtained previously. The concentration of E7 can be obtained by measuring the signal ratio of E7/IS based on the standard curve during MS analysis.

Extraction of peptides/proteins in bio-mixtures. To prepare the mixtures, peptides solutions with different concentrations were pre-mixed with the prepared protein solutions. The E7 peptides or proteins digests were diluted in a step-wise way using water. To prepare the peptides mixture from proteins digests, the digestions of proteins were performed at 37 ºC for 24 h using trypsin with an enzyme/protein ratio of 1/30, w/w. To prepare the serum sample, adult mice (6–8 weeks) were maintained at the Australian

Institute for Bioengineering and Nanotechnology, The University of Queensland by trained laboratory animal technicians. All animal experiments received ethical approval from the University of Queensland Animal Ethics Committee. The mice were sacrificed in CO_2 and the blood sample was directly taken from heart. The serum sample was harvested according to our previous reported methods and was stored at -20 ºC and centrifugated before use.[3]

For a simple PMO extraction, typically 1 μL of PMO material slurry (10 mg/mL) was directly dispersed in 1 mL of mixture solution. After being stirred for 5 min, the mixture was centrifugated to remove the supernatant. Then 1 μL of CHCA matrix solution (10 mg/mL, in ACN/water/TFA, 50:49.9:0.1%, v/v/v) was added to the precipitation to elute the peptides, and the obtained mixtures were deposited on MALDI MPT 384 plate for MS analysis. For a simple MOSF extraction, typically 100 μL of MOSF slurry (10 mg/mL) was dispersed in 1 mL of mixture solution. After being stirred for 5 min, the mixture was centrifugated to remove the deposits. Then 1 μL of CHCA matrix solution (10 mg/mL, in ACN/water/TFA, 50:49.9:0.1%, v/v/v) was mixed with 1 μL of supernant solution, and the obtained mixtures were deposited on MALDI MPT 384 plate for MS analysis. For a combo-pore extraction, the supernatant obtained after MOSF extraction was used for a subsequent PMO extraction. All the samples were analyzed on a Bruker Autoflex TOF/TOF III equipped with Smart Beam system. All the peptides mass spectra were obtained in the RP-HPC-Proteomics mode via an accumulation of 500 laser shots at 10 different sites under a laser intensity of 45% instrument for data collection and calibrated using an auto calibration method. Two standard peptides, Angiotensin II (M.W. 1046.5) and ACTH-Clip (M.W. 2465.7), were used for calibration to reduce variability.

[1] K. Qian, J. J. Wan, X. D. Huang, P. Y. Yang, B. H. Liu, C. Z. Yu, *Chem.-Eur. J.* **2010**, *16*, 822.

[2] S. Z. Qiao, C. Z. Yu, W. Xing, Q. H. Hu, H. Djojoputro, G. Q. Lu, *Chem. Mat.* **2005**, *17*, 6172.

[3] K. Qian, W. Y. Gu, P. Yuan, F. Liu, Y. H. Wang, M. Monteiro, C. Z. Yu, *Small* **2012**, *8*, 231.

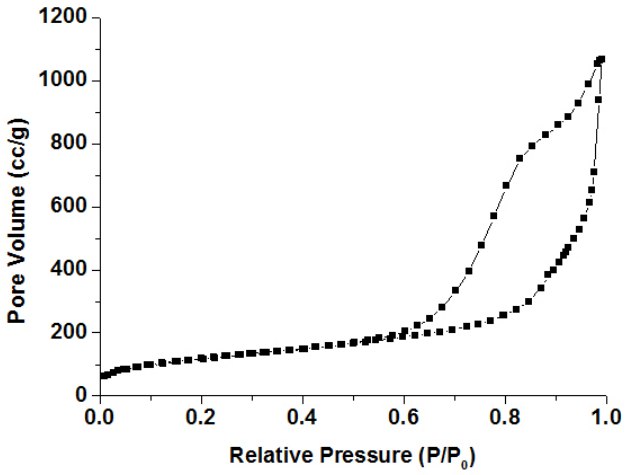

Figure S1. The nitrogen adsorption isotherm of MOSF materials.

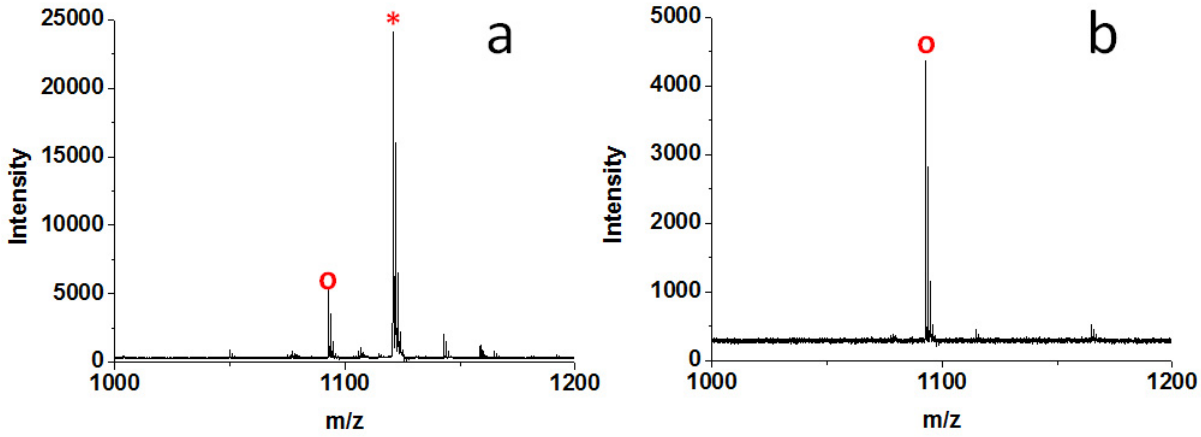

Figure S2. Typical MS spectra after a) MOSF extraction and b) PMO extraction in 10 μM E7 peptides, where the signature peptide is used as the internal standard (IS). The * stands for the E7 peptides signal and the o stands for the IS signal.

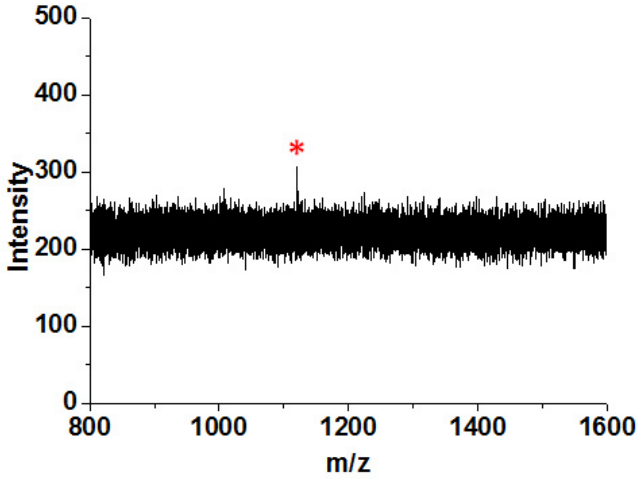

Figure S3. Typical MS spectrum obtained after combo-pore extraction of 4.5 nM E7 in 0.1 mg/mL Cyt-c protein solution.

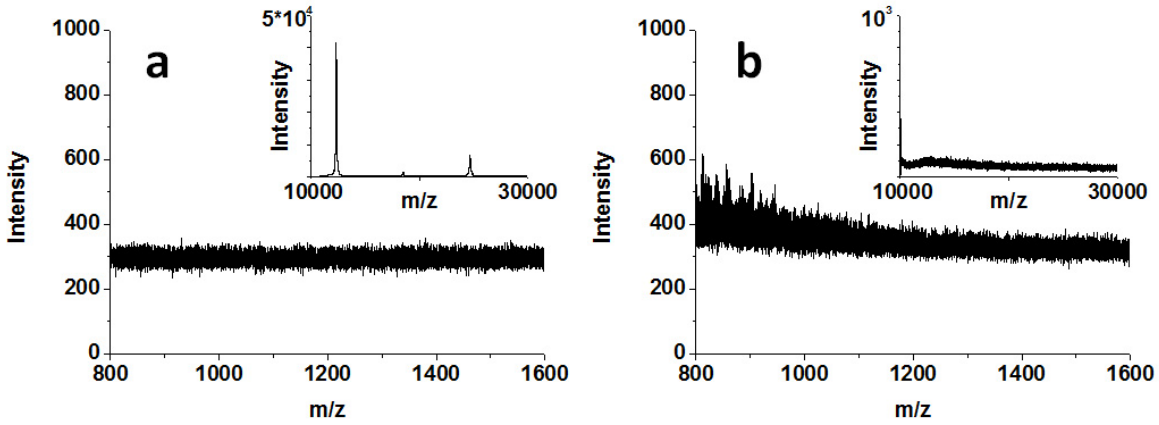

Figure S4. Typical MS spectra obtained from 89 nM E7 in 0.1 mg/mL Cyt-c solution after filtration by commercial MW cut-off membranes 10 kDa a) and 3 kDa b). The inset shows the high mass range spectrum.

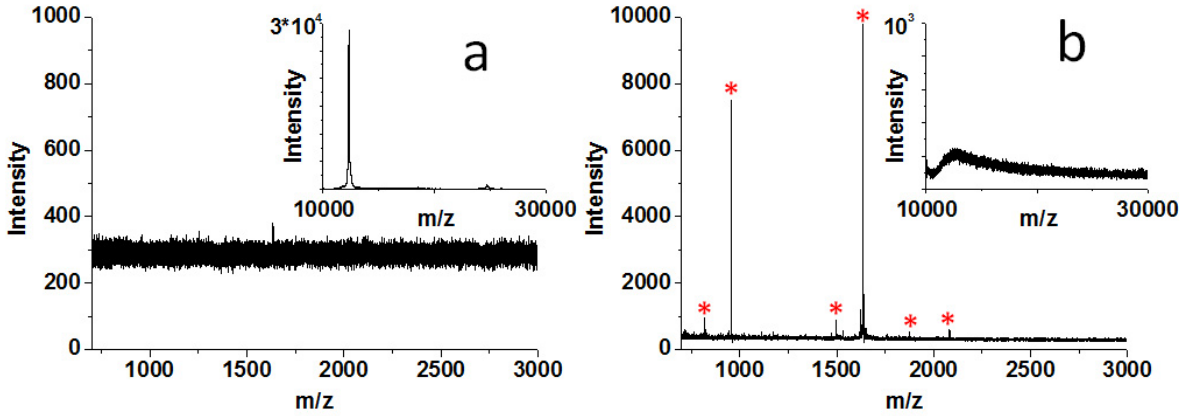

Figure S5. MS spectra from 20 nM Cyt-c digests in 0.1 mg/mL Cyt-c solution a) before treatment and b) after MOSF extraction followed by PMO enrichment (the combo-pore approach). The * stands for the peptide signal and the inset shows the high mass range spectrum.