ELECTRONIC SUPPLEMENTARY INFORMATION

Facile Synthesis of Hybrid Hollow Mesoporous Nanospheres with High Content of Interpenetrating Polymer for Size-selective Peptides/Proteins Enrichment

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Part 1. Synthesis section

a. Chemicals.

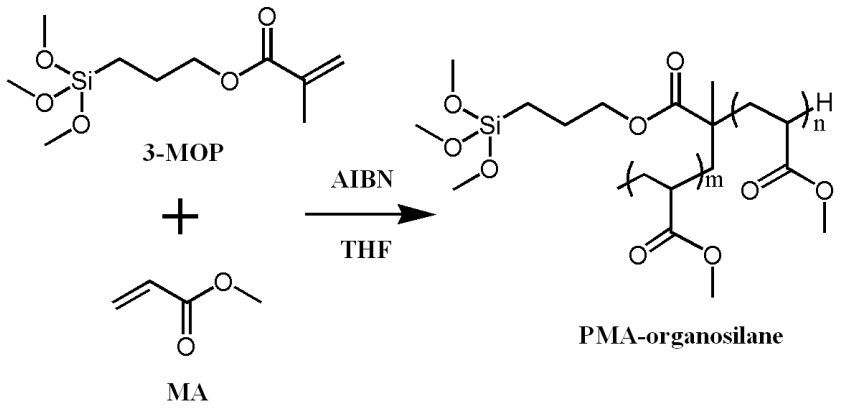
Tetraethoxysilane (TEOS, 99%), cetyltrimethylammonium bromide (CTAB), sodium hydroxide (98%), methyl methacrylate (MMA, 98%), methacrylate (MA, 98%), potassium persulfate ($K_2S_2O_8$), azobisisbutyronitrile (AIBN), tetrahydrofuran (THF), ethylether, trypsin (bovine pancreas), trifluoroacetic acid (TFA), formic acid (FA), acetonitrile (ACN), cytochrome c (cyt c, from horse heart), bovine serum albumin (BSA, from bovine serum) were purchased from Aldrich. Dithiothreitol (DTT) and iodoacetamide (IAA) were from Acros (Morris plains, NJ). Urea was purchased from Invitrogen (Carosbad, CA). [3-(Methacryloxy)propyl]-trimethoxysilane (3-MOP, 98%) were purchased from abcr GmbH & Co. KG. α-Cyano-4-hydroxycinnamic acid (CHCA) was obtained from Bruker Daltonik GmbH (Bremen, Germany). All chemicals were used as received without purification. Deionized water purified by a Milli-Q system (Millipore, Milford, MA) was used in all experiments.

b. Synthesis of PMMA nanoparticles latex.

Polymerization was carried out in a 1 L resin reaction flask with internal stirring. The flask was immersed in a 70 ^{o}C water bath to maintain constant temperature. A water-cooled condenser which is connected to the atmosphere via a wash bottle containing water, to prevent back-diffusion of oxygen into the reaction system, was fixed to the reactor. Nitrogen was bubbled through a thin teflon tube into the reactor. Typically, 650 mL of water and 27.5 mL methyl methacrylate were placed into the reactor and nitrogen was bubbled through to deactivate the inhibitors by excluding oxygen from the reacting system. The system was allowed to sit for at least 15 min to deactivate the inhibitor, attain temperature equilibrium, and saturate the aqueous phase with monomer. Then, a 0.1 g of $K_2S_2O_8$ dissolved in 20 mL water was added. The reaction was sustained for 1 hour. The resulting mixture was latex of PMMA nanoparticles. The number-average molar mass (Mn) of PMMA spheres obtained by size exclusion chromatography is 373000 g mol^{-1}.

c. Synthesis of PMA-organosilane.

As shown in Scheme S1, 13.3 g MA monomer, 3.3 g 3-MOP, 60 mg AIBN as an initiator were dissolved in 300 mL of THF. Argon was bubbled into the reactor. The mixture was stirred for 1 h, and then the polymerization was carried out with stirring at 60 ^{o}C for 8 h under an argon atmosphere. The resulting PMA-Organosilane was dried under reduced pressure at room temperature, and washed three times with ethylether. The number-average molar mass (Mn) of PMA-organosilane obtained by size exclusion chromatography is 2780 g mol^{-1}.



Scheme S1. The synthesis procedure of PMA-organosilane.

d. Synthesis of PMMA-PMA-SiO_2 HMNs.

PMMA-PMA-SiO_2 HMNs were synthesized under basic conditions using CTAB as surfactant micellar templates, PMMA nanoparticles latex as template of hollow spheres, and PMA-organosilane and TEOS as co-condensed precursors. In a typical synthesis, 0.10 g of CTAB was dissolved in 42 g of deionized water under stirring at room temperature. Then 6 mL of PMMA nanoparticles latex and 0.35 mL of NaOH (2 M) were added into the solution. Temperature of the solution was raised to 80 ^{o}C. Then, a mixture of TEOS (0.25 g) and PMA-organosilane (0.60 g) in 2 mL of THF was added sequentially and rapidly via injection. After stirring for an additional 2 h, the reaction mixture was transferred into a Teflon-lined autoclave and aged at 100 ^{o}C under static conditions for 12 h. The solid product obtained was extracted successively with HCl-ethanol and THF to give PMMA-PMA-SiO_2 HMNs.

e. Synthesis of SiO_2 HMNs.

Compared with the synthesis of PMMA-PMA-SiO_2 HMNs, a mixture of TEOS (0.25 g) and PMMA-organosilane (0.60 g) in 2 mL of THF was replaced by 0.6 g TEOS silica precursor. The following procedure is the same as that for PMMA-PMA-SiO_2 HMNs.

f. Characterization.

X-ray diffraction (XRD) measurements were performed on a Rigaku RINT D/Max-2500 powder diffraction system using Cu Ka radiation. Scanning electron microscopy (SEM) images of samples coated with platinum were recorded on a FEI Quanta 200F microscope. Transmission electron microscopy (TEM) and high resolution transmission electron microscope (HRTEM) images were obtained by FEI Tecnai G2 Spirit at an acceleration voltage of 120 kV and FEI Tecnai F30 electron microscope. The powder samples for the TEM measurements were suspended in ethanol and then dropped onto copper grids with holey carbon films. Nitrogen sorption isotherms of samples were obtained by a Micromeritics ASAP 2020 system analyzer at -196 ^{o}C. The Brumauer-Emmett-Teller (BET) surface area was calculated using experimental points at a relative pressure of P/P_o=0.05-0.25. The total pore volume was calculated by the N_2 amount adsorbed at the highest P/P_o ($P/P_o \approx 0.99$). The pore size distribution was calculated by the Non-Local Density Functional Theory. Solid-state ^{13}C (100.5 MHz) cross-polarization magic angle-spinning (CP/MAS) nuclear magnetic resonance (NMR) and solid-state ^{29}Si (79.4 MHz) magic angle-spinning (MAS) NMR experiments were recorded on a Varian infinity-plus 400 spectrometer equipped with a magic-angle spin probe in a 4 mm ZrO_2 rotor. The experimental parameters for ^{13}C CP/MAS NMR experiments were 10-kHz spin rate, 2-s pulse delay, 6-min contact time and 1000-2000 scans and for ^{29}Si MAS NMR experiments were 8-kHz spin rate, 4-s pulse delay, 10-min contact time and 3000-5000 scans. Infrared spectra were recorded on a Thermo Nicolet Nexus 470 Fourier transform infrared (FTIR) spectrometer. Weight changes of the products were monitored using a NETZSCH STA 449F3 analyzer from 25 ^{o}C to 900 ^{o}C under N_2 with a heating rate of 10 ^{o}C/min. Molar masses and molar mass distributions of

polymers were determined by size-exclusion chromatography (SEC) on Agilent Technologies 1200Series with THF as eluent (flow rate=1mL min^{-1}) at 30 ^{o}C using Agilent PLgel 5μm MIXED-C columns.

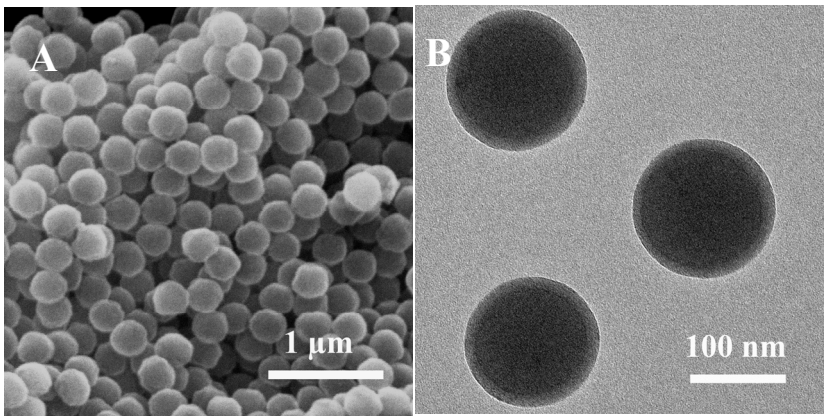


Fig. S1. SEM image (A) and TEM image (B) of PMMA Spheres.

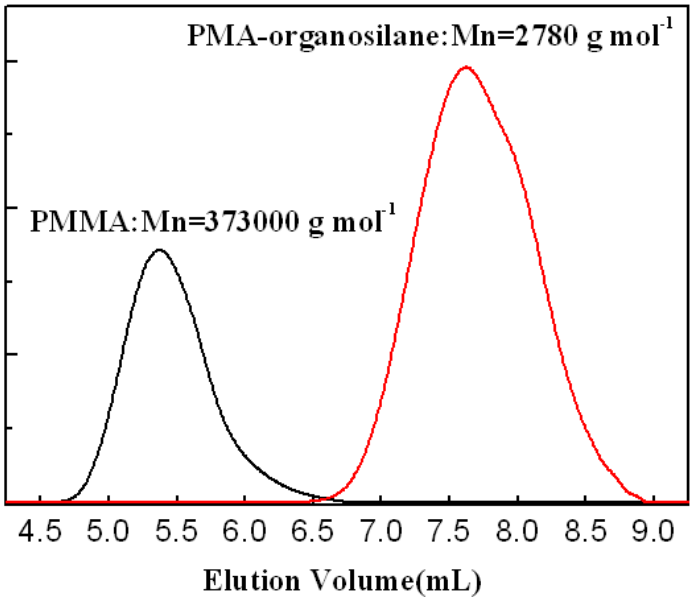


Fig. S2. Evolution of number-average molar mass (Mn) obtained by size-exclusion chromatography for PMMA spheres and PMA-organosilane.

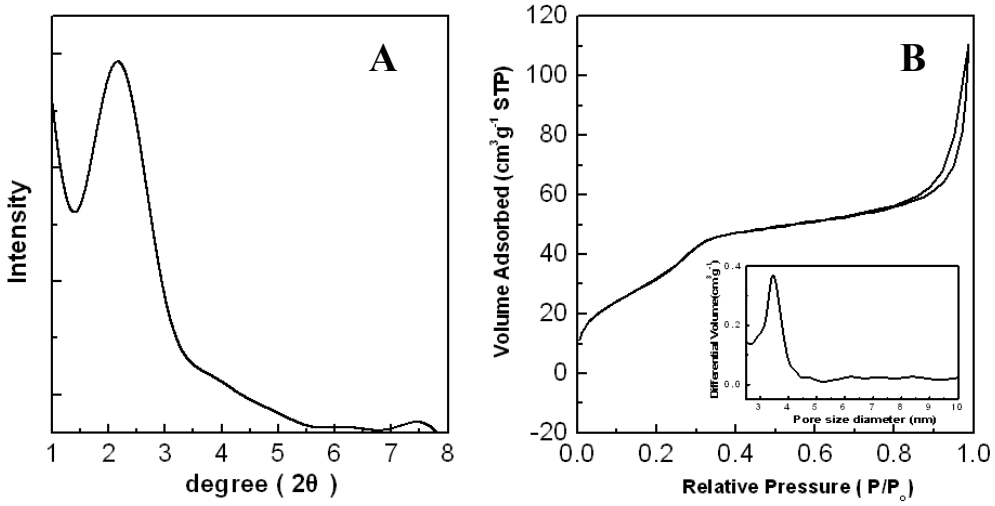


Fig. S3. Low angle XRD patterns (A), N_2 adsorption/desorption isotherms (B) and pore size distribution from adsorption branch (inset of B) of PMMA-PMA-SiO_2 HMNs.

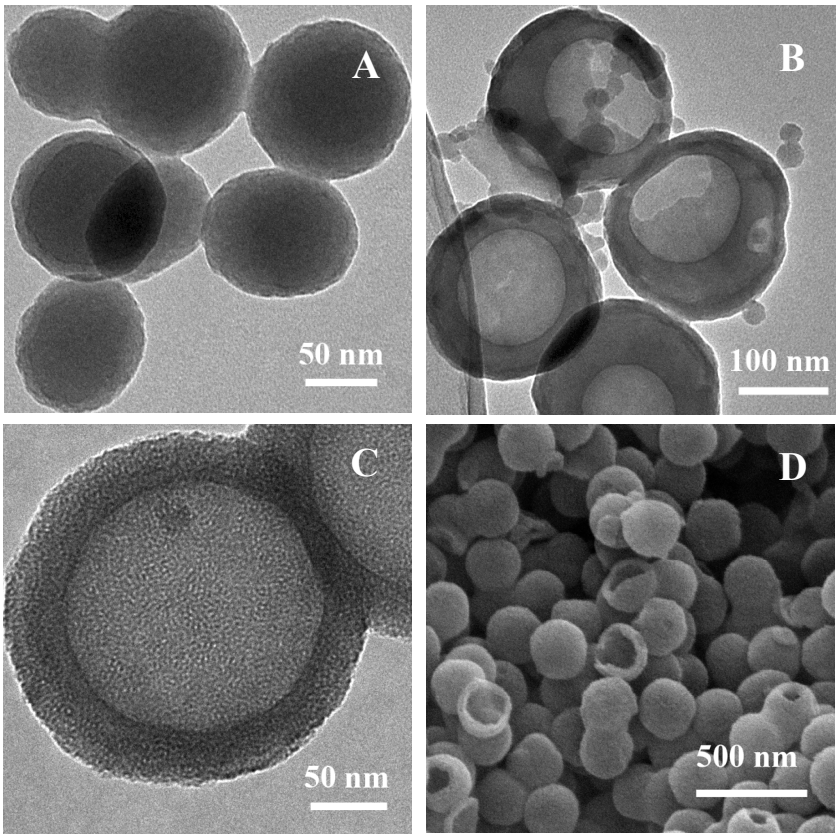


Fig. S4. Cryo-TEM images of samples taken out at different reaction time during the synthesis of PMMA-PMA-SiO_2 HMNs: the sample taken out immediately after the addition of silane precursor (10 s, A), 1 min (B) and 60 min (C); SEM image (D) of samples taken out at 60 min after the addition of silane precursor.

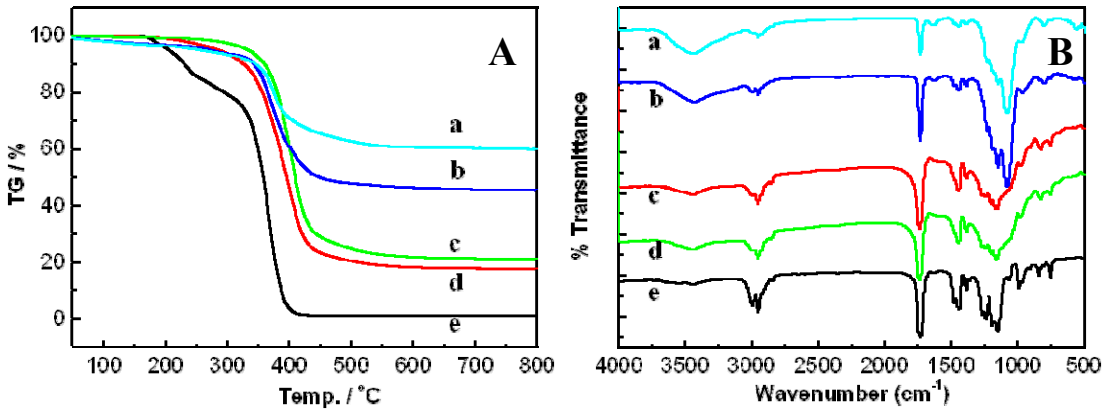


Fig. S5. TG curves (A) and FT-IR spectra (B) of SiO_2 HMNs (a, after THF treatment), SiO_2 HMNs (b, before THF treatment), PMMA-PMA-SiO_2 HMNs (c, after THF treatment), PMMA-PMA-SiO_2 HMNs (d, before THF treatment), and PMMA spheres (e).

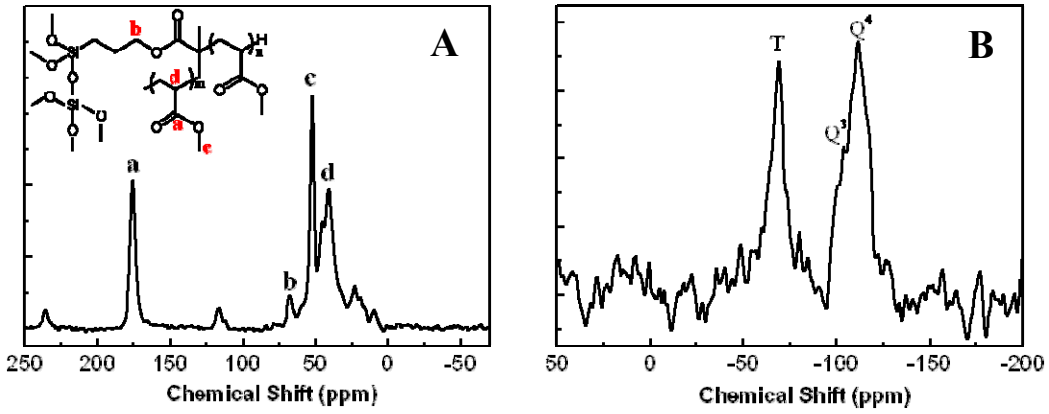


Fig. S6. ^{13}C CP/MAS NMR (A) and ^{29}Si MAS NMR (B) spectrum of PMMA-PMA-SiO_2 HMNs.

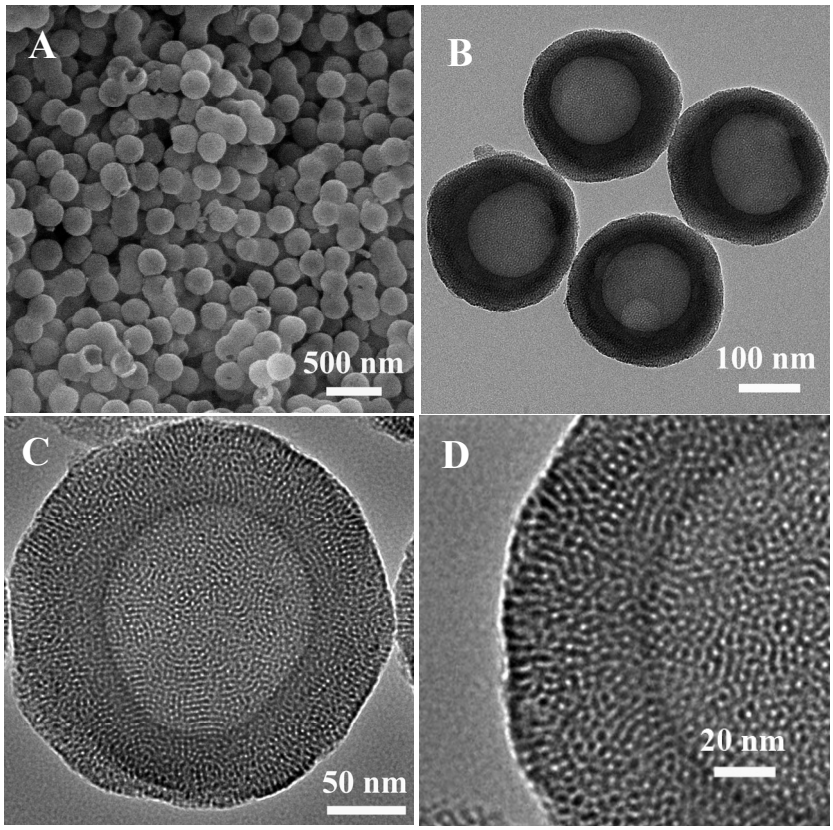


Fig. S7. SEM image (A) and TEM images (B, C and D) of SiO_2 HMNs.

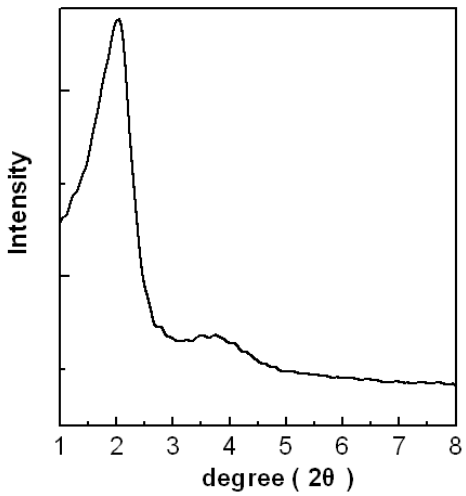


Fig. S8. Low angle XRD pattern of SiO_2 HMNs.

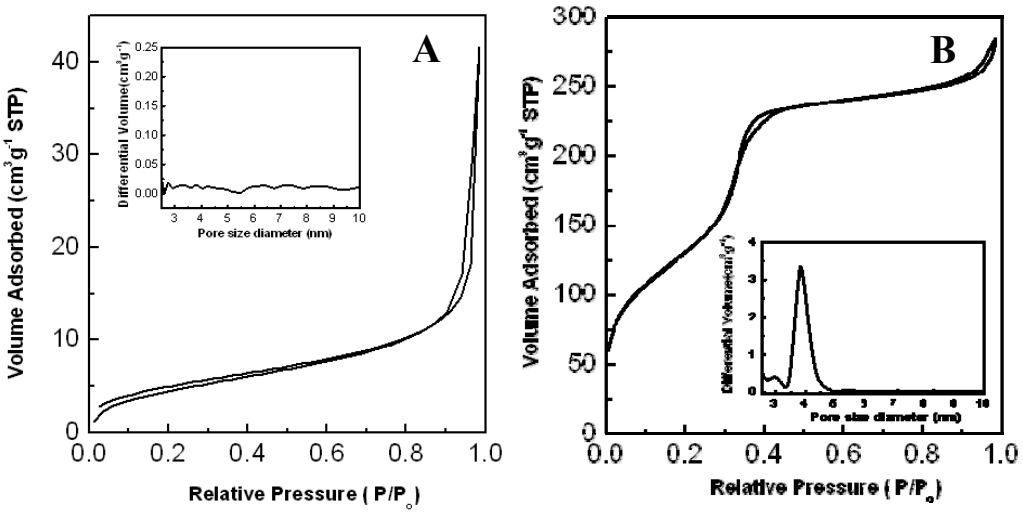


Fig. S9. N_2 adsorption/desorption isotherms and pore size distribution from adsorption branch (inset) of PMMA Spheres (A) and SiO_2 HMNs (B).

Table S1. Physicochemical parameters of extracted samples.

Samples	*d* spacing value (nm)	Surface Area (m^2g^{-1})	Pore Size (nm)	Pore Volume (cm^3g^{-1})
PMMA	-	17	-	0.06
PMMA-PMA-SiO_2 HMNs	4.3	151	3.5	0.13
SiO_2 HMNs	4.4	500	3.8	0.42

Part 2. Enrichment of peptides/proteins

a. Enzymatic digestion protocol.

BSA (1 mg) was dissolved and denatured by 100 μL urea (8 M), followed by addition of 20 μL DTT (100 mM) for the reduction of disulfide linkages at 60 ºC for 1 h. After cooling down to room temperature, 20 μL IAA (200 mM) was added for alkylation in the dark for 30 min, followed by dilution with 50 mM ammonium bicarbonate buffer to decrease the urea concentration below 1 mol L^{-1}. Trypsin digestion was carried out by adding trypsin into the protein solution with an enzyme-to-protein ratio of 1:25 (w/w) at 37 ^{o}C for 24 h. Finally, FA was added to quench the digestion process.

b. Preparation of proteome samples.

Yeast cells and escherichia coli (Strain BLT 5403) were grown on LB culture medium respectively, and were cultured at 37 ºC for 14 h. Then, cells were centrifuged at 4300 g for 10 min at 4 ºC. The precipitated cells were washed with PBS for 3 times. After that, 8 M urea together with 1 mM PMSF and 1 mM protease inhibitor cocktail (set I from MERK) were added into the precipitate with the ratio of 2:1 (v/w), followed by ultrasonication (Cole-Parmer, IL, USA) for 180 s. All those processes were performed in the ice bath. Deer plasma was collected from a 3-year-old female red deer, provided by Dong feng pharmaceutical Co., LTD (Jilin, China).

c. Peptides/proteins enrichment for MALDI-TOF MS analysis.

MALDI-TOF MS experiments were performed on an Ultraflex III MALDI-TOF/TOF instrument (Bruker Daltonics, Bremen, Germany) equipped with a high rep rate (100 Hz) diodepumped all-solid-state SmartBeam laser (third harmonic at 355 nm) to analyze peptides in the positive reflection mode. External calibration of MALDI-TOF MS spectra was performed with ten commercial standard peptides. Spectra were acquired from an accumulation of 1000 laser shots. The voltage was set as following: acceleration, 21.85 kv; lens, 9.2 kv; reflector 1, 26.39 kv; reflector 2, 14.0 kv. Peptide mass fingerprint was searched via MASCOT search engine with SwissProt 57.1 database (462764 sequences; 163773385 residues). Other search parameters were set as follows: enzyme, trypsin; fixed modifications, carbamidomethyl (C); allow up to 2 missed cleavage; peptide tolerance, 100 ppm. A 7 mg/ mL CHCA in 70% (v/v)

aqueous ACN with 0.1% (v/v) TFA solution was prepared by adding CHCA (solid) to the organic solvent, followed by the addition of water and acid. The matrix solution was thoroughly vortexed, leading to a clear working matrix solution.

For the enrichment of peptides and proteins, 2 mg nanomaterials were added to 8 mL solution of standard protein, protein digests or proteins at varied concentrations. The resulting mixtures were agitated for 10 min at room temperature. The peptides/nanomaterials or proteins/nanomaterials obtained by removing the supernate were redispersed in 50 μL CHCA solution. Finally, 0.5 μL of the above slurry was deposited on the MALDI plate and dried, then another 0.5 μL CHCA solution was introduced and applied for MALDI-TOF MS analysis.

For the enrichment of endogenous peptides from proteome sample, 5 mg PMMA-PMA-SiO_2 HMNs were added to 1 mL proteome sample, the resulting mixtures were agitated for 10 min at room temperature. The following procedure is the same as that for the enrichment of standard peptides and proteins.

d. NanoRPLC-ESI-MS/MS analysis and identification.

A nanoRPLC (nanoflow reversed-phase liquid chromatography)-electrospray ionization-tandem mass spectrometry (ESI-MS/MS) system was constructed by combining the nanoRPLC with a Finnigan LTQ XL IT mass spectrometer (Thermo, San Jose, CA). Enriched endogenous peptides from proteome sample were eluted from PMMA-PMA-SiO_2 HMNs by 500 μL aqueous solution containing 70% (v/v) ACN and 0.1% (v/v) TFA. Finally, the eluted peptides were vacuum-lyophilized and resolubilized in 50 mM ammonium bicarbonate buffer for nanoRPLC-ESI-MS/MS analysis.

A 16 cm long capillary (75 μm i.d.) with pulled spray tip was packed with C18 particles (5 μm, 300 Å, Sinochrom ODS-AP) at 5000-7000 psi by a high-pressure pump overnight. Meanwhile, a 2 cm long capillary (75 μm i.d.) packed with the same particles was prepared as the pre-column. The column flow rate was maintained at 160 nL/min for separation and at 7 μL/min for the peptide trap. The ESI voltage was set at 1.8 kV for LTQ, and the spray capillary was heated to 180 ºC. Total ion current chromatograms and mass spectra ranging from m/z 400 to 1800 were recorded with

the Xcalibur software (v 3.1). The MS was set as one full MS scan followed by seven MS/MS scans. Two buffer solutions were (A) H_2O with 2% (v/v) ACN containing 0.1% (v/v) FA and (B) ACN with 2% (v/v) H_2O and 0.1% (v/v) FA, respectively. The gradient was set as follows: 0-10 min, 0% B (v/v); 10 min-80 min, 10%-40% B (v/v); 800 min-100 min, 40%-80% B (v/v); 100-110 min, 20% B (v/v).

Tandem mass spectra detection and database searching were operated by the Sequest database search engine (v.3.1, Thermo Electron Corp.) against the yeast, escherichia coli and deer databases, respectively. Peptides searched using No enzyme constraints. The mass tolerances were 2 Da for parent masses and 1 Da for fragment masses. Identified peptides were filtered via following standards: Xcorr was higher than 1.9 for singly charged peptide, 2.2 for doubly charged peptide, and 3.75 for triply charged peptides. By using above parameters, false discovery rate can be controlled ~ 1%.

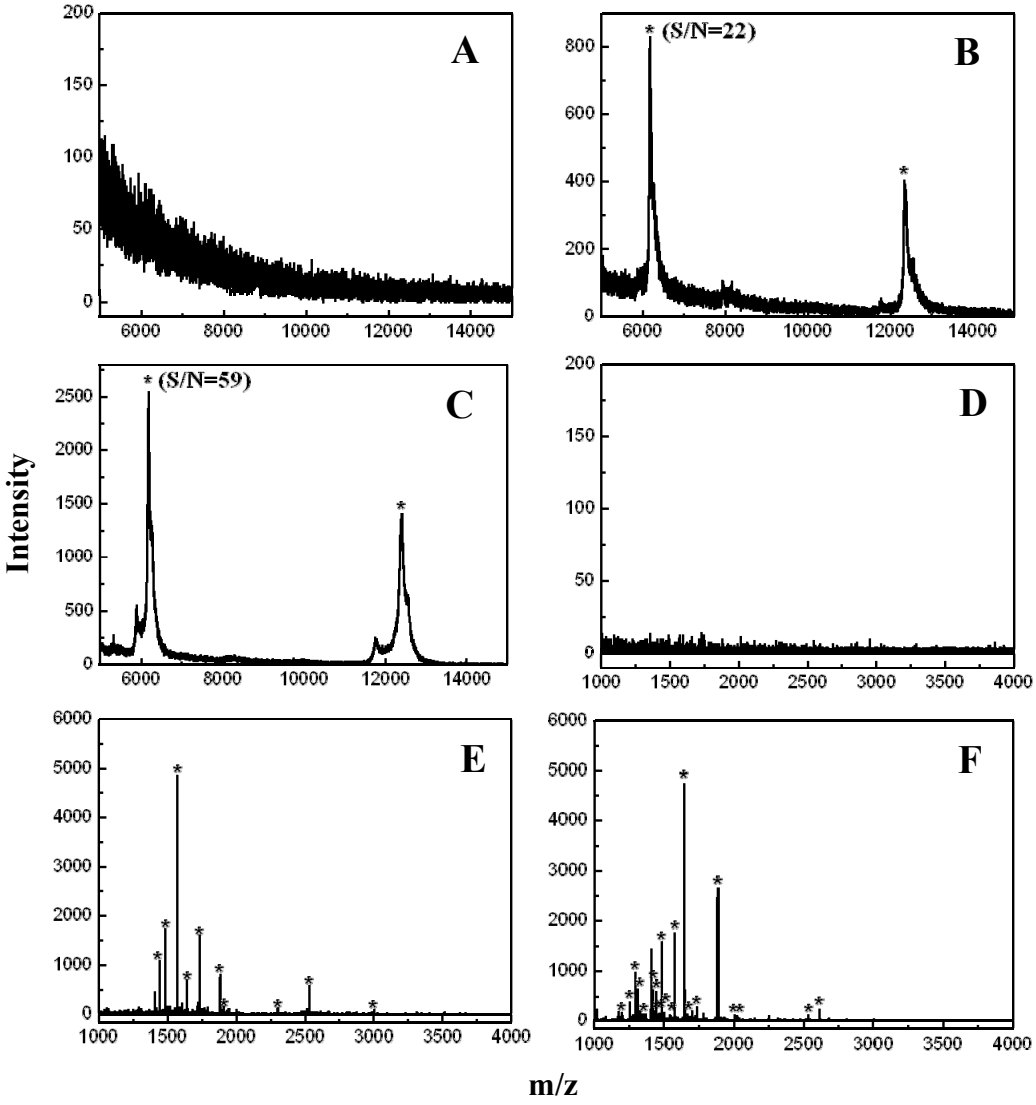


Fig. S10. MALDI-TOF MS spectra of 8 nM cyt c without enrichment (A), after enrichment with PMMA spheres (B) and SiO_2 HMNs (C) ; 1 nM BSA digests without enrichment (D), after enrichment with PMMA spheres (E) and SiO_2 HMNs (F).

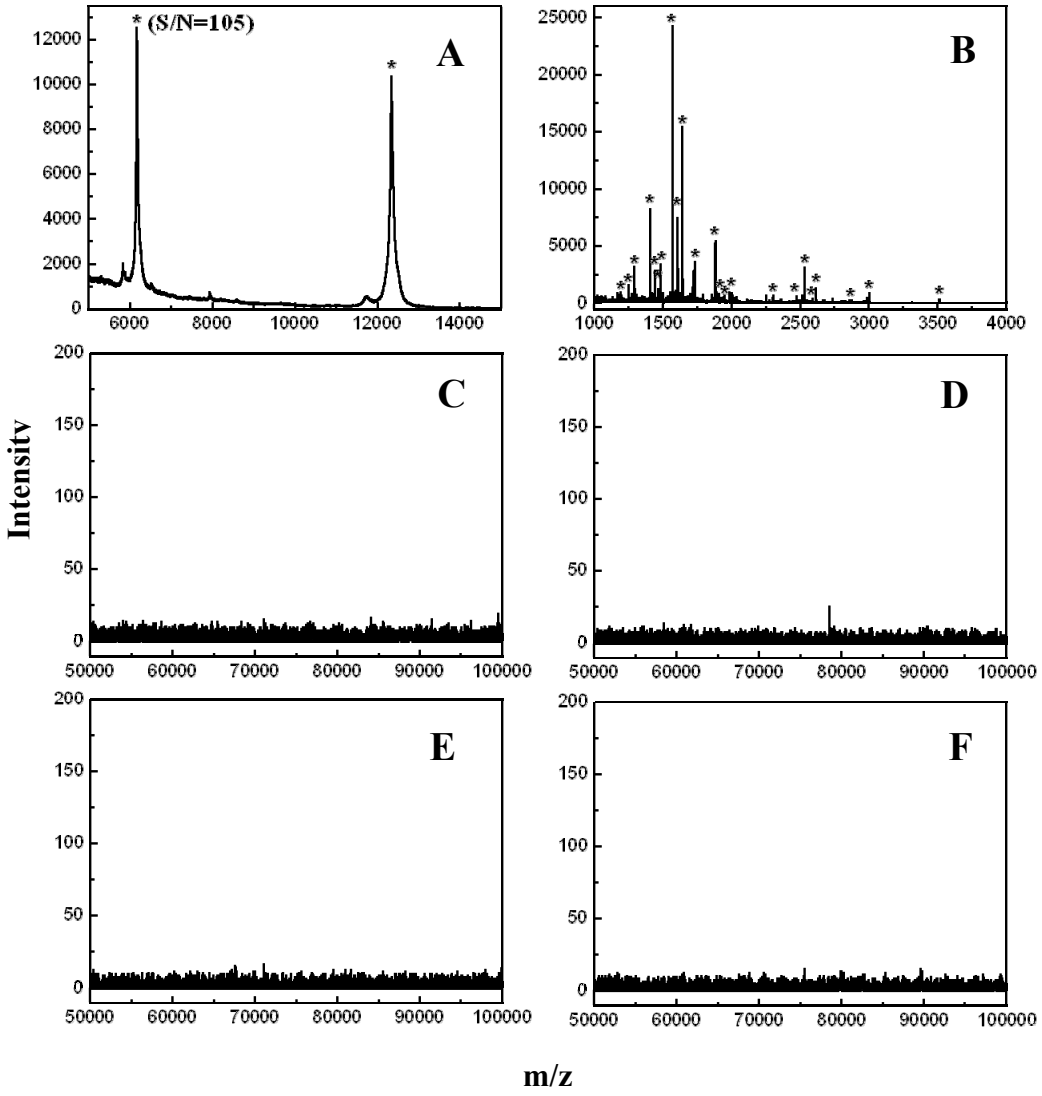


Fig. S11. MALDI-TOF MS spectra of (A) cyt c after enrichment with PMMA-PMA-SiO_2 HMNs from the mixture of cyt c (8 nM) and BSA (1 nM); (B) BSA digests after enrichment with PMMA-PMA-SiO_2 HMNs from the mixture of BSA digests (1 nM) and BSA (1 nM); (C) BSA without enrichment and (D) BSA after enrichment with PMMA-PMA-SiO_2 HMNs from the mixture of cyt c (8 nM) and BSA (1 nM); (E) BSA without enrichment and (F) BSA after enrichment with PMMA-PMA-SiO_2 HMNs from the mixture of BSA digests (1 nM) and BSA (1 nM).

Table S2. The search results of 1 nM BSA digests after enrichment with PMMA spheres, SiO_2 HMNs and PMMA-PMA-SiO_2 HMNs.

Calculated m/z	Database sequence	PMMA spheres	SiO_2 HMNs	PMMA-PMA-SiO_2 HMNs
926.4861	YLYEIAR			√
1192.595	DTHKSEIAHR		√	√
1248.614	FKDLGEEHFK		√	√
1282.703	HPEYAVSVLLR		√	
1304.709	HLVDEPQNLIK		√	√
1348.696	RDTHKSEIAHR		√	
1418.686	SLHTLFGDELCK		√	√
1438.805	RHPEYAVSVLLR	√	√	√
1456.851	ALKAWSVARLSQK		√	
1462.582	TCVADESHAGCEK		√	√
1478.788	LGEYGFQNALIVR	√	√	√
1501.607	EYEATLEECCAK		√	
1531.774	LKECCDKPLLEK		√	
1566.735	DAFLGSFLYEYSR	√	√	√
1638.931	KVPQVSTPTLVEVSR	√	√	√
1660.864	GACLLPKIETMREK		√	
1723.827	MPCTEDYLSLILNR	√	√	√
1879.914	RPCFSALTPDETYVPK	√	√	√
1906.914	LFTFHADICTLPDTEK	√		
1945.009	SLHTLFGDELCKVASLR			√
2002.093	WVTFISLLLLFSSAYSR		√	√
2018.962	LKPDPNTLCDEFKADEK		√	
2300.075	NYQEAKDAFLGSFLYEYSR	√		√
2470.184	RPCFSALTPDETYVPKAFDEK			√
2491.257	GLVLIAFSQYLQQCPFDEHVK			√
2528.212	QNCDQFEKLGEYGFQNALIVR	√	√	√
2585.265	LKECCDKPLLEKSHCIAEVEK			√
2611.158	VHKECCHGDLLECADDRADLAK		√	√
2871.302	CCTKPESERMPCTEDYLSLILNR			√
2999.394	CCTESLVNRRPCFSALTPDETYVPK	√		√
3307.466	TVMENFVAFVDKCCAADDKEACFAVEGPK			√
3510.665	SHCIAEVEKDAIPENLPPLTADFAEDKDVCK			√
Peptides matched		10	21	24
Sequence coverage		20%	42%	52%

Note: √: Peptides matched.

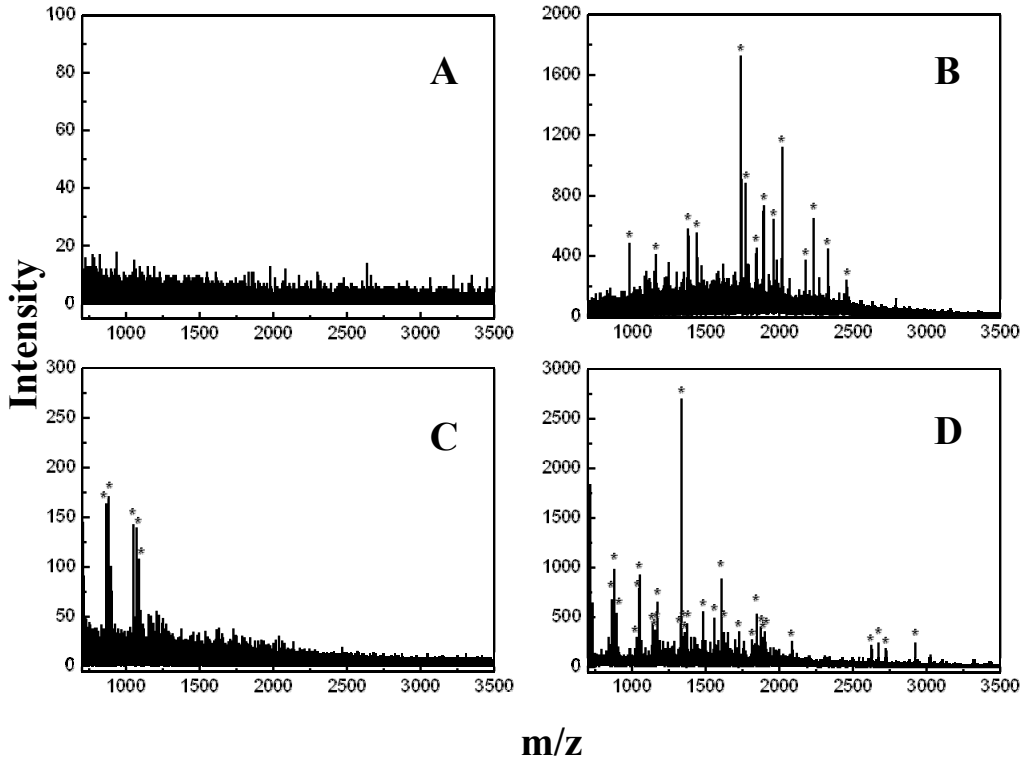


Fig. S12. MALDI-TOF MS spectra of escherichia coli extracts without enrichment (A) and after enrichment with PMMA-PMA-SiO_2 HMNs (B); deer plasma without enrichment (C) and after enrichment with PMMA-PMA-SiO_2 HMNs (D).

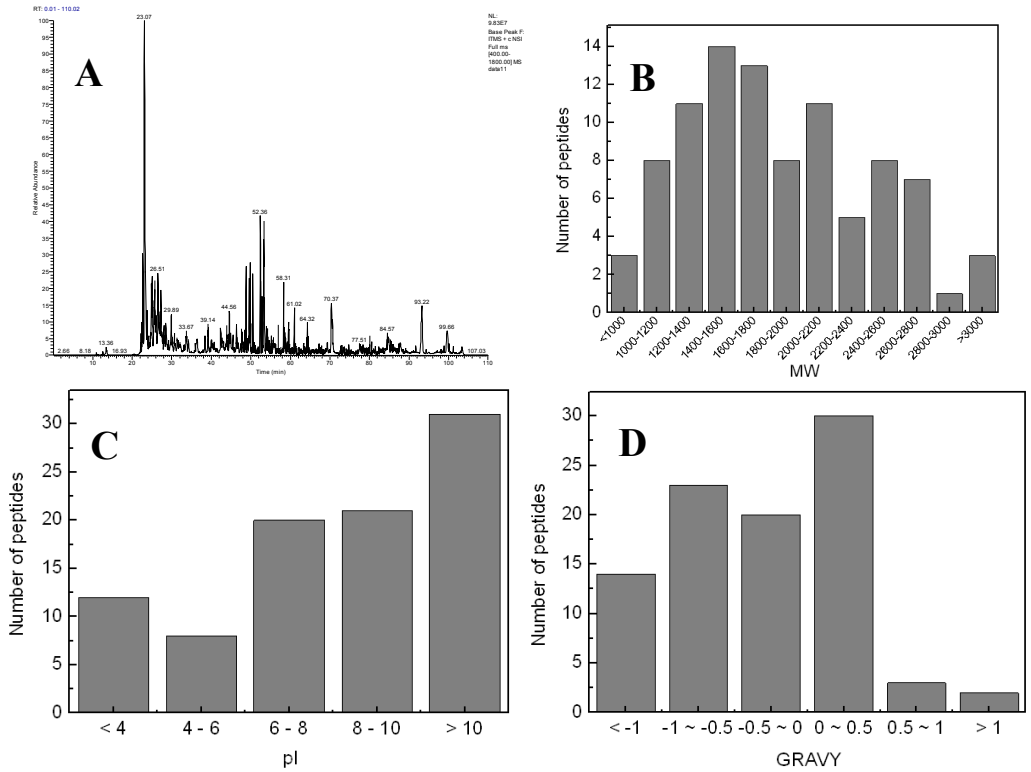


Fig. S13. The base peak chromatogram of endogeneous peptides enriched from yeast cell extracts with PMMA-PMA-SiO_2 HMNs (A); peptide abundances within different MW(B), pI(C) and GRAVY(D).

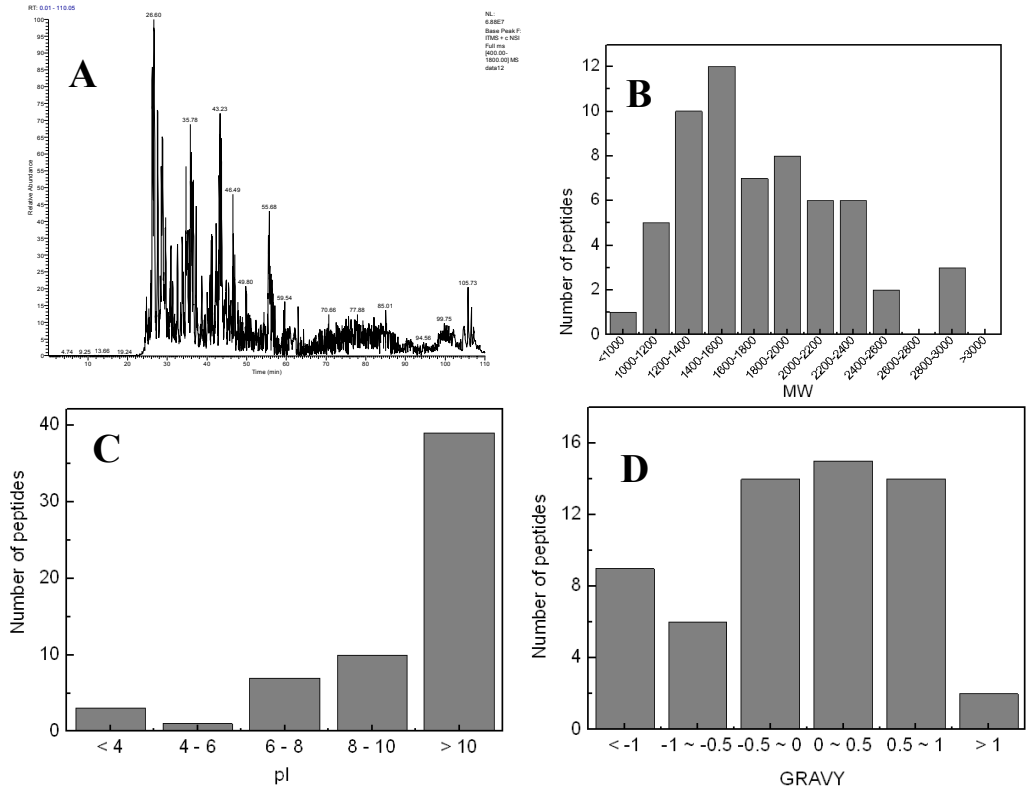


Fig. S14. The base peak chromatogram of endogeneous peptides enriched from escherichia coli extracts with PMMA-PMA-SiO_2 HMNs (A); peptide abundances within different MW(B), pI(C) and GRAVY(D).

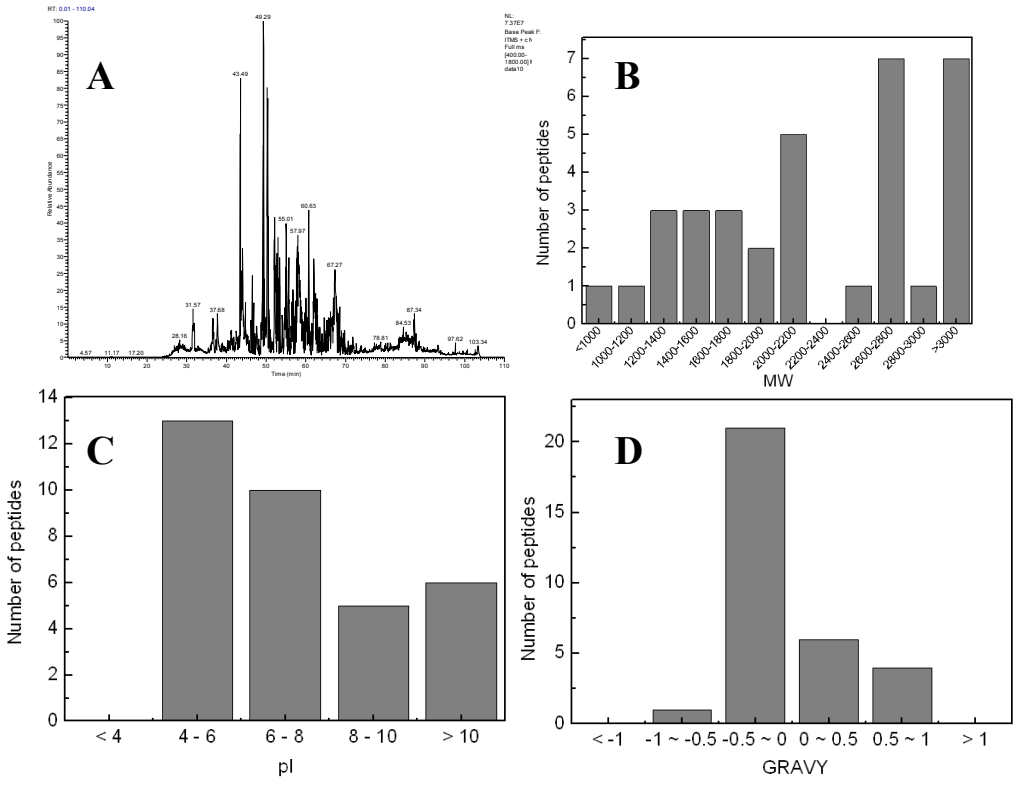


Fig. S15. The base peak chromatogram of endogeneous peptides enriched from deer plasma with PMMA-PMA-SiO_2 HMNs (A); peptide abundances within different MW(B), pI(C) and GRAVY(D).

Table S3. All peptides of yeast cell extracts enriched with PMMA-PMA-SiO_2 HMNs and identifid by NanoRPLC-ESI-MS/MS.

MH+	Peptide	XC	DeltaCn
1525.69200	D.PEAAAAGAAAVANQGKK.-	6.001	0.6
1525.69200	D.PEAAAAGAAAVANQGKK.-	5.929	0.6
2392.65261	S.KPSYYLDPEAAAAGAAAVANQGKK.-	6.096	0.5
1525.69200	D.PEAAAAGAAAVANQGKK.-	5.828	0.5
2531.80194	D.PANLPWGSSNVDIAIDSTGVFKEL.D	6.124	0.5
1397.51909	D.PEAAAAGAAAVANQGK.K	4.615	0.6
1525.69200	D.PEAAAAGAAAVANQGKK.-	5.268	0.5
1525.69200	D.PEAAAAGAAAVANQGKK.-	5.301	0.6
1690.92515	S.HDDKHIIVDGKKIAT.Y	5.248	0.4
1854.09928	S.HDDKHIIVDGKKIATY.Q	4.909	0.5
1525.69200	D.PEAAAAGAAAVANQGKK.-	5.095	0.5
1525.69200	D.PEAAAAGAAAVANQGKK.-	5.386	0.5
3185.61399	N.KETTYDEIKKVVKAAAEGKLKGVLGYTED.A	5.290	0.6
2479.86444	Y.KGGKEVTRVVGANPAAIKQAIASNV.-	5.274	0.4
1860.27637	I.KKVVKAAAEGKLKGVLGY.T	5.715	0.4
2234.41691	T.HGRYAGEVSHDDKHIIVDGK.K	5.189	0.4
1325.44810	S.VPQTFIDFKDD.P	3.776	0.4
2070.28993	D.PQYLVDDLRPEFAGYSKA.A	5.008	0.6
2323.71800	I.IPSSTGAAKAVGKVLPELQGKLTG.M	5.738	0.3
1525.69200	D.PEAAAAGAAAVANQGKK.-	3.256	0.3
2454.91507	I.IPSSTGAAKAVGKVLPELQGKLTGM.A	5.685	0.4
1525.69200	D.PEAAAAGAAAVANQGKK.-	4.861	0.3
2923.27177	M.RIALSRPNVEVVALNDPFITNDYAAY.M	4.639	0.4
1525.69200	D.PEAAAAGAAAVANQGKK.-	3.370	0.3
3433.02449	S.SMPTLIFYKGGKEVTRVVGANPAAIKQAIASNV.-	5.500	0.5
1525.69200	D.PEAAAAGAAAVANQGKK.-	4.737	0.5
2677.13314	N.KETTYDEIKKVVKAAAEGKLKGVLG.Y	4.894	0.5
2157.56349	S.STGAAKAVGKVLPELQGKLTGM.A	5.168	0.5
1525.69200	D.PEAAAAGAAAVANQGKK.-	3.490	0.4
1525.69200	D.PEAAAAGAAAVANQGKK.-	4.679	0.4
2682.17642	G.NIIPSSTGAAKAVGKVLPELQGKLTGM.A	5.408	0.5
1525.69200	D.PEAAAAGAAAVANQGKK.-	4.556	0.4
1469.70841	L.SPKFVKLVSWYD.N	3.533	0.4
1701.94794	G.YSTRVVDLVEHIAKA.-	4.453	0.4
2060.33972	D.PEDIAFKEKQKADAAAKKA.L	4.622	0.3
2281.55831	N.YPGLKTHPNYDVVLKQHRD.A	4.597	0.4
2350.74021	N.KETTYDEIKKVVKAAAEGKLK.G	3.948	0.4
2601.00014	Y.KPVAVPARTPANAAVPASTPLKQEW.M	4.964	0.4
2475.74807	T.HGRYAGEVSHDDKHIIVDGKKI.A	4.654	0.4

1191.44642	L.SPKFVKLVSW.Y	3.339	0.3
2550.97935	G.NIIPSSTGAAKAVGKVLPELQGKLTG.M	4.197	0.5
2453.78400	M.PKLVLVRHGQSEWNEKNLFTG.W	4.190	0.4
2576.84594	K.PSKPSYYLDPEAAAAGAAAVANQGKK.-	4.964	0.4
2052.40387	I.IPSSTGAAKAVGKVLPELQGK.L	4.540	0.3
1525.69200	D.PEAAAAGAAAVANQGKK.-	3.836	0.2
2151.49230	G.NIIPSSTGAAKAVGKVLPELQG.K	4.507	0.4
1187.43475	V.LPELQGKLTGM.A	2.930	0.3
1589.82083	S.HDDKHIIVDGKKIA.T	3.773	0.4
2716.94182	V.SWYDNEYGYSTRVVDLVEHIAKA.-	4.299	0.4
2546.82630	T.HGRYAGEVSHDDKHIIVDGKKIA.T	4.110	0.2
2117.34814	X.STGVFKELDTAQKHIDAGAK.K	4.339	0.4
1690.92515	S.HDDKHIIVDGKKIAT.Y	3.293	0.4
1397.73478	G.KPLVGGGIKKSGKK.-	3.692	0.3
2647.93062	T.HGRYAGEVSHDDKHIIVDGKKIAT.Y	3.992	0.3
2148.45343	M.PKLVLVRHGQSEWNEKNL.F	4.354	0.3
1525.69200	D.PEAAAAGAAAVANQGKK.-	3.243	0.3
1861.17655	A.PPPPPPPPALGGSAPKPAKS.V	3.799	0.3
2052.40387	I.IPSSTGAAKAVGKVLPELQGK.L	3.760	0.5
1489.74763	T.VPKLKPQKDGKNH.V	3.714	0.3
1781.13288	G.AAKAVGKVLPELQGKLTG.M	3.910	0.3
1969.38150	T.GAAKAVGKVLPELQGKLTGM.A	4.064	0.3
2660.92294	D.GKKIATYQERDPANLPWGSSNVDI.A	4.070	0.5
2026.36642	S.STGAAKAVGKVLPELQGKLTG.M	3.938	0.2
1370.49210	D.PANLPWGSSNVDI.A	2.592	0.4
1629.94667	D.AGAKKVVITAPSSTAPM.F	2.494	0.4
1697.10225	I.KKVVKAAAEGKLKGVLG.Y	4.063	0.4
1673.97764	F.DASAGIQLSPKFVKLV.S	3.331	0.3
1513.76427	G.NIIPSSTGAAKAVGKV.L	2.666	0.3
3400.89129	X.STGVFKELDTAQKHIDAGAKKVVITAPSSTAPM.F	4.038	0.3
2146.47422	S.HSSIFDASAGIQLSPKFVKL.V	3.647	0.3
2603.91129	R.IALSRPNVEVVALNDPFITNDYAA.Y	3.610	0.3
1056.23768	V.LPELQGKLTG.M	2.556	0.2
1606.91495	D.MPVNPPFRKPYPY.L	2.897	0.3
1197.40937	R.IALSRPNVEVV.A	2.591	0.3
1492.65724	D.PQYLVDDLRPEF.A	2.750	0.2
1877.89853	G.SLDLIDDAGENSDLEDR.I	3.808	0.2
1626.92251	G.NIIPSSTGAAKAVGKVL.P	2.835	0.2
1756.02234	Q.HGLLSTPAASHIMRTY.E	3.894	0.2
1841.95908	Q.ERDPANLPWGSSNVDIA.I	3.308	0.4
1414.63269	G.NIIPSSTGAAKAVGK.V	2.662	0.3
876.03792	M.VRVAINGF.G	2.366	0.1
1040.11699	D.GPSHKDWRG.G	2.382	0.3

1283.54217	V.GKVLPELQGKLT.G	2.406	0.2
2186.62939	T.VPKLKPQKDGKNHVKKIEV.S	2.872	0.3
1221.25192	V.FDESSAPEIEP.P	2.459	0.1
1414.63269	G.NIIPSSTGAAKAVGK.V	2.752	0.2
1471.79079	V.GKVLPELQGKLTGM.A	2.451	0.2
1354.45922	D.GPSHKDWRGGRT.A	2.810	0.4
1259.48562	M.VRVAINGFGRXG.R	2.390	0.2
818.98314	K.LAQFVQL.F	2.274	0.1
1145.37929	G.IPPAPRGVPQI.E	2.608	0.3
1353.59572	M.RIALSRPNVEVV.A	2.419	0.2
1717.05211	T.VPKLKPQKDGKNHVK.K	2.365	0.1
929.01131	D.PANLPWGSS.N	2.084	0.3
1340.59372	V.GKVLPELQGKLTG.M	2.528	0.2
1970.08886	Y.QERDPANLPWGSSNVDIA.I	2.643	0.3
1229.40823	G.NIIPSSTGAAKAV.G	2.320	0.1
1141.38895	G.KPLVGGGIKKSG.K	2.380	0.3
1736.99229	G.IPPAPRGVPQIEVTFD.V	2.517	0.4
1779.92766	K.PSKPSYYLDPEAAAAGAA.A	2.682	0.3
1767.02316	M.RIALSRPNVEVVALND.P	2.594	0.3
1425.53745	D.GPSHKDWRGGRTA.S	2.615	0.1
1191.31532	I.LWTLSNSLQE.S	2.376	0.2
1441.57033	D.PANLPWGSSNVDIA.I	1.975	0.3
1505.59647	W.HPSPDFGGMHPDPN.L	2.436	0.3
1497.63219	H.DQPVENVIAVSPTQ.I	2.322	0.1
1966.24820	I.SAAQLTYSSMNLNNKIIP.N	2.357	0.3
2147.50042	G.IERVIVSKENYDDLVKIL.N	2.557	0.1
1569.66665	D.GPSHKDWRGGRTASG.N	2.359	0.1
1461.68782	F.DASAGIQLSPKFVK.L	2.465	0.1

Table S4. All peptides of escherichia coli extracts enriched with PMMA-PMA-SiO_2 HMNs and identifid by NanoRPLC-ESI-MS/MS.

MH+	Peptide	XC	DeltaCn
2527.00262	T.GKEIKIAAANVPAFVSGKALKDAVK.-	6.457	0.5
2233.60639	D.VQRHPYKPKLQHIDFVRA.-	5.847	0.5
2853.35240	N.PQTGKEIKIAAANVPAFVSGKALKDAVK.-	7.688	0.5
2527.00262	T.GKEIKIAAANVPAFVSGKALKDAVK.-	5.101	0.4
2275.59029	R.NVVLDKSFGAPTITKDGVSVAR.E	5.346	0.4
1486.83325	S.VIRHANLPVLVVR.-	4.248	0.4
1530.83643	N.VPAFVSGKALKDAVK.-	4.351	0.5
2177.62249	E.VARLPKDVKIEIEAIAVRR.-	4.957	0.4
1479.74474	R.SFTFVTKTPPAAVL.L	3.682	0.5
2021.22706	S.KDEGGRHTPFFKGYRPQ.F	4.848	0.3
2922.11107	Y.LGAVAATVREGRSQDLASQAEESFVEAE.-	4.708	0.5
2275.59029	R.NVVLDKSFGAPTITKDGVSVAR.E	4.825	0.5
2322.67692	A.GVTAPKGKRMGHAGAIIAGGKGTAD.E	4.296	0.4
2092.51114	L.LVKGAVPGATGSDLIVKPAVKA.-	4.830	0.4
1732.06678	N.ASSVIRHANLPVLVVR.-	4.186	0.3
2331.57588	S.KDEGGRHTPFFKGYRPQFY.F	4.734	0.4
2331.57588	S.KDEGGRHTPFFKGYRPQFY.F	4.396	0.3
1648.85175	E.GGRHTPFFKGYRPQ.F	3.917	0.4
2168.40176	S.KDEGGRHTPFFKGYRPQF.Y	4.187	0.3
1573.91090	S.SVIRHANLPVLVVR.-	4.013	0.4
1591.80020	G.GRHTPFFKGYRPQ.F	4.047	0.4
1893.09729	S.KDEGGRHTPFFKGYRP.Q	4.238	0.3
989.14811	L.SSGKVIVEGI.N	2.728	0.3
1963.27687	K.NKAARHKANLTAQINKLA.-	4.150	0.4
2021.22706	S.KDEGGRHTPFFKGYRPQ.F	4.279	0.2
1501.83822	Q.AKYPVDLKLVVKQ.-	3.389	0.4
2216.50321	R.MGHAGAIIAGGKGTADEKFAALE.A	4.451	0.5
1929.25566	A.KGIREKIKLVSSAGTGHF.Y	3.841	0.3
2168.40176	S.KDEGGRHTPFFKGYRPQF.Y	4.266	0.3
1592.90299	R.SFTFVTKTPPAAVLL.K	3.164	0.4
1778.13222	V.RHEIIKTTLPKAKEL.R	3.867	0.4
1959.32336	T.YEPKVLRHFTAKLKEV.-	3.614	0.3
1959.32336	T.YEPKVLRHFTAKLKEV.-	3.448	0.2
2092.42978	A.KGIREKIKLVSSAGTGHFY.T	3.870	0.3
1850.06580	L.SVSDKAGIVEFAQALSAR.G	3.423	0.4
1886.14451	A.KLNIDQNPGTAPKYGIR.G	3.915	0.3
1245.45249	K.IAAANVPAFVSGK.A	2.809	0.4
1315.54103	K.NVLSSGKVIVEGI.N	3.258	0.4
1963.27687	K.NKAARHKANLTAQINKLA.-	3.878	0.3

2480.89081	A.KLNIDQNPGTAPKYGIRGIPTLL.L	3.807	0.2
2919.43911	D.TLRLPMTPITDSGRETVRAALKHAGLL.-	4.311	0.4
1242.53964	V.NTLVVKGKVKR.H	3.218	0.3
1431.70485	V.PAFVSGKALKDAVK.-	2.827	0.2
1374.65653	E.AFTRIGKQLGVIA.-	2.894	0.4
1740.04408	A.IREGGRTVGAGVVAKVLG.-	3.490	0.3
1200.49799	A.AKVKAPVIVQF.S	2.350	0.3
1060.22633	R.GKSVEEILGK.-	2.619	0.2
1394.68452	Q.AKGVYIKKVSIST.T	2.892	0.3
1245.45249	K.IAAANVPAFVSGK.A	2.746	0.3
1466.71098	T.GFFYPVVPKGQAR.I	2.485	0.4
1003.17478	G.KSVEEILGK.-	2.251	0.2
2331.57588	S.KDEGGRHTPFFKGYRPQFY.F	2.796	0.4
1844.15076	A.AAKVPSFRAGKALKDAVN.-	2.449	0.3
1130.27665	R.AQLQEIAQTK.A	2.256	0.2
1770.07019	A.IREGGRTVGAGVVAKVLS.-	3.325	0.3
1159.27163	R.AAIEAAGGKIEE.-	2.297	0.2
1267.45492	R.SFTFVTKTPPAA.V	2.435	0.2
1455.72472	R.RVVEPLITLAKTD.S	2.472	0.3
1247.51152	E.GFTKKLQLVGVG.Y	2.492	0.3
1981.33210	V.LLRGIKREEIERGQVLA.K	2.630	0.2
1019.22550	A.RFGLSLVRA.L	2.325	0.3
1532.72614	R.SGSIKSPIWRSGGVT.F	2.477	0.2
1441.68487	S.RCNILQNNKLQP.T	2.225	0.1
2162.49223	I.EEILQKVEREVMSFVPDI.T	2.322	0.3
1721.07590	R.SFTFVTKTPPAAVLLK.K	2.403	0.3
1366.58650	R.SFTFVTKTPPAAV.L	2.232	0.1
1396.57915	I.EQLKQWIGHRT.L	2.265	0.1
1740.04408	A.IREGGRTVGAGVVAKVLG.-	2.610	0.3
1774.10690	N.FAVRSRRKGLGAASLAL.R	2.240	0.2
2353.72939	S.RVDSSPSKAMTISIYRNKIW.-	2.215	0.2

Table S5. All peptides of deer plasma enriched with PMMA-PMA-SiO_2 HMNs and identifid by NanoRPLC-ESI-MS/MS.

MH+	Peptide	XC	DeltaCn
1957.09009	G.HLDDLPGTLSDLSDLHAH.K	6.343	0.3
2924.26130	T.KAVGHLDDLPGTLSDLSDLHAHKLRVD.P	7.377	0.5
3323.70521	A.NALTKAVGHLDDLPGTLSDLSDLHAHKLRVD.P	7.209	0.5
2184.35144	K.AVGHLDDLPGTLSDLSDLHAH.K	5.763	0.5
1957.09009	G.HLDDLPGTLSDLSDLHAH.K	6.302	0.4
2725.01016	A.VGHLDDLPGTLSDLSDLHAHKLRVD.P	6.357	0.6
2075.31448	V.KAHGEKVANALTKAVGHLDD.L	5.899	0.1
2551.83469	E.KVANALTKAVGHLDDLPGTLSDLSD.L	5.513	0.6
3404.73518	K.AHGEKVANALTKAVGHLDDLPGTLSDLSDLHAH.K	5.989	0.6
2113.27321	A.VGHLDDLPGTLSDLSDLHAH.K	5.525	0.3
1606.85631	Q.KVVAGVANALAHRYH.-	4.552	0.4
1476.61621	D.LPGTLSDLSDLHAH.K	4.084	0.2
2610.98857	B.FTPAVHASLBKFLABVSTVLTSKY.R	4.867	0.6
2641.04141	G.KVGGNAPAFGAEALERMFLSFPTTK.T	5.008	0.5
1209.29073	G.TLSDLSDLHAH.K	3.342	0.4
2088.35316	D.LPGTLSDLSDLHAHKLRVD.P	4.306	0.6
1476.61621	D.LPGTLSDLSDLHAH.K	4.180	0.2
1443.66927	K.FLANVSTVLTSKY.R	3.466	0.3
1552.75735	E.KVANALTKAVGHLDD.L	3.830	0.3
2726.07643	S.DFTPAVHASLDKFLANVSTVLTSKY.R	4.728	0.2
3025.36563	L.TKAVGHLDDLPGTLSDLSDLHAHKLRVD.P	5.613	0.3
1738.92344	H.GEKVANALTKAVGHLDD.L	3.927	0.3
2711.96825	A.NALTKAVGHLDDLPGTLSDLSDLHAH.K	4.861	0.4
3381.78635	K.AVGHLDDLPGTLSDLSDLHAHKLRVDPVNFK.L	4.395	0.5
998.15500	B.VSTVLTSKY.R	2.399	0.5
1959.73424	V.KAHGZKVABALTKAVGHLB.B	4.584	0.5
2796.08839	K.AVGHLDDLPGTLSDLSDLHAHKLRVD.P	4.049	0.5
1957.09009	G.HLDDLPGTLSDLSDLHAH.K	3.242	0.5
1139.28678	A.BALTKAVGHLB.B	3.065	0.5
2625.87859	V.GHLDDLPGTLSDLSDLHAHKLRVD.P	3.842	0.5
3050.45346	H.LPBBFTPAVHASLBKFLABVSTVLTSKY.R	4.787	0.6
1268.93684	A.BVSTVLTSKYR.-	2.856	0.6
1254.37464	A.NALTKAVGHLDD.L	2.833	0.4
3433.92482	G.KVGGNAPAFGAEALERMFLSFPTTKTYFPHF.D	3.823	0.5
3050.45346	H.LPNDFTPAVHASLDKFLANVSTVLTSKY.R	3.924	0.5
2134.33879	F.BLSHGSAZVKAHGZKVABALT.K	3.787	0.5
1606.76511	L.SDLSDLHAHKLRVD.P	2.310	0.2
2641.04141	G.KVGGNAPAFGAEALERMFLSFPTTK.T	2.217	0.4