Supplementary Information

Multi-affinity sites of magnetic guanidyl-functionalized metal-organic framework nanospheres for efficient enrichment of global phosphopeptides

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1. Materials

Iron(III) chloride hexahydrate ($FeCl_3 \cdot 6H_2O$), ammonium acetate (NH_4Ac), zinc nitrate hexahydrate ($Zn(NO_3)_2 \cdot 6H_2O$), L-arginine (Arg), bovine serum albumin (BSA), β-casein (from bovine milk), trypsin (TPCK treated), dithiothreitol (DTT), iodoacetamide (IAA), and 2,5-dihydroxyl benzoic acid (DHB) were purchased from Sigma Aldrich (St. Louis, MO, USA). Commercial TiO_2 microspheres, 2-amino terephthalic acid, 1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC), polyetherimide (PEI, Mw 25,000), and polyvinyl pyrrolidone (PVP, Mw 50,000) were purchased from Aladdin (Shanghai,China). Trisodium citrate dehydrate (Na_3CT), acetic acid (HAC), ethylene glycol (EG), urea, ammonium bicarbonate (NH_4HCO_3), acetonitrile (ACN), anhydrous ethanol, and dimethyl formamide (DMF) were purchased from Forest Science and Technology Development Co. Ltd. (Chengdu, China). HOBT was purchased from GL Biochem (Shanghai, China). N,N-Diisopropylethylamine and trifluoroacetic acid (TFA) were obtained from AstaTech (Chengdu, China). The non-fat milk was obtained from a local supermarket.

2. Synthesis of the SPIOs@PVP-PEI@MOF@Arg nanospheres (SPMA nanospheres)

2.1 Synthesis of the Fe_3O_4 nanoparticles (SPIOs)

The Fe_3O_4 nanoparticles were synthesized by hydrothermal reaction. Briefly, 1.157 g $FeCl_3 \cdot 6H_2O$, 3.303g NH_4Ac, and 0.400 g Na_3CT were dissolved in EG (60 mL) under magnetic stirring. The magnetic stirring bar was removed after stirring for one hour. The mixture was added in the Teflon-line stainless-steel autoclave and maintained at 200 °C for 16 h. Then, the sediment were collected under an external magnetic field. After that, the sediment was thoroughly washed with ethyl alcohol and water for several times. Finally, the Fe_3O_4 nanoparticles were re-dispersed in deionized water (15 mL) for subsequent use.

2.2 Synthesis of the SPIO@PVP-PEI nanospheres

Firstly, 0.4 g PEI and 1.0 g PVP were dissolved in 20 mL deionized water. Then, 5 mL the above SPIOs suspension was added to the above mixed solution under magnetic stirring for 24 h. The products were collected by an external magnetic field and washed with water and DMF alternately for several times. Finally, the SPIO@PVP-PEI nanospheres were re-dispersed in DMF (2 mL) for subsequent use.

2.3 Synthesis of the SPIOs@PVP-PEI@MOF nanospheres

The above SPIO@PVP-PEI nanospheres (400 μL) was dispersed in the mixture solution containing HAC (100 μL), H_2O (2 mL), DMF (13 mL), $Zn(NO_3)_2 \cdot 6H_2O$ (1 g), and 2-amino terephthalic acid (220 mg). Then, the mixture was heated at 100°C for 2 h under vigorous stirring. After that, the brownish yellow products were collected by magnetic separation and washed with DMF and water for several times, respectively. Finally, the obtained SPIOs@PVP-PEI@MOF nanospheres were dispersed in deionized water (1 mL) for further use.

2.4 Synthesis of the SPIOs@PVP-PEI@MOF@Arg nanospheres (SPMA nanospheres)

L-arginine (208 mg), EDCI (345 mg), and HOBT (243 mg) were added into the mixture solution containing H_2O (1.5 mL), DMF (13 mL), and DIPEA (590 μL). The solution was stirred for 1 h, following which the above SPIOs@PVP-PEI@MOF nanospheres (500 μL) was added and continuously stirred for an additional 24 h at room temperature. After that, the products were collected by magnetic separation and washed with DMF and water for several times, respectively. Finally, the obtained SPMA nanospheres were dispersed in deionized water (1 mL) for further use.

2.5 Synthesis of SPIOs@NH_2 nanospheres

The above SPIOs suspension (1 mL) and 2-amino terephthalic acid (1 g) were added in DMF (15 ml). The solution was continuously stirred for 24 h at room temperature. After that, the products were collected by magnetic separation and washed with DMF and water for several times, respectively. Finally, the obtained SPIOs@NH_2 nanospheres were dispersed in deionized water (1 mL) for further use.

3. Phosphopeptide enrichment experiments

3.1 Preparation of tryptic digests of standard proteins

β-casein (1 mg) was dissolved in 1 mL 50 mM NH_4HCO_3 solution at pH 8.2 and digested with trypsin (40:1, w/w) at 37 °C for 16 h. 10 mg of BSA was dissolved in 1 mL 50 mM NH_4HCO_3 solution containing urea (8 mol L^{-1}). The mixture was incubated at 60 °C for 1 h to reduce the disulfide bonds of proteins after the addition of 100 μL of DTT (1 mol L^{-1}). Subsequently, 37 mg of IAA was added and the mixture was incubated at room temperature in the dark for 45 min. Finally, the mixture was diluted with NH_4HCO_3 (50 mmol L^{-1}, pH = 8.2) and incubated at 37 °C for 16 h with trypsin at the mass ratio of enzyme to protein of 1:40 (w/w).

3.2 Preparation of tryptic digests of proteins extracted from nonfat milk

Thirty μL nonfat milk was dissolved in 1 mL 25 mM NH_4HCO_3, and this solution was centrifuged at 14000 rpm for 20 min. The supernate was collected and proceeded at 100 °C for 10 min to make the protein degeneration. The supernate was digested with trypsin (40 μg) at 37 °C for 16 h. This tryptic digest of nonfat milk was diluted by loading buffer for further use.

3.3 Enrichment of phosphopeptides from β-casein tryptic digestions

The above SPIOs@NH_2 nanospheres, SPIOs@PVP-PEI@MOF nanospheres, and SPMA nanospheres in deionized water (1 mL) were separated by magnetic separation and washed with loading buffer 1 (50% ACN-H_2O,

0.1 mol L^{-1} Hac) for three times and then were dispersed in 1ml loading buffer 1 (50% ACN-H_2O, 0.1 mol L^{-1} Hac), respectively. In a typical enrichment process, 20 μL of the material suspension (5 mg/mL) was added to 100 μL of peptide solution (β-casein tryptic digest was diluted with loading buffer1 to different concentrations) and the mixture was then incubated in a shaker at room temperature for 45 min to ensure equilibrium. After magnetic separation and removal of the supernatant, the material were washed three times with loading buffer 1 (400 μL) to remove the nonspecifically adsorbed peptides. The captured phosphopeptides were eluted by 10 μL buffer 2 (50% ACN-H_2O containing 2% TFA) under powerful shaking for 10 min. The eluate was analyzed by MALDI-TOF MS.

3.4 Enrichment of phosphopeptides from the tryptic digest mixtures of β-casein and BSA

The tryptic digest of β-casein and BSA were diluted with loading buffer 1 (50% ACN-H_2O, 0.1 mol L^{-1} Hac) at different molar ratio. Then, 20 μL of SPMA nanospheres suspension (5 mg/mL) was added to 100 μL of the aboved mixture of β-casein and BSA digest. After similar enrichment, washing, and elution protocol was followed, the eluent was analyzed by MALDI-TOF MS.

3.5 Enrichment of phosphopeptides from nonfat milk tryptic digestions

The tryptic digest of nonfat milk was diluted with loading buffer 1 (50% ACN-H_2O, 0.1 mol L^{-1} Hac) at proper concentration. Then, 20 μL of SPMA nanospheres suspension (5 mg/mL) was added to 100 μL of the aboved solution. After similar enrichment, washing, and elution protocol was followed, the eluent was analyzed by MALDI-TOF MS.

3.6 MALDI-TOF MS Analysis

All eluent samples were analyzed in reflector positive mode on AB Sciex 5800 MALDI-TOF/TOF mass spectrometer (AB SCIEX, CA). Matrix DHB was dissolved in 70% ACN-H_2O containing 1% H_3PO_4 (25 mg mL^{-1}). A 1 μL aliquot of the eluent and 1 μL of DHB matrix were sequentially dropped onto the MALDI plate for MS analysis.

3.7 Preparation of tryptic digests of proteins extracted from rat brain lysate

A Sprague Dawley male rat was sacrificed, and its brain was promptly taken out, then cut into bits, and washed with saline. Then, the brain tissues were minced with scissors and homogenized for 30 min. Above sample were diluted in 4% SDS, 100 mM Tris-HCl (pH 8.0), and 100 mM DTT and heated at 100ºC for 5 min. The sample was then cooled to room temperature and loaded onto an ultrafiltration filter followed by centrifugation at 14000×g for 30 min. One hundred microliters of 50 mM iodoacetamide was subsequently added to the filter, and the samples were then incubated for 30 min at room temperature. Next, 100 μL of 25 mM ABC (Applied Biosystems, Foster City, CA, USA) was added to each filter, followed by centrifugation at 14000 × g for 30 min. The protein suspensions were then digested with 40 μL of trypsin buffer at 37ºC for 18 h. Finally, the filter unit was transferred to a new tube, added 40 μL 25 mM ABC and centrifuged at 14000 × g for 30 min. The resulting peptides were collected, and lyophilized for further use.

3.8 Enrichment of phosphopeptides from rat brain lysate digestions

Tryptic digest of rat brain lysate (0.5 mg) was diluted with 500 μL loading buffer 1 (50% ACN-H_2O, 0.1 mol L^{-1} Hac), and the sample processing was same as that of peptide mixture enrichment. The eluted solution was analyzed by Elite-LC-MS/MS.

3.9 Elite-LC-MS/MS Analysis

The samples were analyzed using Easy-nLC nanoflow HPLC system connected to Orbitrap Elite mass spectrometer (Thermo Fisher Scientific, San Jose, CA, USA). One microgram of each sample was loaded onto Thermo Scientific EASY column (two columns) at a flow rate of 200 nL/min. The sequential separation of peptides on Thermo Scientific EASY trap column (100 μm×2 cm, 5 μm, 100 Å, C18) and analytical column (75 μm×25 cm, 5 μm, 100 Å, C18) was accomplished using a segmented 1h gradient from 5% to 28% Solvent B (0.1% formic acid in 100% ACN) for 40 min, followed by 28-90% Solvent B for 2 min and then 90% Solvent B for 18 min. The mass spectrometer was operated in positive ion mode, and MS spectra were acquired over a range of 350-2000 m/z. The dynamic exclusion duration was 30s.

The acquired data from triplicate MS runs for each sample were combined and searched against the uniprot rat protein sequence database (version 1.4.1.1). Proteins were identified using the Andromeda peptide search engine integrated into the MaxQuant environment. A decoy version of the Self-database was used to estimate peptide and protein false discovery rate. We used MaxQuant to calculate LFQ, a measure of protein abundance. The LFQ value is obtained, and is on average highly correlated with protein abundance.

4. Characterization

Scanning electron microscopy (SEM, Hitachi S-4800, Japan) and transmission electron microscopy (TEM, JEM-2010, Japan electronic) were used to analysis the morphologies of the samples. The size distribution and zeta potential were calculated via dynamic light scattering (DLS, Zetasizer Nano ZS90, Malvern Company). Crystal structure with angles from 5° to 70° was tested by powder X-ray diffraction (XRD, X' Pert Pro MPD, Philips, Netherlands). Surface area and pore size analyzer (QuadraSorb SI, America) was employed to study the surface and BJH pore size distribution at 77K. Fourier transform infrared spectra was obtained by spectrometer (FT-IR, PE spectrometer) with wave number range 500-4000 cm^{-1}. The mass loss of sample was analyzed at temperature ranging from 35 to 900 °C with the heating rate of 10K/min by simultaneous thermal analysis (STA449 C Jupiter, NETZSCH). Vibrating sample magnetometer (VSM, model BHV-525, Riken Japanese Electronics Company) was employed to measure the magnetization of the sample with field from 0 Oe to 18000 Oe at 300K.

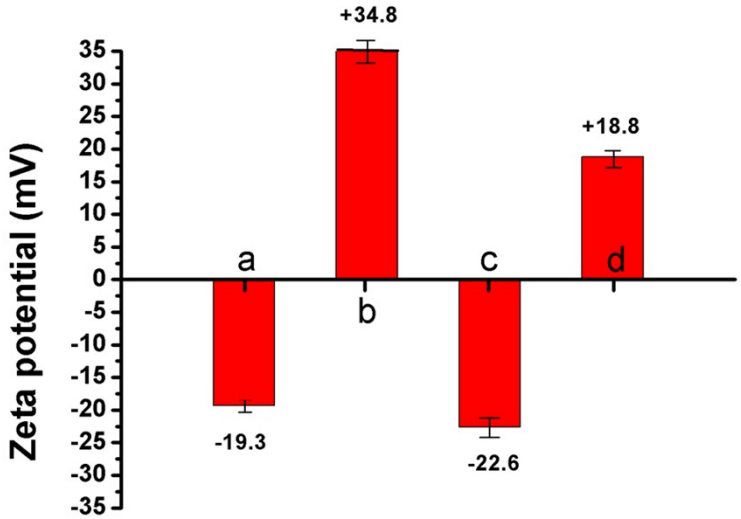

Fig. S1 Zeta potential of the (a) SPIOs, (b) SPIOs@PVP-PEI nanospheres, (c) SPIOs@PVP-PEI@MOF nanospheres, and (d) the SPMA nanospheres.

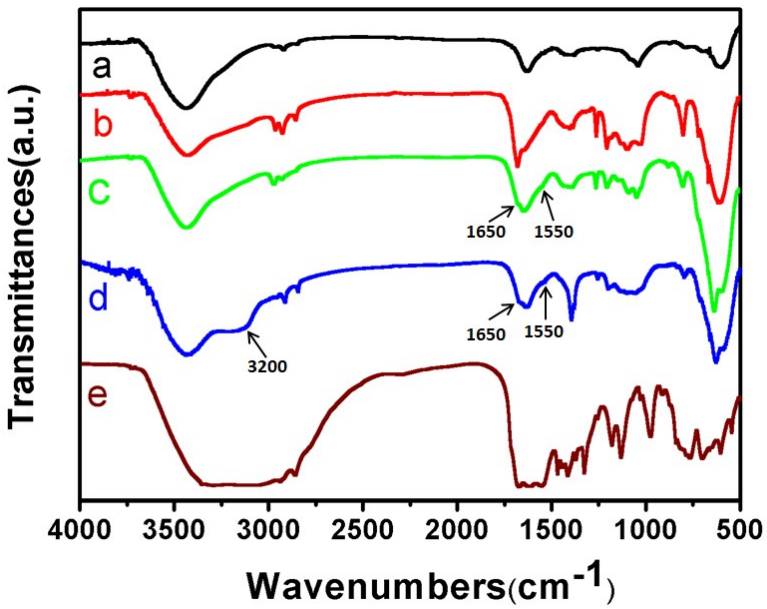

Fig. S2 FTIR spectra of the (a) SPIOs, (b) SPIOs@PVP-PEI nanospheres, (c) SPIOs@PVP-PEI@MOF nanospheres, (d) the SPMA nanospheres, and (e) arginine.

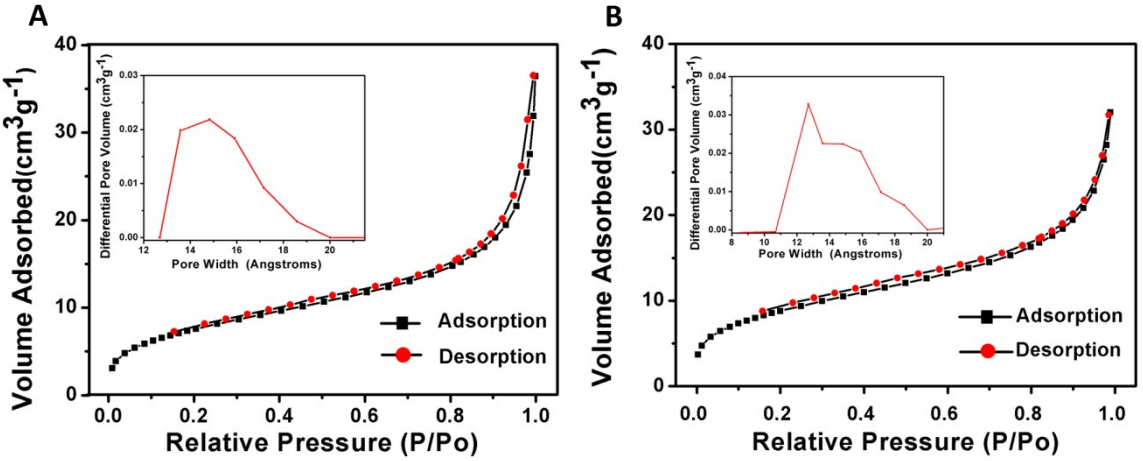

Fig. S3 N_2 adsorption−desorption isotherm and pore size distribution (inset) of the (A) SPIOs@PVP-PEI@MOF nanospheres, and (B) the SPMA nanospheres.

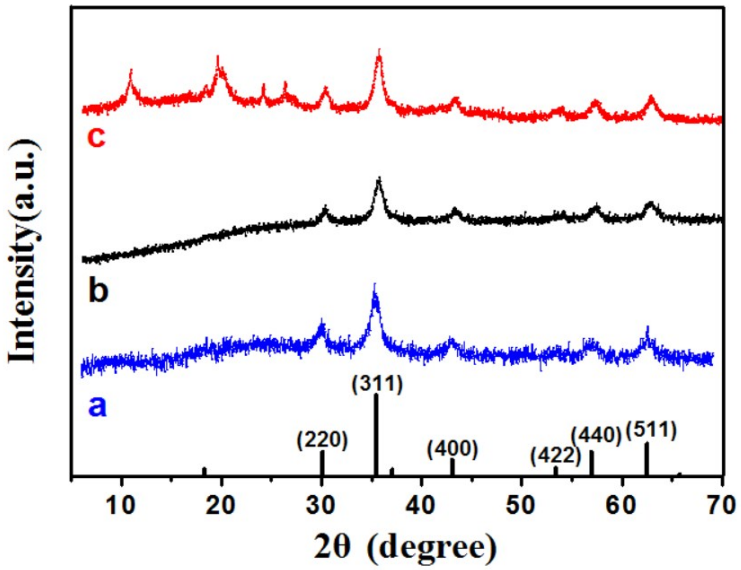

Fig. S4 XRD patterns of (a) SPIOs, (b) SPIOs@PVP-PEI nanospheres, and (c) SPIOs@PVP-PEI@MOF nanospheres. the standard XRD pattern of SPIOs (JCPDS no. 19-06290) is shown by solid bars.

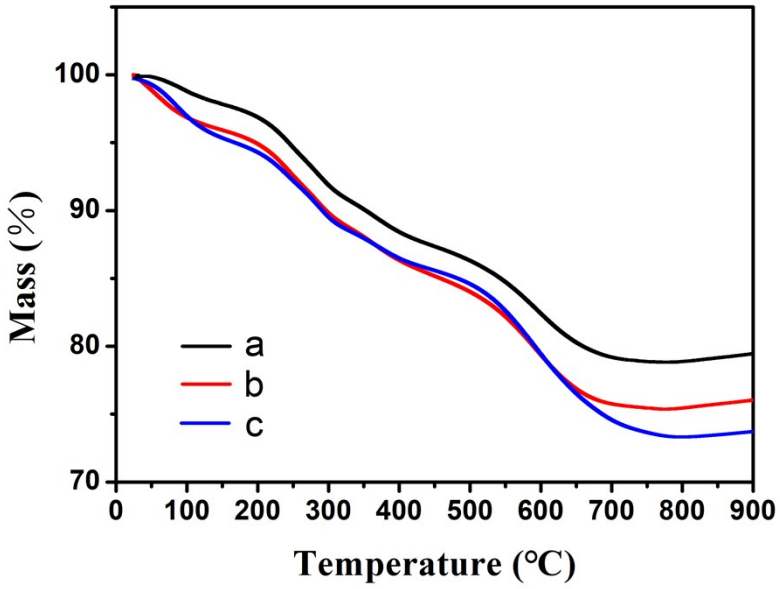

Fig. S5 TGA curves of the (a) SPIOs, (b) SPIOs@PVP-PEI@MOF nanospheres, and (c) the SPMA nanospheres.

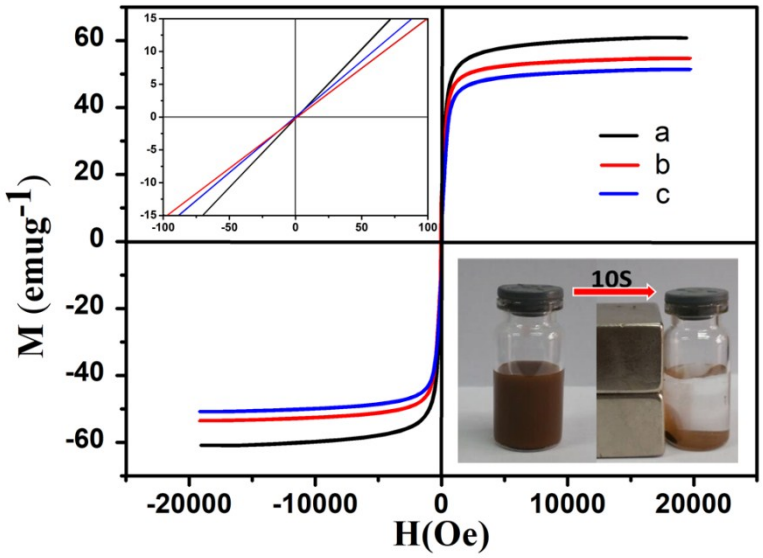

Fig. S6 VSM curves of the (a) SPIOs, (b) SPIOs@PVP-PEI@MOF nanospheres, and (c) SPMA nanospheres, and dispersion and separation process of the SPMA nanospheres (inset).

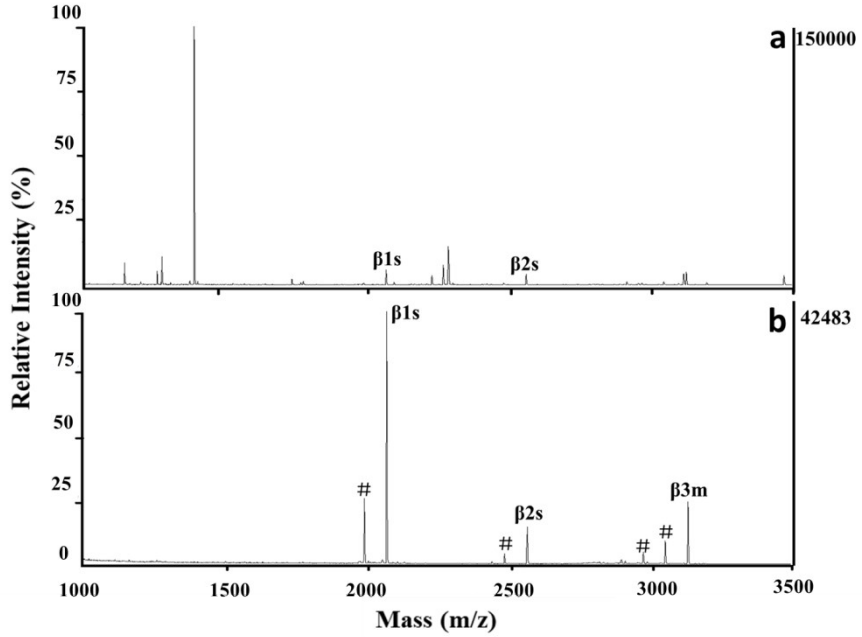

Fig. S7 MALDI-TOF mass spectra of (a) β-casein digest (10^{-6} M) and (b) β-casein digest (10^{-6}M) after enrichment with the SPMA nanospheres. (s, monophosphopeptide; m, multiphosphopeptide; #, dephosphorylated peptide).

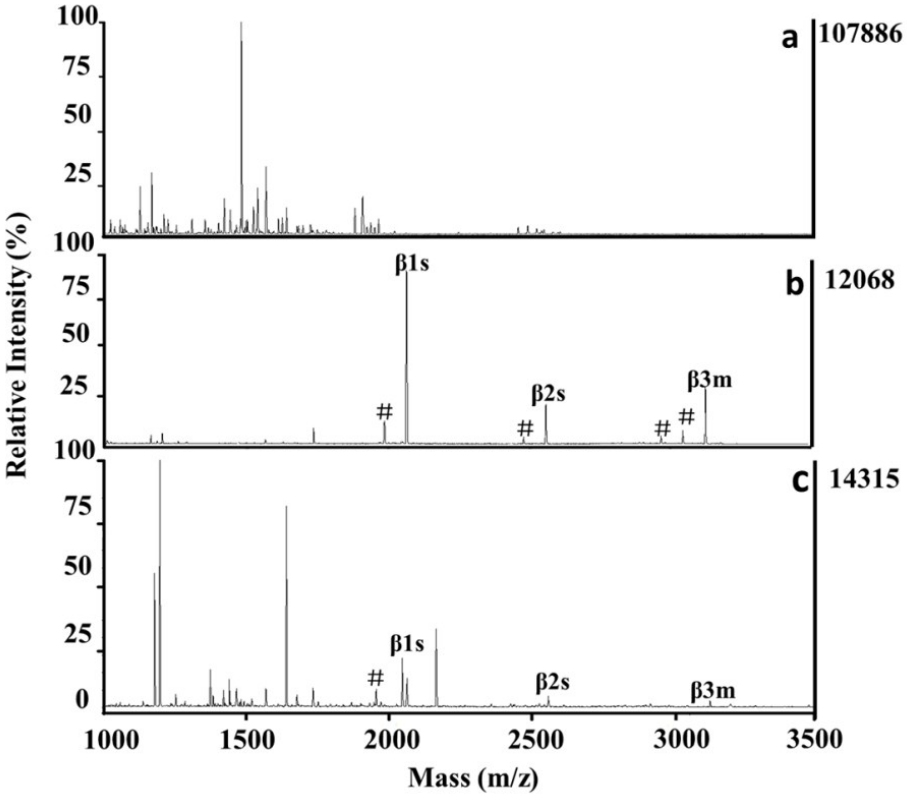

Fig. S8 MALDI-TOF mass spectra of the tryptic digests mixture of β-casein and BSA (with a molar ratio of β-casein to BSA of 1:100): (a) direct analysis, (b) analysis after enrichment by the SPMA nanospheres, and (c) analysis after enrichment using the commercial TiO_2 microspheres. (s, monophosphopeptide; m, multiphosphopeptide; #, dephosphorylated peptide).

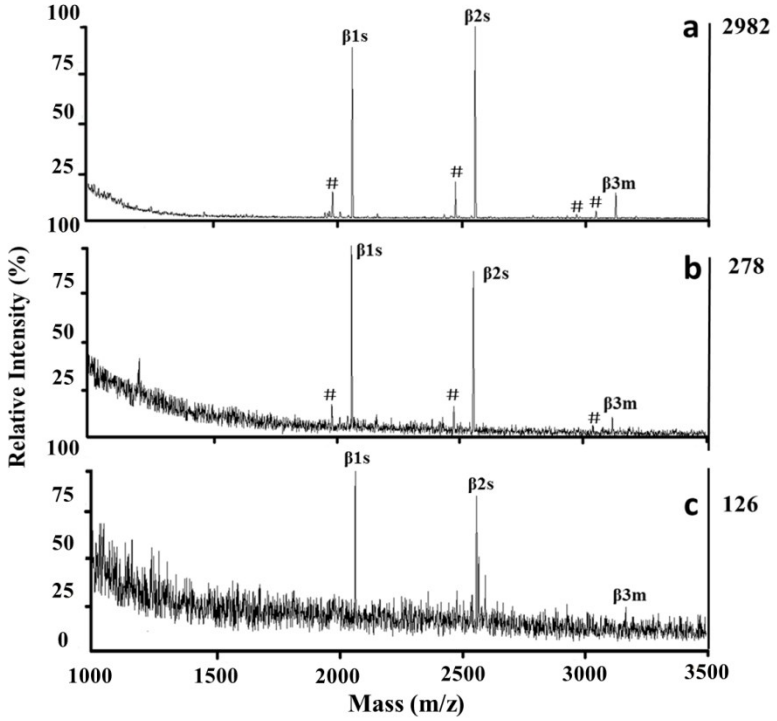

Fig. S9 MALDI-TOF mass spectra of the tryptic digest mixture of β-casein with different concentrations after enrichment with the SPMA nanospheres: (a) 10^{-8} M, (b) 10^{-9} M, and (c) 10^{-10} M. (s, monophosphopeptide; m, multiphosphopeptide; #, dephosphorylated peptide).

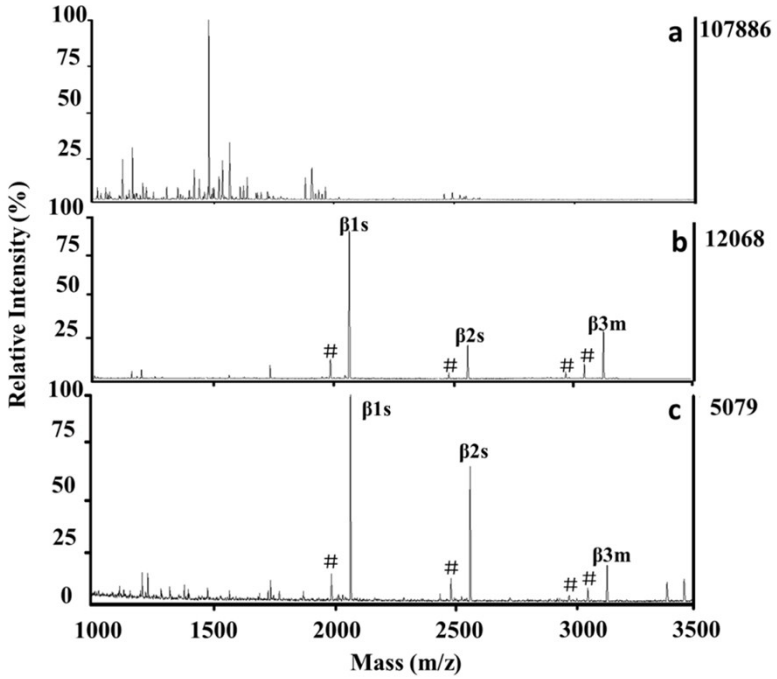

Fig. S10 MALDI-TOF mass spectra of the tryptic digests mixture of β-casein and BSA (with a molar ratio of β-casein to BSA of 1:100): (a) direct analysis,(b) analysis after enrichment by SPMA nanospheres. MALDI-TOF mass spectra of the tryptic digests mixture of β-casein and BSA (with a molar ratio of β-casein to BSA of 1:1000): (c) analysis after enrichment by SPMA nanospheres. (s, monophosphopeptide; m, multiphosphopeptide; #, dephosphorylated peptide).

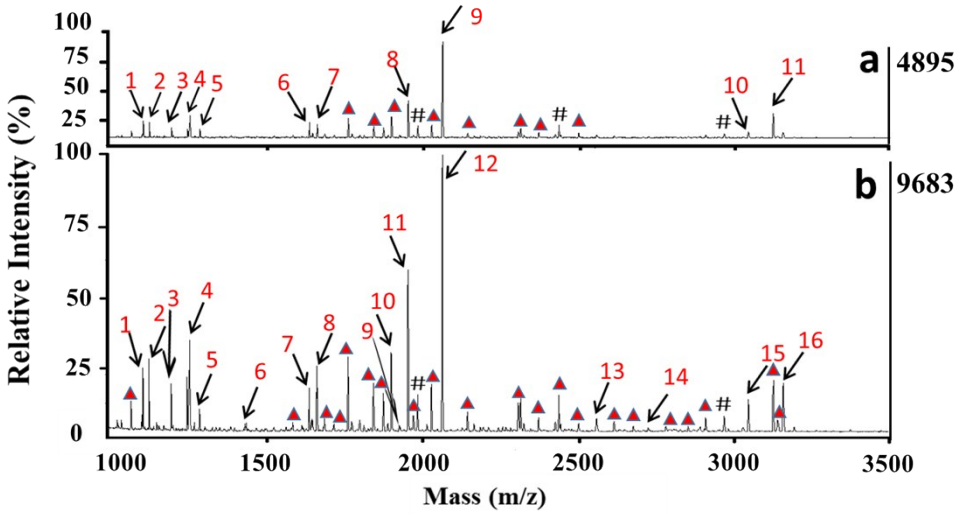

Fig. S11 MALDI-TOF mass spectra of (a) digests of the non-fat milk after enrichment with SPIOs@NH_2 nanospheres and (b) SPIOs@PVP-PEI@MOF nanospheres. Phosphopeptide peaks identified are marked with numbers and non-phosphopeptide peaks are marked with symbol ▲. The dephosphorylated peptide peaks are marked with symbol #.

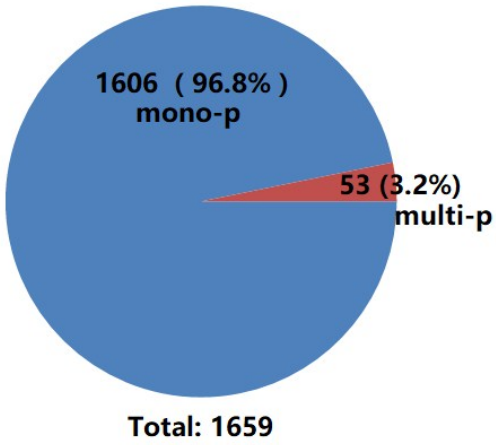

Fig. S12 Number of identified phosphopeptides from tryptic digests of rat brain lysate after enrichment with the SPMA nanospheres. "P" represents phosphopeptide.

Table. S1 Detailed information for the observed phosphopeptides obtained from tryptic digests of β-casein after enrichment by the SPMA nanospheres in MALDI-TOF MS analysis.

No.	Observed m/z	Theoretical m/z	Charge	Peptide sequence	Number of phosphoryl groups	Reported reference
β1s	2061.46	2061.83	-2	FQ***S***EEQQQTEDELQDK	1	1 2 4 5
β2s	2555.48	2556.10	-2	FQ***S***EEQQQTEDELQDKIHPF	1	1 2 3 4 5
β3m	3121.38	3122.27	-3	RELEELNVPGEIVE***S***L***SSS***EESITR	4	1 2 3 4 5

***S*:** phosphorylated site.

Table. S2 List of phosphopeptides from tryptic digests of proteins extracted from non-fat milk after enrichment by the SPMA nanospheres in MALDI-TOF MS analysis.

No.	Observed	Theoretical	Charge	Peptide sequence	Number of phosphoryl groups	Reported reference
1	1103.2	1103.4	-1	KFQ***S***EEQQQT	1	2 3
2	1123.8	1124.1	-2	KEKVNEL***S***KDIG***S***E***S***TEDQA	3	3
3	1197.2	1197.5	-1	KNMAINP***S***KENL	1	2
4	1241.8	1241.3	-1	TIA***S***GEPT***S***TPT	2	2 3
5	1281.6	1281.1	-3	RINKKIEKFQ***S***EEQQQTEDELQDKIHPFAQTQS	1	3
6	1455.3	1455.5	-2	LSKDIG***S***E***S***TEDQA	2	2
7	1522.0	1521.9	-3	VVRNANEEEYSIG***SSS***EE***S***AEV ATEEVKITVDDKHYQKAL	4	3
8	1561.5	1562.0	-2	RELEELNVPGEIVE***S***L***SSS***EESITRI	4	3
9	1634.7	1634.0	-1	ED***S***PEVIESPPEIN	1	2
10	1661.0	1660.8	-2	VPQLEIVPN***S***AEER	1	1 4 5
11	1927.2	1927.7	-2	KDIG***S***E***S***TEDQAMEDIKQ	2	1 2 4 5
12	1952.0	1952.0	-2	YKVPQLEIVPN***S***AEER	1	1 2 4 5
13	2061.4	2061.8	-2	FQ***S***EEQQQTEDELQDK	1	1 2 4 5
14	2080.9	2080.3	-2	KKYKVPQLEIVPN***S***AEER	1	4 5
15	2555.8	2556.1	-2	FQ***S***EEQQQTEDELQDKIHPF	1	1 2 3 4 5
16	2704.0	2703.5	-3	QMEAE***S***I***SSS***EEIVPN***S***VEQK	5	1 4
17	2720.3	2720.9	-3	QMEAE***S***I***SSS***EEIVPN***S***VEQK	5	1 4
18	2965.3	2965.8	-3	ELEELNVPGEIVE***S***L***SSS***EESITR	4	1 2 5
19	3122.0	3122.3	-3	RELEELNVPGEIVE***S***L***SSS***EESITR	4	1 2 3 4 5

***S*:** phosphorylated site.

Table. S3 Detailed information of the observed phosphopeptides obtained from tryptic digests of rat brain lysate.

Master Protein Accessions	Phosphopeptide sequence	Theoretical m/z
D3ZDN9	SSLSYTNEERLLLEGMAASSSSSSAGGVSGSSVPGSGFSVS DLAPPRK	4879.20167
Q9EPJ1	SAGGSAGPGGATGGGIGGGDEPGSPAQGK	2391.02085
Q6TY19	FLLDLERDDTVGAAGGIVTAGGGLSASSGHRGHCFYR	3979.79464
F1M3D2	LGDPDGTGEPPSTPSGLGAGGDR	2189.93466
D3ZIQ9	SRGPFPGVSTDDSAVPPPGGAPHFGHYRTGGGAMGLR	3982.63228
G3V7X9	SAAEAVATSVLTQMASQR	1980.84975
D3ZVD1	QPGTAQAQALGLAQLAAAVPTSR	2380.14217
A0A0G2K9C5	AEAAAAPAVAPGPAQPGHVSPTPATTSPGEKGEAGTPVA AGTTAAAIPR	4507.22531
Q9JKA7	GGGSGGAGGGSSLGHLHDSAEER	2131.87888
D3ZN76	SPDPEMVPQGSPVRHSPPEPR	2376.08022
Q5EIC4	NSSSPVSPASVPGQR	1549.70063
A0A0G2K3Q5	SFESDAEDHKPLK	1582.67849
D4A1D2	EKALDIDSDEEPEPKEQKPEEK	2663.19714
F1LRL9	ASAEGEATAVVSPGVTQAVVEEHCASPEEK	3119.38748
A0A140UHX6	KLSGLER	882.44446
Q6MG07	SSPYGR	746.2869
F1LST4; D3ZKD9	TPPKSPSASK	1159.4796
A0A0G2JW90	YHGHSMSDPGVSYR	1688.6523
F1M4A4	SGTSQEELR	1086.44631
D3ZWV8	FTIDSDAISASSPEKEPQQPPGGGDTDR	2982.30004
B1WC25	SPTGTPAR	866.37677
Q5BJU3	RSLDEDEPPPSPLARYRPLHNAASHEGLAATSGSPPR	4108.90261
Q6AY81	GPTSPTTPR	993.4401
O08560	DPSPEAR	851.32949
A0A0G2K5C6	AELEEMEETHPSDEEGEETKAESFYQK	3268.30353
P62957	YPDHLHISTSPC	1506.60831
A0A0G2K498	EDDGTFGEYSDAEDHKPLK	2232.89688
E9PST5	GLSPLSSTADTK	1256.57699
Q4FZT2	QCEGITSPESSK	1402.5556
A0A0G2K2M9	SRTSPVTR	983.46699
D4A8V2	RTSPSPPAR	1048.49353
M0R4V3	ATWGDGGDSSPSNVVSK	1743.72215
R9PXV2	LSMPQSAAVNTTPPHNR	1900.87352
D4A6C5	SSSPEPVTHLK	1261.58241
D3ZT20	EPSSPEAQK	1052.4296
P09951	QASQAGPGPR	1048.45715
D3ZWS0	VSPTGAAGR	895.40332

A0A0G2K2C0	VAAGSGAGGGGGAGGGGGGGGGNRAGGGGAGGAGGGGGGGGGSR	3120.30843
D3ZGL1	DAPLSPPAQK	1103.51327
P10362	IETQTQEEVRDSKENTEK	2244.00274
P62494	ENDMSPSNNVVPIHVPPTTENKPK	2724.26987
Q5BJT1	RHSMQTEQIR	1365.60931
A0A0G2K6I5	GFSEEQLR	1045.43502
F1MAQ8	QRSPIALPVK	1188.65004
Q63092	SATPATDGR	955.38807
A0A0G2K613	GDVTAEEAAGASPAKANGQXNGHVK	2490.10789
Q3MUI2	EAAAAPAVAPGPAQPGHVSPTPATTSPGEK	2924.32271
F1LM42	QPPISPTSK	1034.4918
O88954	LTGSQEVK	941.43395
F1LM42	QKEESPQGSEEK	1455.59991
Q5M9G6	LSPEPVAHR	1085.51394
A0A0G2K7T6	AAPQSPSVPK	1061.5027
Q75Q39	KTPEGR	767.34475
A0A0G2K2M9	HSLSGSSPGIK	1149.52998
D3ZG21	LSTSPATR	912.41864
F1LRL9	VISPLR	764.40662
A0A0G2JVB6	AGSRLSAEDR	1141.49974
G3V7T1	QASPVAFK	927.43356
D4A720	SGSIIGSR	856.39242
A0A0G2K2F4	HLCSPSDHR	1188.46158
Q7TP42	EDSKKEETPGTPK	1525.67816
F1LMT5	GSPGLTR	767.34475
A0A0G2K9C5	KAEAAAAPAVAPGPAQPGHVSPTPATTSPGEK	3123.45479
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P97603	LELKPIDKSPDPNPVMTDTPIPR	2653.33068
E9PU02	FNFESVPESPGEK	1546.64613
Q5M7A3	EDYQEILDSPIK	1529.6771
A0A0G2K1Y8	KLDPAQSASRENLLEEQGSIALR	2605.29813
D3ZSZ4	ELLGLEDESPAH	1389.59337
B4F7A3	LDDGHLNNSLGSPVQADVYFPR	2494.13984
P47942	NLHQSGFSLSGAQIDDNIPR	2249.03465
F1LNK0	LEETSKVSIEETVAKEEESLK	2458.18479
M0R3Z8	LLLLERPSPVR	1372.77121
F1M3W5	FGDVEADSPVEQTIIQDHSAFK	2513.12319
Q5QD51	EGITPWASFKK	1343.63953
Q4G008	NNRPAFFSPSLK	1457.69369
A0A0G2K1Y8	LDPAQSASRENLLEEQGSIALR	2477.20317
Q9Z2L0	LTFDSSFSPNTGK	1480.63557
A0A0H2UHP5	DDVEGMMSQGQRPTTPIPDVQSTKGGSLAAGAKK	3553.66624
F1M1Y0	WVHFSDASPEHVTPELTPR	2285.03867
A0A0G2K5C6	KPSPFLSPSGDHEANGPGETSLNPPGFVTATAEK	3516.63188
F8WFS9	VTMILQSPSFR	1358.6538

G3V9D7	VTQILQSPAFR	1339.67698
P31016	EQLMNSSLGSGTASLR	1730.77789
O88794	GLATGDSPLGPMTHHGEEDWVYER	2734.16032
Q6MG08	QLSVPASDEEDEVPVPVPR	2142.99547
Q03351	GPVAVISGEEDSASPLHHINHGITTPSSLDAGPDTVVIGMTR	4315.07005
A0A0G2K0S7	QELANSSDVTLPDRPLSPPLTAPPTMK	2955.45331
P83868	DWEDDSDEDMSNFDR	1955.62732
B1WC16	ELFDYSPPLHK	1425.64501
P25122	LALSDSPDGRPGGFWR	1810.82722
Q02294	EVAEVSPMSAANISIAAR	1895.89325
G3V8J5	LEASTSDPLPAGGGSVLPGSR	2047.96959
Q5FVJ0	ELDDISLTPDPEPTHEDPNYLMANER	3091.32382
F1LRL9	LGGDGSPTQVDVSQFGSFK	2005.89028
P08050	MGQAGSTISNSHAQPFDFPDDNQNAK	2857.18833
A0A096MJN4	LDPYDSSEDDKEYVGFATLPNQVHR	2975.30949
F1LMW7	LSGFSFK	865.38555
A0A0G2K613	LSGLSFK	831.4012
Q5U300	ATLPSPDKLPGFK	1450.73416
A0A0G2K5C6	WLAESPVGLPPEEEDKLTR	2246.07406
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F1LRL9	SLMSSPEDLTK	1287.55381
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F1M5M9	NDLQSPTEHISDYGFGGVMGR	2360.0013
D4ABP4	GDFSPFGNTQGPSR	1546.63221
Q9Z1T4	GSESPNSFLDQEYR	1708.68504
A0A0G2K0F3	MLASPEDFETVR	1474.62837
F1LQN3	LSASPQELGKPYLESFQPNLHSTK	2751.33894
Q5M7V8	IDISPSTFR	1115.51327
Q9Z1T4	LGDSLQDLYR	1259.56676
Q64548	EQDSPPMKPGVLDAIR	1832.86123
B1WC16	IDISPSALR	1051.51835
S4VP54	LKISPEQHWDFTAEDLK	2137.00016
F1LRL9	QGFSDKESPVSDLTSDLYQDK	2439.05992
Q7TP75	HAFSPVASVESASGEVLHSPK	2216.03834
D4ADX8	QMASQFPPPPTPPPVDSQPLKPLPASVTPQSPPAVK	3808.93836
F8WFW5	RLSCDGLGPSDLLGKPLAR	2105.0573
B2RZ79	AASALLLR	894.48084
F1LN49	RQSLGGFLK	1085.55032
O35987	KKSPNELVDDLFK	1612.79822
Q9WU70	KLSLPTDLKPDLDVK	1761.93979
Q5PQP2	KLSGDQITLPTTVDYSSVPK	2229.10502

Q5YLM1	LLSPVAAASAAR	1206.62421
Q9QY02	GISPIVFDR	1083.52344
D3ZWQ0	RGSQPDAELDGAGTSLLR	1922.89676
F1LQZ9	AQSPLLPEPLK	1272.65993
A0A096MKC0	ALSSGGSITSPPLSPALPK	1859.95142
P48768	GISALLLNQGDGDRK	1636.80543
D4A6H8	HISPVQALSEFK	1435.69811
F1MA89	SASADNLILPR	1236.59839
F1LNK0	EESTETPDIPAIPSDVTQPQPEAVVSEPAEVR	3497.62071
A0A0G2K2D6	RLSLESEGANEGAAAAPELSALEEAFR	2868.34112
A0A0G2K0F3	DKSDSETEGLVFAR	1633.71052
A0A0U1RRX0	RLSPPGGSGSGVPGGGPLLPTAGR	2224.12341
Q9JMB3	VISQTNLITTVTPEK	1723.88776
Q1M168	DEWQDEDLPRPLPEDTGEDHLGGTVEDSSSPPSTLNLSGAHR	4636.03836
Q64548	SPPVAMETASTGVAAVPDALDHSSSPTLK	2932.36456
A0A0G2JYE0	VPLAAVAGSEGPEQLQPPCPSQTGSPPVGLIK	3261.62251
Q5XI21	GDLSQHATPLPTPAVLPGDSPITPTPEQIGK	3214.60315
I7FKL4	FSWGAEGQKPGFGYGGR	1880.81157
F1LRL9	QSPDHPTVGAGMLHITENGPTEVDYSPSDIQDSSLSHK	4126.83357
G3V8P8	GSPSGLAPILR	1147.5871
F1LRL9	ESVASGDDRAEEDMDEALEK	2275.89081
Q75Q39	ASPALGSGPDGSGDSLEMSSLDR	2285.95916
D3ZGQ8	LSPIPEEVVR	1218.61298
F1M6V8	ASPGPGGLSGGESLLVK	1605.78838
S4VP54	ISPEQHWDFTAEDLK	1895.82114
A0A0G2QC21	MSGFIYQGK	1110.46896
F1LRL9	ESTAAYQTSSSPPIDAAAAEPYGFR	2667.16103
A0A0G2JSL4	ASGPLSPPTGPPSPVPTGPAVR	2119.05835
D3ZMS5	ESDILSDEEEDFHHPLK	2119.88559
Q9JKA9	DSASPGAASGLDPLDSAR	1766.75926
D3ZYW8	ALDPAPLAQPTPVGSVQTSPELEHR	2690.31854
Q6J4I0	IAESHLQTISNLSENQASEEEDELGELR	3221.44817
Q63622	NLSQIENVHGYVLQSHISPLK	2456.23335
A0A0G2K0S7	KQELANSSDVTLPDRPLSPPLTAPPTMK	3083.54828
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A0A0H2UHZ2	EFITGDVEPTDAESAWHSENEEDDK	2930.15238
A0A0G2JXG5	SAGGPCPAGAGSGPGGASCPVGVSPGGVSMFR	2925.24777
P08050	VAAGHELQPLAIVDQRPSSR	2224.12341
A0A0G2K2M9	AAEIPAVASCWVGPQVSPEHK	2313.07334

Q80VJ4	LTLEGLEEDDDDDDKASPTVLHK	2635.16584
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G3V7G0	DFQEYVEPGEDFPASPQR	2190.90157
B5DFK6	VDIITEEMPENALPSDEDDKDPNDPYR	3197.35043
F1LS01	FDWGPAPPTTFKPNSPDLAK	2266.05801
Q9JIR4	LQTHDESSLPLPQPSPFMPR	2357.09956
A0A0G2JTR4	GPPEGSETMPYIDESPTMSPQLSAR	2757.17834
O08618	LGIAVIHGEAQDAESDLVDGRHSPPMVR	3065.45102
O08618	LGIAVIHGEAQDAESDLVDGRHSPPMVR	3049.45611
Q68FP9	MADTSGEVAAVPASGAANGLSNGAGATPAQPNNPLSR	3517.60133
F1LRL9	DVMSDETNNEETESPSQEFVNITK	2823.15502
Q5QD51	VIETVVISETGESPECVGAHLLPAEK	2844.37367
D3ZT07	FGIHVYQFPECDSDEDEDFKQQDR	3084.23534
D3ZJR1	TVFAGAVPVLPASPPPK	1727.91319
Q5PPM8	IINLGPVHPGPLSPEPQPMGVR	2385.21486
P31044	VLTPTQVMNRPSSISWDGLDPGK	2578.23711
A0A0G2JYE0	EVDGLLTSDPMGSPVSSK	1898.8453
O35314	WWQQEEQLEPEESREEVSFPDR	2913.23632
O88778	EPELEMESLTGSPEDR	1898.77253
G3V9Y1	QLHIEGASLELSDDDTESK	2166.94383
F1LRL9	SAGFIPIKEDFSPEKK	1872.91431
Q66X93	VSVTVDYIRPASPATETVPAFSER	2672.29674
F1M1Y0	LPPGKPGVGDVSRPFSPPIHSSSPPPIAPLAR	3307.73511
Q56R18	NVPQEESLEDSDVDADFK	2116.85943
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A0A0G2K2P5	DDISEIQSLASDHSVR	1851.81203
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Q03555	EVHDELEDLPSPPPPLSPPPTTSPHK	2893.36555
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O08838	IDVESTELASSESPQAAELEAGAPQEK	2866.28775
D3ZBU7	SPPEMSLLHDVGPGPAIK	1924.92383
Q5QD51	SPEQPAGSDTPSELVLSGHGPAAEASGAAGDPADADPATK	3808.68214
B2GUY4	STSPPPSPEVWAESR	1706.74216
Q64548	SPPVAMETASTGVAAVPDALDHSSSPTLK	2916.36964

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