Supporting Information

Dual-Metal Centered Zirconium−Organic Framework: A Metal-Affinity Probe for Highly Specific Interaction with Phosphopeptides

Jiaxi Peng,[ac] Hongyan Zhang,[ac] Xin Li,[ac] Shengju Liu,[ac] Xingyun Zhao,[ac] Jing Wu,[d] Xiaohui Kang,[a] Hongqiang Qin,[a] Zaifa Pan[b] and Ren'an Wu*[a]*

[a] CAS Key Lab of Separation Sciences for Analytical Chemistry, National Chromatographic R&A Center, Dalian Institute of Chemical Physics, Chinese Academy of Sciences, Dalian, 116023, China [b] College of Chemical Engineering and Materials Science, Zhejiang University of Technology, Hangzhou 310014, China [c] University of Chinese Academy of Sciences, Beijing, 100049, China [d] Wenzhou Institute of Biomaterials and Engineering, Chinese Academy of Sciences, Wenzhou, 325000, China

*E-mail: wurenan@dicp.ac.cn. Tel: +86-411-84379828

*E-mail: panzaifa@zjut.edu.cn. Tel: +86-571-88320797

Table S1. Contact angles of UiO-66-NH$_2$, UiO-66-NH-PO$_3$, and DZMOF.

Materials	Contact angle / degree	Images
UiO-66-NH$_2$	35.7	
UiO-66-NH-PO$_3$	29.1	
DZMOF	20.2	

Table S2. ICP-OES results of UiO-66-NH$_2$, UiO-66-NH-PO$_3$, and DZMOF.

Materials	Zr (wt%)
UiO-66-NH$_2$	22.2
UiO-66-NH-PO$_3$	20.0
DZMOF	24.3

Table S3. Comparison of our proposed DZMOF to the reported MOF based affinity probes in the past three years.
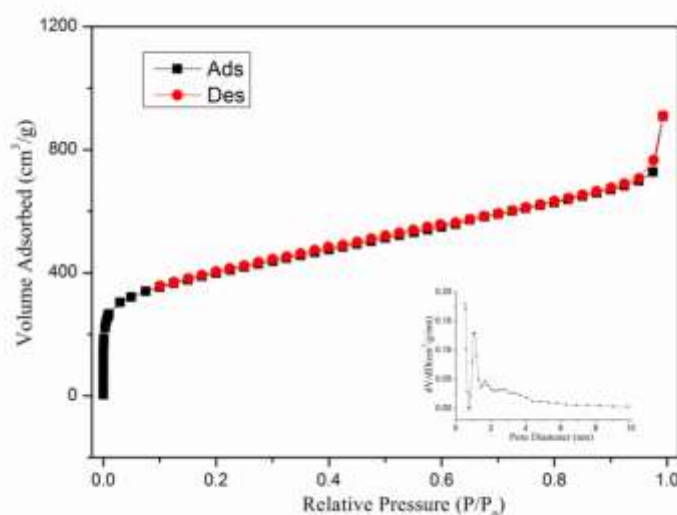
MOF Affinity Probes	Year of Publication	Sensitivity	Selectivity β-casein:BSA(n:n)	Ref
Fe$_3$O$_4$@PDA@Zr-MOF	2014	1 fmol	1:500	1
magG@PDA@Zr-MOFs	2015	100 fmol	1:1000	2
Fe$_3$O$_4$@MIL-100 (Fe)	2015	0.5 pmol	1:500	3
UiO-66 and UiO-67	2015	15 fmol	1:200	4
MIL-101(Cr)-UR$_2$	2016	20 fmol	1:200	5
Fe$_3$O$_4$@PDA@Er(btc)	2016	4 fmol	1:500	6
UiO-66-(OH)$_2$	2016	10 pmol	1:100	7
DZMOF	Proposed Probe	10 fmol	1:5000	Our work

Table S4. Identification of phosphopeptides from tryptic digests of β-casein by MALDI-TOF MS with enrichment of DZMOF.
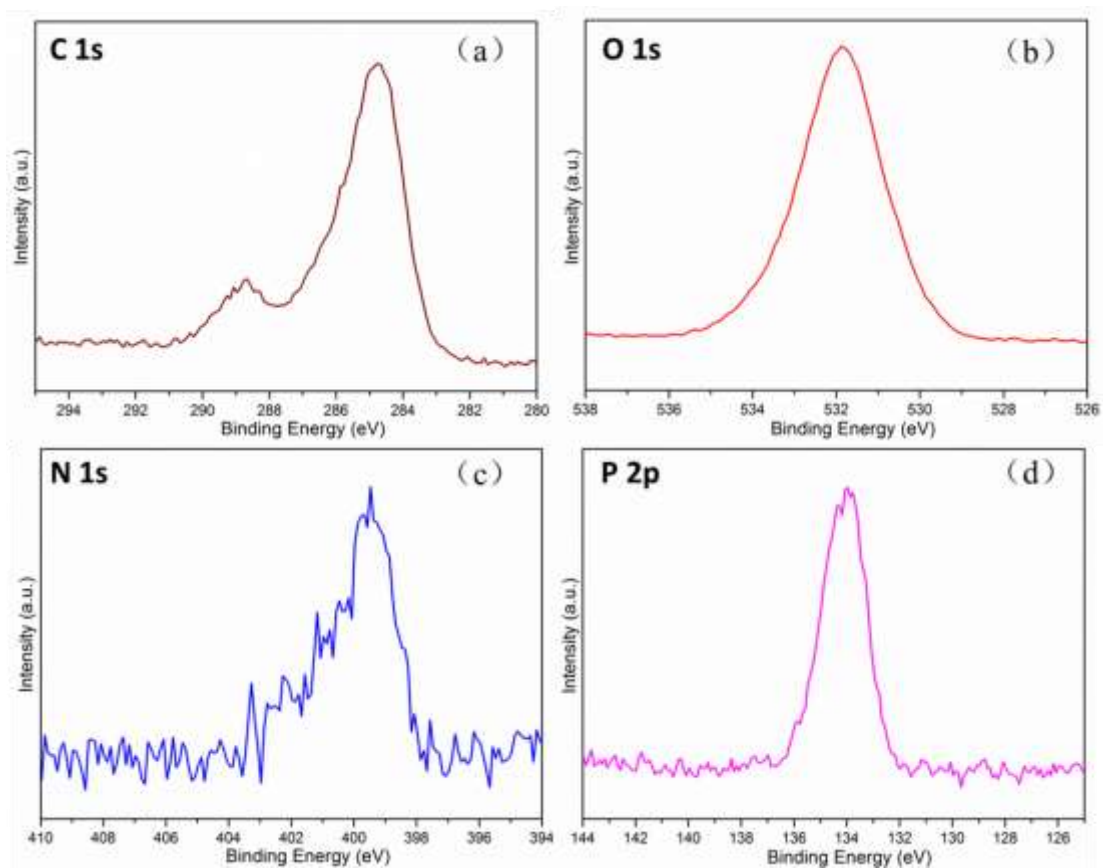
Protein	Peak	Amino Acid Sequence	No. of Phosphorylation Sites	[M+H]$^+$
β-casein	β$_1$	FQ[pS]EEQQQTEDELQDK	1	2061.7798
	β$_2$	FQ[pS]EEQQQTEDELQDKIHPF	1	2556.0596
	β$_3$	RELEELNVPGEIVE[pS]L[pS][pS][pS]EESITR	4	3122.2236

Table S5. Recovery of a standard phosphopeptide (GRRNpSIGK) treated by DZMOF. The recovery of standard phosphopeptide was calculated by the peak intensity ratio of eluted light isotope-labeled standard phosphopeptides to subsequently added heavy isotope-labeled standard phosphopeptides.

Repeated Experiments	Intensity Ratio (L/H)	Average Recovery ± SD （%, n=5）
1	95.6%	
2	89.5%	
3	93.6%	91.4±3.2
4	87.4%	
5	90.8%	

Figure S1. The nitrogen sorption/desorption isotherms of UiO-66-NH$_2$. The inset shows the pore distribution obtained by DFT.
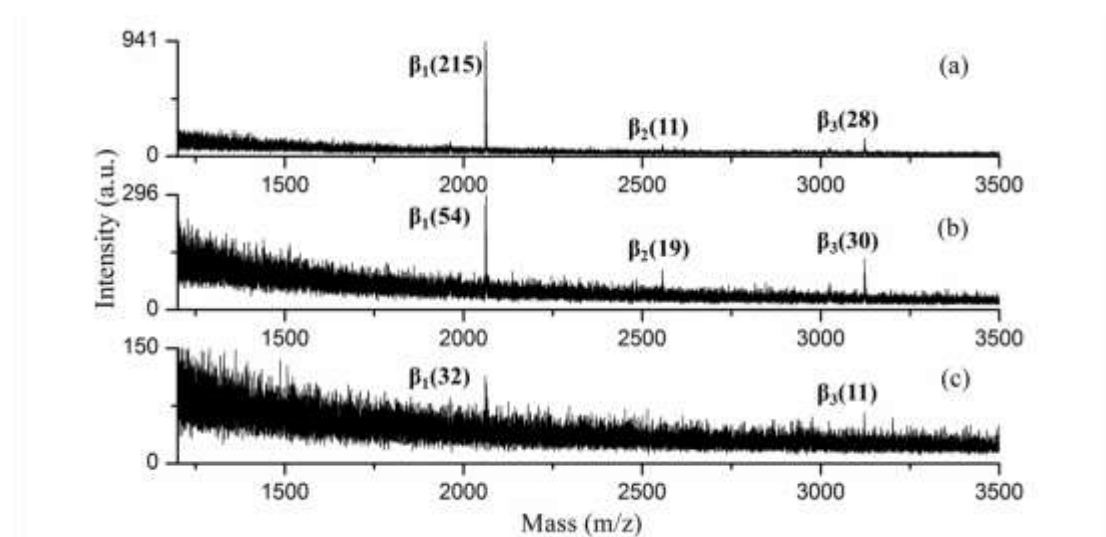
Figure S2. High-resolution XPS spectra of (a) C 1s, (b) O 2p, (c) N 1s, and (d) P 2p regions taken from DZMOF.
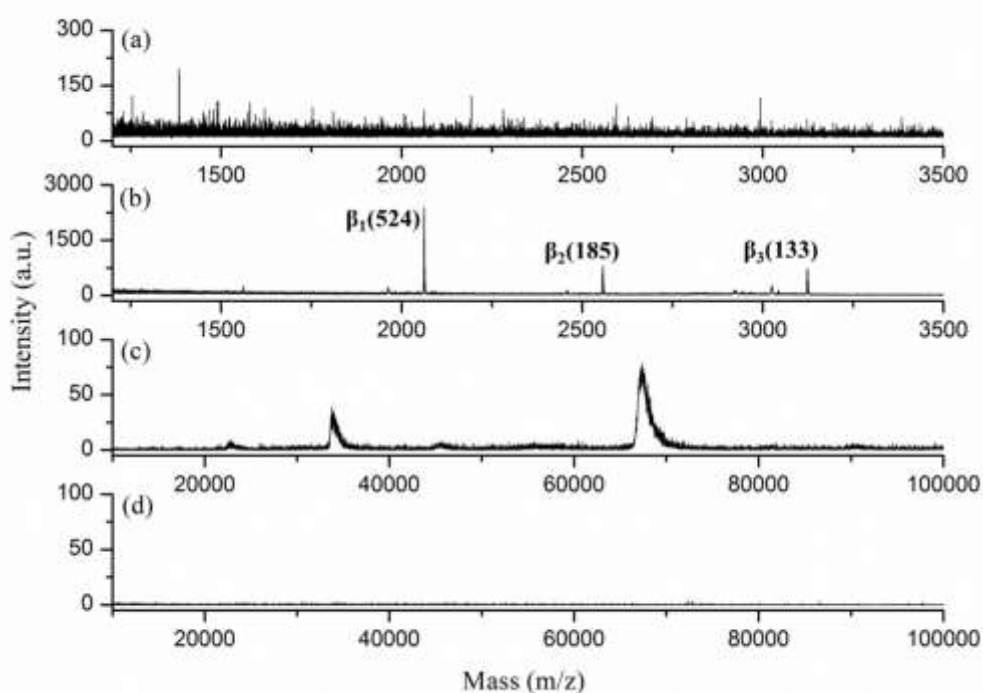

Figure S3. Digital photograph of a homemade pipette tip column.
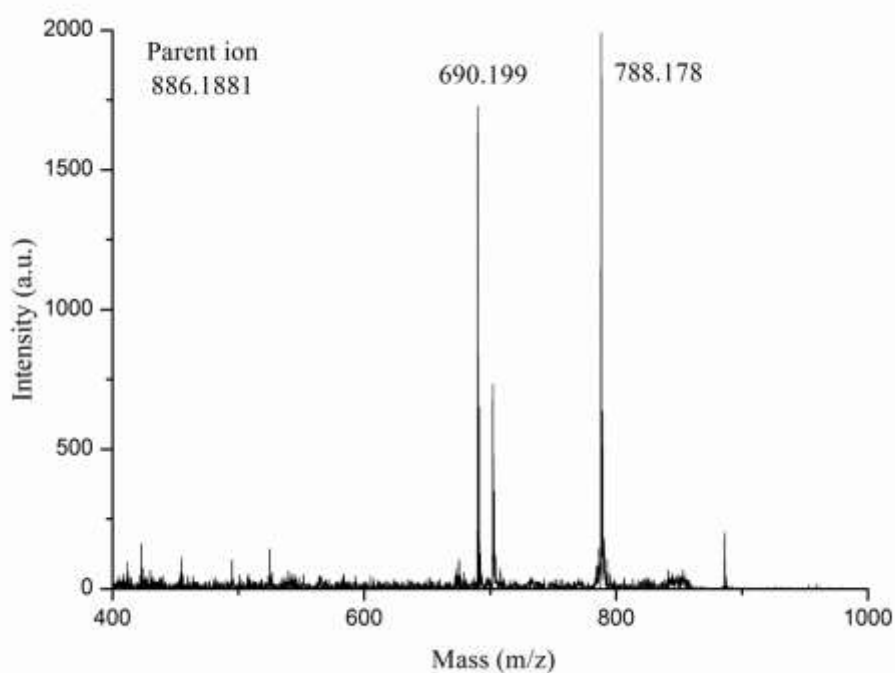
Figure S4. MALDI-TOF mass spectra of 10 pmol of β-casein tryptic digests (a) before and after enrichment by (b) DZMOF and (c) UiO-66-NH$_2$. The numbers in parentheses represent the S/N of the peaks; "#" denoted dephosphorylated fragments.
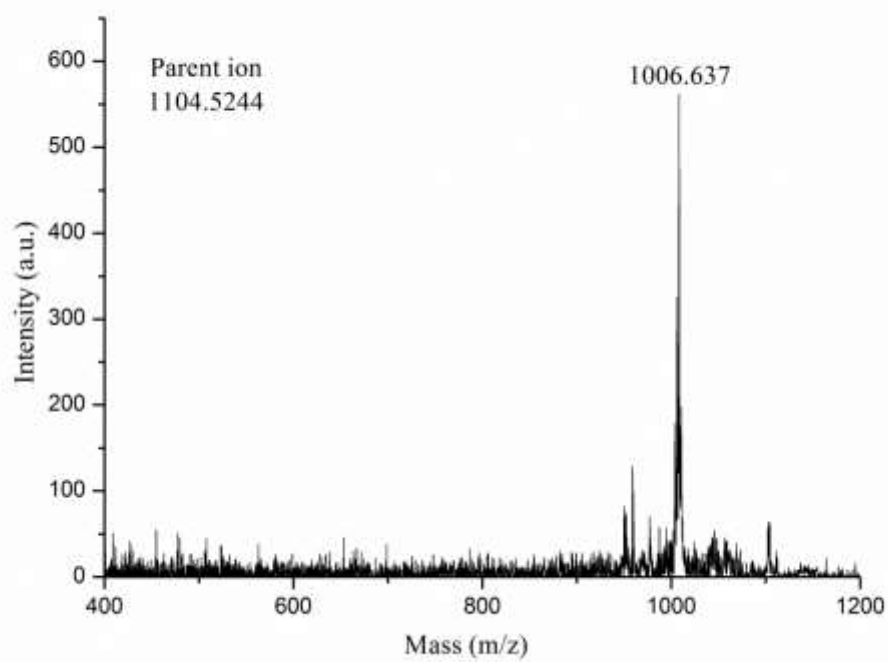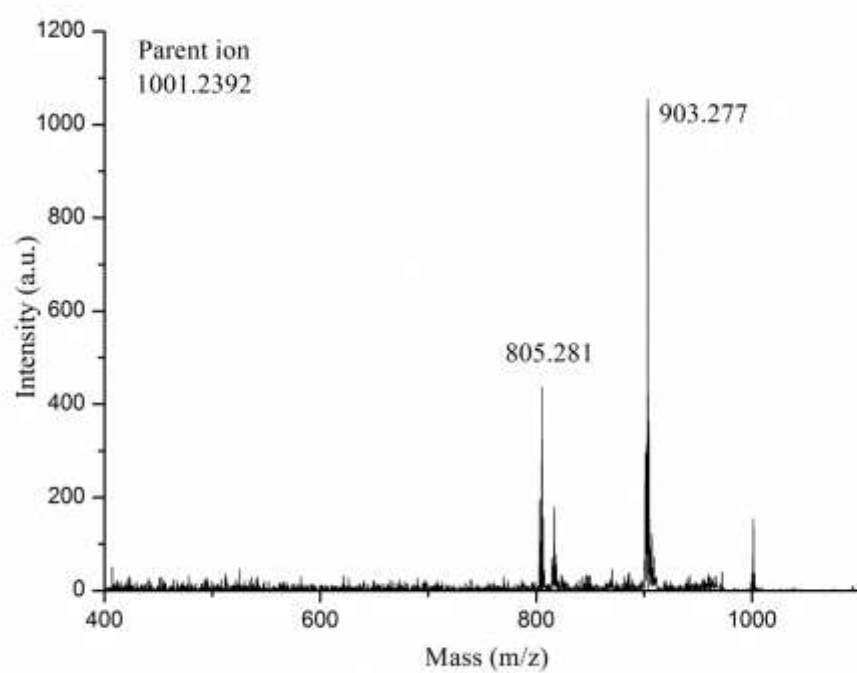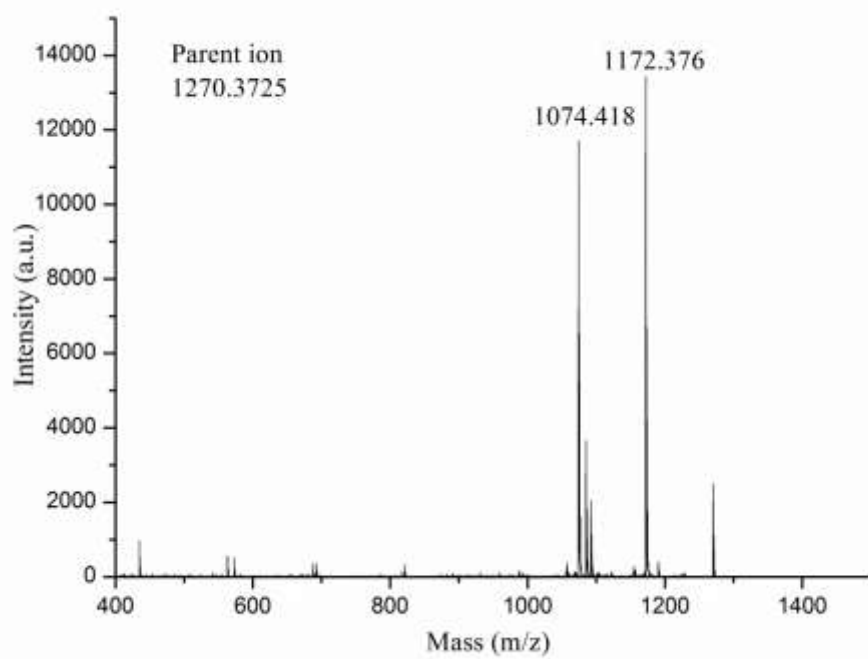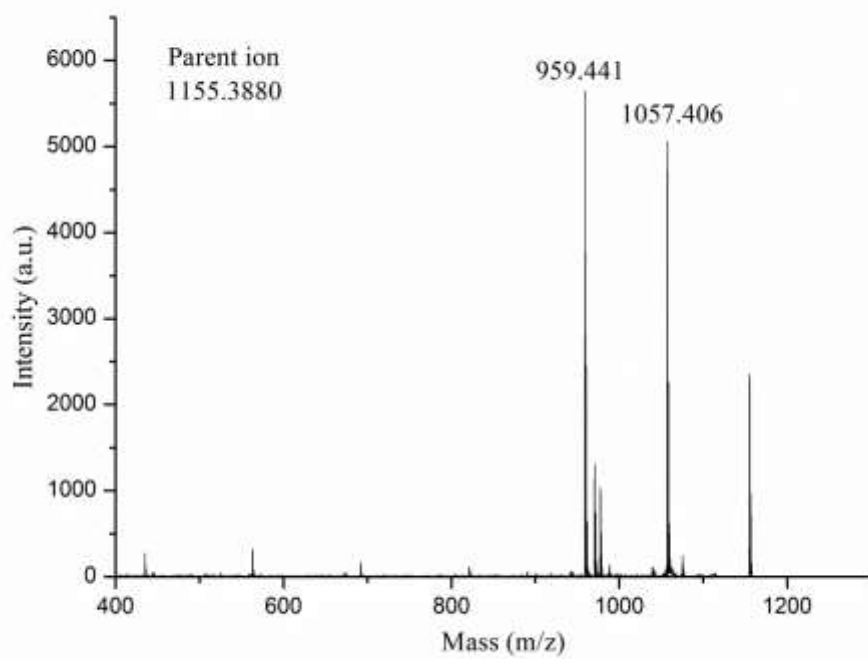

Figure S5. MALDI-TOF mass spectra of the tryptic digests of β-casein with a concentration of (a) 1.3 fmol/μL, (b) 0.67 fmol/μL, and (c) 0.07 fmol/μL, treated by DZMOF respectively. The numbers in parentheses represent the S/N of the peaks.
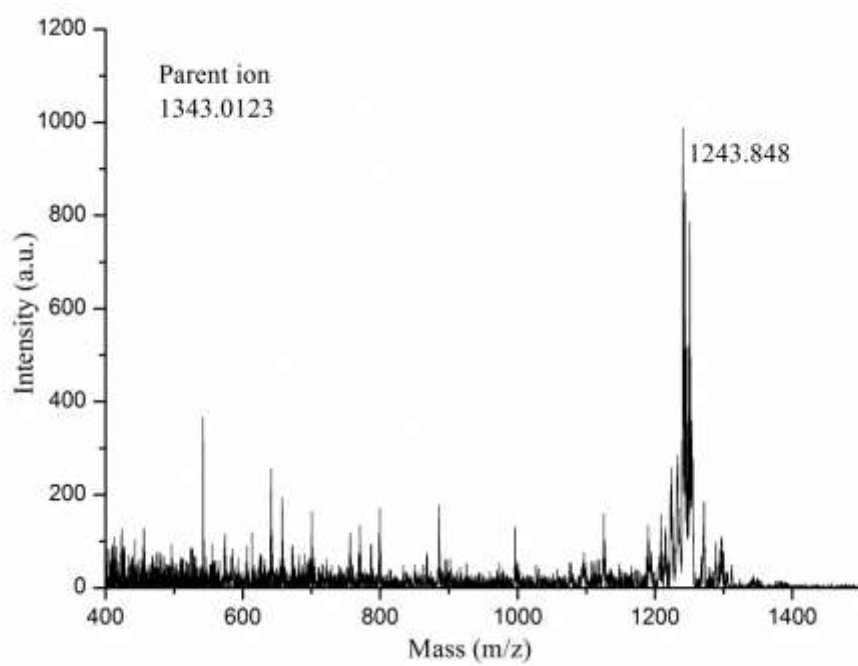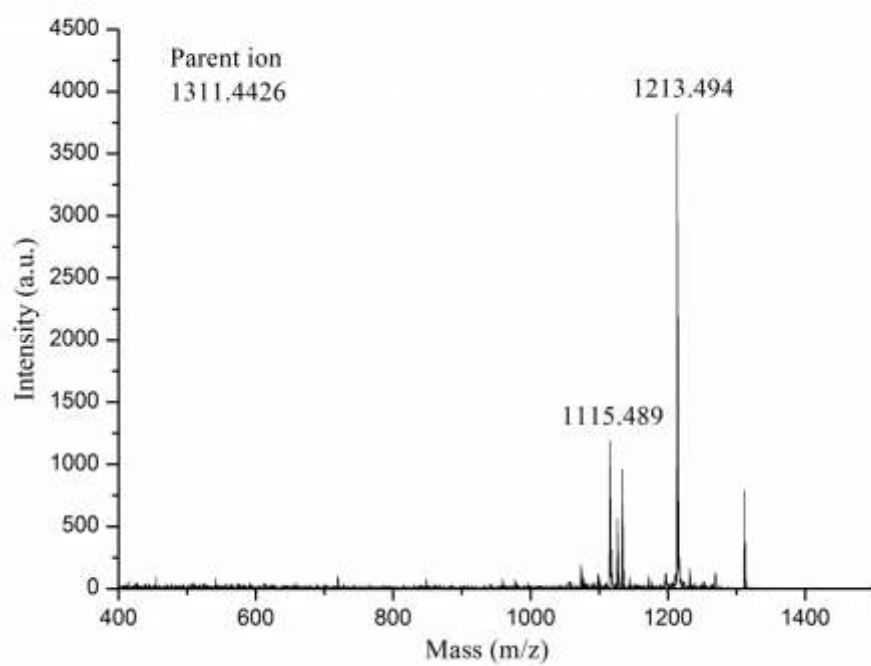
Figure S6. MALDI-TOF mass spectra of β-casein tryptic digests and BSA standard protein with a molar ratio of 1:1000 by direct analysis at (a) low molecular weight region, (c) high molecular weight region and enrichment with DZMOF at (b) low molecular weight region, (d) high molecular weight region. The numbers in parentheses represent the S/N of the peaks.
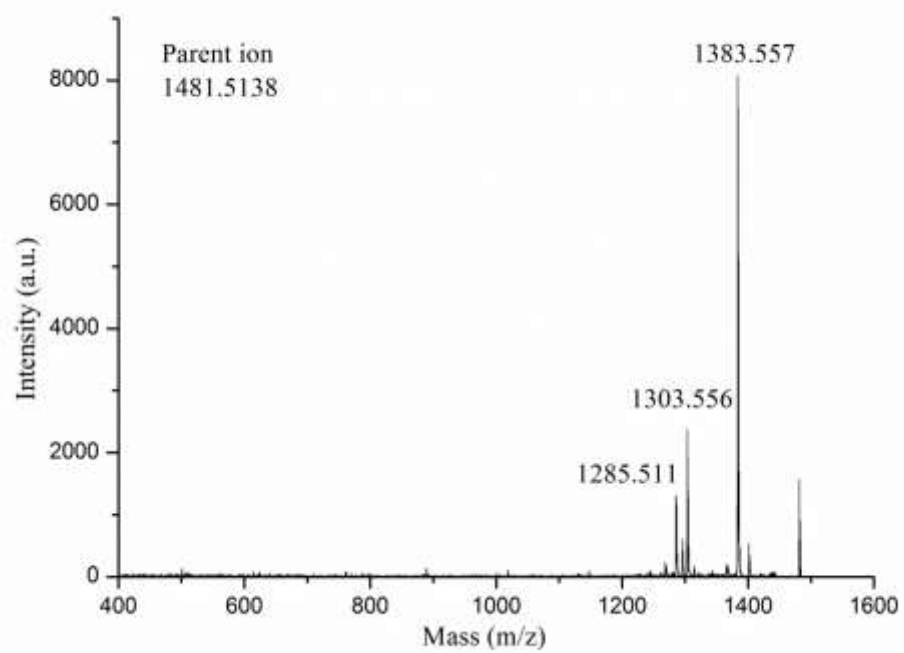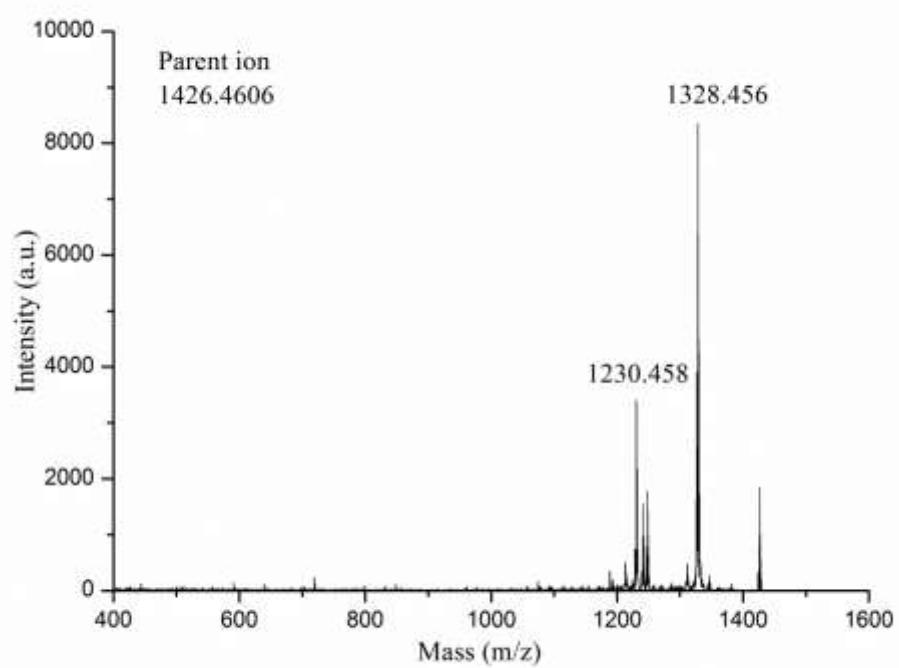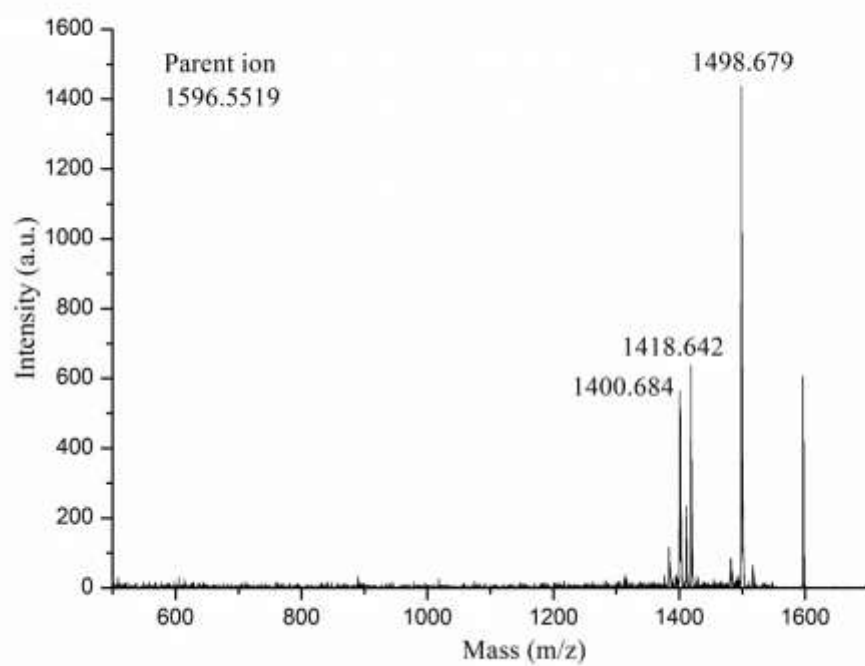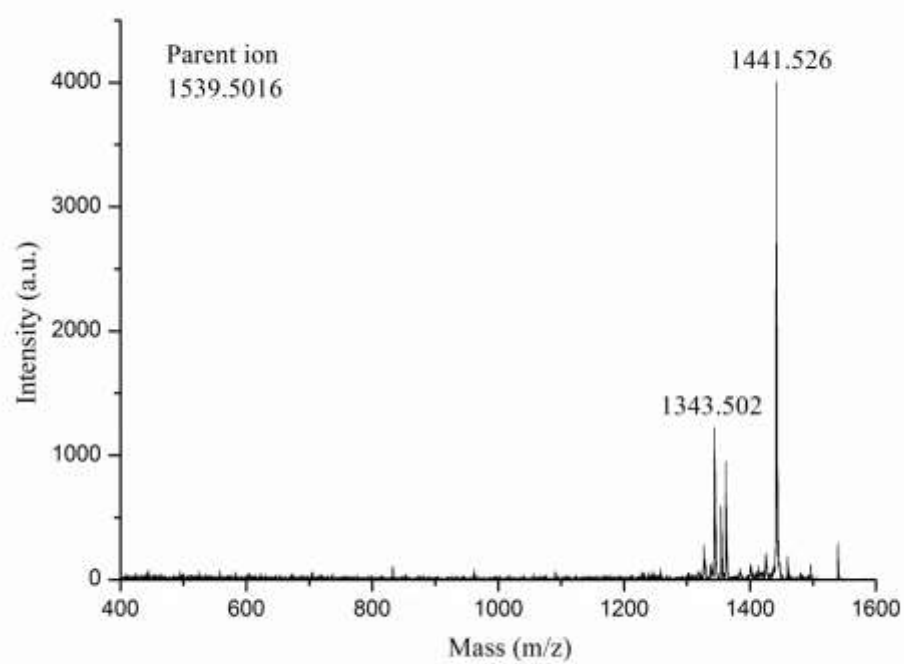
Parent ion
1001.2392

805.281

903.277

Intensity (a.u.)

Mass (m/z)

Parent ion
1104.5244

1006.637

Intensity (a.u.)

Mass (m/z)

Parent ion
1311.4426

1213.494

1115.489

Parent ion
1343.0123

1243.848

Figure S7. MALDI-TOF MS/MS spectra of phosphorylated peptides in human saliva after enrichment with DZMOF (dephosphorylated fragments about [M-98]⁺, [M-98-80]⁺ or [M-98×2]⁺).

References

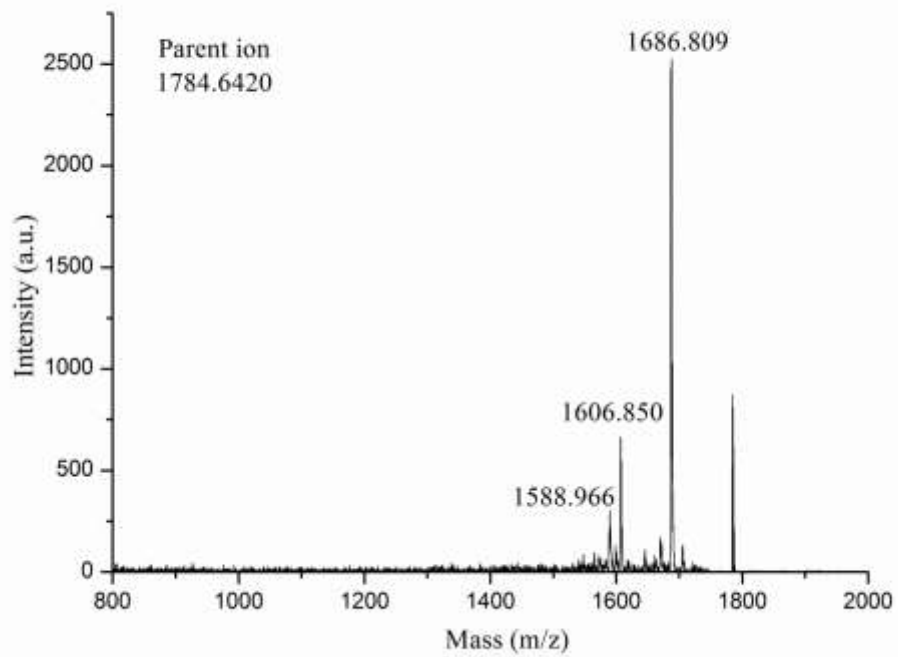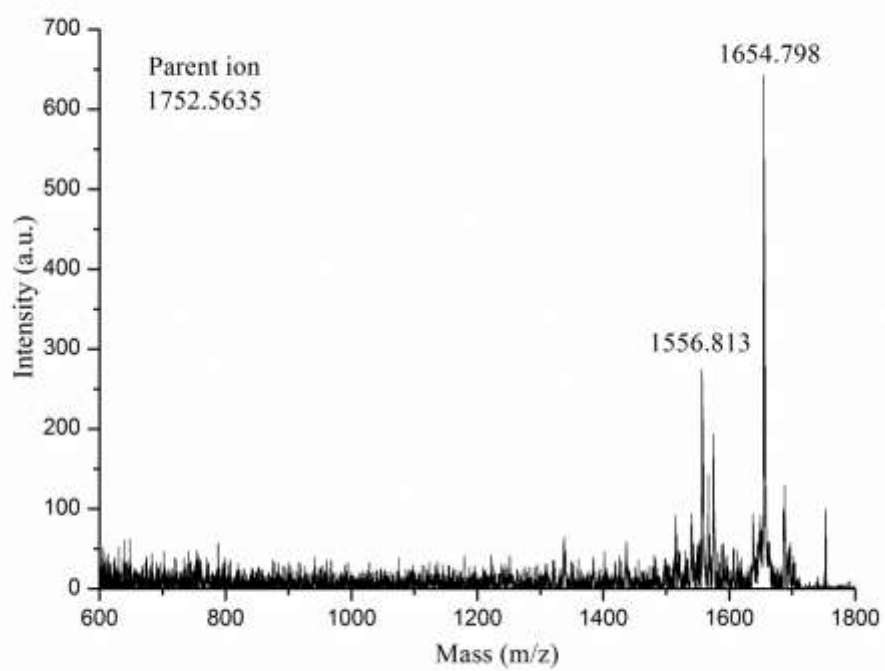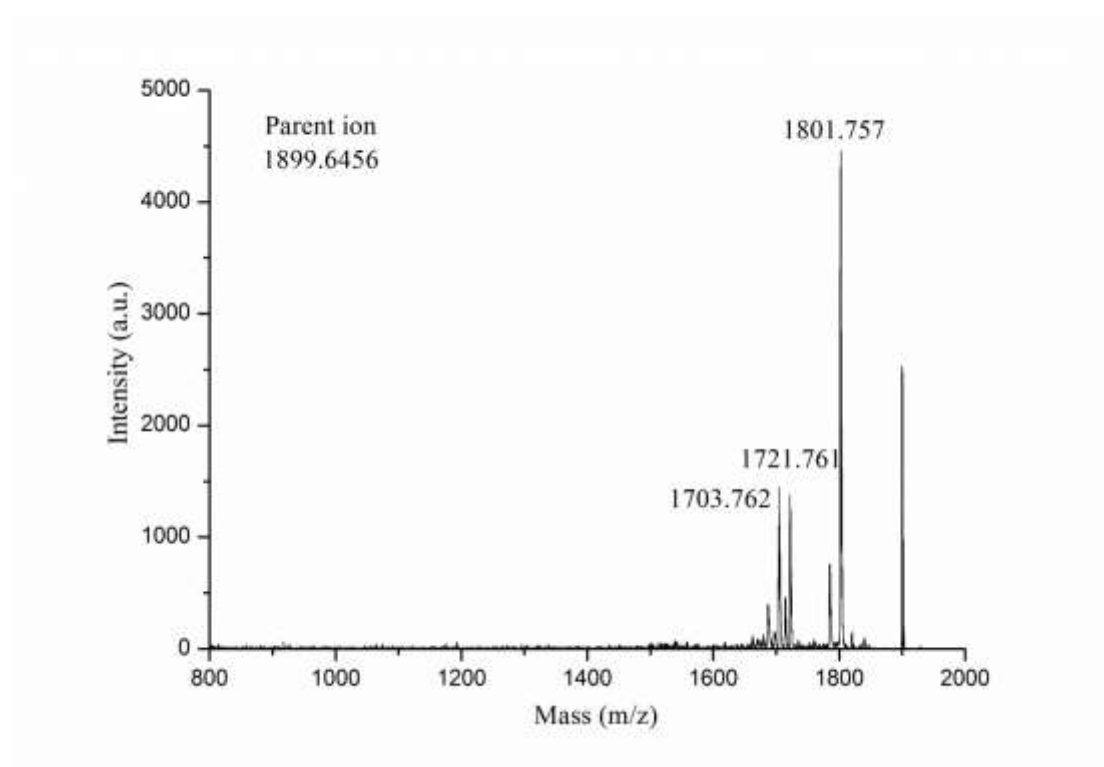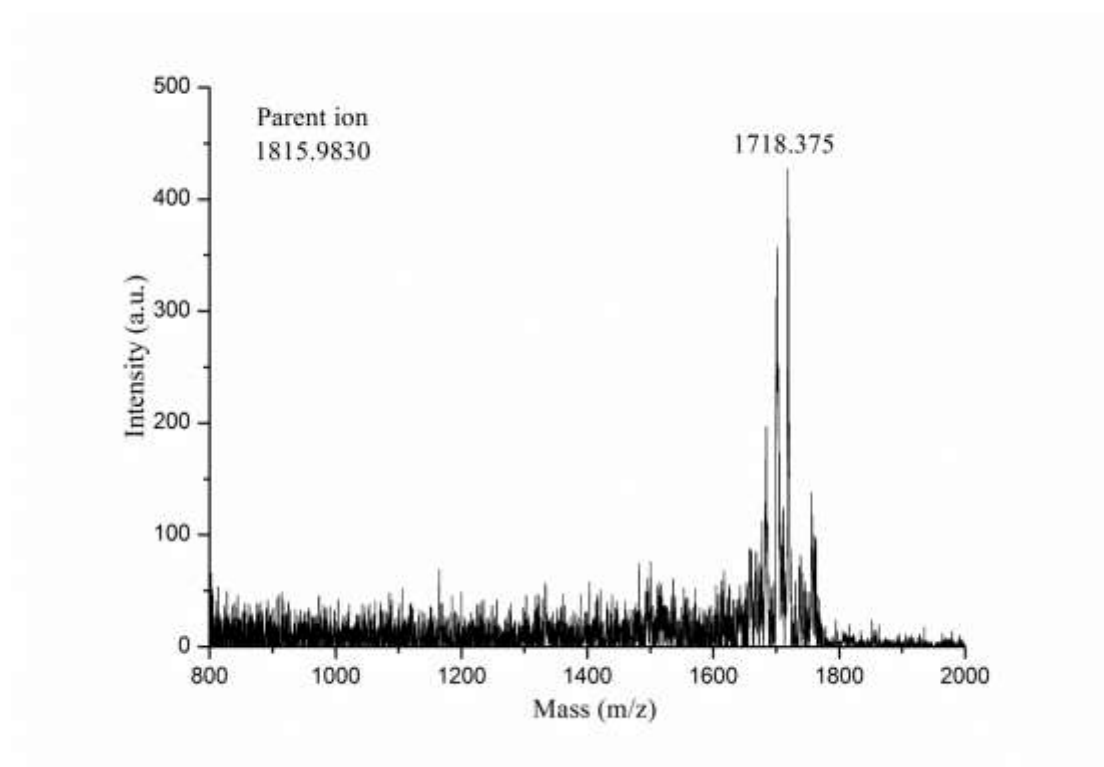(1) Zhao, M.; Deng, C.; Zhang, X. The Design and Synthesis of a Hydrophilic Core-Shell-Shell Structured Magnetic Metal-Organic Framework as a Novel

Immobilized Metal Ion Affinity Platform for Phosphoproteome Research. *Chem. Commun.* **2014,** *50*, 6228-6231.

(2) Zhao, M.; Zhang, X.; Deng, C. Facile Synthesis of Hydrophilic Magnetic Graphene@Metal–Organic Framework for Highly Selective Enrichment of Phosphopeptides. *RSC Adv.* **2015,** *5*, 35361-35364.

(3) Chen, Y.; Xiong, Z.; Peng, L.; Gan, Y.; Zhao, Y.; Shen, J.; Qian, J.; Zhang, L.; Zhang, W. Facile Preparation of Core-Shell Magnetic Metal-Organic Framework Nanoparticles for the Selective Capture of Phosphopeptides. *ACS Appl. Mater. Interfaces* **2015,** *7*, 16338-16347.

(4) Zhu, X.; Gu, J.; Yang, J.; Wang, Z.; Li, Y.; Zhao, L.; Zhao, W.; Shi, J. Zr-Based Metal–Organic Frameworks for Specific and Size-Selective Enrichment of Phosphopeptides with Simultaneous Exclusion of Proteins. *J. Mater. Chem. B* **2015,** *3*, 4242-4248.

(5) Yang, X.; Xia, Y. Urea-Modified Metal-Organic Framework of Type MIL-101(Cr) for the Preconcentration of Phosphorylated Peptides. *Microchim. Acta* **2016,** *183*, 2235-2240.

(6) Xie, Y.; Deng, C. Highly Efficient Enrichment of Phosphopeptides by a Magnetic Lanthanide Metal-Organic Framework. *Talanta* **2016,** *159*, 1-6.

(7) Chen, L.; Ou, J.; Wang, H.; Liu, Z.; Ye, M.; Zou, H. Tailor-Made Stable Zr(IV)-Based Metal-Organic Frameworks for Laser Desorption/Ionization Mass Spectrometry Analysis of Small Molecules and Simultaneous Enrichment of Phosphopeptides. *ACS Appl. Mater. Interfaces* **2016,** *8*, 20292-20300.