Supporting Information

Enrichment of Phosphorylated Peptides with Metal-Organic Framework Nanosheets for Serum Profiling of Diabetes and Phosphoproteomics Analysis

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The synthesized procedure of Ti-based MOF nanosheets, Zr-BTB nanosheets, MIL-125, NH_2-MIL-125.

Ti-based MOF nanosheets.[S1, S2] H_4DOBDC (0.95 g, 5 mmol) and DEF (3 mL) were mixed in a Teflon lined Paar autoclave of 25 mL. The mixture was stirred at room temperature and 0.35 mL of titanium isopropoxide (1.16 mmol) were added. The reactor was sealed and heated at 200 ºC for 20 h (heating rate: 170 ºC h^{-1}). Then the mixture was naturally cooled down to room temperature. Finally, the resulting dark solid was repeatedly washed with DEF and dried at room temperature. The Ti-based MOF nanosheets were obtained via a top-down liquid ultrasonication exfoliation method. The dried powders of Ti-based MOF (10 mg) were added to isopropanol (10 mL) by ultrasonicating for about 2 days at room temperature. Then the suspension was centrifugated at 3000 rpm for 5 min to get the upper-layer colloidal suspension, after that it was centrifugated at 12000 rpm for 20 min to get the Ti-based MOF nanosheets.

Zr-BTB nanosheets.[S3] $ZrCl_4$ (10.12 mg, 0.045 mmol) and H_3BTB (12.5 mg, 0.028 mmol) were dissolved in a mixture of HCOOH (1,113 mg), DMF (5 mL) and deionized (DI) water (60 μL) in a pyrex vial of 25 mL. Then the pyrex vial was kept at 120 ºC for 48 h. After cooling to room temperature, white precipitates were obtained and washed three times using DMF and twice using methanol respectively. Finally, the solid white samples were calcined overnight in a vacuum oven.

MIL-125.[S4] In a typical experiment, a solution of titanium isopropoxide (0.6 mL, 2.0 mmol) and H_2BDC (498 mg, 3.0 mmol) were added to a mixture of DMF and methanol (V_{DMF}:$V_{methanol}$ = 9:1). The above mixture was stirred for 20 min at room temperature and was transferred to a 25 mL Teflon liner and heated at 150 ºC for 20 h. After cooling down to room temperature, the resultant suspension was washed with DMF and methanol respectively. Finally, the solid white product was activated under vacuum at 150 ºC for 12 h.

NH_2-MIL-125.[S5] The highly porous NH_2-MIL-125(Ti) nanoparticles was synthesized by a solvothermal route in a methanol-DMF mixed with solvent with NH_2-BDC and titanium isopropoxide as the organic linker and the metal source. In brief, NH_2-BDC (272 mg, 1.5 mmol) was dissolved in methanol and DMF mixed solvent ($V_{methanol}$: V_{DMF}

= 1:9). Then, titanium isopropoxide (0.13 mL, 0.4 mmol) was added to the NH_2-BDC solution. After stirring for 20 minutes, the mixture was transferred to a 20 mL Teflon-lined autoclave and placed in an oven at 150 ^oC for 72 h. After cooling to room temperature, a yellow powder product was obtained and washed three times using DMF and twice using methanol. Finally, the solid yellow samples were calcined overnight in a vacuum oven at 150 ^oC for 12 h.

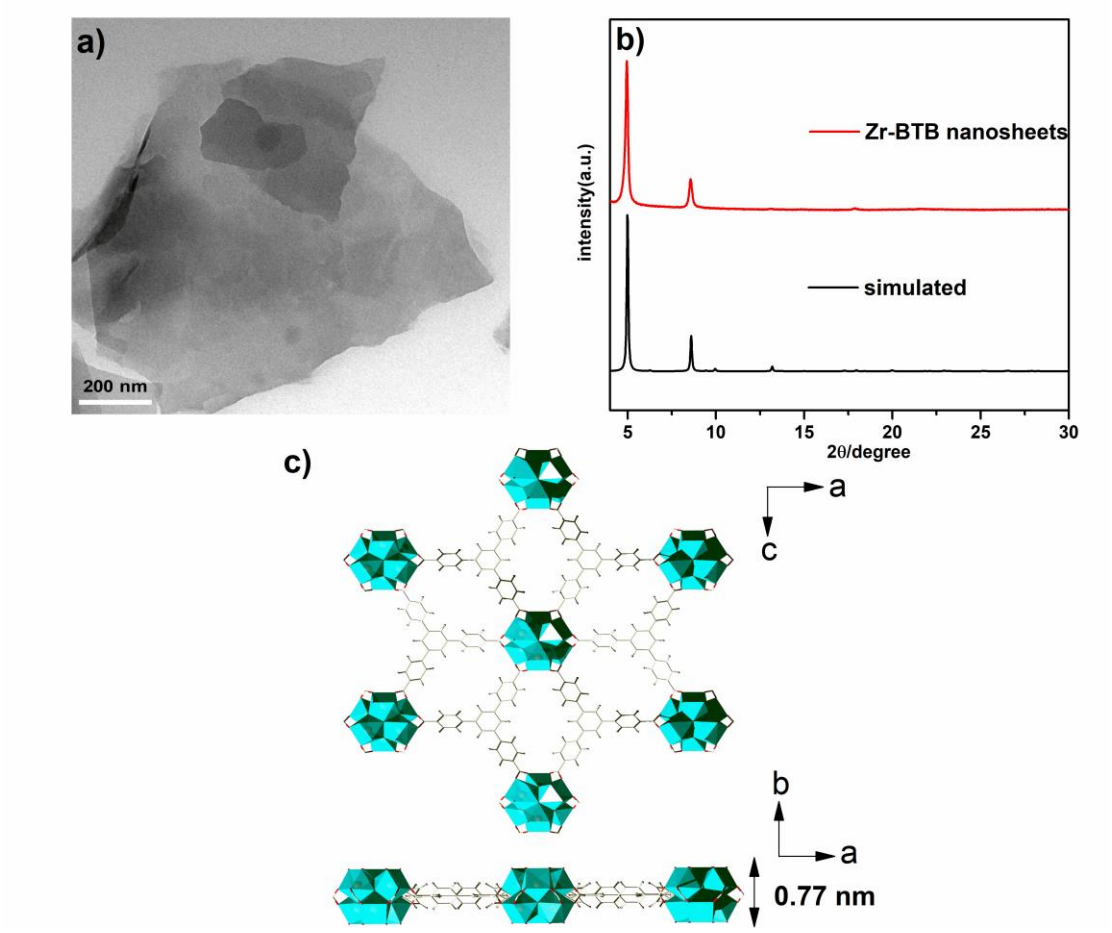


Figure S1. Characterization of Zr-BTB nanosheets. (a) TEM image of Zr-BTB nanosheets. (b) Powder XRD data for simulated Zr-BTB nanosheets (Powder Pattern Settings: Include preferred orientation, *h*=0, *k*=1 and *l*=0, respectively. March-Dollase parameter: 4.5), and Zr-BTB nanosheets. (c) The crystal structure of a single layer of Zr-BTB nanosheet. The Zr, C, and O atoms are shown in orange, grey, and red, respectively.

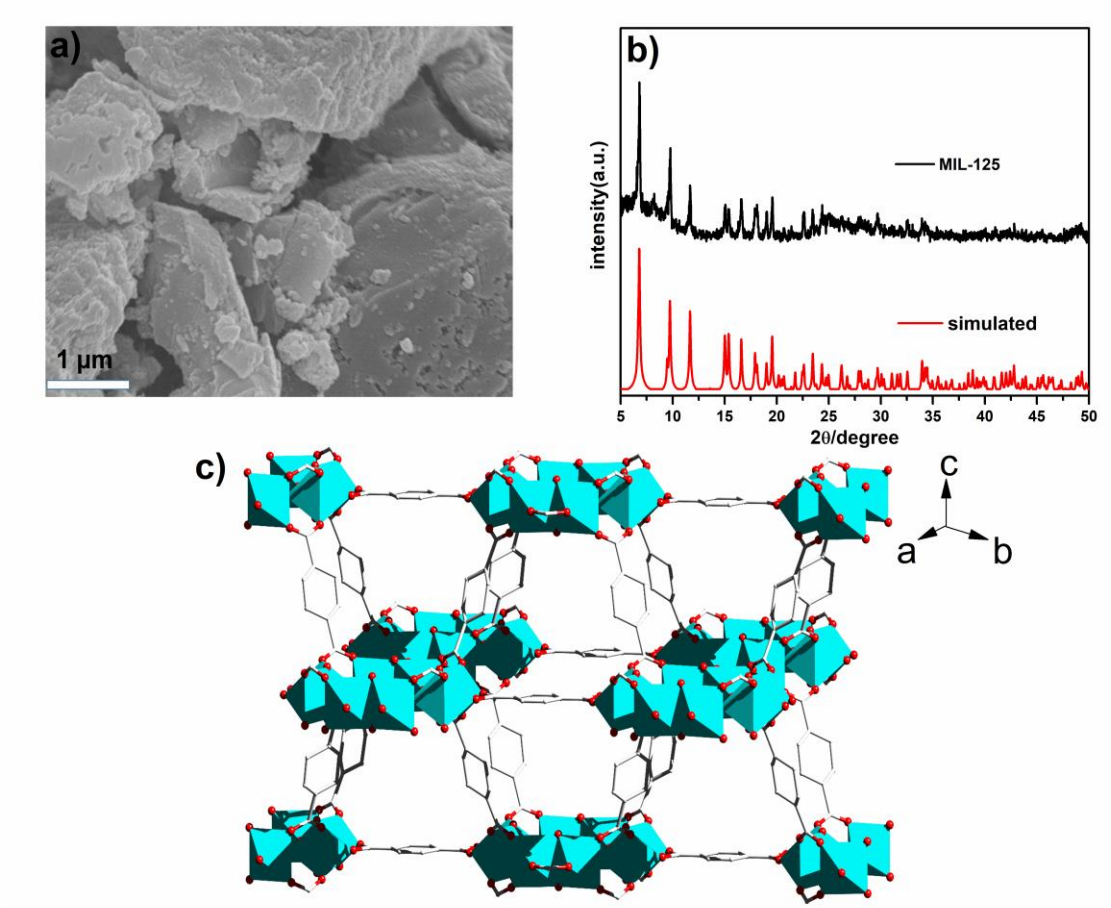


Figure S2. Characterization of MIL-125. (a) SEM image of MIL-125. (b) Powder XRD data for simulated MIL-125, and MIL-125. (c) The crystal structure of MIL-125. The Ti, C, and O atoms are shown in blue, grey, and red, respectively.

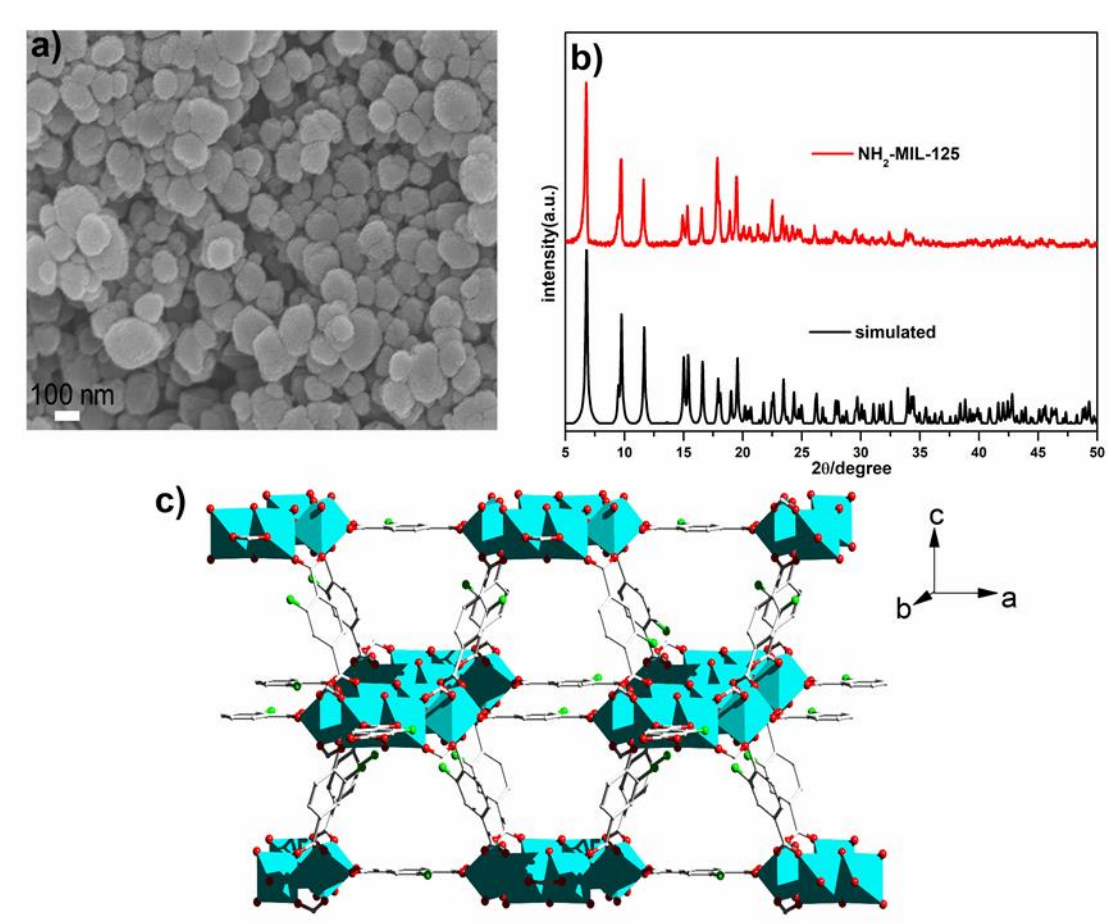


Figure S3. Characterization of NH_2-MIL-125. (a) SEM image of NH_2-MIL-125. (b) Powder XRD data for simulated NH_2-MIL-125 and NH_2-MIL-125. (c) The crystal structure of NH_2-MIL-125. The Ti, C, O, and N atoms are shown in blue, grey, red, and green, respectively.

Table S1. Detailed information of detected phosphopeptides enriched from the tryptic digest of α-casein, β-casein and non-fat milk.

Peak No.	m/z	No. of phosphorylation	Peptide sequences
α1	1237.2	1	TVDMEpSTEVF
α2	1661.3	1	VPQLEIVPNpSAEER
α3	1927.2	2	DIGpSEpSTEDQAMEDIK
α4	1943.5	2	DIGpSEpSTEDQAoMEDIK
α5	1952.8	1	YKVPQLEIVPNpSAEER
α6	2618.2	4	NTMEHVpSpSpSEESIIpSQETYK
α7	2635.2	4	NToMEHVpSpSpSEESIIpSQETYK
α8	2677.8	3	VNELpSKDIGpSEpSTEDQAMEDIK
α9	2703.5	5	Q*MEAEpSIpSpSpSEEIVPNpSVEQK
α10	2720.4	5	QMEAEpSIpSpSpSEEIVPNpSVEQK
α11	2735.9	5	QoMEAEpSIpSpSpSEEIVPNpSVEQK
α12	2935.6	3	EKVNELpSKDIGpSEpSTEDQAMEDIK
α13	3007.3	4	NANEEEYpSIGpSpSpSEESAEVATEEVK
α14	3087.9	5	NANEEEYpSIGpSpSpSEEpSAEVATEEVK
β1	2061.6	1	FQpSEEQQQTEDELQDK
β2	2556.4	1	FQpSEEQQQTEDELQDKIHPF
β3	2965.7	4	ELEELNVPGEIVEpSLpSpSpSEESITR
β4	3122.1	4	RELEELNVPGEIVEpSLpSpSpSEESITR

Q*, pyroglutamylation on the N-terminal Gln.

Ao, To, Qo, oxidation on methionine.

Table S2. Detailed information of the enrichment efficiency of Ti-based MOF nanosheets from tryptic digests of β-casein that compared to Zr-BTB nanosheets, other two Ti-MOFs (MIL-125 and NH_2-MIL-125) and TiO_2 nanoparticles.

	Ti-based MOF nanosheets	Zr-BTB nanosheets	MIL-125	NH_2-MIL-125	TiO_2 nanoparticles
β1	√	√	√	√	√
β2	√		√	√	√
β3	√		√	√	√
β4	√		√	√	√
$β4^{2+}$	√				
nonphosphopeptides		√	√	√	√
number of detected dephosphorylated peptides	5	nd	3	3	4
NL	4.0E+4	4.0E+3	8.0E+3	1.4E+4	1.4E+4

$β4^{2+}$: The tetra-phosphorylated peptide with two units of positive charge.

NL : normalized level.

nd : not detected.

Table S3. The comparison of 2-D Ti-based MOF nanosheets with other MOFs materials in terms of the selectivity and the detection limits.

	2-D Ti-based MOF nanosheets	Zr-BTB nanosheets	MIL-125	NH_2-MIL-125
Selectivity	100%	10%	70%	70%
Detection limits (S/N)	0.5 fmol μL^{-1}	2000 fmol μL^{-1}	400 fmol μL^{-1}	20 fmol μL^{-1}

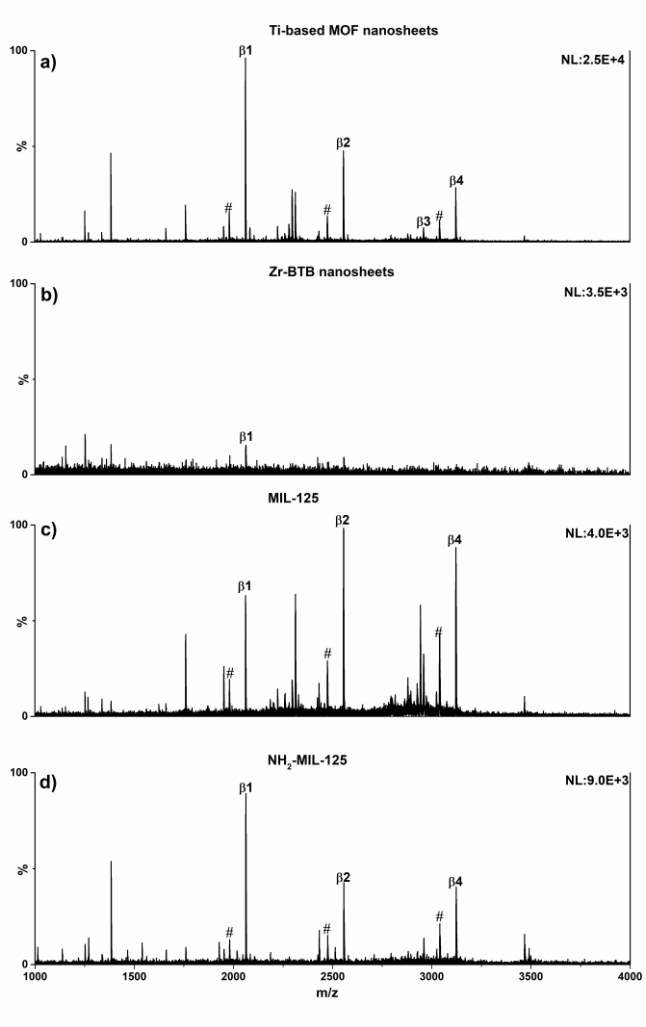


Figure S4. Mass spectrum of phosphopeptides from tryptic digest of β-casein (4× 10^{-6} M) enrichment with different materials. (a) Ti-based MOF nanosheets, (b) Zr-BTB nanosheets, (c) MIL-125 and (d) NH_2-MIL-125 (#: dephosphorylated residue of corresponding peptides (m/z: $[M+H]^+$-80); all unmarked peaks are from non-phosphopeptides; β1: No. 1 phosphopeptide from β-casein; NL: Normalized Level). The detected phosphopeptides with m/z value and amino acid sequences are listed in Table S1.

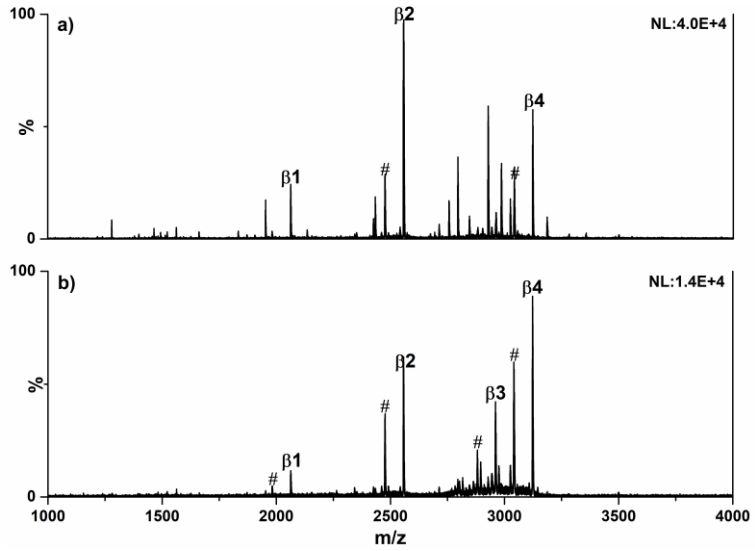


Figure S5. Mass spectrum of phosphopeptides from tryptic digest of β-casein (4× 10^{-6} M) enrichment with TiO_2 nanoparticles by different treated process. (a) unpretreated TiO_2 nanoparticles. (b) pretreated by β-glycerophosphate disodium TiO_2 nanoparticles (#: dephosphorylated residue of peptides (m/z: $[M+H]^+$-80); all unmarked peaks are from non-phosphopeptides; β1: No. 1 phosphopeptide from β-casein; NL: Normalized Level). The detected phosphopeptides with m/z value and amino acid sequences are listed in Table S1.

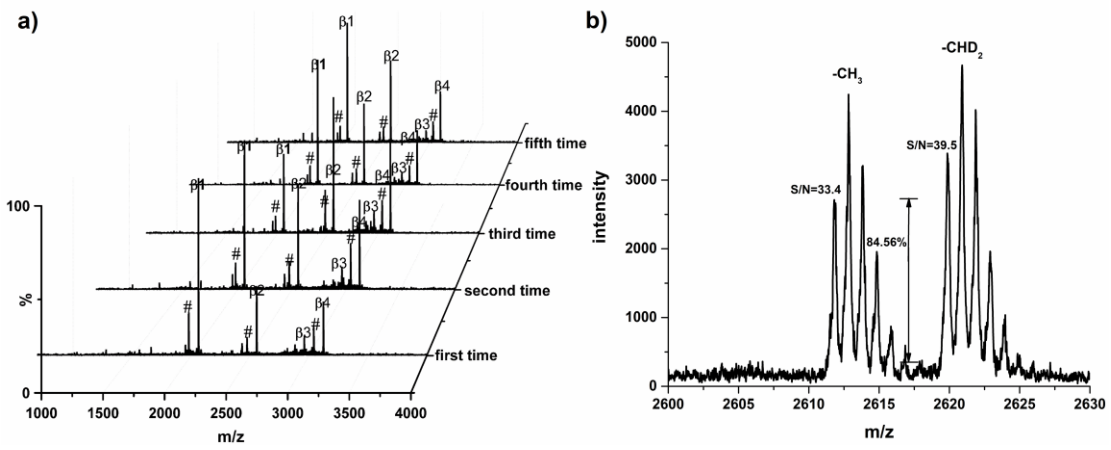


Figure S6. Mass spectrum of phosphopeptides from tryptic digest of β-casein (4×10^{-6} M) enriched with Ti-based MOF nanosheets to show (a) the reproducibility and (b) the recovery. (#: dephosphorylated residue of peptides (m/z: $[M+H]^+$-80); all unmarked peaks are from non-phosphopeptides; β1: No. 1 phosphopeptide from β-casein; NL: Normalized Level). Recovery was calculated from the measurements of 1:1 mixture of the D-labelled and H-labelled β2 peaks.[S6]

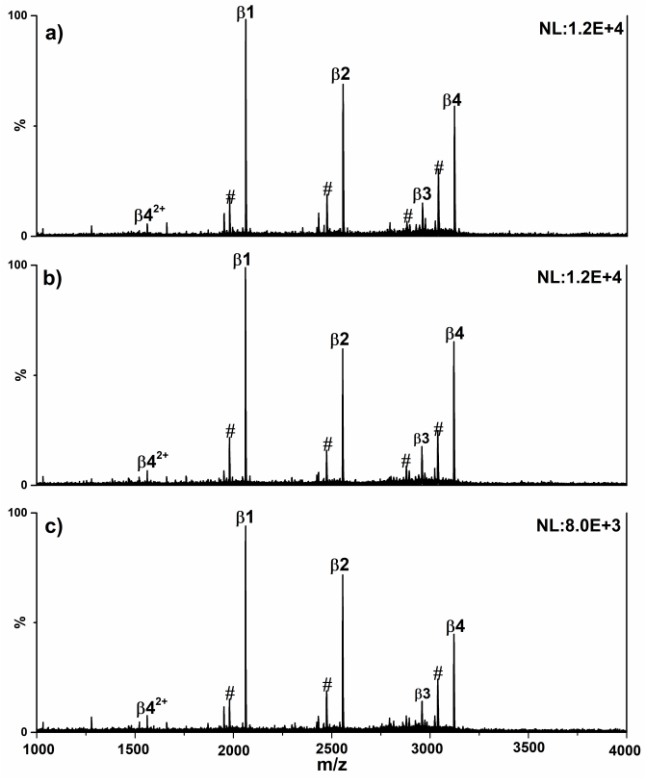


Figure S7. **Mass spectra of phosphopeptides from tryptic digest of β-casein (4×10-6 M) enriched with Ti-based MOF nanosheets by different pretreated solutions** (a) 10 mM β-glycerophosphate disodium. (b) 10 mM NaCl and (c) 10 mM KCl (#: dephosphorylated residue of corresponding peptides (m/z: $[M+H]^+$-80); all unmarked peaks are from non-phosphopeptides; β1: No. 1 phosphopeptide from β-casein; NL: Normalized Level). The detected phosphopeptides with m/z value and amino acid sequences are listed in Table S1.

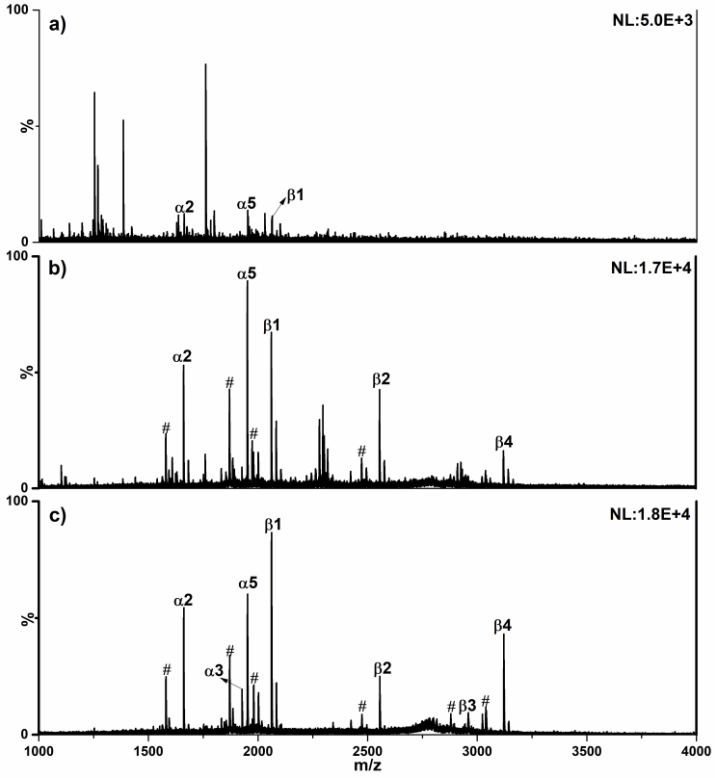


Figure S8. Direct Analysis of tryptic digest of nonfat milk and enrichment with Ti-based MOF nanosheets by different treated process. (a) direct analysis and enrichment with (b) unpretreated Ti-based MOF nanosheets and (c) pretreated by β-glycerophosphate disodium Ti-based MOF nanosheets (#: dephosphorylated residue of peptides (m/z: $[M+H]^+$-80); all unmarked peaks are from non-phosphopeptides; β1: No. 1 phosphopeptide from β-casein; NL: Normalized Level). The detected phosphopeptides with m/z value and amino acid sequences are listed in Table S1.

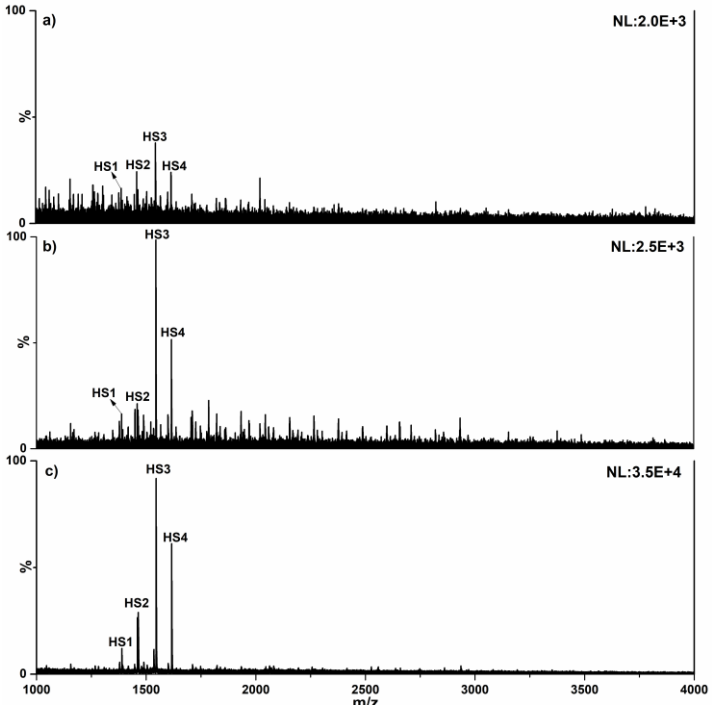


Figure S9. Direct Analysis of human serum and enrichment with Ti-based MOF nanosheets by different treated process. (a) direct analysis and enrichment with (b) unpretreated Ti-based MOF nanosheets and (c) pretreated by β-glycerophosphate disodium Ti-based MOF nanosheets (#: dephosphorylated residue of peptides (m/z: $[M+H]^+$-80); all unmarked peaks are from non-phosphopeptides; β1: No. 1 phosphopeptide from β-casein; NL: Normalized Level). The detected phosphopeptides with m/z value and amino acid sequences are listed in Table 1.

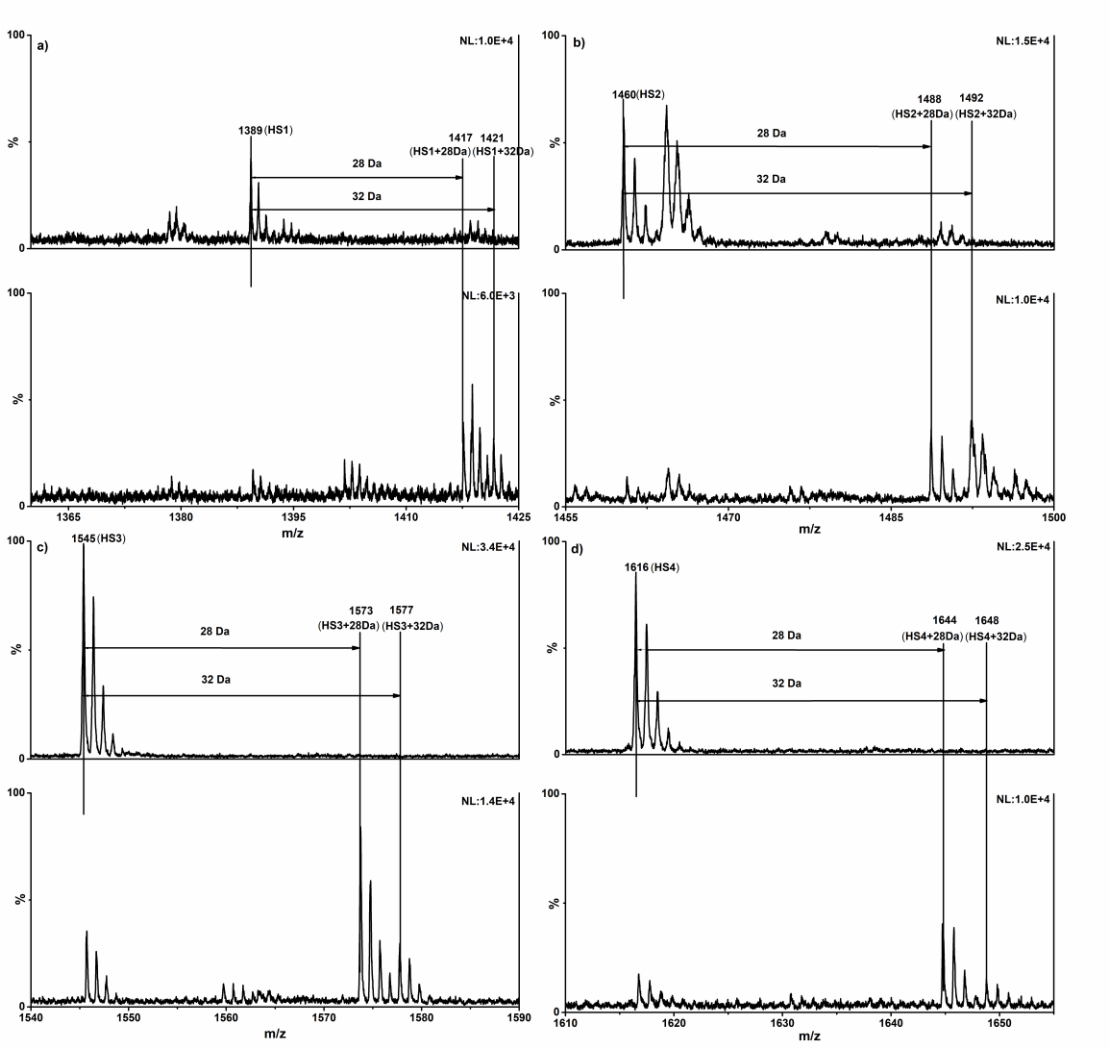
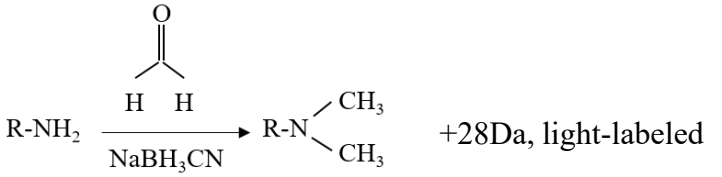
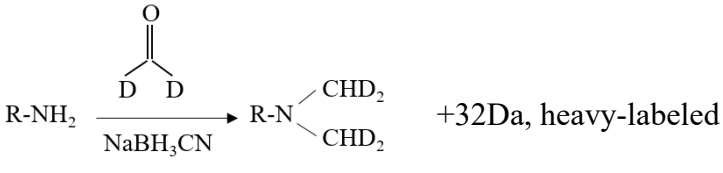


Figure S10. Representative MALDI-TOF MS spectra of FPA from serum phosphopeptides labeled *in situ* by CH_2O and CD_2O, respectively and the labeling reactions are as follows. (a) HS1. (b) HS2. (c) HS3. and (d) HS4.



+28Da, light-labeled



+32Da, heavy-labeled

Table S4. Peak area information of endogenous phosphopeptides captured from human serum using 2-D Ti-based MOF nanosheets and labeled by CH_2O (HV1, healthy volunteers) and CD_2O (DP1, diabetes patients) according the *in situ* labeling strategy at different pipette ratio, every peak area was calculated from the isotope peak that labeled by CH_2O and CD_2O, respectively.

pipette ratio / NO.	1:4		1:1		4:1	
	peak area	peak area	peak area	peak area	peak area	peak area
HS1	630	2161	3320	8554	1926	3578
HS2	2902	1013	6225	2947	2203	979
HS3	468	4531	1771	10427	1120	2799
HS4	160	1776	227	2076	118	957

Table S5. Peak area information of endogenous phosphopeptides captured from human serum using 2-D Ti-based MOF nanosheets and labeled by CH_2O (HV2, healthy volunteers) and CD_2O (DP2, diabetes patients) according the *in situ* labeling strategy at different pipette ratio, every peak area was calculated from the isotope peak that labeled by CH_2O and CD_2O, respectively.

pipette ratio / NO.	1:4		1:1		4:1	
	peak area	peak area	peak area	peak area	peak area	peak area
HS1	939	2588	1566	2431	3240	3220
HS2	3859	709	4077	528	4555	482
HS3	1366	6134	2737	5224	5212	3660
HS4	497	1838	589	1589	549	1108

Table S6. Peak area information of endogenous phosphopeptides captured from human serum using 2-D Ti-based MOF nanosheets and labeled by CH_2O (HV3, healthy volunteers) and CD_2O (DP3, diabetes patients) according the *in situ* labeling strategy at different pipette ratio, every peak area was calculated from the isotope peak that labeled by CH_2O and CD_2O, respectively.

pipette ratio / NO.	1:4		1:1		4:1	
	peak area	peak area	peak area	peak area	peak area	peak area
HS1	484	4347	1142	3266	389	1870
HS2	3440	567	2268	583	2620	104
HS3	1544	4266	4152	4815	1828	5430
HS4	856	2320	946	1224	1009	662

Table S7. Detailed information of identified phosphopeptides from mouse brain neocortex lysate by 2-D Ti-based MOF nanosheets. (A separate Excel file)

Table S8. Unique phosphorylation sites information identified from mouse brain neocortex lysate by 2-D Ti-based MOF nanosheets. (A separate Excel file)

Table S9. Detailed information of identified phosphopeptides from mouse spinal cord lysate by 2-D Ti-based MOF nanosheets. (A separate Excel file)

Table S10. Unique phosphorylation sites information identified from mouse spinal cord lysate by 2-D Ti-based MOF nanosheets. (A separate Excel file)

Table S11. Detailed information of identified phosphopeptides from mouse testis lysate by 2-D Ti-based MOF nanosheets. (A separate Excel file)

Table S12. Unique phosphorylation sites information identified from mouse testis lysate by 2-D Ti-based MOF nanosheets. (A separate Excel file)

References

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