Supporting Information

Two-dimensional Magnetic Bimetallic Organic Framework Nanosheets for Highly Efficient Enrichment of Phosphopeptides

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MALDI-TOF MS Analysis

The sample (1 μL) and 1 μL of matrix (DHB 25 $mg \cdot mL^{-1}$, ACN/H_2O/H_3PO_4 , 70:29:1, v/v/v) were blended, deposited and air-dried on a plate. All MALDI-TOF MS data were performed by autoflex maX (Bruker Scientific, USA in reflector positive mode with a Nd : YAG laser) at 383 nm. The MALDI-TOF MS analysis was obtained in positive ion mode with a mass range of 1000-3500 m/z on the repetition rate of 1000 Hz, and an acceleration voltage of 20 kV.

LC-MS/MS Analysis and Data Search

Phosphopeptides of breast cancer cells lysate were analyzed on an EASY-nLC 1200 system (Thermo Scientific, CA) coupled with Obitrap Fusion Lumos MS (Thermo Finnigan, San Jose, CA) with nano-electrospray ion source. Lyophilized samples were dissolved in 20 μL TFA (1%) and loaded on thermo scientific analytical column (100 μm x 2 cm, 5 μm, 100 Å, C18) and eluted and separated with analytical columns (75 μm x 25 cm, 5 μm, 100 Å, C18, Thermo Co.) by mobile phase A (0.1% FA in H_2O) and mobile phase B (0.1% FA in 100% ACN) for 60 min. The linear gradient elution was carried out as follows: 5-28% mobile phase B for 0-40 min; 28-90% mobile phase B for 40-42 min; 90% mobile phase B for 42-60 min. The flow rate was 300 $nL \cdot min^{-1}$. The MS was operated in positive mode. The full MS scan spectra (m/z 375-1800) were performed in Orbitrap with a resolution of 120000 (m/z 200). MS2 spectra were acquired with a resolution of 50,000 (m/z 200).

Raw data were analyzed with Proteome Discoverer 2.4. The database was Unipro_ Human_20210621_202249. fasta. Search parameters were as follows: Monoisotopic mass, trypsin;

Maximum missed cleavages, 2; Charge number of peptide: 2+, 3+, and 4+, Carbamidomethylation (C) was set as fixed modification; Variable modifications (M); Acethyl(protein N-term), Phospho (STY); Precursor-ion mass tolerance, 10 ppm; Fragment-ion mass tolerance, 0.05 Da.

DFT Theoretical Calculations

All of spin-polarized calculations based on density functional theory (DFT) were performed by utilizing Dmol3 package [1]. The generalized gradient approximation (GGA) in the Perdew-Burke-Ernzerhof form and Semicore Pseudopotential method (DSPP) with the double numerical basis sets plus the polarization functional (DNP) were adopted [2-3]. The double numerical basis sets plus the polarization functional (DNP) were adopted. A DFT-D correction with Grimme scheme was used to account for the dispersion interaction [4]. The SCF convergence for each electronic energy was set as 1.0×10^{-6} Ha, and the geometry optimization convergence criteria were set up as follows: 1.0×10^{-6} Ha for energy, 0.001 Ha·Å^{-1} for force, and 0.001 Å for displacement, respectively. Bond energy were examined by linear and quadratic synchronous transit methods in combination with the conjugated gradient (CG) refinement. Finally, the adsorption energies (E_{ads}) are calculated as $E_{ads}=E_{ad/sub}-E_{ad}-E_{sub}$, where $E_{ad/sub}$, E_{ad} and E_{sub} are the optimized adsorbate/substrate system, the adsorbate in the structure and the clean substrate respectively. Moreover, the bonding energy of all structures were calculated.

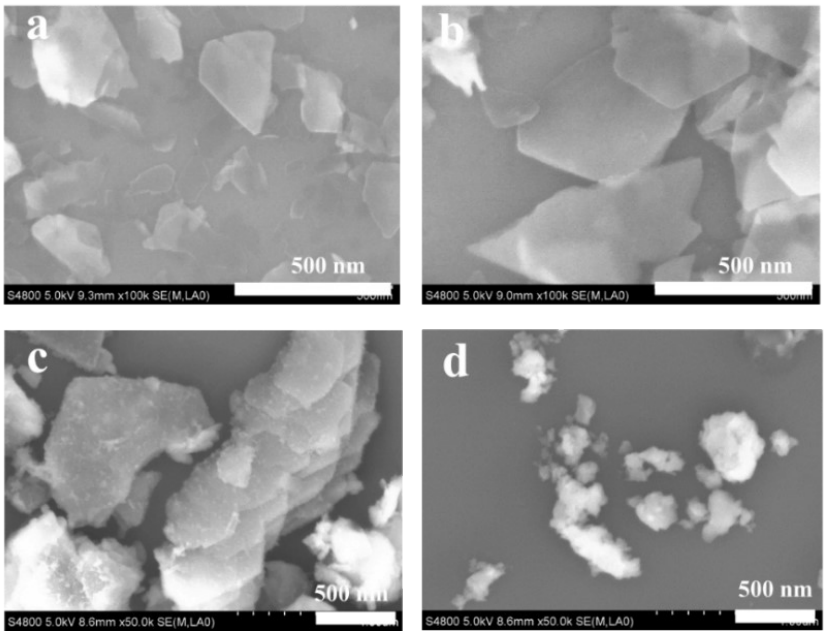

Figure S1. SEM images of the Zr-Ti BPDC nanosheets (a, $nZr^{4+}/nTi^{4+}=1/1$; b, $nZr^{4+}/nTi^{4+}=1/5$; c, $nZr^{4+}/nTi^{4+}=1/10$;

d, nZr^{4+}/nTi^{4+}=1/20)

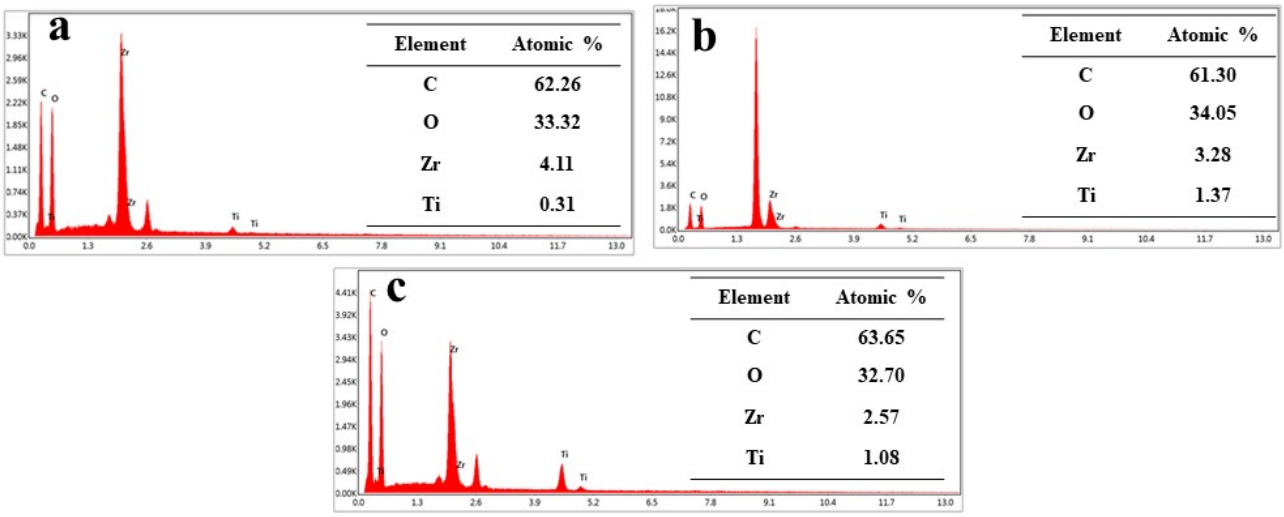

Figure S2. EDX images of the Zr-Ti BPDC nanosheets (a, nZr4+/nTi4+=1/1; b, nZr4+/nTi4+=1/5; c, nZr4+/nTi4+=1/10)

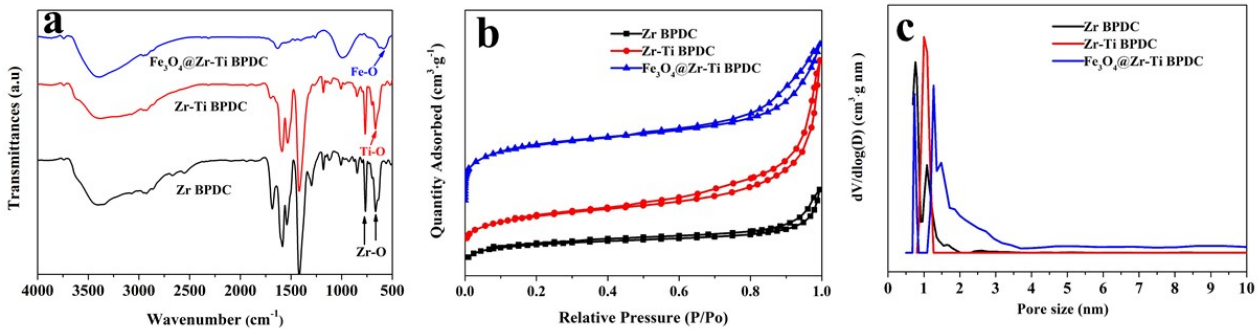

Figure S3. FTIR spectra (a) and N_2 adsorption-desorption isotherms (b) and pore size distributions (c) of Zr BPDC nanosheets, Zr-Ti BPDC nanosheets and Fe_3O_4@Zr-Ti BPDC nanosheets

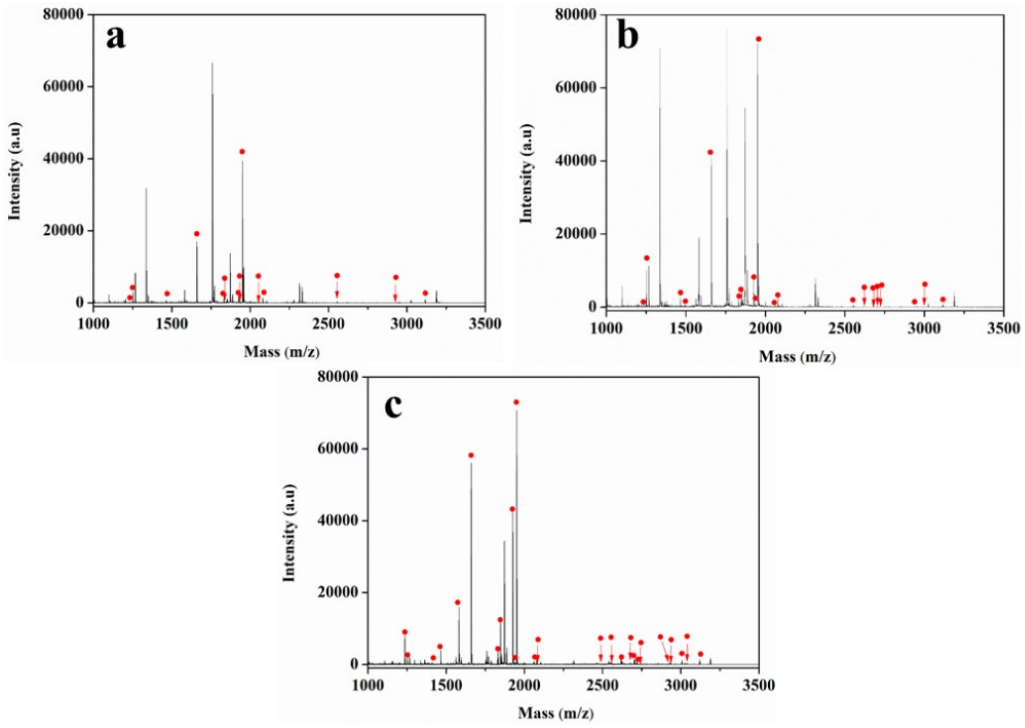

Figure S4. MALDI-TOF mass spectra of α-casein digestion (4×10^{-6} M) after enrichment by loading buffer (ACN/TFA=50%/0.1%) and elution (10% $NH_3\cdot H_2O$), (a) Fe_3O_4@Zr-Ti BPDC nanosheets (nZr^{4+}/nTi^{4+}=1/1) (b) (nZr^{4+}/nTi^{4+}=1/5), (c) (nZr^{4+}/nTi^{4+}=1/10)

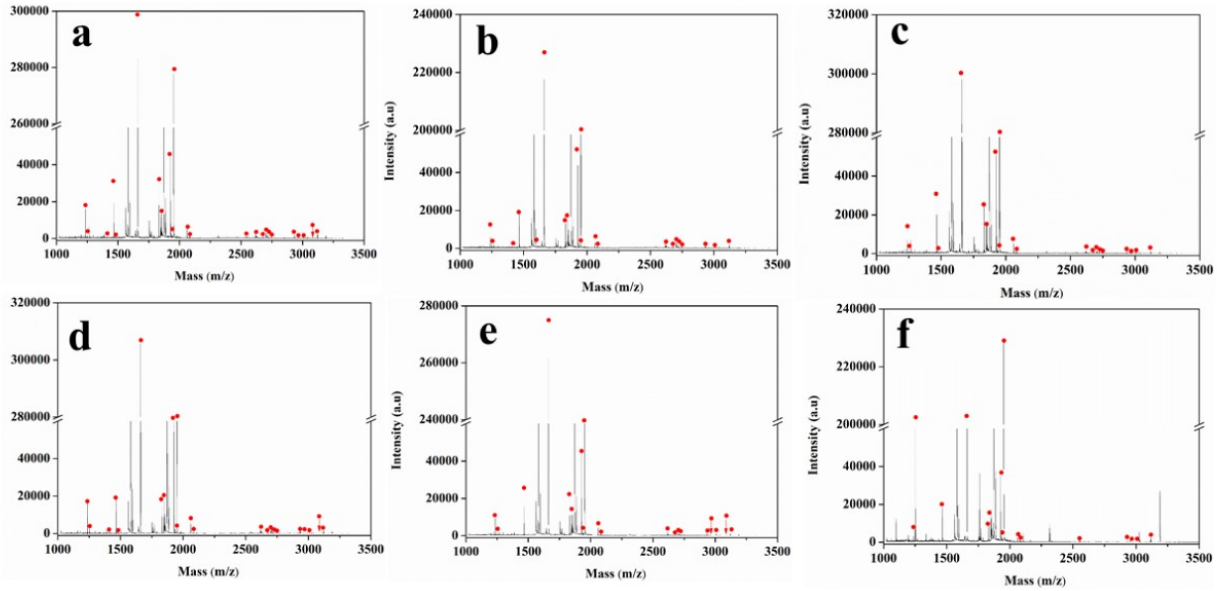

Figure S5. MALDI-TOF mass spectra of α-casein digestion (4×10^{-6} M) after enrichment by Fe_3O_4@Zr-Ti BPDC nanosheets in (a, 50% ACN/0.1% TFA and 10% $NH_3\cdot H_2O$; b, 50% ACN/0.25% TFA and 10% $NH_3\cdot H_2O$; c, 50% ACN/0.5% TFA and 10% $NH_3\cdot H_2O$; d, 75% ACN/0.1% TFA and 10% $NH_3\cdot H_2O$; e, 75% ACN/0.25% TFA and 10% $NH_3\cdot H_2O$; f, 75% ACN/0.5% TFA and 10% $NH_3\cdot H_2O$)

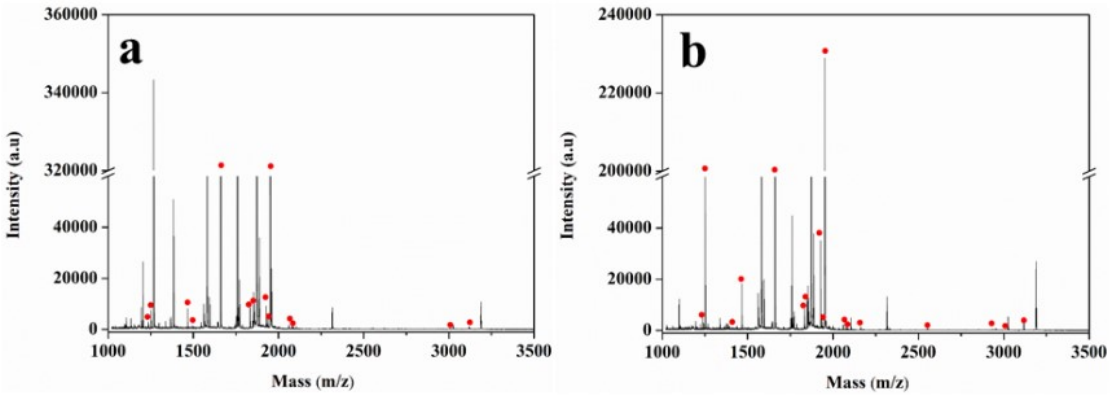

Figure S6. MALDI-TOF mass spectra of α-casein digestion (4×10^{-6} M) after enrichment by Fe_3O_4@Zr-Ti BPDC nanosheets in (a, 50% ACN/0.1%TFA and 2% $NH_3\cdot H_2O$; b, 50% ACN/0.1% TFA and 5% $NH_3\cdot H_2O$)

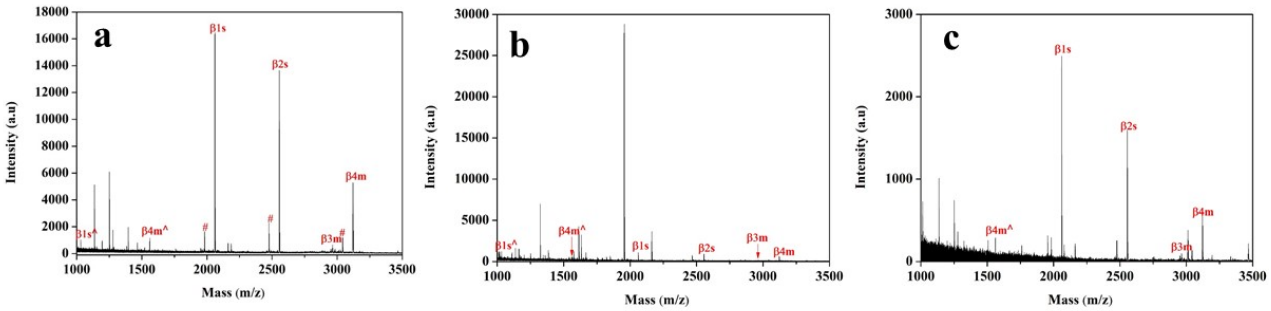

Figure S7. MALDI-TOF mass spectra of β-casein digest (4×10^{-6} M) after enrichment by Fe_3O_4@Zr-Ti BPDC nanosheets. (a, cycling 1st; b, 3rd; c, 5th) (#, dephosphopeptide)

Table S1. Detail information of the observed phosphopeptides obtained from tryptic digest of α-casein after enrichment by Fe_3O_4@Zr-Ti BPDC nanosheets in MALDI-TOF MS analysis

Observed m/z	Peptides sequence	Number of phosphorylation
1237.59	TVDME[pS]TEVF	1
1253.58	TVD[Mo]E[pS]TEVF	1
1412.60	EQL[pS]T[pS]EENSK	2
1467.31	TVDME[pS]TEVFIK	1
1662.02	VPQLEIVPN[pS]AEER	1
1833.33	YLGEYLIVPN[pS]AEER	1
1847.61	DIGSE[pS]TEDQAMEDIK	1
1927.90	DIG[pS]E[pS]TEDQAMEDIK	2
1943.62	DIG[pS]E[pS]TEDQA[Mo]EDIK	2
1952.09	YKVPQLEIVPN[pS]AEER	1

2062.09	FQ[pS]EEQQQTEDELQDK	1
2080.56	KYKVPQLEIVPN[pS]AEER	1
2490.02	AMKPWIQPKTKVIP[pY]VR[pY]L	2
2556.45	FQ[pS]EEQQQTEDELQDKIHPF	1
2618.90	NTMEHV[pS][pS][pS]EESII[pS]QETYK	4
2678.36	VNEL[pS]KDIG[pS]E[pS]TEDQAMEDIK	3
2703.10	QMEAE[pS]I[pS][pS][pS]EEIVPN[pS]VEQK	5
2720.68	Q[Mo]EAE[pS]I[pS][pS][pS]EEIVPN[pS]VEQK	5
2746.90	HIQKEDVP[pS]ER[pY]LGYLEQLLR	2
2926.70	RELEELNVPGEIVESL[pS][pS][pS]EESITR	3
2934.32	KEKVNEL[pS]KDIG[pS]E[pS]TEDQAMEDIKQ	3
2966.06	RELEELNVPGEIVE[pS]L[pS][pS][pS]EESITR	4
3007.89	NANEEEYSIG[pS][pS][pS]EE[pS]AEVATEEVK	4
3087.81	NANEEEY[pS]IG[pS][pS][pS]EE[pS]AEVATEEVK	5
3122.02	RELEELNVPGEIVE[pS]L[pS][pS][pS]EESITR	4

Table S2. Detail information of the detected phosphopeptides obtained from tryptic digest of β-casein after enrichment by Fe_3O_4@Zr-Ti BPDC nanosheets in MALDI-TOF MS analysis

Position	Observed m/z	Peptides sequence	Number of phosphorylation
β1s^	1031.66	FQ[pS]EEQQQTEDELQDK	1
β4m^	1561.88	RELEELNVPGEIVE[pS]L[pS][pS][pS]EESITR	4
β1s	2062.28	FQ[pS]EEQQQTEDELQDK	1
β2s	2556.58	FQ[pS]EEQQQTEDELQDKIHPF	1
β3m	2965.82	ELEELNVPGEIVE[pS]L[pS][pS][pS]EESITR	4
β4m	3122.93	RELEELNVPGEIVE[pS]L[pS][pS][pS]EESITR	4

"^" denotes doubly charged peak; "s" denotes mono-phosphopeptides; "m" denotes multi-phosphopeptides.

Table S3. List of phosphopeptides from nonfat milk digests after enrichment by Fe_3O_4@Zr-Ti BPDC nanosheets in MALDI-TOF MS analysis.

m/z(Da)	Number of phosphate groups	Peptide sequence
3122.19	4	RELEELNVPGEIVE[pS]L[pS][pS][pS]EESITR
3042.30	3	RELEELNVPGEIVESL[pS][pS][pS]EESITR

3024.62	3	RELEELNVPGEIVESL[pS][pS][pS]EESITR
2966.25	4	ELEELNVPGEIVE[pS]L[pS][pS][pS]EESITR
2951.17	3	KEKVNEL[pS]KDIG[pS]E[pS]TEDQA[Mo]EDIKQ
2779.12	1	KIEKFQ[pS]EEQQQTEDELQDKIHPF
2735.06	5	Q[Mo]EAE[pS]I[pS][pS][pS]EEIVPN[pS]VEAQK
2555.88	1	FQ[pS]EEQQQTEDELQDKIHPF
2456.62	4	KNTMEHV[pS][pS][pS]EESII[pS]QET
2160.46	2	[pT]V[pY]QHQKA[Mo]KPWIQPK
2106.86		FLLQEPVLGPVRGPFPIIV
2060.75	1	FQ[pS]EEQQQTEDELQDK
1981.88	1	NMAINP[pS]KENLCSTFCK
1951.92	1	YKVPQLEIVPN[pS]AEER
1943.87	2	DIG[pS]E[pS]TEDQA[Mo]EDIK
1927.07	2	DIG[pS]E[pS]TEDQAMEDIK
1847.94	1	DIGSE[pS]TEDQAMEDIK
1832.82	1	YLGEYLIVPN[pS]AEER
1760.15		HQGLPQEVLNENLLR
1660.83	1	VPQLEIVPN[pS]AEER
1561.33	4	RELEELNVPGEIVE[pS]L[pS][pS][pS]EESITR
1466.62	2	TVDME[pS]TEVFTK
1454.80	2	LSKDIG[pS]E[pS]TEDQA
1282.74	1	KKIEKFQ[pS]EEQQQTEDELQDKIHPFAQ
1252.82	1	TVD[Mo]E[pS]TEVF
1194.92	1	NMAINP[pS]KEN
1155.60	2	[pS][pS]EEKFLR
1031.32	1	FQ[pS]EEQQQTEDELQDK

Table S4. The enrichment effect of Fe_3O_4@TiO_2, Fe-MOF and Zr-MOF

Material	LODs	Selectivity	Ref
Fe_3O_4@mTiO_2-80	5 fmol·μL^{-1}	β-casein/BSA=1/500	5
Fe_3O_4@MIL-101(Fe)	8 fmol	β-casein/BSA=1/1000	6
Mag-MOF (Fe) nanofibers	0.5 fmol	β-casein/BSA=1/400	7
SPIO@SiO_2@MOF	0.1 fmol·μL^{-1}	β-casein/BSA=1/400	8

SiO_2@PDA@Zr-MOF	4 fmol	β-casein/BSA=1/1000	9
Fe_3O_4@PDA@Zr-MOF	1 fmol	β-casein/BSA=1/500	10
Fe_3O_4@Zr-Ti BPDC nanosheets	0.4 fmol·μL^{-1}	β-casein/Cyt C=1/1000	This work

Table S5. Comparison of Fe_3O_4@Zr-Ti BPDC nanosheets and previously reported materials for enrichment of phosphopeptides from biological sample

Materials	LODs	Real biological Samples	Number of phosphopeptides	Ref
$Bi_{0.15}Fe_{0.15}TiO_2$	2×10^{-9} M	human liver lysate	116	11
MAX-Ti_3AlC_2	5 fmol	BEL7402 cells lysate	830	12
mP5SOF-Arg	0.1 fmol	A594 cells lysate, mouse liver tissue lysate	450 and 1445	13
THZr-MOFs	0.05 fmol·μL^{-1}	human serum, HeLa cell lysate	1432	14
G@mSiO_2-PFIL-Ti^{4+}	0.15 fmol	mouse brain lysate	924	15
Fe_3O_4@Zr-Ti BPDC nanosheets	0.4 fmol·μL^{-1}	breast cancer cells lysate	2101	This work

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