Electronic Supplementary Material (ESI) for RSC Advances.

Supporting Information

Facile Synthesis of Hydrophilic Magnetic Graphene @Metal-organic Framework for Highly Selective Enrichment of Phosphopeptides

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Part 1 Synthesis and characterizations

1.1 Materials and chemicals. Graphene was purchased from Shanghai Boson Technology Co., Ltd. Dopamine hydrochloride and zirconium chloride ($ZrCl_4$) were purchased from Aladdin Chemistry Co., Ltd. (USA). 1,4-benzenedicarboxylic acid was purchased from Ourchem Chemical Reagent Co., Ltd. (Shanghai, China). The NdFeB magnet was purchased from PCCW (Beijing, China), 2 cm long, 2 cm wide, 1 cm high, with surface magnetic field strength of 1000 Gauss. All other chemicals and reagents are of the highest grade commercially available and used as received.

1.2 Synthesis of magG@PDA@Zr-MOFs. Firstly, 400 mg of graphene was dispersed in 50 mL of HNO_3, and the dispersion was mechanically stirred at 60 ℃ for 7 hours. The HNO_3-treated graphene obtained was washed with deionized water until the supernatant turned into neutral, and was then dried in vacuum at 50 ℃.

Magnetic graphene sheets (magG) were prepared via a solvothermal reaction, in which the ethylene glycol solution of HNO_3-treated graphene and $FeCl_3 \cdot 6H_2O$ was heated at 200 ℃ for 10 hours. In detail, 300 mg of the pretreated graphene and 405 mg of $FeCl_3 \cdot 6H_2O$ were dispersed in 40 mL of ethylene glycol under ultrasonication. Then, 0.15 g of trisodium citrate, 1.8 g of sodium acetate and 1.0 g of poly(ethylene glycol)-20000 were dissolved in the precursor under magnetic stirring for 2 hours. After that, the mixture was sealed in a Teflon-lined stainless-steel autoclave and was submitted to a solvothermal reaction. The intermediate product was collected by magnetic separation and washed with deionized water. The as-synthesized magG was dried in vacuum at 50 ℃.

Polydopamine-coated magG was synthesized through an oxidative polymerization reaction. First of all, 10 mg of magG was dispersed in 20 mL of Tris buffer (10 mM, pH=8.5), and 20 mL of ethanol was added afterwards. The aqueous solution was adequately blended under ultrasonication. After that, 80 mg of dopamine hydrochloride was added into the dispersion, and the polymerization of dopamine was performed under continuous mechanical stirring for 6-20h at room temperature. The

as-prepared magG@PDA was isolated by magnetic separation, and washed with deionized water and ethanol several times. Eventually, the magG@PDA composites were dried in vacuum at 50 ℃.

The magG@PDA@Zr-MOFs composites were fabricated according to a previous report with minor modifications[1]. A solid mixture of 0.156 g zirconium chloride, 0.1g 1,4-benzenedicarboxylic acid and 0.054 g ammonium formate was dissolved in N,N-dimethylformamide (DMF) by ultrasonication treatment. The magG@PDA nanosheets were placed in a Teflon-lined stainless steel autoclave which was filled with 75 mL of the synthesis solution, and heated at 85 °C in an air oven for 24 h. After the solvothermal reaction and cooling to room temperature, the resulting magG@PDA@Zr-MOFs were washed with ethanol several times, and then dried in vacuum at 50 ℃.

1.3 Characterizations of magG@PDA@Zr-MOFs. Transmission electron microscope (TEM) images and energy dispersive X-ray (EDX) spectra were recorded on a JEOL 2011 microscope (Japan) operated at 200 kV. Samples were dispersed in ethanol beforehand and were then collected by carbon-film-covered copper grids for analysis. Scanning electron microscope (SEM) images were acquired with a Philips XL30 electron microscope (Netherlands) operating at 20 kV. A thin gold film was sputtered on samples before measurement. Powder X-ray diffraction (XRD) patterns were taken on a Bruker D4 X-ray diffractometer with Ni-filtered Cu K_{α} radiation (40 kV, 40 mA). Nitrogen adsorption-desorption isotherms were measured at 77 K with a Micrometrics Tristar 3000 analyzer (USA). The samples were degassed in vacuum at 200 ℃ for 8 h prior to measurement. The Brunauer-Emmett-Teller (BET) method was utilized to calculate the specific surface area using adsorption data in a relative pressure range from 0.01 to 0.38. Fourier transform infrared (FT-IR) spectra were collected on Nicolet Fourier spectrophotometer (USA) using KBr pellets. Raman spectra were measured on a LabRam-1B Raman spectrometer with a laser at an excitation wavelength of 632.8 nm at room temperature. Zeta potential measurements were performed on a Nano ZS90 zeta analyzer (Malvern Instruments Ltd.).

Part 2 Enrichment experiments

2.1 Materials and chemicals. L-1-tosylamido-2-phenylethylchloromethyl ketone (TPCK) treated trypsin (from bovine pancreas) and 2,5-dihydroxybenzoic acid (DHB) were purchased from Sigma Chemical (St. Louis, MO, USA). Bovine β-casein and bovine serum albumin (BSA) were obtained from Bio Basic (Toronto, Canada). The human serum sample originated from a hepatocellular carcinoma patient was offered by Shanghai Zhongshan Hospital. Acetonitrile (ACN) and trifluoroacetic acid (TFA) were purchased from Merck (Darmstadt, Germany). Distilled water was purified by a Milli-Q system (Millipore, Bedford, MA, USA). All other chemicals and reagents are of the highest grade commercially available and used as received.

2.2 Preparation of standard protein tryptic digests. Bovine β-casein and bovine serum albumin (BSA) were dissolved in 25 mM ammonium bicarbonate (NH_4HCO_3) buffer (pH = 8.3) and treated with trypsin (2.5%, w/w) for 16 h at 37 °C, respectively. The tryptic digests were diluted with 25 mM NH_4HCO_3 for subsequent enrichment and MS analysis. Before the digestion, BSA was reduced with DTT and carboxamidomethylated with iodoacetamide. Bovine β-casein was digested directly.

2.3 Preparation of the human serum sample and the lysate of mouse liver. The human serum originated from a hepatocellular carcinoma patient was diluted 10 fold with deionized water before the enrichment of phosphopeptides.

Mice were sacrificed and the livers were quickly removed and placed in an ice-cold homogenization buffer consisting of 7 M urea, 2 M thiourea and a mixture of protease inhibitor (1mM phenylmethanesulfonylfluoride) and phosphatase inhibitors (0.2 mM Na_3VO_4, 1 mM NaF). After mincing with scissors and washing to remove blood, the livers were homogenized in a Potter-Elvejhem homogenizer with a Teflon piston, and 1 g of tissue required 5 mL of the homogenization buffer. The suspension was homogenized for approximately 2 min, vortexed at 0 °C for 30 min, and centrifuged at 22 000 g for 1.5 h. As a result, the supernatant contained the total liver proteins. Appropriate volume of protein sample was precipitated as above, lyophilized to dryness, and redissolved in reducing solution (6 M guanidine hydrochloride, 100 mM

NH_4HCO_3, pH = 8.3) with the protein concentration adjusted to 2 μg/μL. Then, 500 μg of this protein sample (with a volume of 20 μL) were mixed with 50 μL of 50 mM DTT. The mixture was incubated at 60 °C for 1 h, and 50 μL of 125 mM 2-iodoacetamide was added and incubated for an additional 30 min at 37 °C in darkness afterwards. The resulting protein mixtures were exchanged into 50 mM NH_4HCO_3 buffer (with the final pH = 8.3), and incubated with trypsin (2.5%, w/w) at 37 °C for 16 h.

2.4 Enrichment of phosphopeptides. Before the enrichment of β-casein tryptic digests, 10 mg of magG@PDA@Zr-MOFs composites were suspended in 1 mL of deionized water with the help of a vortex. Firstly, β-casein tryptic digests were diluted with 50%ACN/0.1%TFA (V/V) and 10 μL of magG@PDA@Zr-MOFs suspension was added into 200 μL of the dilution in a 0.6 mL centrifuge tube. The mixtures were then vibrated in a shaker at 37 °C for 30 min to ensure equilibrium. After magnetic separation and the removal of the supernatant, the magnetic MOF materials were rinsed with 200 μL of 50% ACN/0.1% TFA (V/V) buffer three times. After that, 10 μL of 0.4 M ammonia was added into the tube and vibrated for 10 min to elute the captured peptides. The supernatant of the eluent was pipetted onto a MALDI sample target and dried. Later on, 0.8 μL of DHB matrix was pipetted on it. The sample target was left at room temperature for the evaporation of the solvent. Eventually, the substrates were submitted to MALDI-TOF MS for analysis.

To enrich phosphopeptides from tryptic digest mixtures of β-casein and BSA, a suspension of magG@PDA@Zr-MOFs (10 mg/mL, 10 μL) was added into 200 μL of the tryptic digest mixture of β-casein and BSA at a certain molar ratio. After similar enrichment, washing and elution procedure was followed, the eluent was deposited on a MALDI target using dried droplet method and 0.8 μL of DHB matrix was introduced in the same way. Finally, the adduct was analyzed by MALDI-TOF MS.

To identify endogenous phosphopeptides originated from human serum, 10 μL of magG@PDA@Zr-MOFs dispersion was added into 200 μL of 50%ACN/0.1% TFA solution which contained 2 μL of human serum dilution. After conventional enrichment and washing protocol was followed, the nanocomposites were eluted by

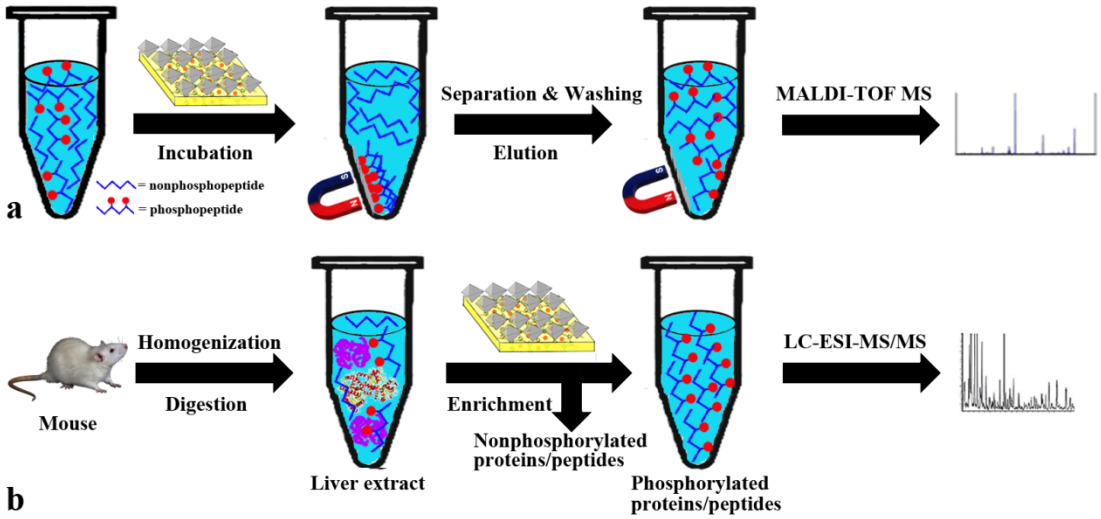
0.4 M ammonia. The eluent was pipetted onto a MALDI target and dried, followed by the introduction of DHB matrix. The substrates were subjected to MALDI-TOF MS for phosphopeptides identification at last.

The tryptic digest of mouse livers was lyophilized and then dissolved in 50% ACN/0.1% TFA (V/V) buffer. Approximately 400 μL of diluted mouse liver digest was incubated with 2.0 mg of magG@PDA@Zr-MOFs at 37 °C for 30 min. Then, the magG@PDA@Zr-MOFs containing adsorbed phosphopeptides were washed with 50% ACN/0.1% TFA (V/V) buffer three times and eluted by 0.4 M ammonia. The eluent was lyophilized and dissolved in 35 μL of loading phase. Finally, the solution was submitted to Nano-LC–ESI-MS/MS analysis.

2.5 MALDI-TOF-MS analysis. Mass spectra were acquired in positive reflective mode on a 5800 Proteomics Analyzer (Applied Biosystems, Framingham, MA, USA) with the Nd: YAG laser at 366 nm, the repetition rate of 200 Hz and the acceleration voltage of 20 kV. All the spectra were taken from signal-averaging of 800 laser shots with the laser intensity kept at a proper constant.

2.6 Nano-LC-ESI-MS/MS analysis and database searching. The peptides eluted from magG@PDA@Zr-MOFs was thoroughly lyophilized and redissolved in 5%ACN/0.1%TFA (V/V) aqueous solution, and was then separated by nano-LC and analyzed by online electrospray tandem mass spectrometry. The experiments were performed on a Nano Aquity UPLC system (Waters Corporation, MA, USA) connected to a quadrupole-Orbitrap mass spectrometer (Q-Exactive) (Thermo Fisher Scientific, Bremen, Germany) equipped with an online nano-electrospray ion source (Haochuang Biotech Corporation, Zhejiang, China). An amount of 10 μL peptide sample was loaded onto the Thermo Scientific Acclaim PepMap C18 column (100 μm × 2 cm, 5 μm, Thermo) with a flow of 10 μL/min for 3 min, and was separated by the analytical column (Acclaim PepMap C18, 75 μm × 15 cm, 2 μm, 100 Å) with a linear gradient from 2% D to 45% D in 105 min subsequently. The column was re-equilibrated under initial conditions for 15 min. The column flow rate was maintained at 300 nL/min and the column temperature was maintained at 40℃. The electrospray

voltage of 1.5 kV versus the inlet of the mass spectrometer was used. The Q-Exactive mass spectrometer was operated in the data-dependent mode to switch automatically between MS and MS/MS acquisition. Survey full-scan MS spectra (m/z 350-1200) were acquired with a mass resolution of 70K, followed by 15 sequential high energy collisional dissociation (HCD) MS/MS scans with a resolution of 17.5 K. In all cases, one microscan was recorded by using dynamic exclusion of 30 s. Thermo Scientific Proteome Discoverer software version 1.4.0.288 with the MASCOT™ v2.3.2 searching engine was used for the searching of the database. The database was Mouse UniProtKB/Swiss-Prot database (Release 2012_12, with 16,648 sequences). Raw files generated by the Q-Exactive instrument were searched directly using a 10 ppm precursor mass tolerance and a 20 mmu fragment mass tolerance. The enzyme specificity with trypsin was used. Up to two missed cleavages were allowed and peptides with at least 7 amino acids were retained. Oxidation (M), phosphorylation (STY), acetylization (protein N-term) and deamidation (NQ) were set as variable modifications. The phosphoRS 3.0 algorithm was used to calculate the probability of phosphorylation site. Based on the above parameters, the target-decoy-based strategy was applied to control peptide level false discovery rates (FDR) lower than 1%, demonstrating the reliability of the identified results in this investigation.



Scheme S1 a) The workflow of phosphopeptides enrichment by using magG@PDA@Zr-MOFs; b) The schematic illustration for the enrichment of phosphopeptides from mouse livers by using magG@PDA@Zr-MOFs.

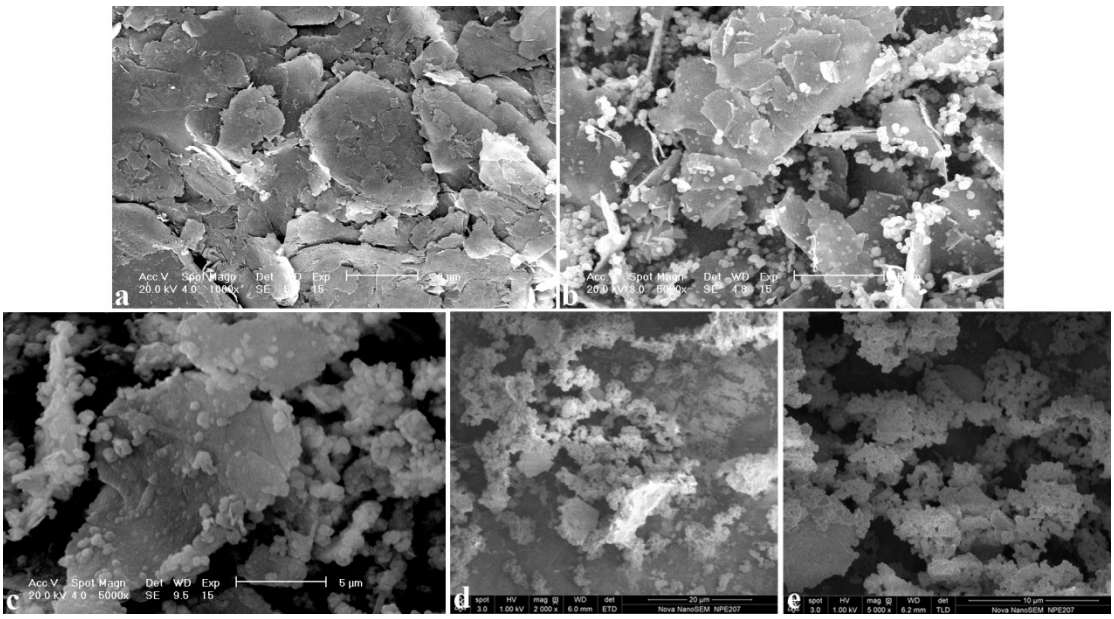


Fig. S1 The SEM images of (a) HNO_3-treated graphene, (b) magG, (c) magG@PDA and (d), (e) magG@PDA@Zr-MOFs.

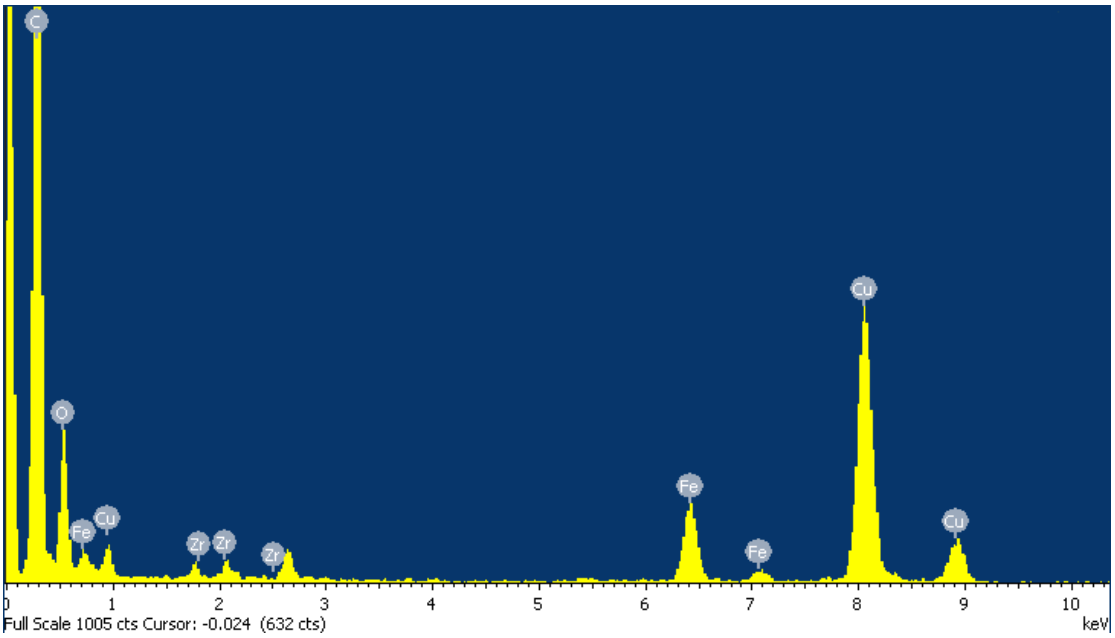


Fig. S2 The energy dispersive X-ray (EDX) spectrum of magG@PDA@Zr-MOFs.

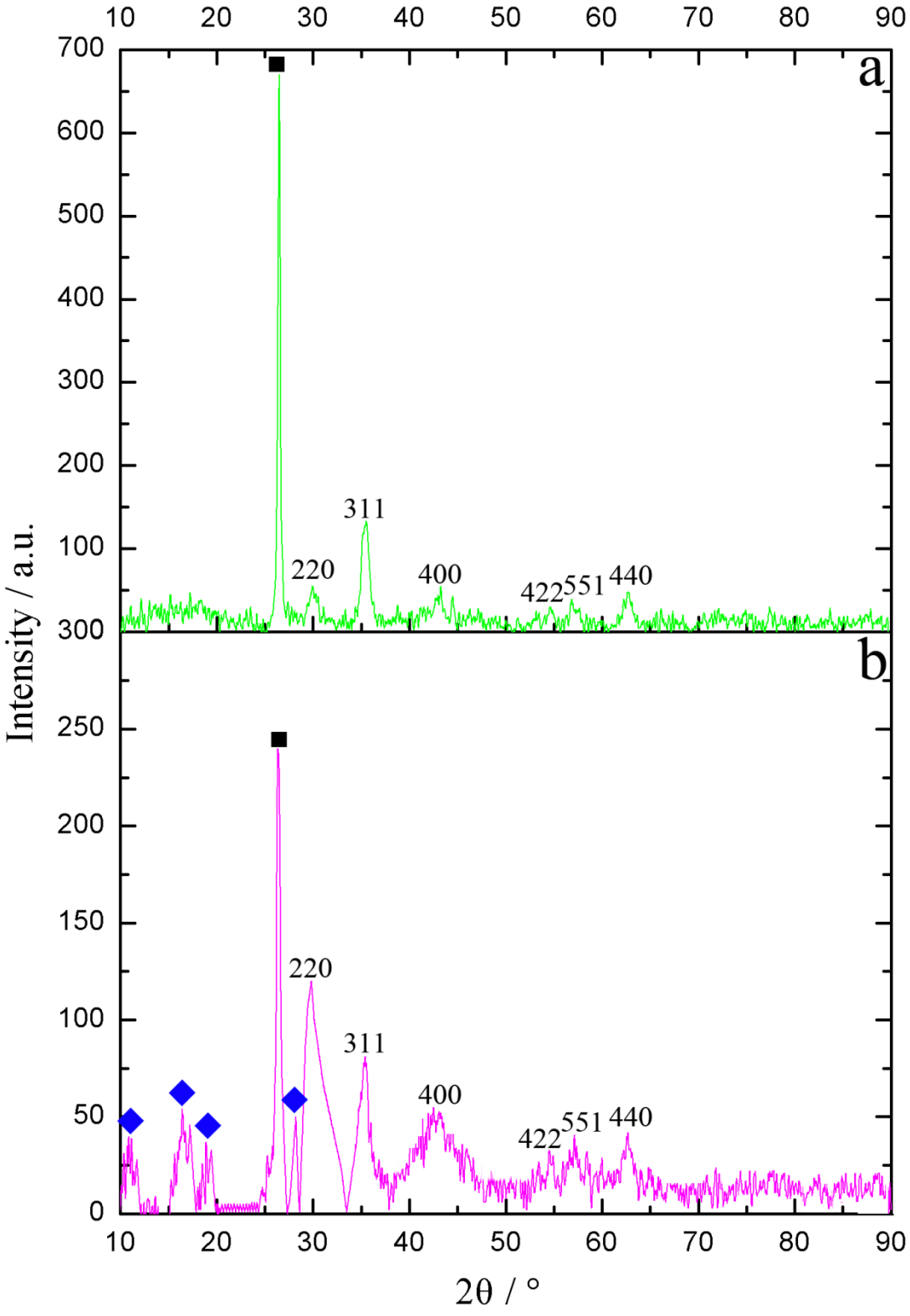


Fig. S3 The XRD patterns of a) magG@PDA and b) magG@PDA@Zr-MOFs. Peaks originated from Zr-bdc MOFs are marked with the symbol ◆ in blue, those originated from Fe_3O_4 are marked with miller indexes and the one assignable to graphene is

marked with the symbol ■.

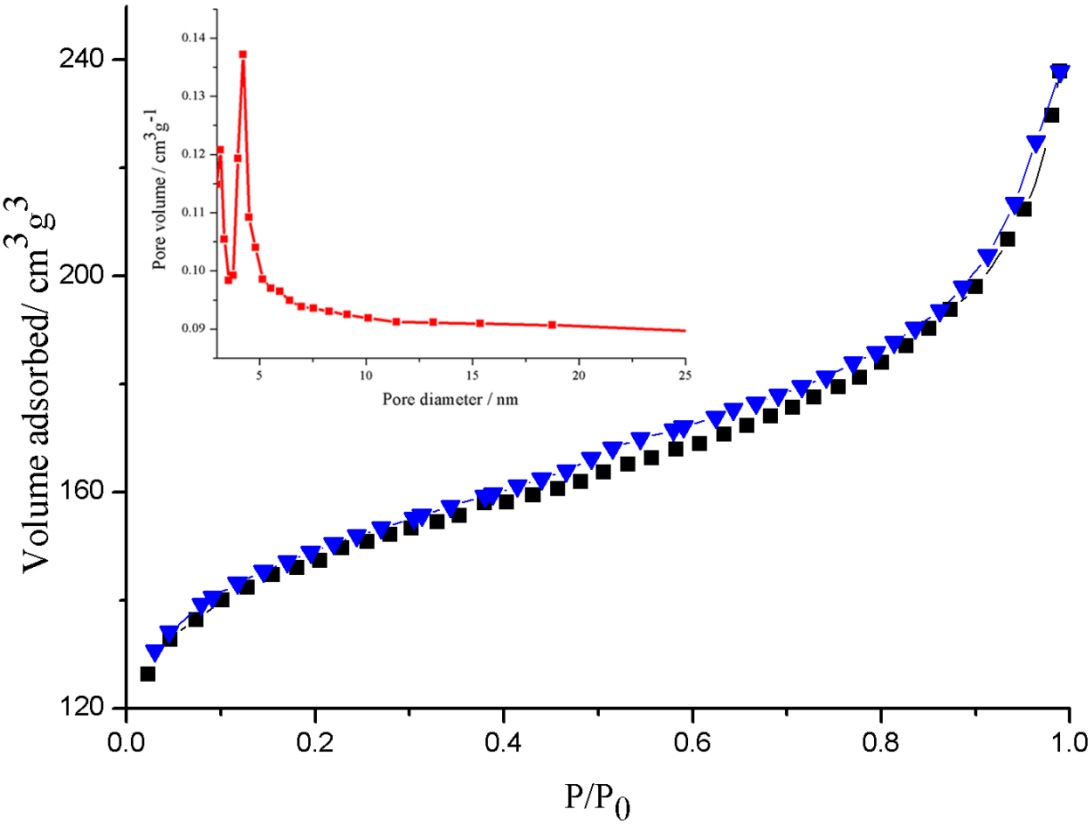


Fig. S4 The N_2 adsorption-desorption isotherms of magG@PDA@Zr-MOFs measured at 77 K. The inset shows corresponding pore size distribution analysis obtained using the Barrett-Joyner-Halenda (BJH) method.

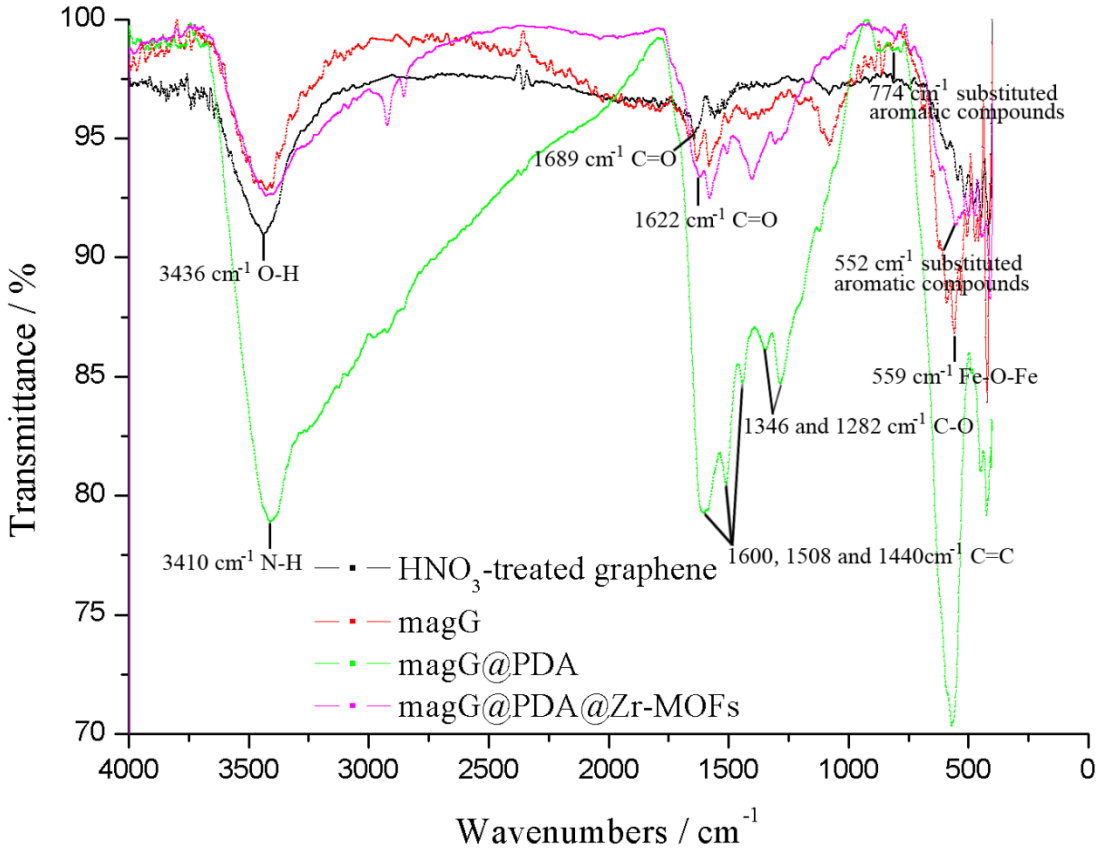


Fig. S5 The FT-IR spectra of HNO_3-treated graphene, magG, magG@PDA and magG@PDA@Zr-MOFs.

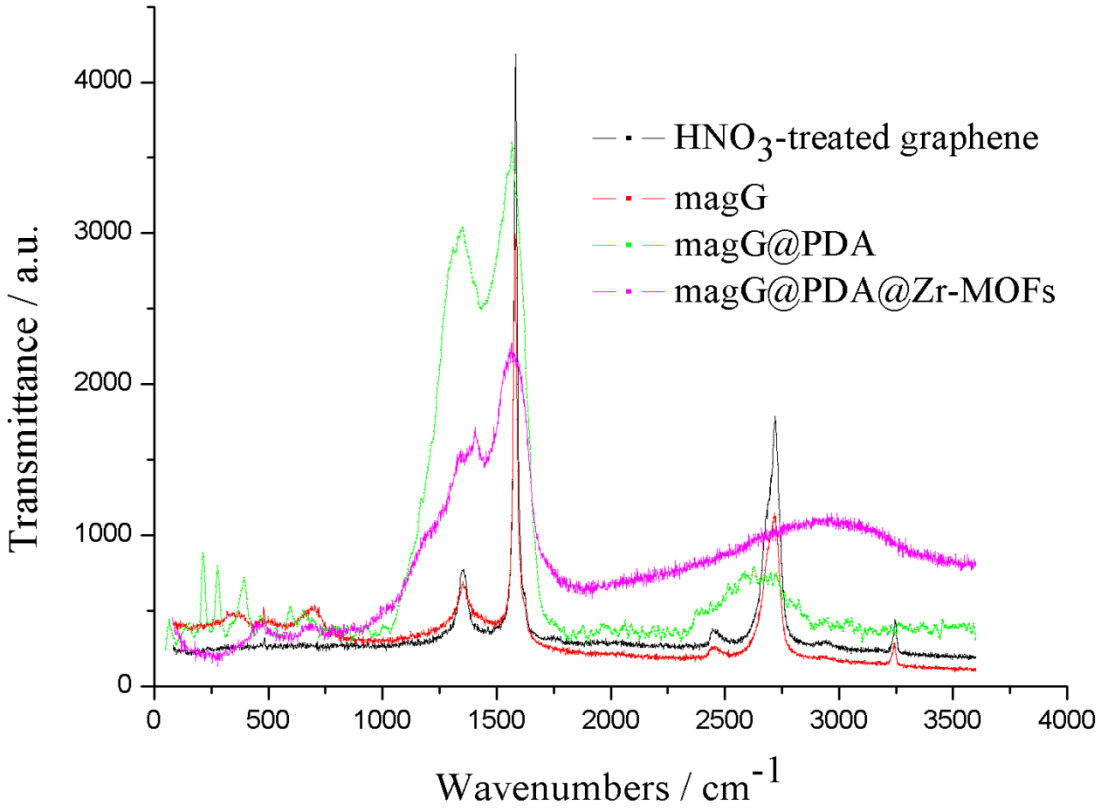


Fig. S6 The Raman spectra of HNO_3-treated graphene, magG, magG@PDA and magG@PDA@Zr-MOFs.

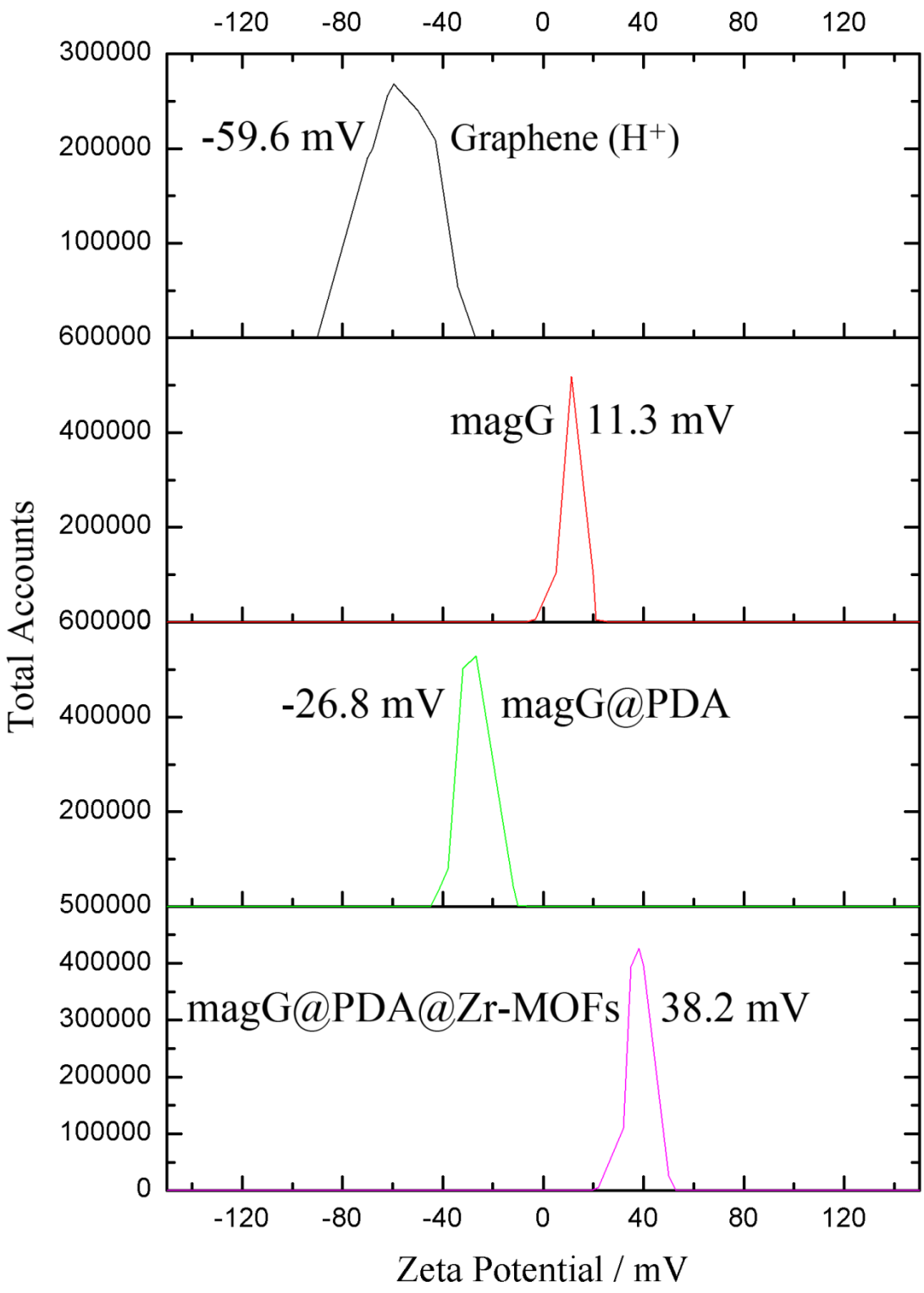


Fig. S7 The zeta potential distributions of HNO_3-treated graphene, magG, magG@PDA and magG@PDA@Zr-MOFs.

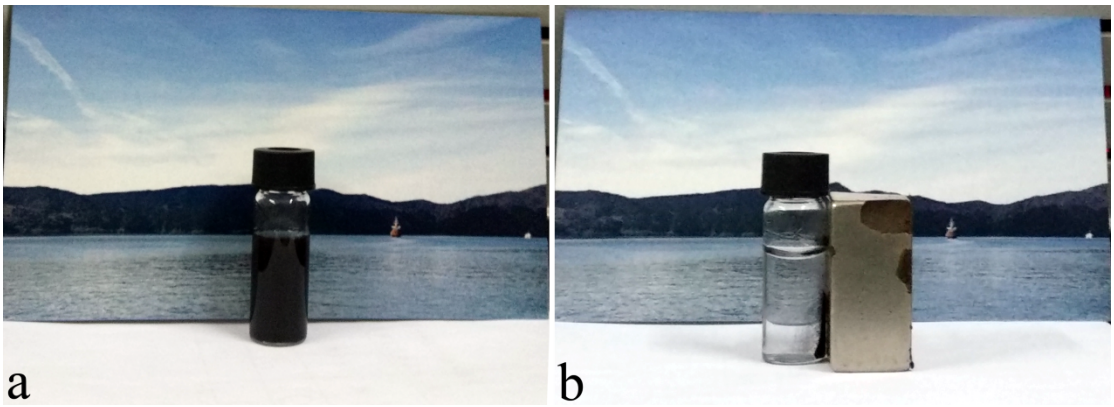


Fig. S8 The photos of the aqueous dispersion of magG@PDA@Zr-MOFs composites: (a) before and (b) after separation with a magnet for 5s.

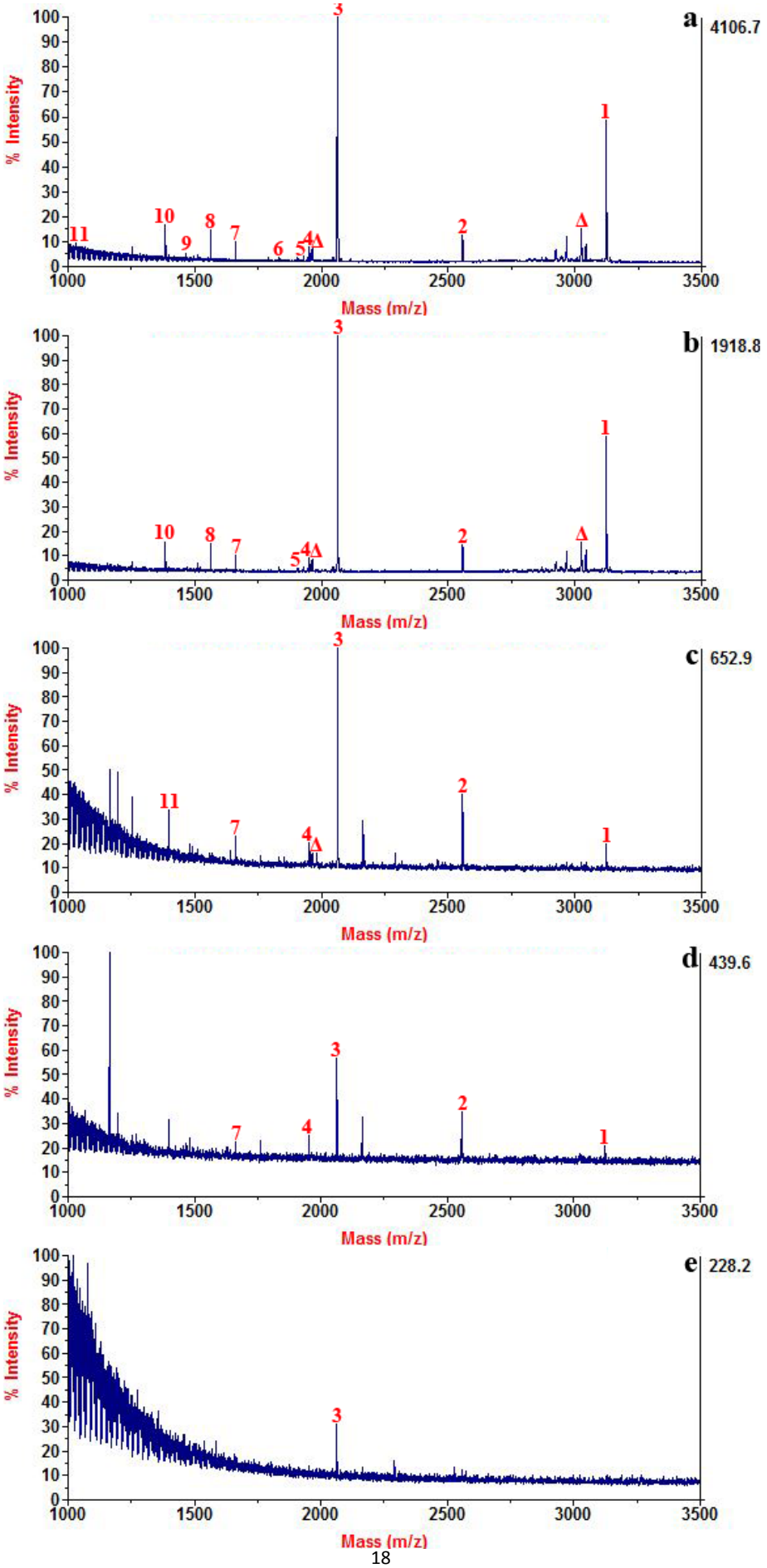

a
4106.7
b
1918.8
c
652.9
d
439.6
e
228.2
% Intensity
Mass (m/z)
1
2
3
4
4Δ
5
6
7
8
9
10
11
Δ

Fig. S9 MALDI-TOF mass spectra of the peptides derived from β-casein tryptic digest with various concentrations enriched by magG@PDA@Zr-MOFs: a) 10^{-6} M, b) 10^{-7} M, c) 10^{-8} M, d) 10^{-9} M and e) 10^{-10} M. Phosphopeptides identified are marked with numbers and dephosphorylated fragments of phosphopeptides through loss of H_3PO_4 are marked with the symbol Δ.

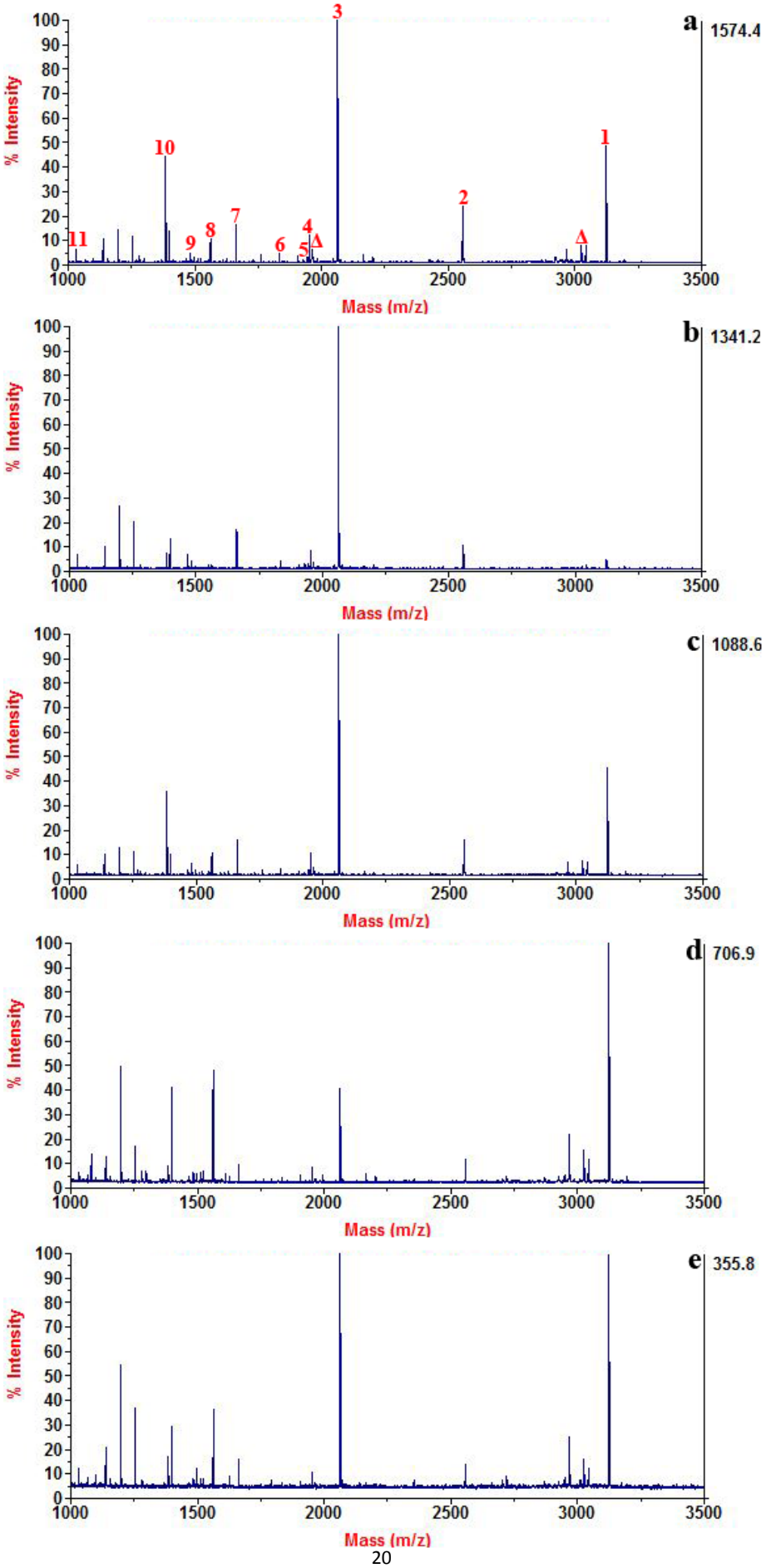

a
1574.4
b
1341.2
c
1088.6
d
706.9
e
355.8
% Intensity
Mass (m/z)
1
2
3
4
5
6
7
8
9
10
11
Δ

Fig. S10 MALDI-TOF mass spectra for the peptides derived from 10^{-6} M β-casein tryptic digest after treatment with magG@PDA@Zr-MOFs used: a) for the first time, b) the 2nd time, c) the 3rd time, d) the 4th time and 5) the 5th time. Phosphopeptides identified are marked with numbers and dephosphorylated fragments of phosphopeptides are marked with the symbol Δ.

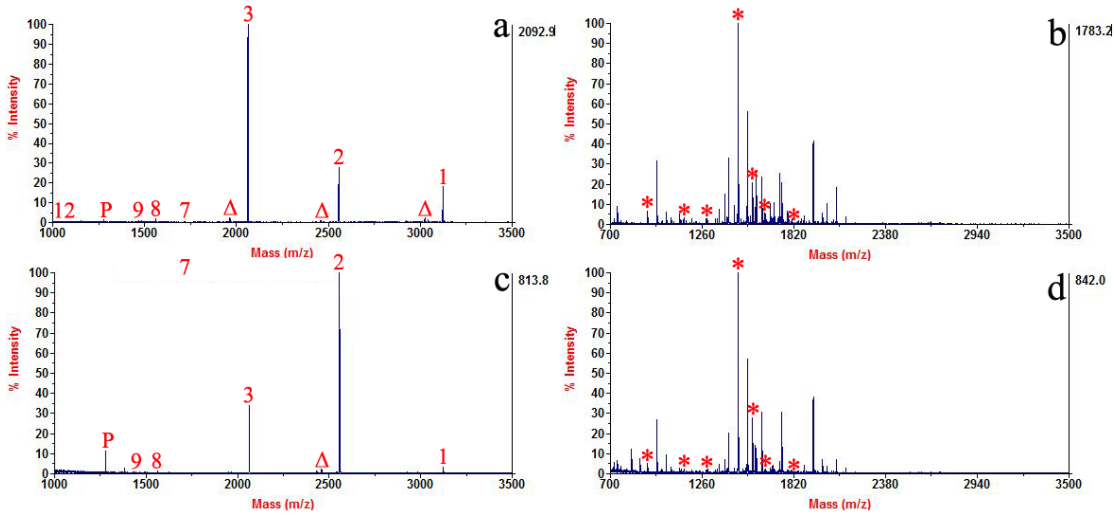


Fig. S11 MALDI-TOF mass spectra for the peptides derived from 10^{-6} M β-casein tryptic digests after treatment with magG@PDA@Zr-MOFs used: a) for the first time, and c) the 3rd time; MS spectra for the peptides derived from 7.5×10^{-4} M BSA tryptic digests after treatment with magG@PDA@Zr-MOFs used: b) the 2nd time and d) the 4th time. Phosphopeptides identified are marked with numbers or captical P, and dephosphorylated fragments of phosphopeptides are marked with the symbol Δ. Peptides originated from BSA are marked with the symbol *.

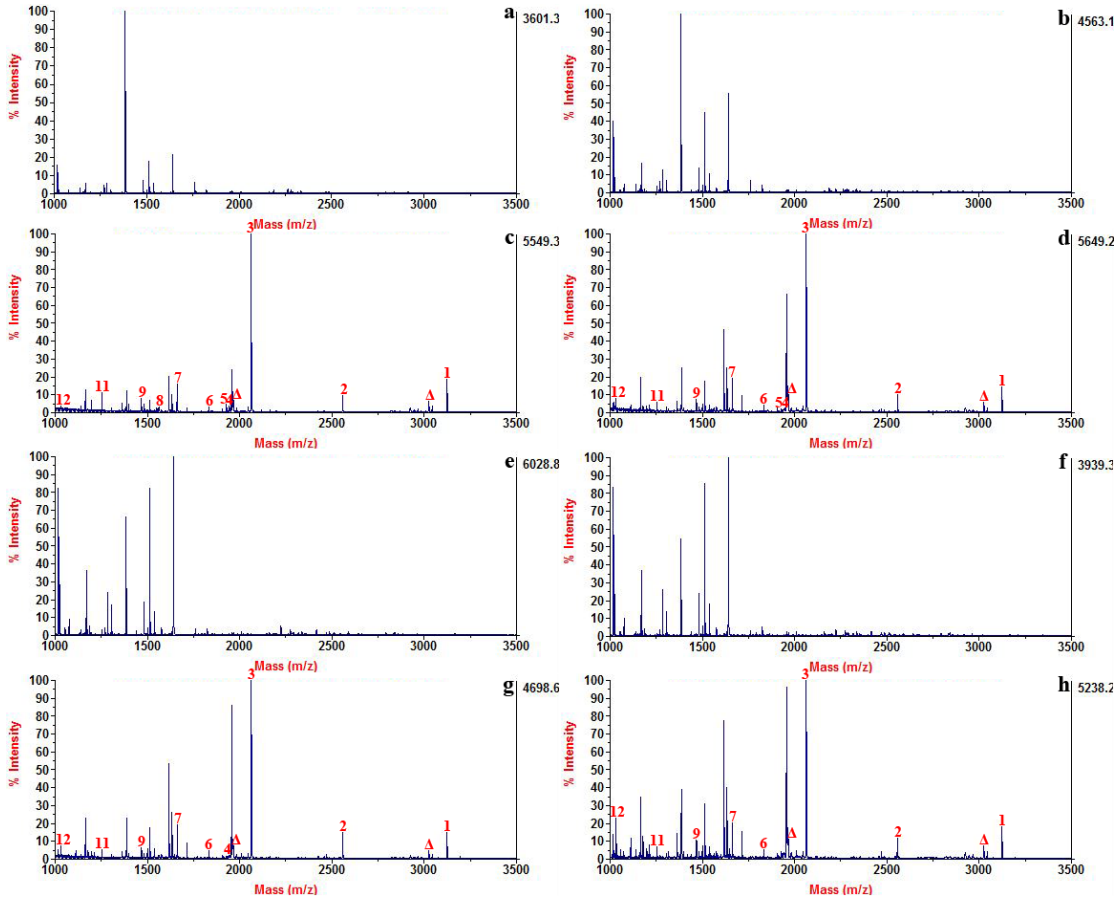


Fig. S12 MALDI-TOF mass spectra for the peptides derived from the tryptic digest mixtures of β-casein and BSA (at molar ratio of 1:100, 1:200, 1:500 and 1:1000): (a), (b), (e) and (f) before; (c), (d), (g) and (h) after enrichment with magG@PDA@Zr-MOFs. Phosphopeptides identified are marked with numbers and dephosphorylated fragments of phosphopeptide peaks are marked with the symbol Δ.

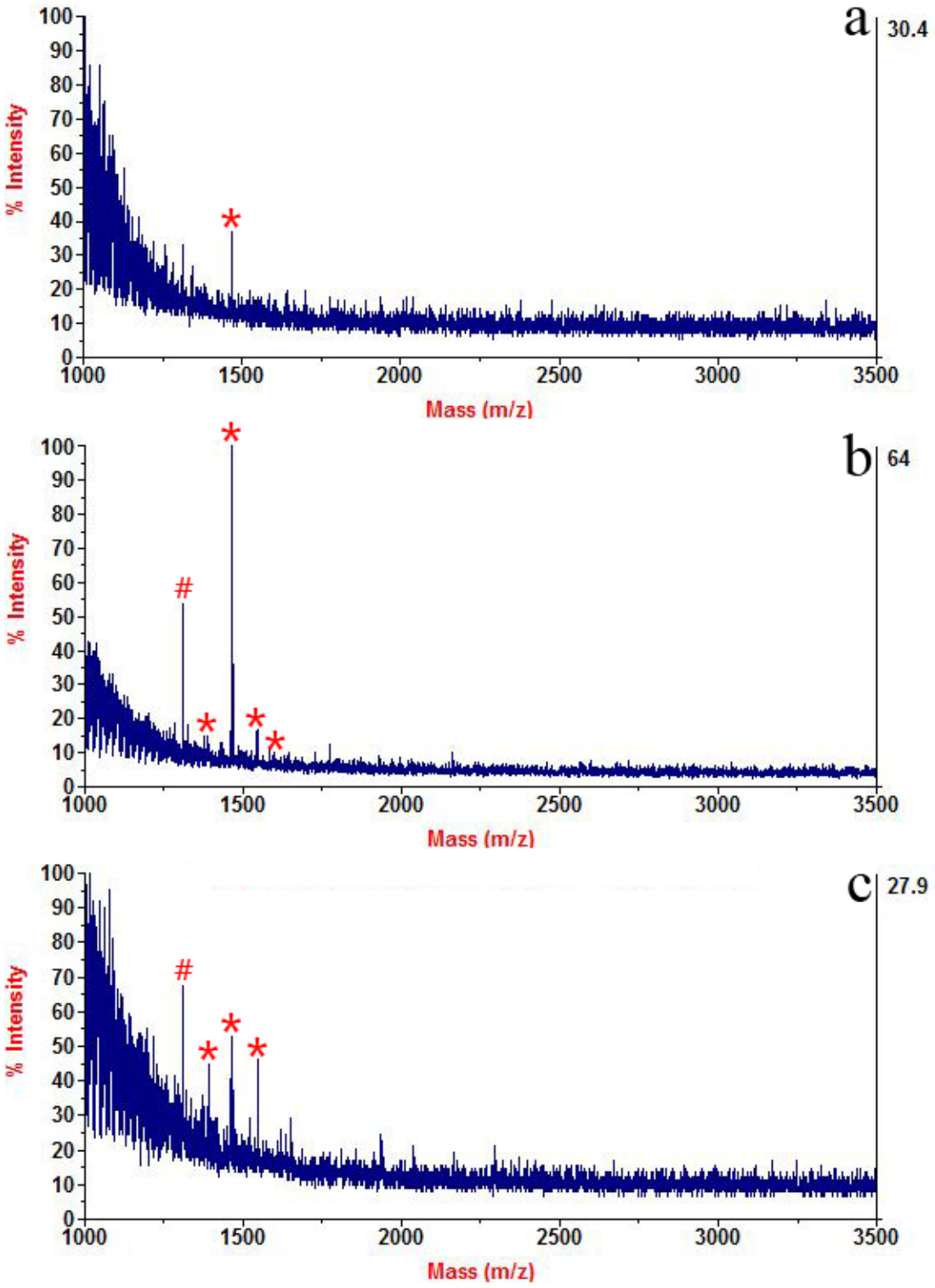


Fig. S13 MALDI-TOF mass spectra of peptides derived from human serum dilution in one trial: (a) before, (b) after enrichment with magG@PDA@Zr-MOFs and (c) after enrichment with Fe_3O_4@PDA@Zr-MOFs. The peaks marked with asterisks represent phosphopeptides and that marked with pound sign represent a dephosphorylated fragment.

Table S1. The Zeta potential changes throughout the fabrication of magG@PDA@Zr-MOFs

Sample	Zeta potential / mV
HNO_3- treated graphene	-59.6
magG	11.3
magG@PDA	-26.8
magG@PDA@Zr-MOFs	38.2

Table S2. Detailed information for the phosphopeptides identified from tryptic digests of β-Casein after enrichment with magG@PDA@Zr-MOFs

Peak No.	Theoretical *m/z*	aa	Peptide Sequence
1	3122.27	*β*/1-25	RELEELNVPGEIVE[pS]L[pS][pS][pS]EESITR
2	2556.09	*β*/33-52	FQ[pS]EEQQQTEDELQDKIHPF
3	2061.83	*β*/33-48	FQ[pS]EEQQQTEDELQDK
4	1951.95[a]	α-S1/119-134	YKVPQLEIVPN[pS]AEER
5	1927.69	α-S1/43-58	DIG[pS]E[pS]TEDQAMETIK
6	1832.85[a]	α-S1/104-119	YLGEYLIVPN [pS]AEER
7	1660.75[a]	α-S1/106-119	VPQLEIVPN[pS]AEER
8	1561.60	α-S2/126-137	EQL[pS]T[pS]EENSKK
9	1466.61[a]	α-S2/153-164	TVDME[pS]TEVFTK
10	1,384.70	α -S1/38–49	FFVAPFPEVFGK
11	1253.11[a]	α-S2/106-115	TVD[Mo]ME[pS]TEVF[b]
12	1030.91	*β*/33-48	FQ[pS]EEQQQTEDELQDK

Table S3. Detailed information for the phosphopeptides enriched from the tryptic digest of mouse livers using magG@PDA@Zr-MOFs

Sequence	Modifications	Ion Score	Exp Value	Charge	MH+ [Da]	ΔM [ppm]
EGEEPTVySDDEEPKDETARK	Y8(Phospho)	26	0.059322077	3	2504.03647	0.61
yMLVRYEDLAR	Y1(Phospho)	33	0.005296584	2	1508.68657	-6.77
yRSmLKR	Y1(Phospho)	28	0.019201092	2	1049.50591	9.23
mFMSELSGNVIDIcPVGALTSKPYAFtARPWEtR	T33(Phospho) T27(Phospho)	45	0.002076717	4	4020.85805	11.03
cGtMIDFGRDEAPEPtQFPIPK	T16(Phospho) T3(Phospho)	25	0.086123272	2	2666.13091	9.00
LPAPQEDTASEAGtPQGEVQTR	T14(Phospho)	64	8.03818E-06	2	2362.05718	0.52
LARHStGLQSLGFtLR	T14(Phospho) T6(Phospho)	32	0.013024276	3	1916.92636	6.23
GIPLPTGDtsPEPELLPGDPLPPPK	T9(Phospho) S10(Phospho)	39	0.001889211	3	2694.28562	5.18
KSYGLsLTtAALGNEEKK	T9(Phospho)	39	0.00538339	3	2069.94870	-3.37

	S6(Phospho)					
RVSVcAEtFNPDEEEEDNDPR	T8(Phospho)	36	0.004492918	3	2588.00809	-6.30
DEILPTtPISEQK	T7(Phospho)	62	9.17432E-06	2	1550.73821	2.06
SGVAVPtSPK	T7(Phospho)	28	0.011457625	2	1022.49443	2.52
ctGtAtNsR	T6(Phospho)	14	0.040641526	3	1287.28727	-3.31
	T4(Phospho)					
	T2(Phospho)					
	S8(Phospho)					
AKsPtPSLsPAR	T5(Phospho)	29	0.008743606	2	1451.56853	-3.30
	S9(Phospho)					
	S3(Phospho)					
KPtKAPK	T3(Phospho)	47	0.000192815	2	849.45091	-10.02
KEtEsEAEDDNLDDLER	T3(Phospho)	64	3.99142E-06	2	2167.79057	-2.18
	S5(Phospho)					
KQtPPASPsPQPIEDRPPSSPIYEDAAPFK	T3(Phospho)	37	0.0035249	3	3407.57663	4.94
	S9(Phospho)					

KtPRLmK	T2(Phospho)	29	0.010166176	2	969.49822	3.15
mtDTPKEGKLTR	T2(Phospho)	26	0.040955222	2	1472.68132	-0.15
FSEMMDHMGGDEDVDLPEVDGADDDSQDsDDEKMPDLE	S29(Phospho)	44	0.000348989	3	4310.60520	7.80
FSEMMDHMGGDEDVDLPEVDGADDDsQDsDDEKMPDLE	S29(Phospho) S26(Phospho)	28	0.008523249	3	4390.53183	-1.39
FSEMMDHMGGDEDVDLPEVDGADDDsQDsDDEK	S29(Phospho) S26(Phospho)	70	4.06718E-07	3	3805.28061	-2.74
YGPSSVEDTTGSGAADAKDDDDIDLFGsDDEEESEEAK	S28(Phospho)	41	0.002029886	3	4046.58615	0.73
VEMGTSSQNDVDMSWIPQETLNQINKAsPR	S28(Phospho)	41	0.001689248	3	3455.57444	3.93
YGPSSVEDTTGSGAADAKDDDDIDLFGsDDEEESE	S28(Phospho)	31	0.022935661	3	4174.67099	-1.72

EAKK						
YMAENPTAGVVQEEEEDNLEYDsDGNPIAPSKK	S23(Phospho)	50	0.000208176	3	3719.59238	-0.54
MLPHAPGVQMQAIPEDAIPEEsGDEDEEDPDKR	S22(Phospho)	55	5.42212E-05	4	3725.61562	4.60
VQGEAVSNIQENTQTPTVQEEsEEEEVDETGVEVK	S22(Phospho)	41	0.002774307	3	3940.71494	-4.96
mNSGGGGGLPPPSAAASPSSssLAAAVAVAVAASSGVGGVPGGPAAAAGVKLK	S22(Phospho)	35	0.009545324	4	4617.26870	5.73
	S21(Phospho)					
SKEsLQEAGKSDANTDLIGGSPK	S21(Phospho)	45	0.0217	3	2413.12955	6.80
	S15(Phospho)					
	S11(Phospho)					
	S4(Phospho)					
	S1(Phospho)					

FPSPGNAGVsGLAEGILDLFsVK	S21(Phospho) S10(Phospho)	27	0.055710879	3	2435.13059	0.38
GATPAEDDEDKDIDLFGsDEEEEDKEAAR	S18(Phospho)	66	4.8075E-06	3	3276.30796	-4.45
LsRsRtASLTSAASIDGSR	S18(Phospho) S14(Phospho) S11(Phospho) S10(Phospho) S8(Phospho) S6(Phospho) S4(Phospho) S2(Phospho)	55	0.000173	3	2175.91971	-0.01
AKPAAQSEEETATsPAAsPTPQSAER	S18(Phospho) S14(Phospho)	34	0.008297263	3	2772.16074	-5.59
SDLIEDEELEDTGKGsEDEWEQVGPK	S16(Phospho)	33	0.011986626	3	3014.25107	-5.47

KEDsDEDEDEEDEDDsDEDEDDEEEDEFEPPIVK	S16(Phospho) S4(Phospho)	71	3.6576E-07	3	4220.39060	-4.16
GHPSAGAEEEGGsDGsAAEAEPR	S16(Phospho) S13(Phospho) S1(Phospho)	28	0.014470471	2	2327.84990	2.08
VDIITEEMPENALPsDEDDKDPNDPYR	S15(Phospho)	65	6.15226E-06	3	3197.36496	4.50
KLEKEEEEGISQEssEEEQ	S15(Phospho) S14(Phospho) S11(Phospho)	64	1.73E-05	2	2397.92143	4.47
KVEEEQEADEEDVsEEEAEDREGASK	S14(Phospho)	81	1.23884E-07	3	3046.24436	8.98
KVEEEQEADEEDVsEEEAEDR	S14(Phospho)	70	1.10083E-06	2	2574.00591	6.65
AKPAAQSEEETATsPAASPTPQSAER	S14(Phospho)	49	0.000225981	3	2692.20609	-1.42

TGEPDEEEGTFRSsIR	S14(Phospho)	29	0.021469695	2	1889.78325	-4.30
DGELPVEDDIDLsDVELDDLEKDEL	S13(Phospho)	51	0.000131542	2	2910.25664	0.47
DGELPVEDDIDLsDVELDDLEK	S13(Phospho)	47	0.000394777	2	2553.11211	4.10
ESDDKPEIEDVGsDEEEEEKKDGDK	S13(Phospho)	40	0.001295293	3	2931.17905	0.08
ESDDKPEIEDVGsDEEEEEKK	S13(Phospho)	39	0.00147808	3	2515.99436	-5.61
ESDDKPEIEDVGsDEEEEEK	S13(Phospho)	34	0.004502251	3	2387.91483	0.56
TAsLTSAASIDGSR	S13(Phospho)	71	1.91E-06	2	1496.60246	-0.37
	S9(Phospho)					
	S6(Phospho)					
	S5(Phospho)					
	S3(Phospho)					
	S1(Phospho)					

QKsDAEEDGVTGsQDEEDSKPK	S13(Phospho) S3(Phospho)	46	0.000392069	3	2538.96054	-6.01
NLAKPGVTSTsDsEDEDDQEGEK	S13(Phospho) S11(Phospho) S10(Phospho) S9(Phospho) S8(Phospho)	41	0.006	3	2895.19461	0.55
DLGHPVEEEDEsGDQEDDDDEIDDGDKDQDI	S12(Phospho)	60	9.13722E-06	3	3568.31943	4.31
LLKEGEEPTVYsDDEEPKDETAR	S12(Phospho)	55	9.64568E-05	3	2730.19004	-4.78
EGVILTNENAAsPEQPGDEDAK	S12(Phospho)	40	0.001674948	2	2364.04131	7.32
VMHTQcHSTPDsAEDVR	S12(Phospho)	27	0.029947632	2	2049.80840	-3.46
IVEPEVVGEsDsEVEGDAWR	S12(Phospho) S10(Phospho)	50	0.000125796	2	2361.94805	-1.88
APDRtPPSEEDSAEAER	S12(Phospho)	63	1.99E-05	3	1936.79148	-0.33

	S8(Phospho) S5(Phospho)					
IEDVGsDEEDDSGKDK	S12(Phospho) S6(Phospho)	36	0.00736	2	1817.69585	-0.17
AELGMNDsPSQsPPVK	S12(Phospho) S8(Phospho)	34	0.004824076	2	1816.72405	0.84
IEDVGsDEEDDSGKDKK	S12(Phospho) S6(Phospho)	33	0.0189	3	945.79038	-0.38
FIIGSVSEDNsEDEISNLVK	S11(Phospho)	93	9.46113E-09	2	2275.04350	2.49
NVPQEESLEDsDVDADFK	S11(Phospho)	87	2.387E-08	2	2116.87432	6.98
RGGGSGGGDEsEGEEVDED	S11(Phospho)	28	0.006956007	2	1917.65349	-4.39
FIIGSVsEDNsEDEISNLVK	S11(Phospho) S7(Phospho)	57	3.08238E-05	2	2355.00664	1.05
FIDKDQQPsGsEGEDDDA	S11(Phospho)	40	0.001676475	3	2753.12217	-0.24

EAALKK	S9(Phospho)					
MESEAGADDsAEEGDLLDDDDNEDRGDDQLELK	S10(Phospho)	118	2.28101E-11	3	3691.43772	3.86
MESEAGADDsAEEGDLLDDDDNEDR	S10(Phospho)	65	1.66242E-06	2	2792.96269	-7.56
DDDDIDLFGsDDEEESEEAK	S10(Phospho)	57	1.95624E-05	2	2352.84892	3.79
ATWGDGGDNsPSNVVSK	S10(Phospho)	48	0.000194942	2	1770.74053	4.18
AEAKEESEEsDEDMGFGLFD	S10(Phospho)	47	0.000130781	2	2314.84477	-5.82
VVDYSQFQEsDDADEDYGR	S10(Phospho)	37	0.003055025	2	2317.87480	-0.93
AEEDEILNRsPR	S10(Phospho)	34	0.005185092	2	1508.67217	-1.32
GIPLPTGDTsPEPELLPGDPLPPPK	S10(Phospho)	31	0.009974815	3	2614.31522	3.79
AEAKEEsEEsDEDMGFG	S10(Phospho)	43	0.000342589	2	2394.82231	-0.94

LFD	S7(Phospho)					
AESSDsGAEsEEEEAQEELK	S10(Phospho) S6(Phospho)	32	0.002836232	2	2313.81035	-2.80
EGEEPTVYsDDEEPKDETARK	S9(Phospho)	59	2.00233E-05	3	2504.04233	2.95
IYHLPDAEsDEDEDFK	S9(Phospho)	59	8.05E-05	3	2517.04502	-0.16
EGEEPTVYsDDEEPKDETAR	S9(Phospho)	45	0.000488947	2	2375.93364	-2.66
IYHLPDAEsDEDEDFKEQTR	S9(Phospho)	42	0.002474369	3	2517.04648	0.42
ESLKEEDEsDDDNM	S9(Phospho)	39	0.000417363	2	1735.58708	-1.05
TDSREDEIsPPPPNPVVK	S9(Phospho)	39	0.002284536	2	2056.96245	1.78
ESLKEEDEsDDDNm	S9(Phospho)	38	0.000523965	2	1751.57610	-4.41
EGEEPTVYsDDEEPK	S9(Phospho)	34	0.00301409	2	1803.67156	-7.18
RAGDVLEDsPKRPK	S9(Phospho)	32	0.0244	4	1647.82046	-0.64
ILEGLVSSsHPLPLK	S9(Phospho)	29	0.015428474	2	1669.89250	-0.01
AGGAGGEGsDDDTSLT	S9(Phospho)	25	0.019789189	2	1489.53325	0.37

HTGPNsPDTANDGFVR	S9(Phospho) S6(Phospho) S2(Phospho)	43	0.00197	2	1764.73894	2.92
ENPPsPPTsPAAPQPR	S9(Phospho) S5(Phospho)	25	0.040639032	2	1802.73735	-7.63
TPEELDDsDFETEDFDVR	S8(Phospho)	72	1.00081E-06	2	2238.86601	2.72
HSSLPTEsDEDIAPAQR	S8(Phospho)	69	1.62637E-06	2	1932.83904	2.83
AELGMNDsPSQSPPVK	S8(Phospho)	61	1.07366E-05	2	1736.75896	1.60
DSDQVAQsDGEESPAAEEQLLGER	S8(Phospho)	51	0.000128818	3	2640.09977	1.96
SDEEDEDsDFGEEQR	S8(Phospho)	51	3.04931E-05	2	1866.63603	9.33
GILAADEsVGTMGNR	S8(Phospho)	47	0.000294392	2	1570.69939	3.94
AGDVLEDsPK	S8(Phospho)	45	0.000220136	2	1110.47099	-0.47
TALPTSGsSTGELELLAGEVPAR	S8(Phospho)	43	0.00083422	2	2336.14751	3.97
EIITEEPsEEEADMPKPK	S8(Phospho)	32	0.009297539	2	2151.94121	0.36
AGDVLEDsPKRPK	S8(Phospho)	36	0.00701	3	1491.72049	0.07

VIENTDGsEEEMDAR	S8(Phospho)	30	0.011232231	2	1774.67168	-6.84
RsPsPYYSR	S8(Phospho)	42	0.000738	2	1272.48015	-0.68
	S7(Phospho)					
	S6(Phospho)					
	S4(Phospho)					
	S2(Phospho)					
RDsVLAASR	S8(Phospho)	38	0.00253	2	1054.50469	0.51
	S3(Phospho)					
SVPTVDsGNEDDDSSFK	S7(Phospho)	63	5.88071E-06	2	1878.71904	-4.65
DIDLFGsDEEEEDK	S7(Phospho)	63	4.05459E-06	2	1720.65056	1.84
NLLEDDsDEEEDFFLR	S7(Phospho)	53	0.000369	3	2617.10611	-1.15
KGTGDcsDEEVDGKADGADAK	S7(Phospho)	45	0.000431243	3	2204.85477	-4.67
KEESEEsDDDMGFGLFD	S7(Phospho)	44	0.000355221	2	2029.71672	-4.45
ASVSDLsPR	S7(Phospho)	41	0.00076355	2	1011.44774	-2.94
KEESEEsEDDMGFGLFD	S7(Phospho)	42	0.000438035	2	2043.72478	-8.13
DIDLFGsDEEEEDKEAAR	S7(Phospho)	35	0.003665663	2	2147.84868	-7.77

VVPSPDsDSDTDLEDPSPR	S7(Phospho)	35	0.004186505	2	2107.87114	0.34
QLSVPAsDEEDEVPAPIPR	S7(Phospho)	32	0.015050128	2	2128.96660	-6.26
KEEsEEsDDDMGFGLFD	S7(Phospho) S4(Phospho)	49	6.98489E-05	2	2109.68266	-4.46
KEEsEEsEDDmGFGLFD	S7(Phospho) S4(Phospho)	41	0.000406535	2	2139.70806	2.53
SLDsDEsEDEDDDYQQK	S7(Phospho) S4(Phospho)	39	0.000272727	2	2177.67949	-7.40
GSAEGssDEEGKLVIDEPAK	S7(Phospho) S6(Phospho)	37	0.00261417	2	2177.88725	-0.72
RIDIsSALR	S7(Phospho) S5Phospho)	37	0.00515	2	1207.61968	0.13
KEESEEsDDDMGFGLFD	S7(Phospho) S4(Phospho)	35	0.001253987	2	2125.67388	-6.17
KAEDsDsEPEPEDNVR	S7(Phospho)	26	0.015884912	2	1976.70500	-5.52

	S5(Phospho)					
KEEsEEsEDDMGFGLFD	S7(Phospho) S4(Phospho)	25	0.009328719	2	2123.70659	-0.54
DWEDDsDEDMSNFDR	S6(Phospho)	54	8.88644E-06	2	1955.61150	-8.14
IEDVGsDEEDDSGKDK	S6(Phospho)	50	0.000113658	2	1817.68852	-4.20
YGMGTsVER	S6(Phospho)	49	8.98116E-05	2	1079.42461	1.69
DMDEPsPVPNVEEVTLPK	S6(Phospho)	39	0.002034591	2	2075.92900	2.22
IEDVGsDEEDDSGK	S6(Phospho)	38	0.001123425	2	1574.56243	-7.50
IEDVGsDEEDDSGKDKK	S6(Phospho)	37	0.00343442	3	1945.79770	3.38
VATLNsEEENDPPTYK	S6(Phospho)	36	0.003381645	2	1886.81157	3.16
QGDNIsDDEDEVR	S6(Phospho)	27	0.016387747	2	1571.58965	2.45
QPLLLsEDEEDTKR	S6(Phospho)	26	0.055393384	2	1752.79155	-7.81
EEsEEsEDDMGFGLFD	S6(Phospho) S3(Phospho)	33	0.000891497	2	1995.60527	-3.75
EEsEEsDDDMGFGLFD	S6(Phospho) S3(Phospho)	28	0.002567343	2	1981.57829	-9.50

EEsEEsDEDMGFGLFD	S6(Phospho) S3(Phospho)	24	0.006920221	2	1995.60527	-3.75
sLSVTsLGGLPVWEAER	S6(Phospho) S1(Phospho)	76	3.96274E-07	2	1960.88494	1.60
NSTPsEPDSGQGPPAEEE EGEEEAAKEEAEAQGVR	S5(Phospho)	57	3.52372E-05	3	3720.55332	6.09
NSTLsEEDYIER	S5(Phospho)	48	0.000168548	2	1535.63725	7.22
KETEsEAEDDNLDDLER	S5(Phospho)	39	0.001778823	2	2087.81890	-4.82
DKEVsDDEAEEK	S5(Phospho)	38	0.001115554	2	1473.57305	6.86
VSGPsSSENQEGTLTDSM K	S5(Phospho)	37	0.003297407	2	2033.83330	-1.83
WLDEsDAEMELR	S5(Phospho)	31	0.007966223	2	1573.61931	-3.05
KVMDsDEDDADY	S5(Phospho)	28	0.006721295	2	1482.49272	-3.48
SLsYsPVER	S5(Phospho) S4Phospho) S3Phospho) S1Phospho)	36	0.00299	2	1197.45891	0.01

SASsDTSEELNSQDSPK	S4(Phospho)	73	6.07835E-07	2	1861.72429	-4.99
SKEsLQEAGKSDANTDLIGGSPK	S4(Phospho)	53	0.000101166	3	2412.12979	0.28
KEEsEESDDDMGFGLFD	S4(Phospho)	46	0.000181757	2	2029.70818	-8.66
EEAsDDDMEGDEAVVR	S4(Phospho)	40	0.00063544	2	1846.67754	4.85
KEEsEESEDDMGFGLFD	S4(Phospho)	36	0.00169915	2	2043.75640	7.34
SQSsDTEQPSPTSGGGK	S4(Phospho)	31	0.010163373	2	1729.67754	-7.96
IDIsPSTFR	S4(Phospho)	30	0.010334153	2	1115.51384	0.47
TcDsPQNPVDFISGPVPDSPFPR	S4(Phospho)	29	0.037704439	2	2609.13530	-1.00
EDssEEEEEEIDDEEIER	S4(Phospho)	50	3.27204E-05	2	2370.78813	-1.07
	S3(Phospho)					
VPssDEEVVEEPQSR	S4(Phospho)	40	0.000631961	2	1846.70098	-7.29
	S3(Phospho)					
SAsPDDDLGSSNWEAADLGNEER	S3(Phospho)	77	3.38468E-07	2	2514.98564	-1.49
SQsGEDESLNQPGPIK	S3(Phospho)	77	2.92254E-07	2	1765.76714	1.72

SAsSDTSEELNSQDSPK	S3(Phospho)	68	2.00294E-06	2	1861.72600	-4.07
AEsPETSAVESTQSTPQK	S3(Phospho)	60	1.73587E-05	2	1956.85002	3.35
SLsVTSLGGLPVWEAER	S3(Phospho)	58	2.95666E-05	2	1880.91875	1.75
SEsPEPGYVVTSSGLLLP VLLPR	S3(Phospho)	53	4.19232E-05	2	2490.30132	4.85
STsPAPADVAPAQEDLR	S3(Phospho)	51	0.000134851	2	1804.80132	-5.58
SAsSDTSEELNSQDSPKR	S3(Phospho)	45	0.000535149	2	2017.81853	-8.01
RNsLTGEEGELVK	S3(Phospho)	37	0.003085236	2	1511.71636	4.07
GWsPPPEVR	S3(Phospho)	33	0.004518595	2	1104.48760	0.14
SHsLPNSLDYAQASER	S3(Phospho)	33	0.010123982	2	1854.80925	3.99
TAsFSESR	S3(Phospho)	33	0.003752373	2	964.37956	2.45
ADsHGELDLAR	S3(Phospho)	30	0.011558597	2	1263.53789	1.03
EIsDDEAEEEK	S3(Phospho)	29	0.00634156	2	1373.50188	1.90
EEsEESDEDMGFGLFD	S3(Phospho)	29	0.00383278	2	1915.63909	-3.84

EEsEESEDDMGFGLFD	S3(Phospho)	28	0.005552824	2	1915.63909	-3.84
KNsATIPESDDL	S3(Phospho)	27	0.022335056	2	1369.58306	-3.86
RIsLDRTGR	S3(Phospho)	26	0.036575163	2	1153.57573	-7.00
AGsPQLDDIR	S3(Phospho)	25	0.025847652	2	1151.51030	0.87
SAsADNLILPR	S3(Phospho)	84	8.90E-08	2	1236.59892	0.38
	S1(Phospho)					
sPsPAPPPPPPPPPR	S3(Phospho)	42	0.000827834	2	1744.77629	-5.59
	S1(Phospho)					
sASPDDDLGSSNWEAADLGNEER	S3(Phospho)	41	0.000520401	2	1564.59746	0.38
	S1(Phospho)					
sPsPEPIYNSEGK	S3(Phospho)	41	0.000520401	2	1564.59746	0.38
	S1(Phospho)					
SsPPPLSGASEVDAGELGSER	S2(Phospho)	94	6.65882E-09	2	2121.94023	3.07
AsPALGSGHHDGSGDSLEMSSLDR	S2(Phospho)	40	0.001458737	3	2463.02378	-0.23

sASPDDDLGSSNWEAADLGNEER	S1(Phospho)	78	1.9208E-07	2	2515.01250	9.18
sAEDLTDGSYDDILNAEQLK	S1(Phospho)	64	6.18754E-06	2	2276.98564	2.17
sTSPAPADVAPAQEDLR	S1(Phospho)	53	0.000103082	2	1804.81828	3.82
sGDETPGSEAPGDK	S1(Phospho)	39	0.001002183	2	1426.53435	-1.89

References

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