Supporting Information

Alkali-resistant zirconium-biligand organic framework with dual-metal center in highly selective capture of phosphopeptides

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Experiment details

Materials and Reagents

All reagents were obtained from commercial sources and used without further purification. Bovine serum albumin (BSA), β-casein (from bovine milk), trypsin, dithiothreitol (DTT), and iodoacetamide (IAA), 2,5-dihydroxybenzoic acid (DHB), zirconium oxychloride ($ZrOCl_2 \cdot 8H_2O$), and trifluoroacetic acid (TFA) were purchased from Sigma-Aldrich (St. Louis, MO). 2-Amino-benzenedicarboxylic acid and benzoic acid were obtained from J&K Scientific Ltd. (Beijing, China). Zirconium tetrachloride ($ZrCl_4$), Nitroterephthalic acid, 2,4,6-collidine were obtained from Aladdin (Shanghai, China). Phosphorus oxychloride ($POCl_3$), phosphoric acid and sodium chloride (NaCl) were purchased from Sinopharm Group Chemical Regent Co., Ltd. (Shanghai, China). Methanol, and dimethylformamide (DMF) were obtained from Tianjin Chemical Plant (Tianjin, China). Ammonium bicarbonate was purchased from BioRad (Hercules, CA). Acetonitrile was HPLC grade and purchased from Merck (Darmstadt, Germany). Deionized water was purified using a Milli-Q water system (Millipore, USA).

Characterization of materials

The nitrogen sorption/desorption isotherms were measured at 77 K using an Autosorb iQ2 adsorptometer (Quantachrome Instruments). Zeta potential measurement was conducted by a Zetasizer Nano Series (Malvern). Fourier transform infrared (FTIR) spectra were observed with a Bruker Hyperion 3000 (Bruke Optics). Powder X-ray diffraction (XRD) patterns were recorded on an Empyrean XRD system (PANalytical, Almelo) with Cu–Kα radiation (λ = 1.54056 Å) over a 2θ range of 5-90°. Transmission electron microscope (TEM) images were acquired by using a JEOL 2000 EX electronic microscope with an accelerating voltage of 120 keV. Elemental mapping images were carried out on an FEI Tecnai F30 microscope with an accelerating voltage of 300 kV. X-ray photoelectron spectroscopy (XPS) was recorded on a Thermo Scientific ESCALAB 250Xi system equipped with a dual X-ray source using Al–Kα. The contact angles of the particle's surface with deionized water were detected by a drop shape analyzer (DSA100). Inductively coupled plasma-optical emission spectroscopy (ICP-OES) was performed on a PerkinElmer Optima 7300 DV series. Thermogravimetric analysis (TGA) was carried out on a simultaneous thermal analyzer (STA 449 F3, NETZSCH) under flowing artificial air. MALDI-TOF-MS analysis results were obtained using a MALDI-TOF/TOF 5800 System (AB SCIEX, Foster City, CA) equipped with a 1 kHz OptiBeam on-axis laser.

Sample preparation

For the digestion of α-casein and β-casein, 1 mg of α-casein and β-casein was dissolved, respectively, in 1 mL of NH_4HCO_3 solution (50 mM) and digested with trypsin by using a mass ratio of trypsin to stand protein at 1:40 (w/w) at 37 °C for 16 h.

Human saliva was collected from the morning salivary of a healthy male volunteer. Informed consent was obtained from all human subjects. Briefly, the volunteer was not allowed to eat or drink for at least 2 h before collection and rinsed his mouth with pure water before taking sample. About 2 mL saliva was taken and centrifuged for 5 min at 14000 g. The supernatant was stored in -20 °C before use.

MALDI-TOF Analysis

All MALDI-TOF mass experiments were carried out on an AB Sciex 5800 MALDI-TOF/TOF mass spectrometer (AB Sciex, CA) equipped with a pulsed Nd/YAG laser at 355 nm. MALDI-TOF analysis adopted a sample-first method. Briefly, each 0.5 μL of eluate and 2,5-dihydroxybenzoic acid matrix (25 mg/mL, 70% ACN, and 1% H_3PO_4) were sequentially dropped onto the MALDI stainless-steel target for mass analysis.

Nano-RPLC-ESI-MS/MS Analysis

The nano-RPLC-ESI-MS/MS experiments were performed on an LTQ-Orbitrap Elite mass spectrometer coupled with Dionex UltiMate 3000 RSLC-nano System (Thermo, San Jose, CA). The acidified sample eluate was automatically loaded onto a 3 cm C18 trap column (200 μm i.d.) at a flow rate of 3 μL/min. The separation was performed on a 15 cm C18 column (150 μm i.d.) with a flow rate of 600 nL/min. Water with 0.1% FA (buffer A) and 80% acetonitrile with 0.1% FA (buffer B) were used as mobile phase to generate a 176 min gradient, set as follows: 0% B for 10 min, 0-3% B for 3 min, 3-30% B for 135 min, 30-45% B for 15 min, 45-99% B for 2 min, and 99% B for 11 min. The LTQ-Orbitrap Elite mass spectrometer was operated in a positive, data-dependent MS/MS acquisition mode. The ion-transfer capillary temperature was set at 275 °C, and the spray voltage was 2.5 kV. The full mass scan acquired in the Orbitrap mass analyzer was from m/z 350 to 1650 with a resolution of 60 000 and the top 20 parent ions with charge states ≥2 from the full scan were fragmented by collision-induced dissociation with 35% normalized collision energy. The dynamic exclusion function was set as follows: repeat count 1, repeat duration 30 s,

and exclusion duration 90 s.

Supporting figures

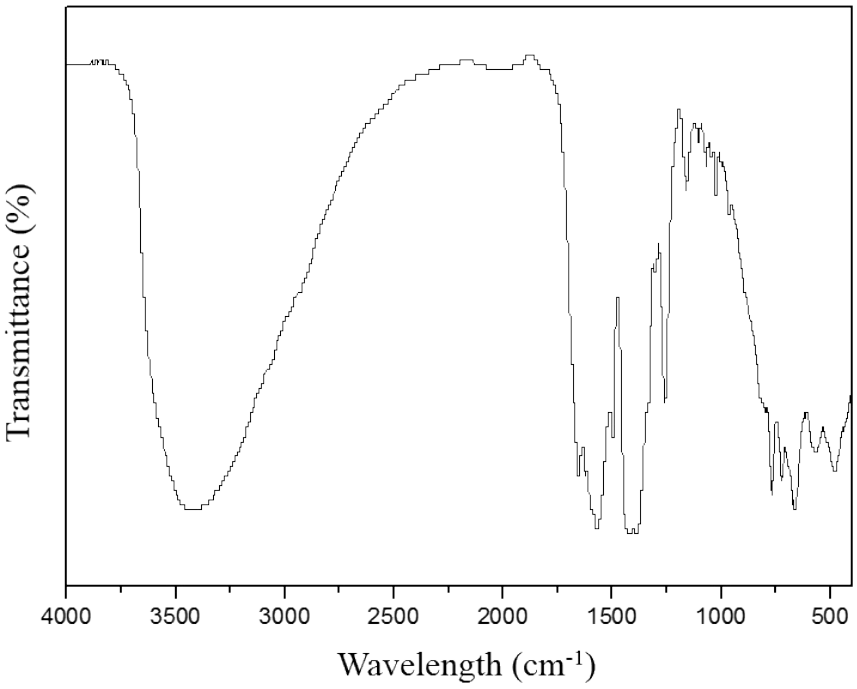

Figure S1. FT-IR spectra of as-synthesized UIO-66-NH_2.

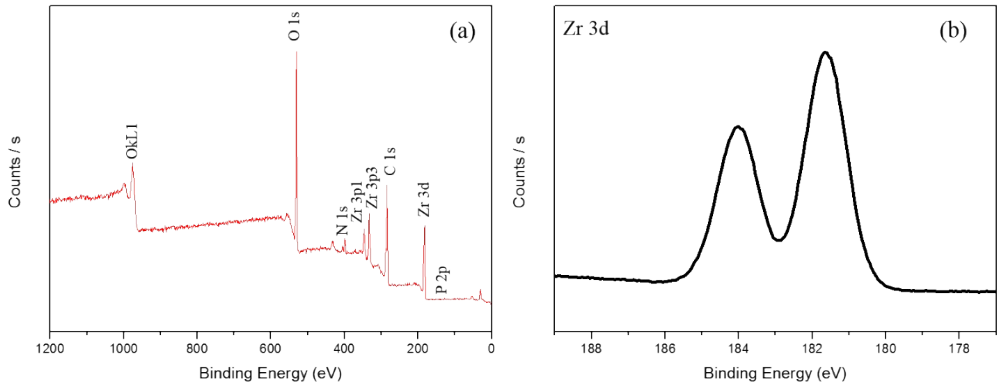

Figure S2. High-resolution XPS spectra of (a) C1s, (b) O2p, (c) N 1s, and (d) P 2p regions taken from biUIO-66-NH_2NO_2.

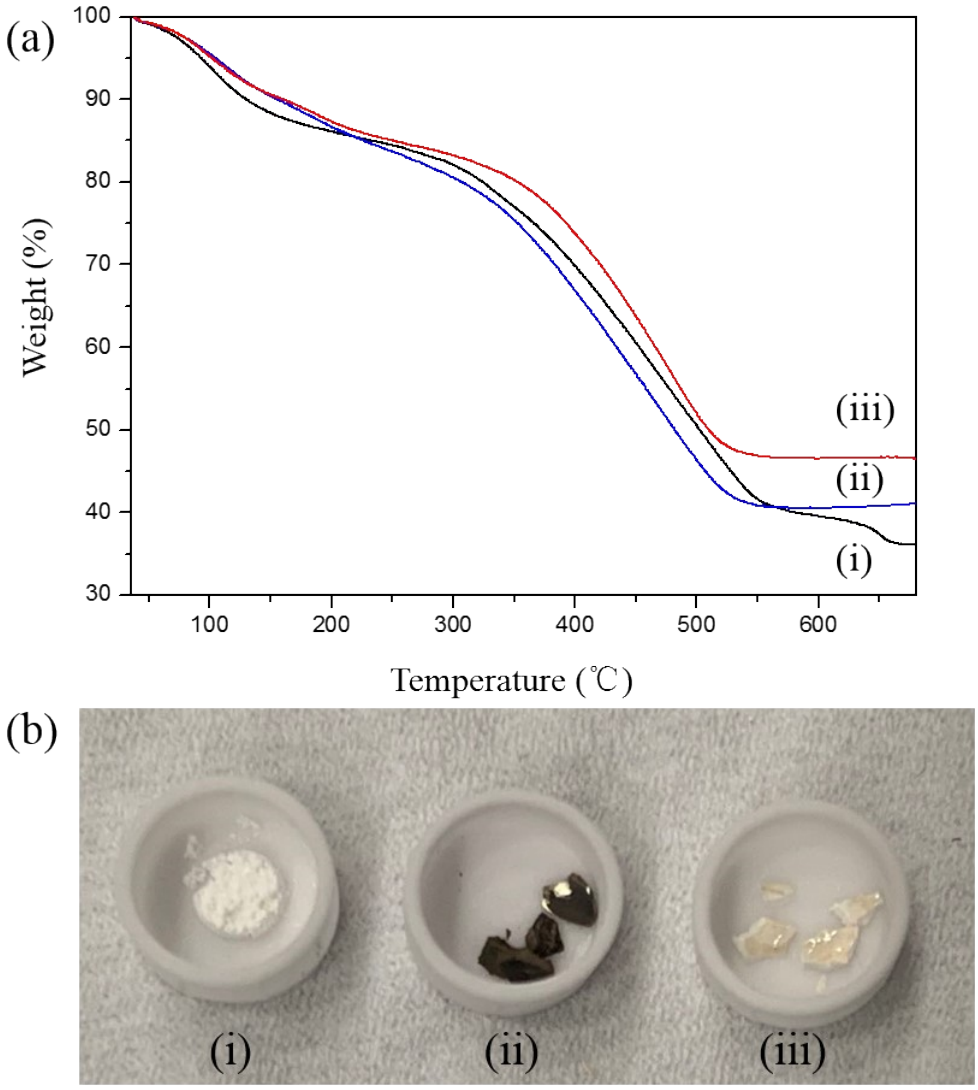

Figure S3. (a) TGA curves and (b) the picture of residues at 700 ℃ after TGA, for (i) biUIO-66-NH_2NO_2, (ii) biUIO-66-NH_2NO_2-PO_3, (iii) biDZMOF respectively.

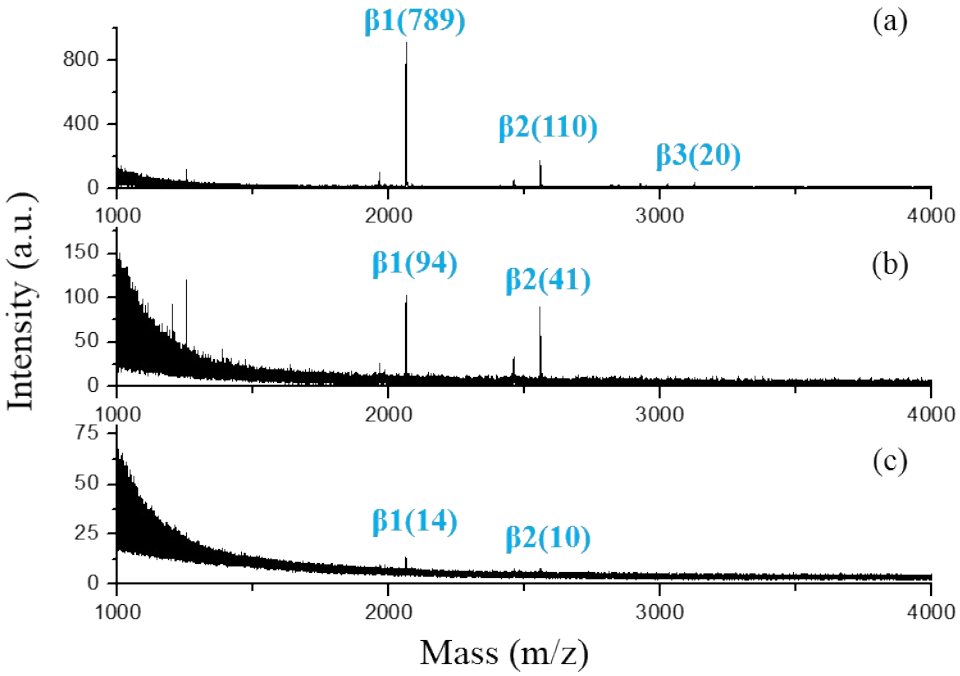

Figure S4. MALDI-TOF mass spectra of the tryptic digests of β-casein with a concentration of (a) 10 fmol/μL, (b) 0.5 fmol/μL, and (c) 0.05 fmol/μL, treated by biDZMOF respectively. The numbers in parentheses represent the S/N of the peaks.

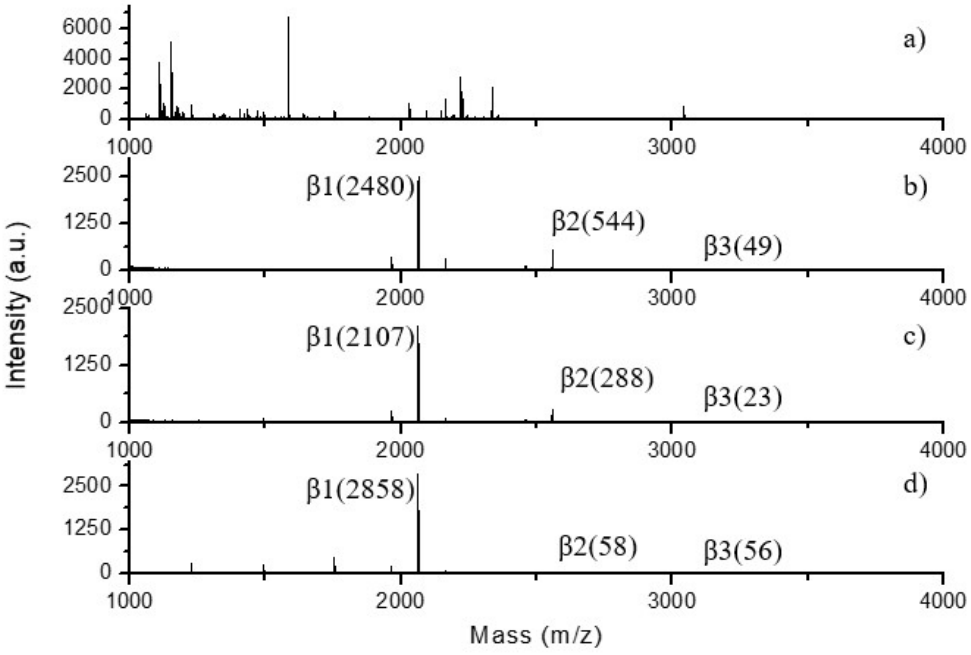

Figure S5. MALDI-TOF mass spectra of peptide mixtures of tryptic digests of β-casein and BSA with a molar ratio of (a) 1:500 without enrichment and (b) 1:1000, (c) 1:2000, and (d) 1:5000 treated by biDZMOF, respectively. The numbers in parentheses represent the S/N of the peaks.

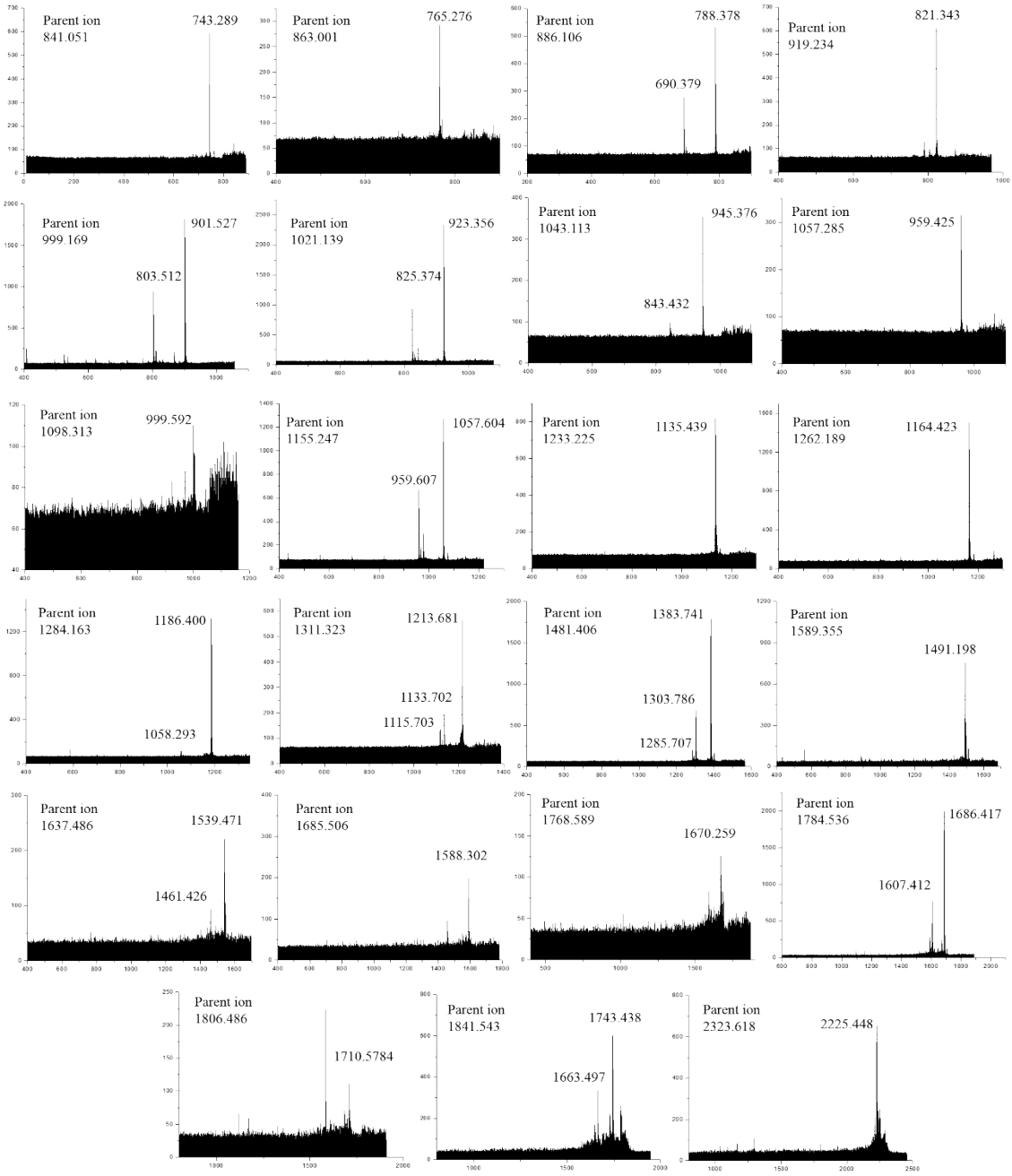

Figure S6. MALDI-TOF MS/MS spectra of phosphorylated peptides in human saliva after enrichment with biDZMOF (dephosphorylated fragments about $[M-98]^+$, $[M-98-80]^+$ or $[M-98\times2]^+$).

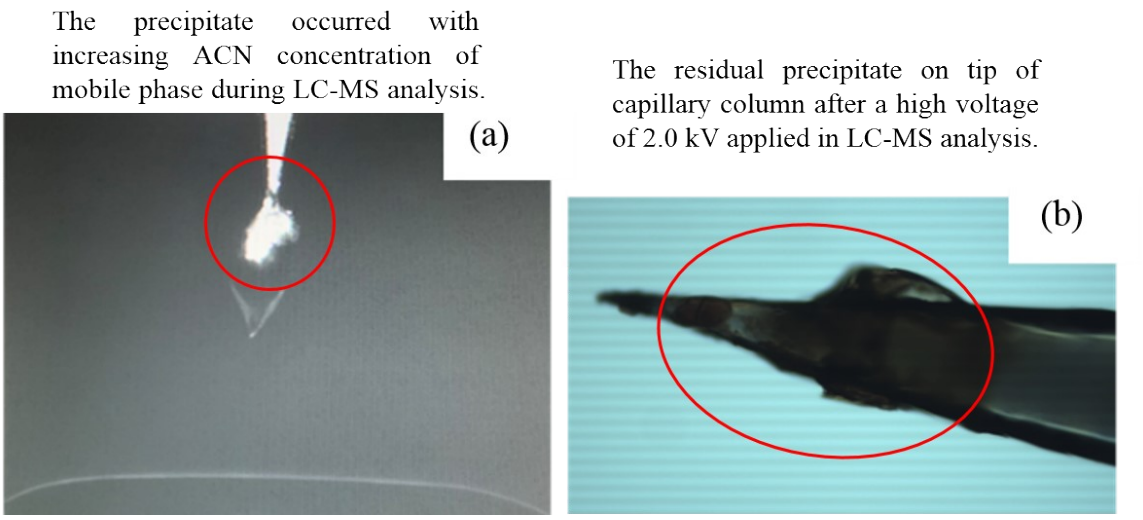

Figure S7 (a) The surveillance camera screen of Nano-RPLC-ESI-MS/MS system during the analysis of the eluate of UIO-66-NH_2 which was injected into nano-HPLC directly after acidized by FA. (b) The picture of microscope of the tip of capillary column after the direct injection of the eluate of UIO-66-NH_2.

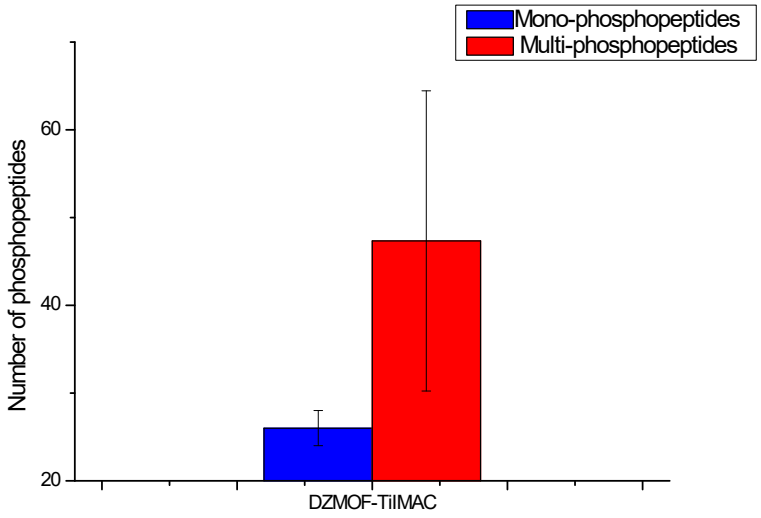

Figure S8 Phosphopeptides identified from saliva after enrichment by DZMOF-TiIMAC.

Supporting tables

Table S1. Contact angles of biUIO-66-NH_2NO_2, biUIO-66-NH_2NO_2-PO_3, and biDZMOF.

Materials	Contact angle (degree)	Images
biUIO-66-NH_2NO_2	24.3	
biUIO-66-NH_2NO_2-PO_3	28.3	
biDZMOF	21.0	

Table S2. ICP-OES results of biUIO-66-NH_2NO_2, biUIO-66-NH_2NO_2-PO_3, and biDZMOF.

Materials	Zr (wt%)
biUIO-66-NH_2NO_2	22.5
biUIO-66-NH_2NO_2-PO_3	22.3
biDZMOF	25.4