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Electronic Supplementary Information (ESI)

Porous anatase TiO_2 derived from a titanium metal-organic framework as a multifunctional phospho-oriented nanoreactor integrating accelerated digestion of proteins and *in situ* enrichment

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Experimental section

1. Materials and reagents

Terephthalic acid, N,N-dimethylformamide (DMF) and anhydrous methanol were purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). Titanium (Ⅳ) isopropoxide was purchased from Sigma Chemical (St. Louis, MO, USA).

Bovine β-casein, ammonium bicarbonate (NH_4HCO_3), L-1-tosylamido-2-phenylethylchloromethyl ketone (TPCK) treated trypsin (from bovine pancreas), α-cyano-4-hydroxy-cinnamic acid (α-CHCA), 2,5-dihydroxybenzoic acid (DHB) and trifluoroacetic acid (TFA) were purchased from Sigma Chemical (St. Louis, MO, USA). Acetonitrile (ACN) was purchased from Merck (Darmstadt, Germany). Deionized water was purified by a Milli-Q system (Milford, MA, USA).

All other chemicals and reagents were of the highest grade commercially available and used as received.

2. Synthesis of MIL-125 (Ti) and HPT

Since the hierarchical porous anatase TiO_2 (HPT) is obtained through the thermal decomposition of the titanium metal-organic framework MIL-125 (Ti), MIL-125 (Ti) MOFs were synthesized at the very beginning. MIL-125 (Ti) MOFs were prepared by a hydrothermal reaction. Detailedly, 3.0 g of terephthalic acid was firstly dispersed in 60 mL of DMF, followed by the addition of 9 mL anhydrous methanol and 3 mL titanium (Ⅳ) isopropoxide. Next, the mixture was transferred into a 200 mL Teflon-lined stainless steel autoclave and ultrasonicated for 5 min. After that, the autoclave was sealed and heated at 150 ℃ for 24 h. After cooling down to room temperature, the resulting white precipitation was gathered by centrifugation, and sequentially washed with methanol and DMF. The products were dried in vacuum at 50 ℃ for characterization and future use.

HPT was synthesized by the hydrolysis of the MIL-125 (Ti) MOFs precursor and subsequent calcination in air. In detail, 500 mg of MIL-125 (Ti) powders were dispersed in 200 mL of deionized water and refluxed at 90 ℃ for 3 h. The pretreated MOFs were washed with deionized water three times and then with ethanol three times. After washing and drying, the MIL-125 (Ti) powders were calcined at 400 ℃ for 4 h. The heating temperature was raised from room temperature to 400 ℃ at a heating rate of 5 ℃ min^{-1}.

3. Characterizations of MIL-125 (Ti) and HPT

Various characterization technologies were used to demonstrate the successful synthesis of MIL-125 (Ti)

MOFs and HPT.

The scanning electron microscope (SEM) images and the energy dispersive X-ray (EDX) spectra were taken to reveal the morphology and chemical composition of MIL-125 (Ti) and HPT. The SEM images were recorded on a Nova NanoSem 450 electron microscope (FEI, USA) operated at 15 kV, and the EDX spectra were collected on a Phenom Prox electron microscope (Phenom, Netherlands) operating at 15 kV.

The transmission electron microscope (TEM) images were taken to observe the structure of MIL-125 (Ti) and HPT. The TEM observation was characterized by a JEM-2011 electron microscope (JEOL, Japan) operated at 200 kV. The samples were dispersed in ethanol beforehand and collected for analysis by using carbon film-covered copper grids.

The powder X-ray diffraction (XRD) patterns were collected to confirm the crystal structure of MIL-125 (Ti) and HPT. The XRD patterns were identified using a D8 Advance X-ray diffractometer (Bruker, Germany) with Ni-filtered CuKα radiation (40 kV, 40 mA).

The nitrogen adsorption-desorption isotherms were measured to estimate the surface area and the pore size of HPT. The N_2 adsoprtion-desorption isotherms were recorded at 77 K with a Micromeritcs Tristar 3000 analyzer (Tristar, USA). The samples were degassed in vacuum at 200 ℃ for 8 h prior to measurement. The Brunauer-Emmett-Teller (BET) method was adopted to calculate the surface area and the Barrett-Joyner-Halenda (BJH) method was used to determine the average pore size.

The Fourier transform infrared (FT-IR) spectra and the Raman spectra were recorded to show the characteristic groups of MIL-125 (Ti) and HPT. The positions and intensities of the peaks in the spectra change with the collapse of MIL-125 (Ti) and the formation of HPT. The FT-IR spectra were collected on a Nexus 470 Fourier spectrophotometer (Nicolet, USA) using KBr pellets. The Raman spectra observation was performed at room temperature on a LabRam-1B Raman spectrometer (JY, France) with a laser at an excitation wavelength of 632.8 nm.

4. Tryptic digestion of bovine β-casein and the commercial nonfat bovine milk

To prepare the NH_4HCO_3 solution of bovine β-casein, we dissolved 5 mg of β-casein in 1 mL of deionized water and denatured it in a boiling water bath for 5 min. The β-casein aqueous solution was exchanged into 25 mM NH_4HCO_3 buffer (with the final pH of 8.3 and the final concentration of 1 μg/μL). For the in-solution digestion of β-casein, we incubated the β-casein solution with trypsin at 37 ℃ for 16 h. The weight (of trypsin) to weight (of protein) ratio was 1:40. The resultant tryptic digest was diluted with

25 mM NH_4HCO_3 solution to lower concentrations for the investigation on low-concentration β-casein tryptic digests.

Before the in-solution and the HPT-assisted digestion of commercial nonfat bovine milk, 30 μL of the milk was diluted with 900 μL of 25 mM NH_4HCO_3 solution. The solution was then centrifugated at 10000 rpm for 25 min, and the supernatant was collected for tryptic digestion.

5. Activation of HPT

To enrich phosphopeptides from β-casein tryptic digests or to accelerate the digestion process of β-casein protein, we dispersed 10 mg of HPT in 1 mL of 50% ethanol (v/v) aqueous solution with the help of ultrasonication in advance. And we activated the HPT by washing it with 100 μL of 50%ACN/0.1%TFA (v/v) buffer three times before the enrichment or the assisted digestion.

6. Preparation of α-CHCA and DHB matrix

To prepare the α-CHCA matrix solution, we dissolved 8 mg of α-CHCA in 1 mL of 50%ACN/0.1%TFA (v/v) buffer. Meanwhile, 6 mg of ammonium hydrogen citrate was dissolved in 10 mL of 50%ACN/0.1%TFA buffer. The two solutions of equal volume were mixed to obtain the α-CHCA matrix.

To prepare the DHB matrix solution, we dissolved 20 mg of DHB in 1 mL of 50% ACN (v/v) aqueous solution containing 1% H_3PO_4 (v/v).

7. Enrichment of phosphopeptides from β-casein tryptic digests

Firstly, the β-casein tryptic digest was diluted to different concentrations with 50%ACN/0.1%TFA. Next, 200 μg of the preactivated HPT was added into each dilution (with the volume of 200 μL). The mixtures were vibrated in a shaker at 37 ℃ for 30 min to ensure equilibrium. After centrifugation and removal of the supernatant, the HPT was rinsed with 200 μL of 50%ACN/0.1%TFA three times. Afterwards, 10 μL of 0.4 M ammonium was added and vibrated for 10 min to elute the phosphopeptides captured in the HPT. The eluent was deposited on a MALDI sample target (Applied Biosystems/MDS SCIEX, Foster City, CA, USA) and dried at room temperature. For comparison, the β-casein tryptic digests without enrichment were also dropped on the same sample target. Later on, 0.8 μL of DHB matrix was deposited on it and dried. Four replicate spots were taken for every sample. The substrates were submitted to MALDI-TOF MS for analysis.

8. In-solution digestion in the presence of HPT

Before the HPT-assisted digestion, 200 μg of HPT was activated with 50%ACN/0.1%TFA and was then added into 200 μL of β-casein in-solution digestion systems (with the concentrations of 1 μg/μL, 0.1 μg/μL and 0.01μg/μL) or the nonfat bovine milk in-solution digestion system (with the concentration of 1 μg/μL). The mixtures were incubated at 37 ℃ for 30min. After incubation, HPT was separated from the supernatant by centrifugation and rinsed with 200 μL of 50%ACN/0.1%TFA three times. After that, 10 μL of 0.4 M ammonium was added to elute the phosphopeptides captured in HPT. The supernatant and the eluent were deposited on a MALDI sample target and dried at room temperature, followed by the addition of α-CHCA (for the supernatant) or DHB (for the eluent) matrix.

For comparison, in-solution digestions with an enzyme to protein ratio of 1:40 (w/w) for 16 h and 30 min were also carried out under the same condition. The tryptic digestion products were dropped on a MALDI sample target and dried. Finally, the α-CHCA matrix was dropped on the sample spots.

9. MALDI-TOF MS analyses

MALDI-TOF MS analyses were performed in positive reflective mode on a 5800 Proteomics Analyzer (Applied Biosystems, Framingham, MA, USA) with the Nd: YAG laser (383 nm) operated at a repetition rate of 200 Hz and an acceleration voltage of 20 kV. All the spectra were taken from signal-averaging of 1000 laser shots with the laser intensity kept at a proper constant.

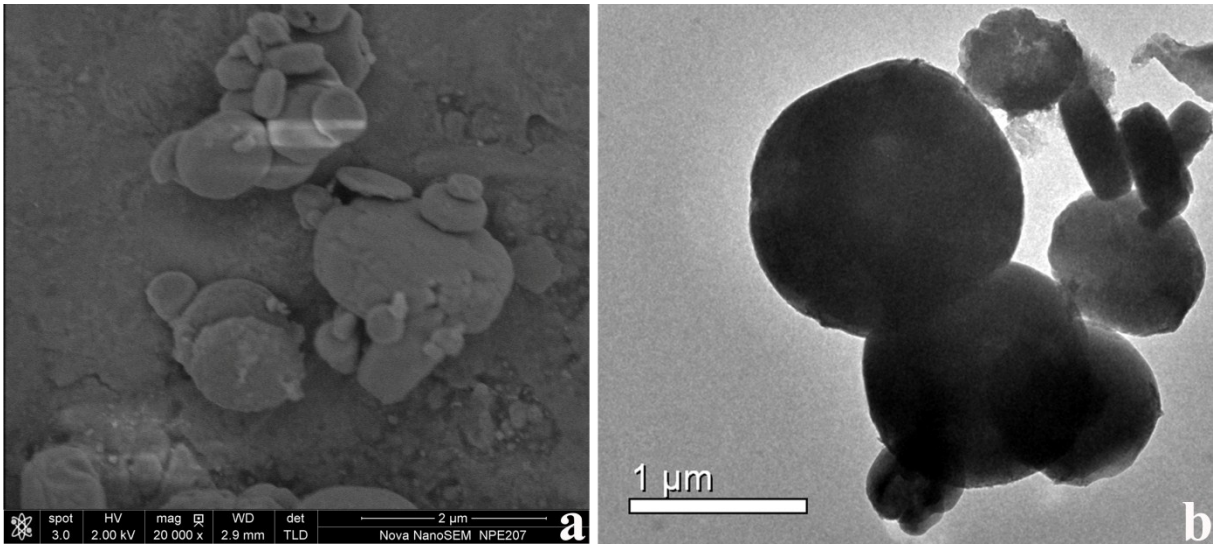


Fig. S1 The SEM image (a) and TEM image (b) of MIL-125 (Ti) MOFs.

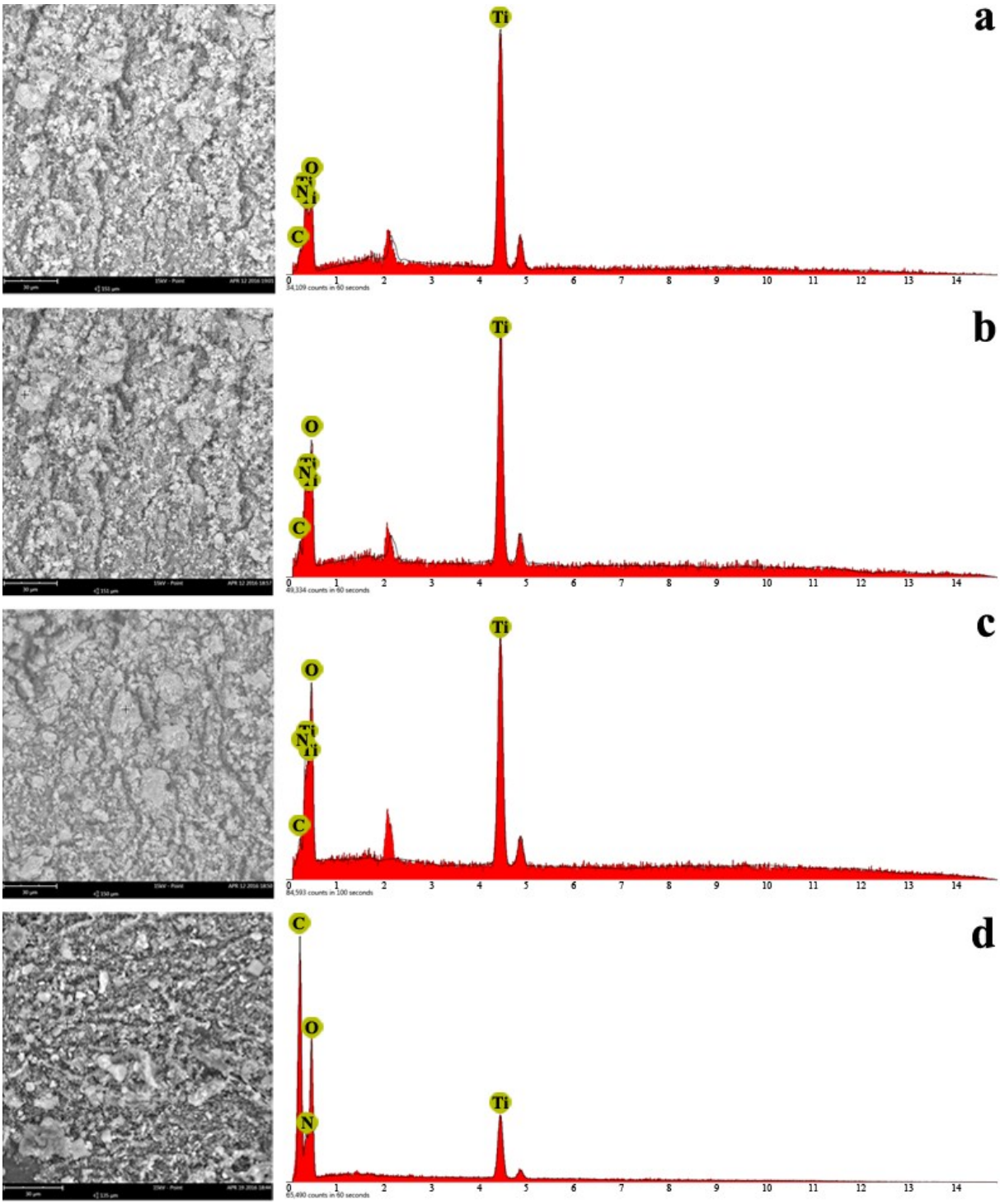


Fig. S2 The energy dispersive X-ray (EDX) spectra of HPT (a, b and c) and MIL-125 (Ti) (d) and the corresponding regions chosen for EDX analysis. Three different regions were selected for the EDX analysis of HPT.

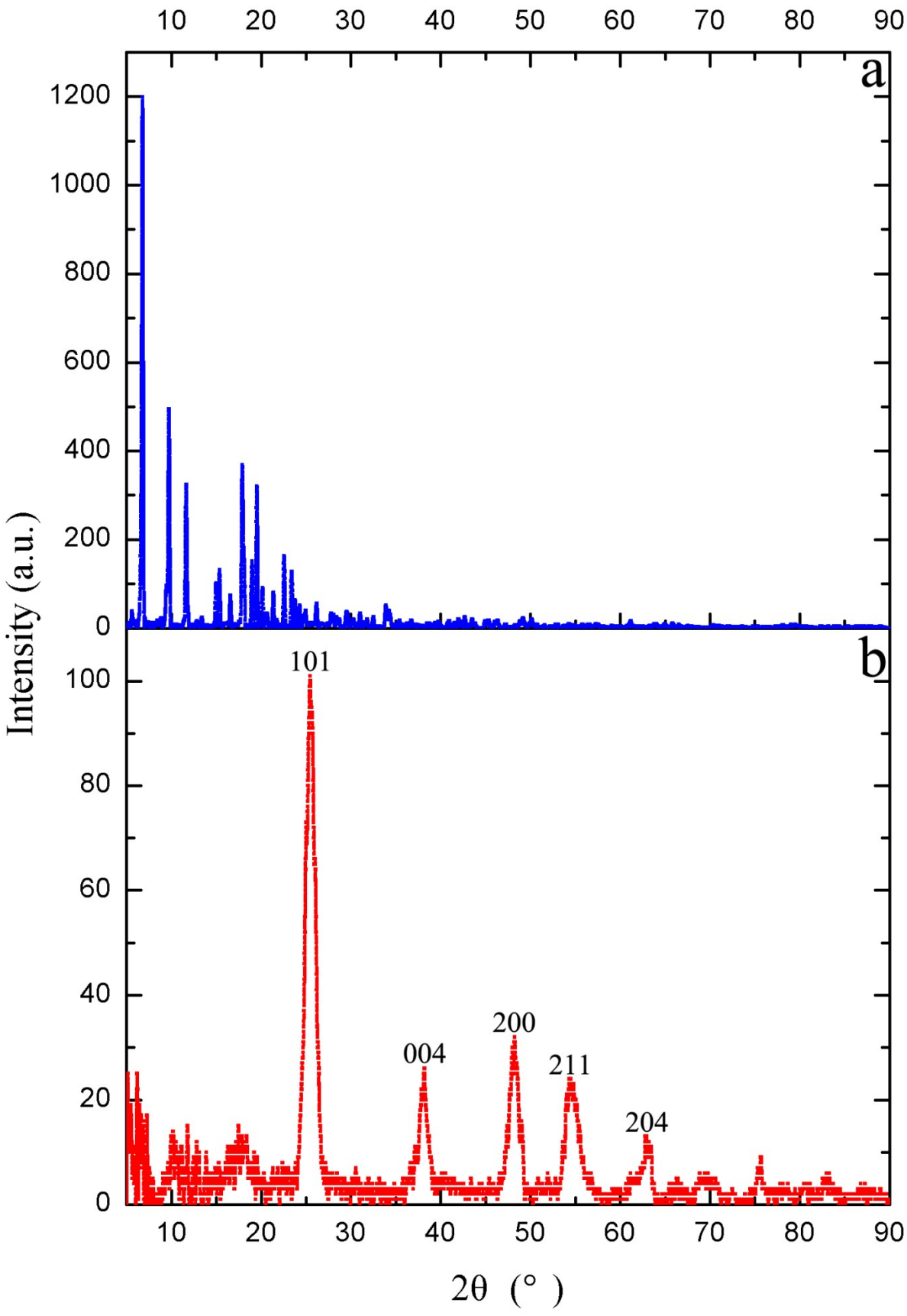
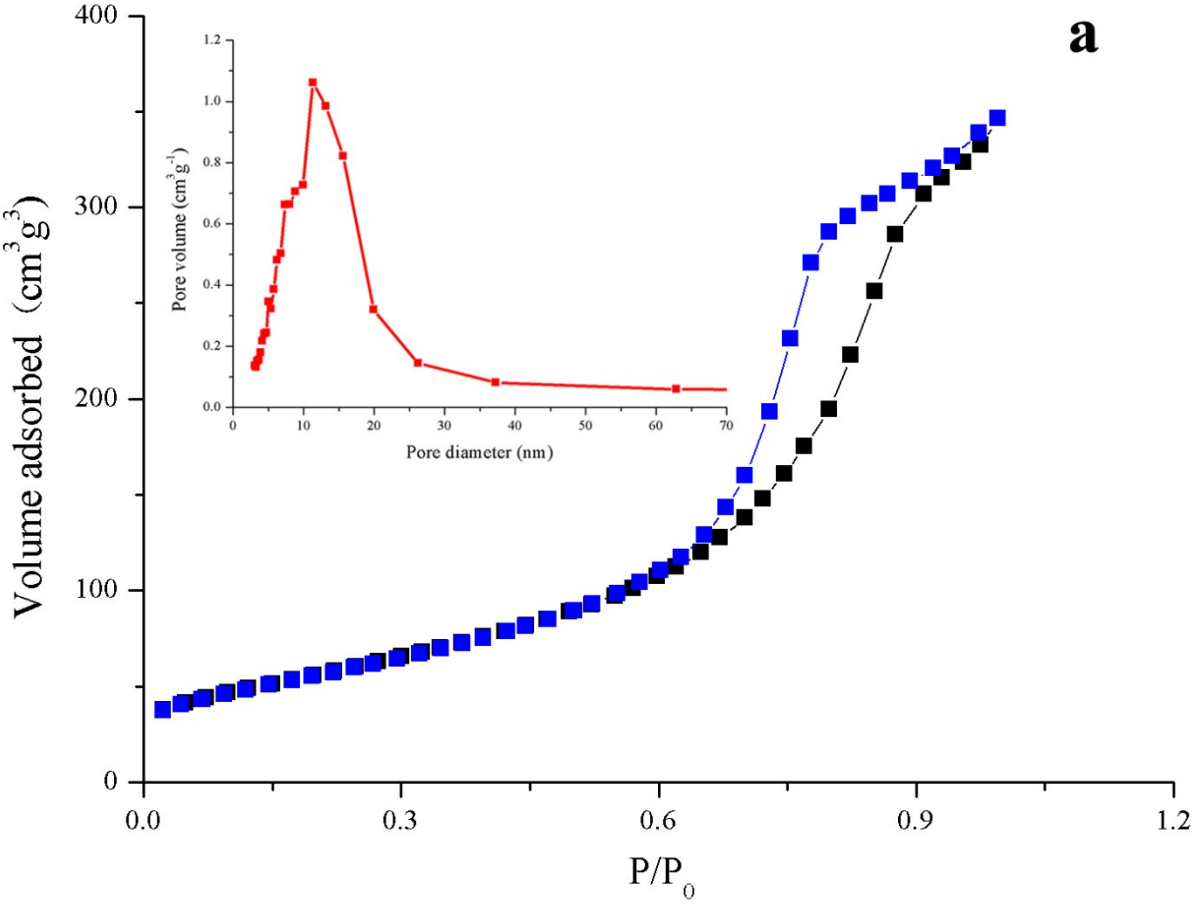


Fig. S3 The XRD patterns of MIL-125 (Ti) MOFs (a) and HPT (b). Peaks originated from HPT are marked with miller indexes.


a
Volume adsorbed (cm3 g-1)
P/P0
Pore volume (cm3 g-1)
Pore diameter (nm)

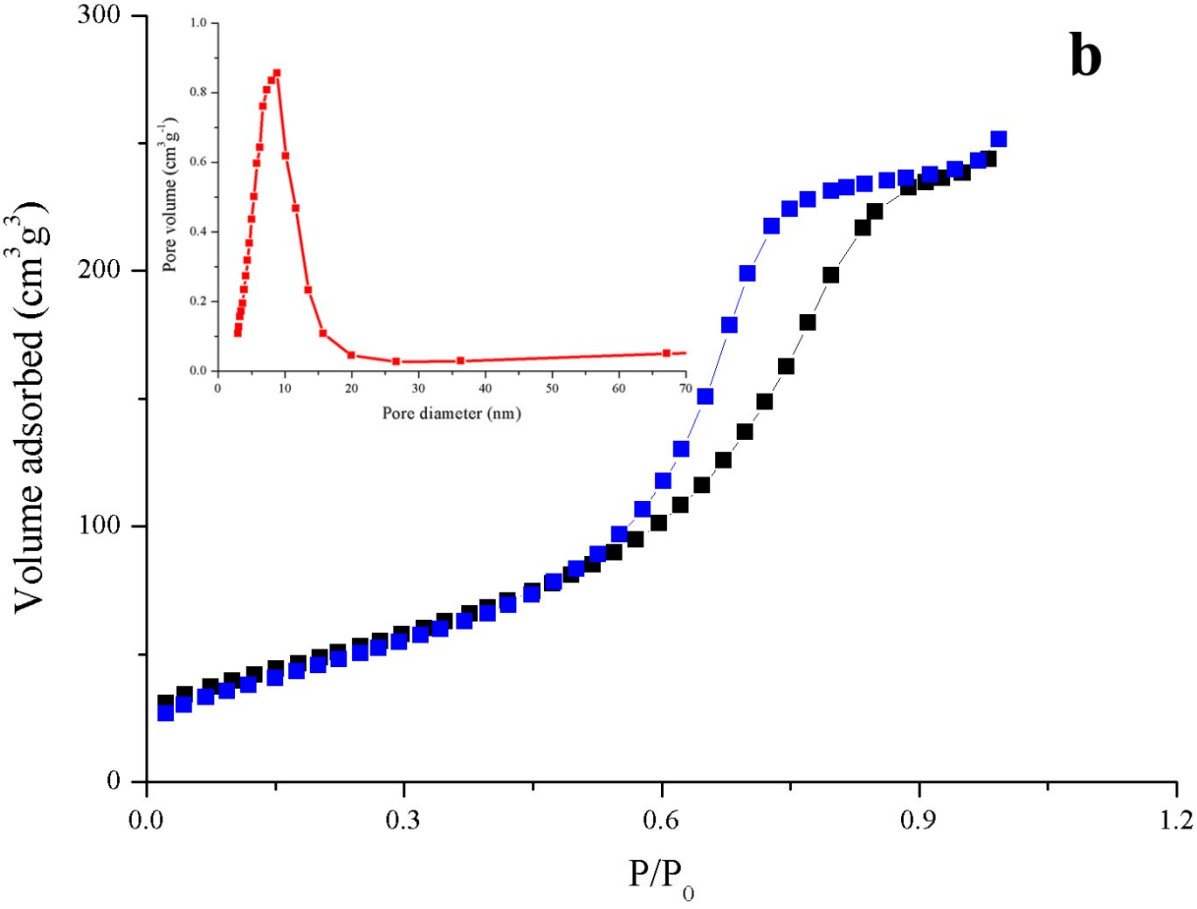

b
Volume adsorbed (cm3 g-1)
P/P0
Pore volume (cm3 g-1)
Pore diameter (nm)

Fig. S4 The nitrogen adsorption-desorption isotherms of HPT with (a) and without (b) the hydrolysis of MIL-125 (Ti) MOFs measured at 77 K. The inset shows the corresponding pore size distribution analysis obtained using the Barrett-Joyner-Halenda (BJH) method.

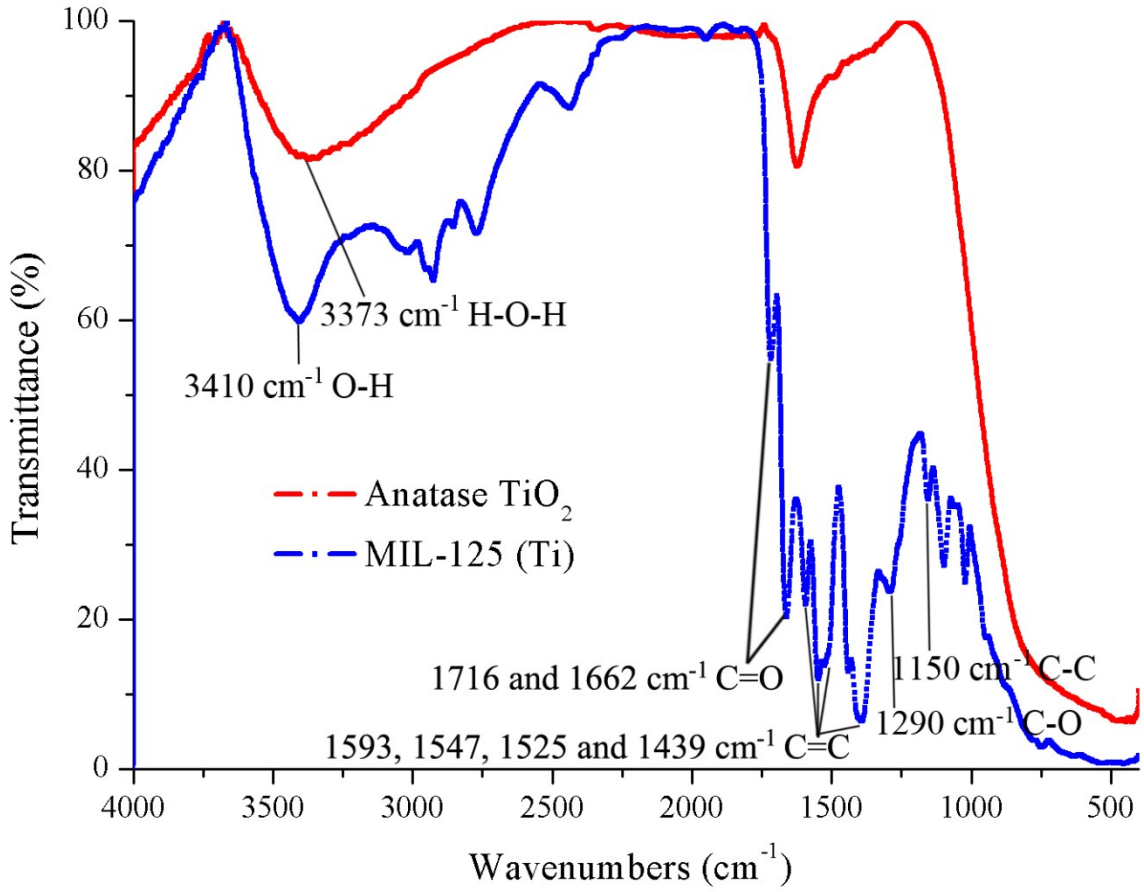


Fig. S5 The FT-IR spectra of MIL-125 (Ti) and HPT.

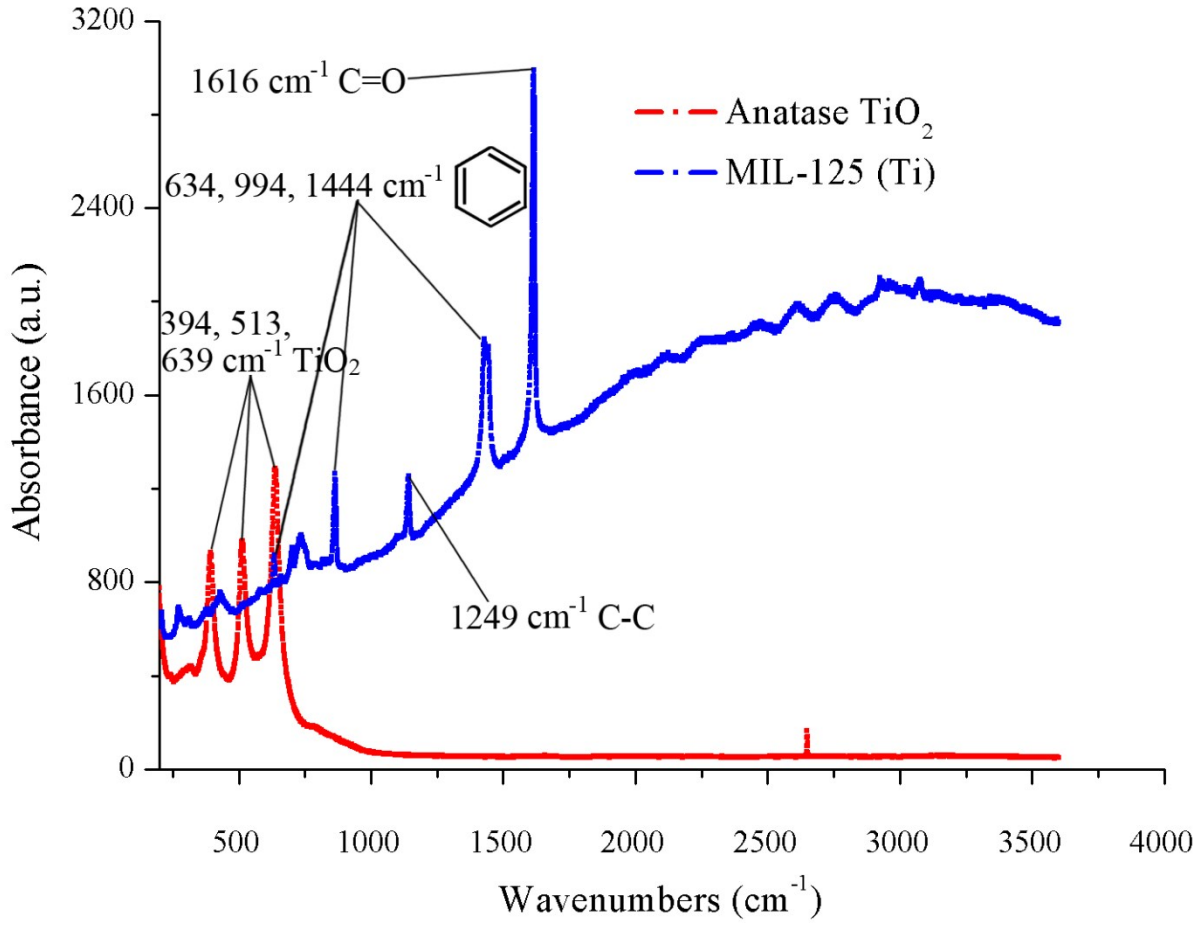


Fig. S6 The Raman spectra of MIL-125 (Ti) and HPT.

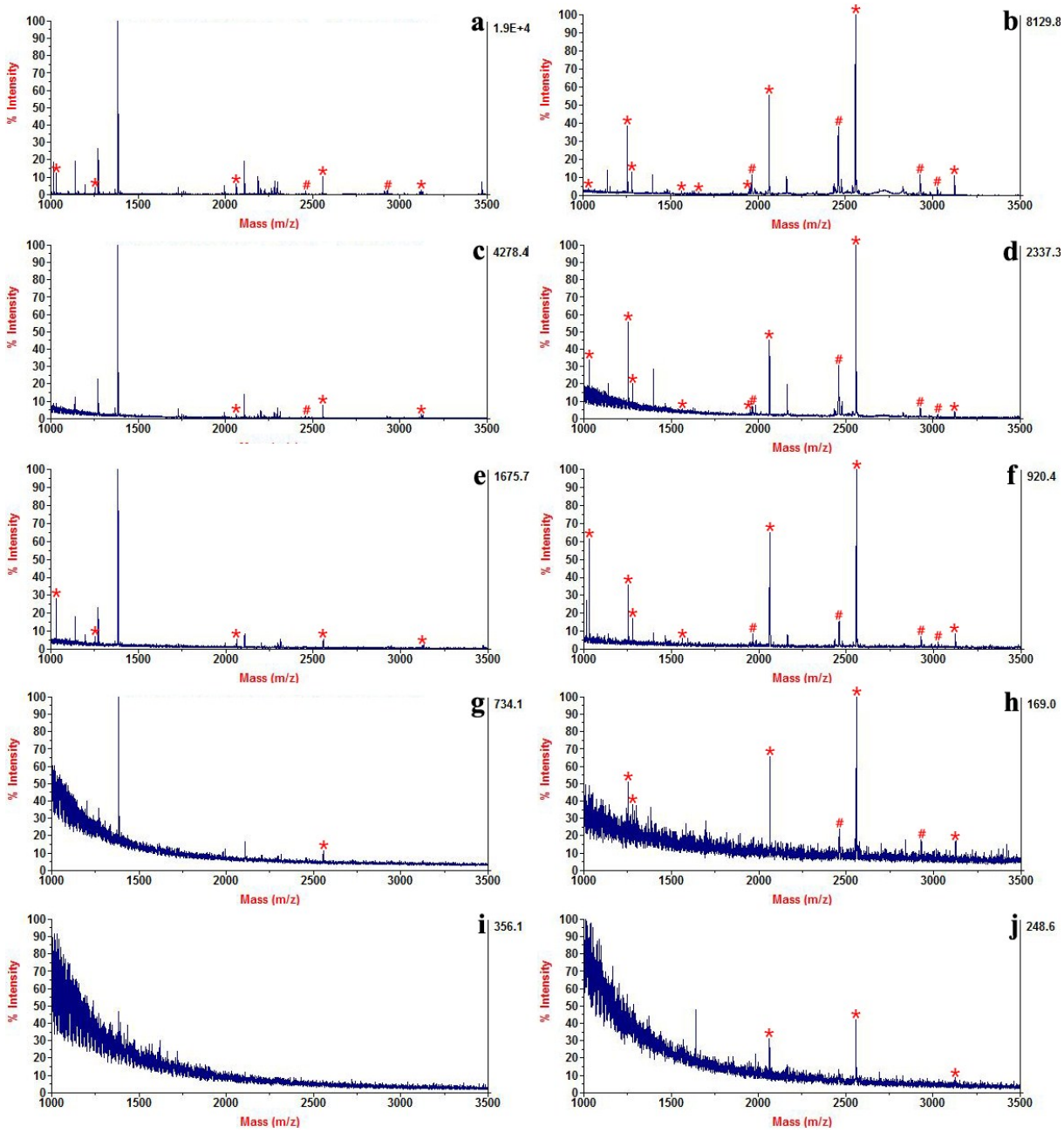


Fig. S7 MALDI-TOF mass spectra of the β-casein tryptic digests with various concentrations before enrichment: (a) 4×10^{-7} M, (c) 4×10^{-8} M, (e) 4×10^{-9} M, (g) 4×10^{-10} M and (i) 4×10^{-11} M; and after enrichment with HPT: (b) 4×10^{-7} M, (d) 4×10^{-8} M, (f) 4×10^{-9} M, (h) 4×10^{-10} M and (j) 4×10^{-11} M. The peaks marked with asterisks represent phosphopeptides and those marked with pound signs represent dephosphorylated peptides.

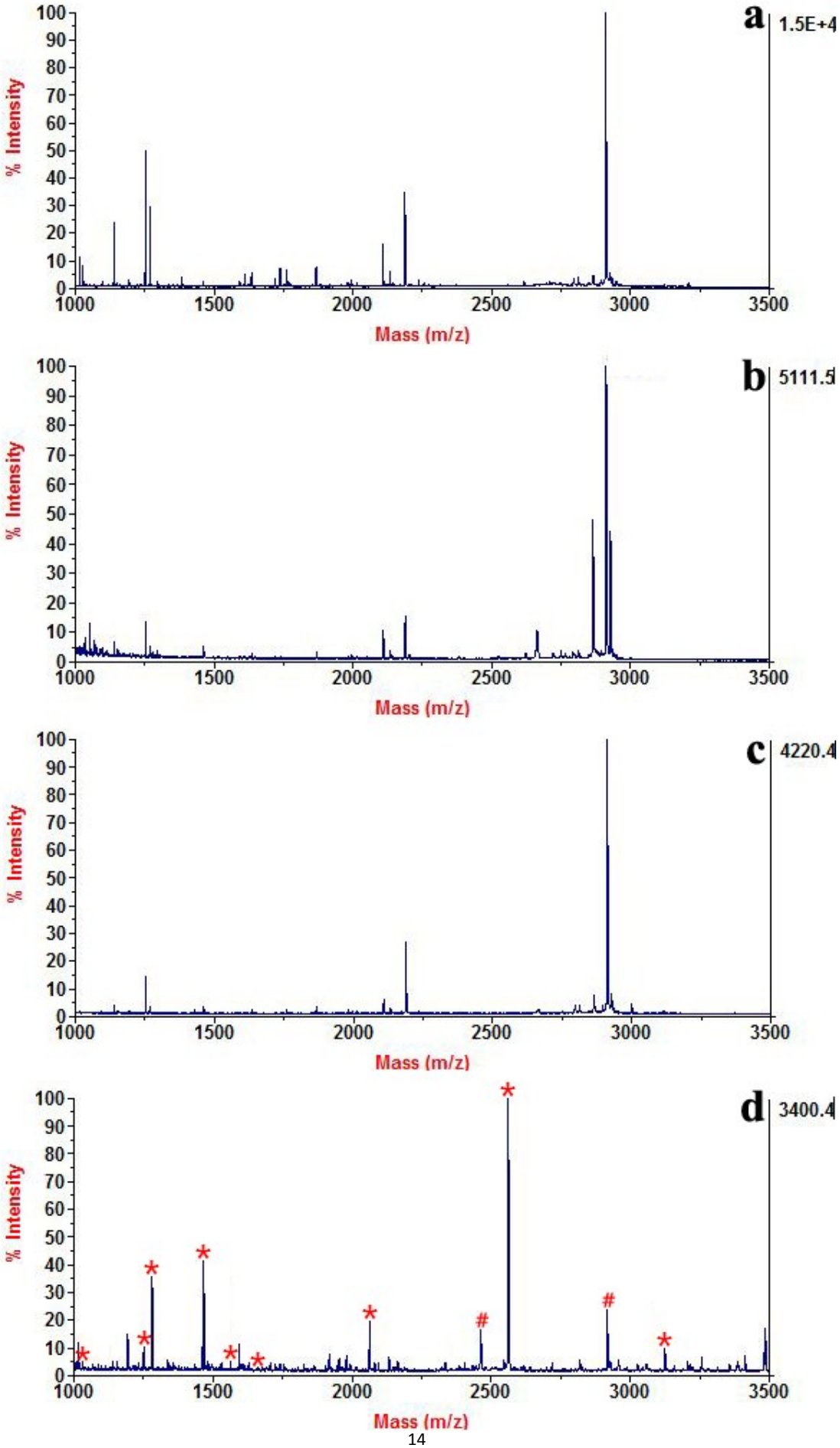

a
1.5E+4
b
5111.5
c
4220.4
d
3400.4
% Intensity
Mass (m/z)
100
90
80
70
60
50
40
30
20
10
0
1000
1500
2000
2500
3000
3500
*
#

Fig. S8 MALDI-TOF mass spectra of the β-casein solution (0.1 μg/μL) (a) after 16 h in-solution tryptic digestion and (b) after 30 min in-solution digestion; (c) the supernatant and (d) the eluent of the β-casein solution (0.1 μg/μL) after 30 min HPT-assisted digestion.

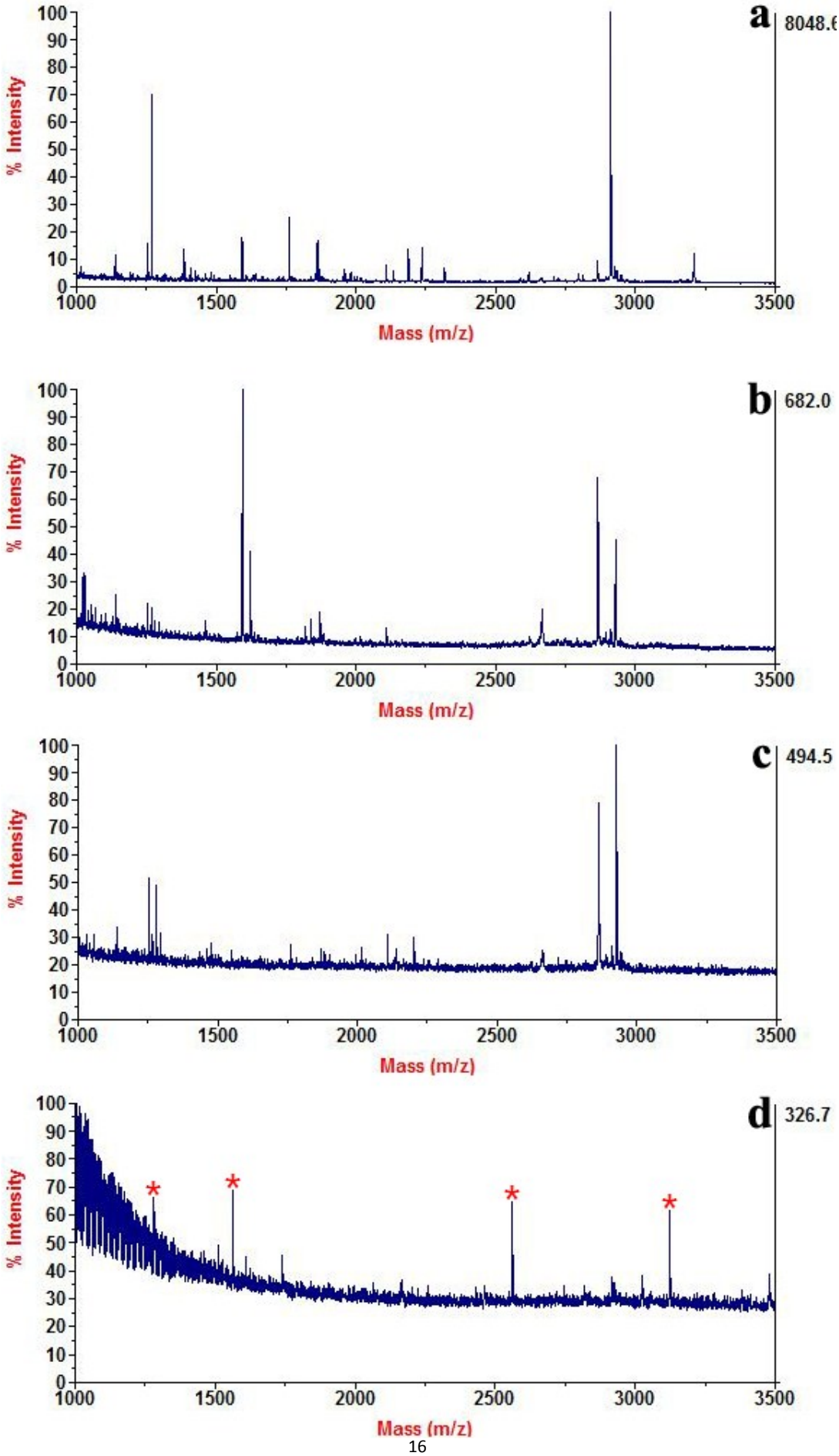

a
8048.6
b
682.0
c
494.5
d
326.7
% Intensity
Mass (m/z)
100
90
80
70
60
50
40
30
20
10
0
1000
1500
2000
2500
3000
3500
*

Fig. S9 MALDI-TOF mass spectra of the β-casein solution (0.01 μg/μL) (a) after 16 h in-solution tryptic digestion and (b) after 30 min in-solution digestion; (c) the supernatant and (d) the eluent of the β-casein solution (0.01 μg/μL) after 30 min HPT-assisted digestion. The peaks marked with asterisks represent phosphopeptides.

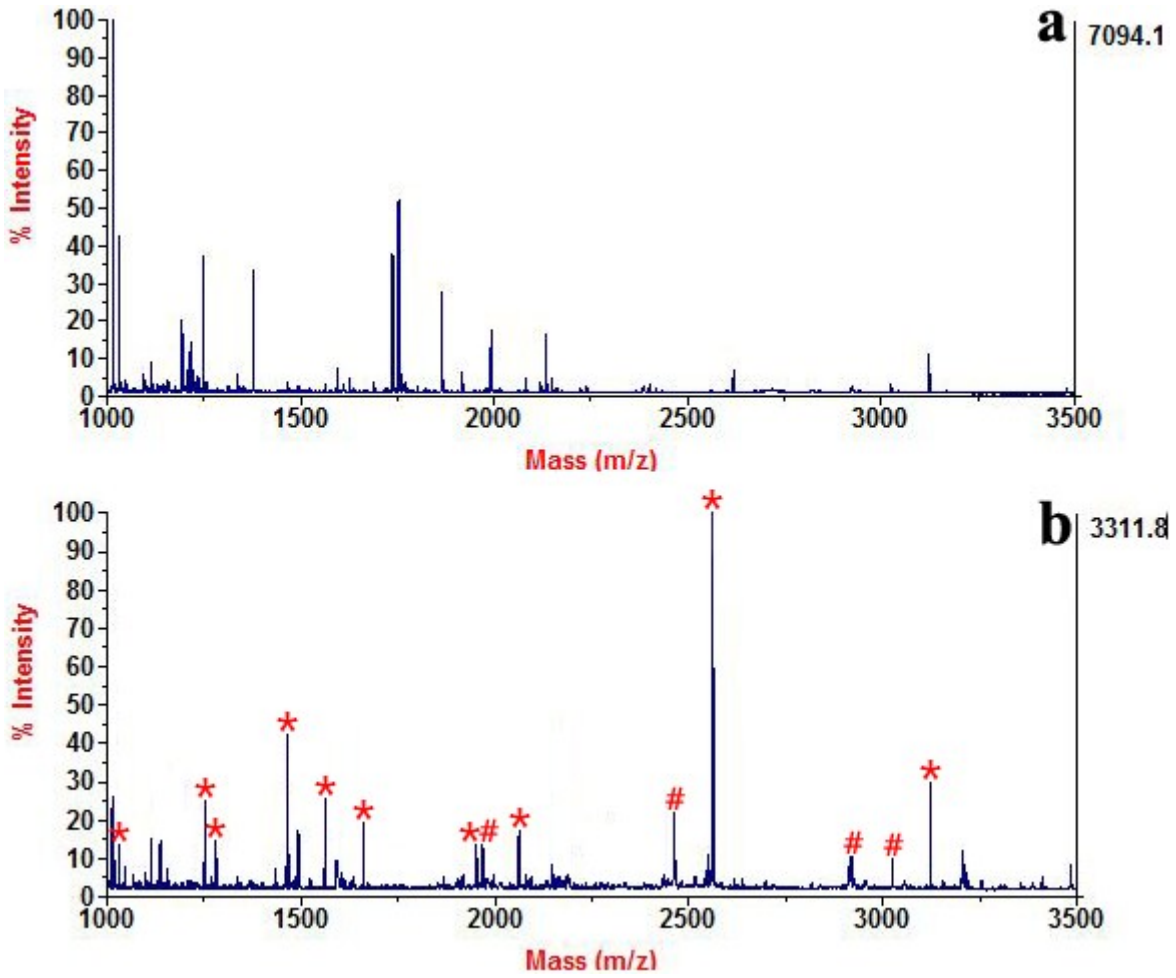


Fig. S10 MALDI-TOF mass spectra of the β-casein solution (1 μg/μL) after 30 min digestion and *in situ* enrichment: (a) with HPT derived from the MIL-125 (Ti) MOFs without hydrolysis; (b) with HPT derived from the MIL-125 (Ti) MOFs with hydrolysis.

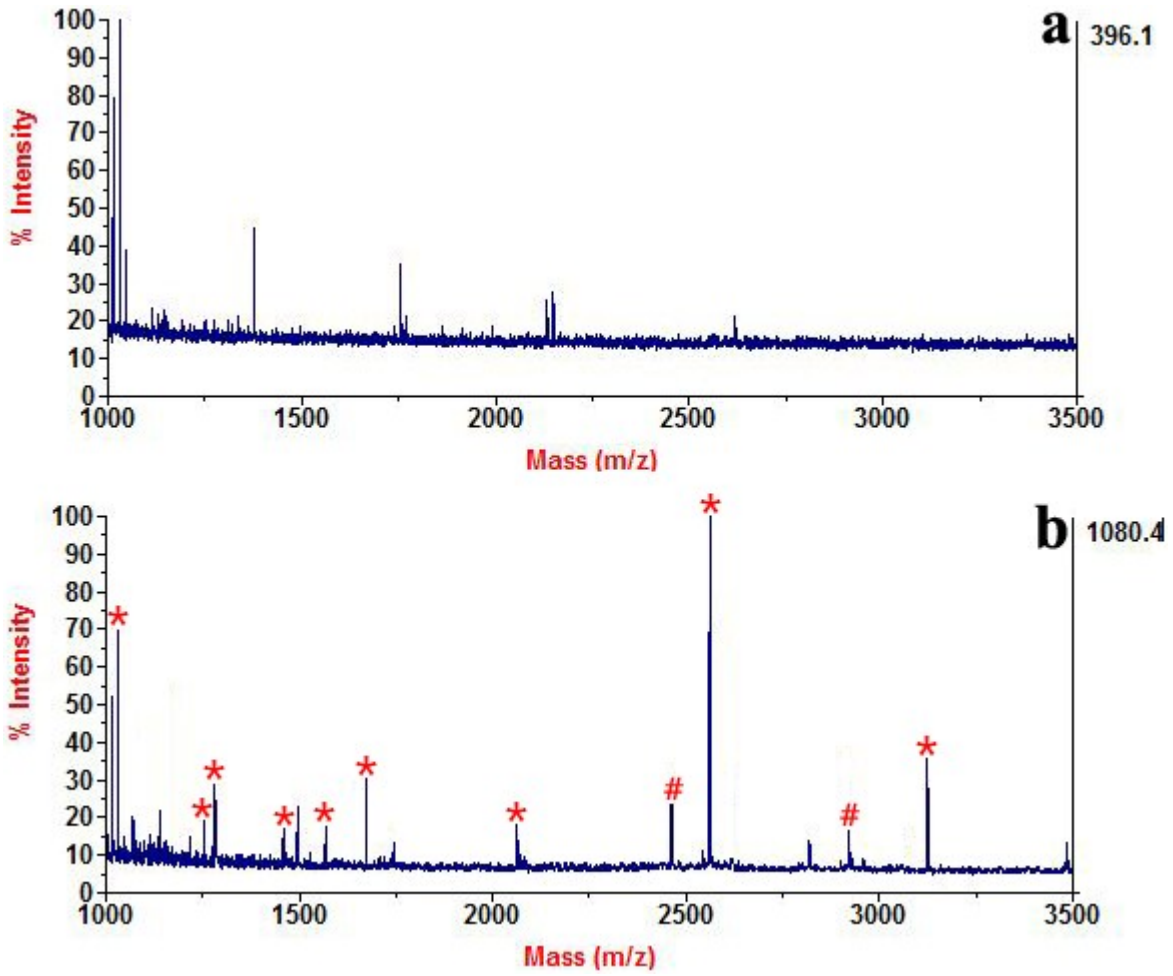


Fig. S11 MALDI-TOF mass spectra of the β-casein solution (0.1 μg/μL) after 30 min digestion and *in situ* enrichment: (a) with HPT derived from the MIL-125 (Ti) MOFs without hydrolysis; (b) with HPT derived from the MIL-125 (Ti) MOFs with hydrolysis.

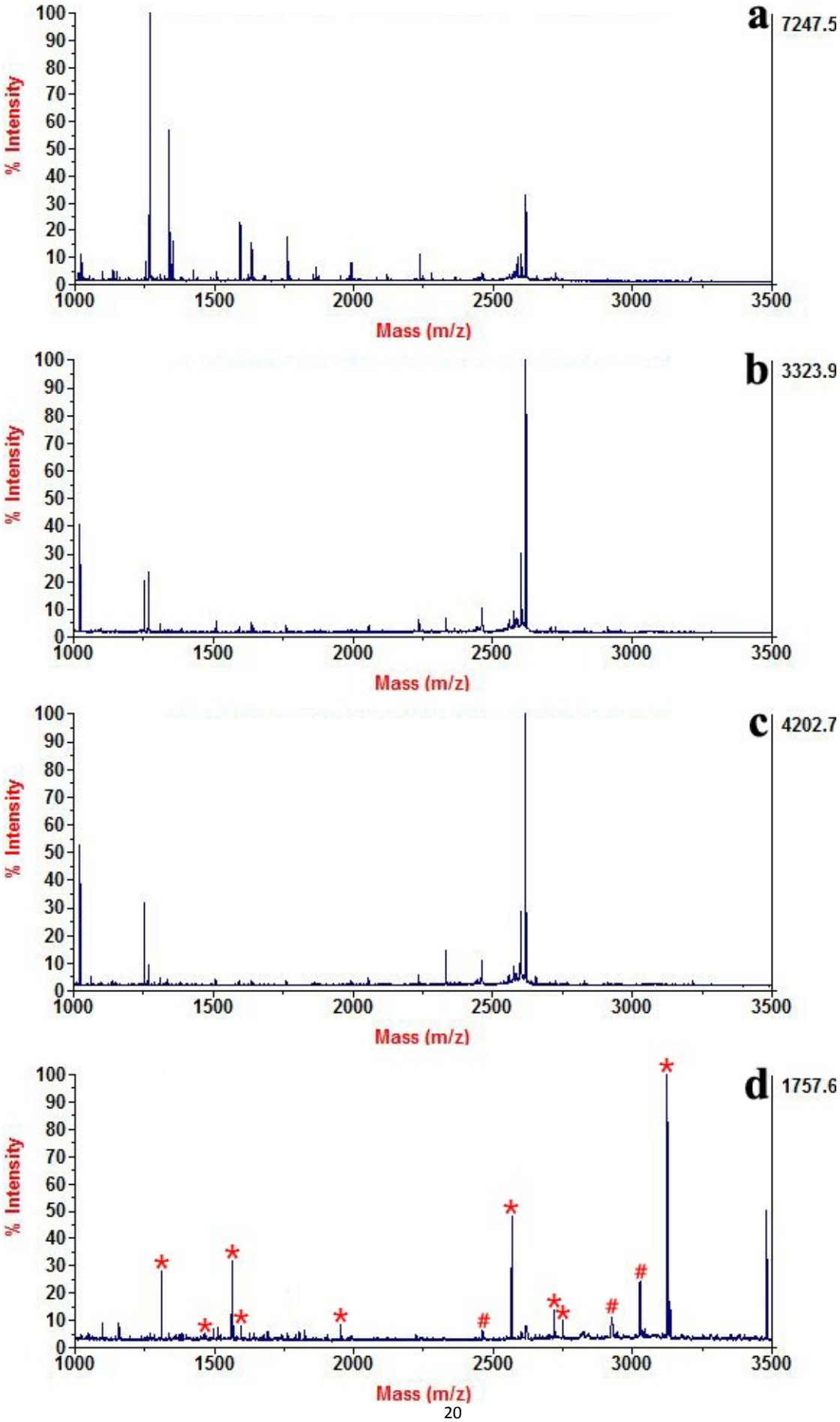

a
7247.5
% Intensity
Mass (m/z)
b
3323.9
% Intensity
Mass (m/z)
c
4202.7
% Intensity
Mass (m/z)
d
1757.6
% Intensity
Mass (m/z)

Fig. S12 MALDI-TOF mass spectra of the nonfat bovine milk solution (1 μg/μL) after (a) 16 h in-solution tryptic digestion and (b) after 30 min in-solution digestion (b); (c) the supernatant and (d) the eluent of the nonfat bovine milk solution after 30 min HPT-assisted digestion.

Table S1. The EDX analysis results of HPT and MIL-125 (Ti)

Sample	Run	Element symbol	Weight concentration / %	Error
HPT	1	Ti	35.4	0.1
		O	42.9	0.2
		N	16.4	2.3
		C	5.3	2.0
	2	Ti	29.0	0.0
		O	48.1	0.4
		N	17.8	2.9
		C	5.1	1.6
	3	Ti	26.2	0.1
		O	53.8	0.1
		N	15.8	3.3
		C	4.1	1.2
MIL-125 (Ti)	1	C	32.0	0.9
		O	39.4	0.0
		Ti	6.4	0.1
		N	22.2	3.8

Table S2. Detailed information of the phosphopeptides identified from β-casein tryptic digests after enrichment with HPT and from β-casein solutions after 30 min HPT-assisted digestion

Theoretical m/z	aa	Peptide sequence
3122.27	β/1-25	RELEELNVPGEIVE[pS]L[pS][pS][pS]EESITR
2556.09	β/33-52	FQ[pS]EEQQQTEDELQDKIHPF
2061.83	β/33-48	FQ[pS]EEQQQTEDELQDK

1951.95	α-S1/104-119	YKVPQLEIVPN[pS]AEER
1660.75	α-S1/106-119	VPQLEIVPN[pS]AEER
1561.70	α-S2/126-137	EQL[pS]T[pS]EENSKK
1466.51	α-S2/138-149	TVDME[pS]TEVFTK
1278.60	β/33–52	FQ[pS]EEQQQTEDELQDKIHPF
1253.11	α-S2/138-147	TVD[Mo]ME[pS]TEVF
1030.91	β/33-48	FQ[pS]EEQQQTEDELQDK