Supplementary Material (ESI) for Chemical Communications
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Supporting Information

Experimental details

Chemicals and reagents

Pseudoboehmite (78.4 wt% Al_2O_3), phosphoric acid (85 wt%), triethylamine (TEA, 99%), tetrabutyl titanate (IV) (99%) and glucose (AR) were obtained from Tianjin Kermel Chemical Reagent Development Center (Tianjin, China) without further purification. α- and β-caseins (from bovine milk), trypsin (from porcine pancreas, TPCK treated), bovine serum albumin (BSA) and 2,5-dihydroxybenzoic acid (2,5-DHB) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Urea, ammonium bicarbonate, dithiothreitol (DTT), and iodoacetamide (IAA) were obtained from BioRad (Hercules, CA, USA). Acetonitrile (ACN) and trifluoroacetic acid (TFA) were purchased from Merck (Darmstadt, Germany). All the water used in experiments was prepared using a Milli-Q purification system (Millipore, Bedford, MA, USA). All the other chemicals and reagents were of analytical grade and were obtained from Shanghai Chemical Reagent.

Synthesis of hierarchical TiAPO-5

Pseudoboehmite treated with KOH was prepared with the following procedure: 20 g of pseudoboehmite was dispersed in 50 mL of ethanol solution of KOH (0.1 mol/L) at refluxing condition and the mixture was stirred for 2 h. Then the solid was filtrated and washed with ethanol until the filtrate was neutral. Finally, it was dried at room temperature for 48 h. TiAPO-5H-*x* was prepared with the following molar composition: 1.0 Al: 1.0 P: 0.77 TEA: 25 H_2O: *x* Ti. In a typical synthesis, the calculated amount of KOH treated-pseudoboehmite was added to deionized water in a plastic beaker under stirring, followed by the addition of glucose. And then phosphoric acid was added into the solution. After stirring for 1 h, the addition of tetrabutyl titanate was carried out. TEA was added dropwise after stirring for 20 h. The obtained mixture was stirred for another 1 h. Finally, the obtained gel was transferred into a stainless-steel autoclave lined with polytetrafluorethylene and crystallized statically at 180 °C for 48 h. After the hydrothermal process, the solid product was filtered, washed with deionized water, dried at 120 °C for 12 h, and calcined at 550 °C in air for 24 h. The ordinary TiAPO-5-0.05 was synthesized as comparision without the addition of glucose.

Characterizations

Powder X-ray diffraction (XRD) was performed on a Panalytical X'pert PRO instrument with Cu Kα (λ = 0.15418 nm) radiation. Tube voltage and tube current are 40 kV and 40 mA, respectively. The adsorption/desorption isotherms were measured with a Quantachrome Autosorb using N_2 as adsorbate at 77 K. Samples were outgassed at 150 °C for 1.5 h prior to measurements. Total surface area was calculated according to BET method, and pore size distributions were calculated from the desorption branch of the isotherm based on BJH method. UV-Vis DRS were collected

on Shimadzu UV-2550 UV-Vis spectrophotometer equipped with a diffuse reflectance attachment. Thermogravimetric analysis (TG) was performed on a NETZSCH STA 409 PC thermal analyzer under air atmosphere.

Preparation of standard protein digests

α- and β-caseins (1 mg) were dissolved in a 1 mL of ammonium bicarbonate buffer (50mM, pH 8.2) respectively, and digested at 37 °C for 16 h with trypsin at the ratio of enzyme to substrate at 1:25 (w/w). BSA (6 mg) was dissolved in 1 mL denaturing buffer containing 8M urea and 50mM ammonium bicarbonate respectively, then the protein was reduced by 20 μL DTT (20 mM) at 37 °C for 2 h and alkylated by 40 μL IAA (40 mM) at room temperature in the dark for 40 min. The solution was then diluted 10-fold with 50mM ammonium bicarbonate buffer (pH 8.2) and incubated at 37 °C for 16 h with trypsin at the ratio of enzyme to substrate at 1:25 (w/w). Finally, the obtained tryptic digest was diluted to the target concentration with 0.1% TFA in 50% (v/v) ACN aqueous solution.

The capture of phosphopeptides by hierarchical TiAPO-5

Protein digests were diluted with loading buffer containing 0.1% TFA in 50% (v/v) ACN. A protein digest solution (2 pmol, 2 μL) was added into a 100 μL suspension of TiAPO-5H (10 mg/mL) in loading buffer, and incubated at room temperature for 10 min. The supernatant was removed after centrifugation at 13500×g for 5 min and the TiAPO-5H-*x* with captured phosphopeptides were washed with 100 μL of the loading buffer solutions containing 200 mM NaCl and 100 μL of buffer solutions containing 0.1% TFA in 30% (v/v) ACN, respectively. The captured phosphopeptides were then eluted with 10 μL 10% $NH_3.H_2O$ for 10 min. Finally, the supernatant was collected and lyophilized to dryness after centrifugation at 13500×g for 5 min. 2 μL of DHB solution (25 mg/mL in 50% ACN) containing 1% H_3PO_4 (v/v) was used to redissolve the dired residue and 1 μL of resulting solution was deposited on the sample target for subsequent MALDI-TOF MS analysis.

Mass spectrometry

All MALDI-TOF mass spectra were acquired by a Bruker Autoflex^{TM} time-of-flight mass spectrometer (Bruker, Bremen, Germany) equipped with a delayed ion-extraction device and a 377 nm pulsed nitrogen laser. The MALDI uses a ground-steel sample target with 384 spots. The range of laser energy was adjusted to slightly above the threshold for obtaining good resolution and signal-to-noise ratio. All measurements were carried out in linear positive-ion mode with delayed ion extraction. The delay time for ion extraction and the extraction voltage were set at 90 ns and 20 kV, respectively. Each mass spectrum was acquired by the accumulation of 50 laser shots.

Figure and Table

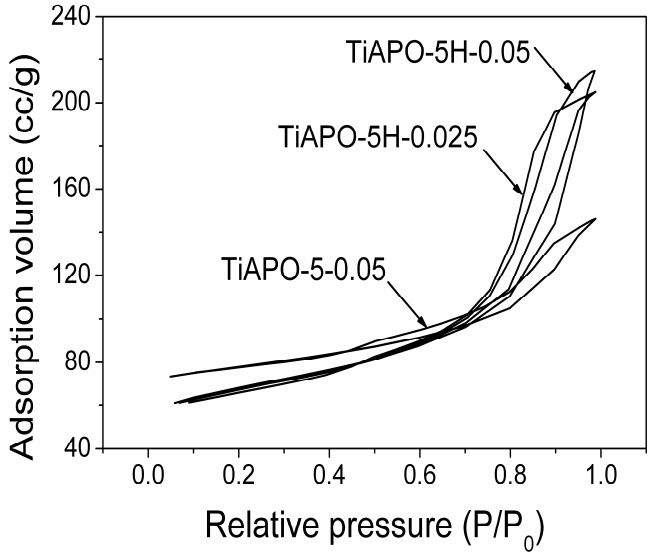


Figure S1. N_2 adsorption–desorption isotherms of TiAPO-5H-*x* and TiAPO-5-0.05.

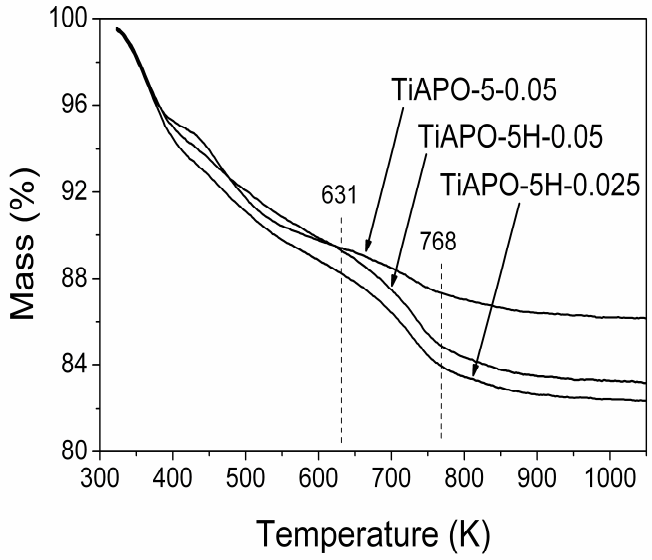
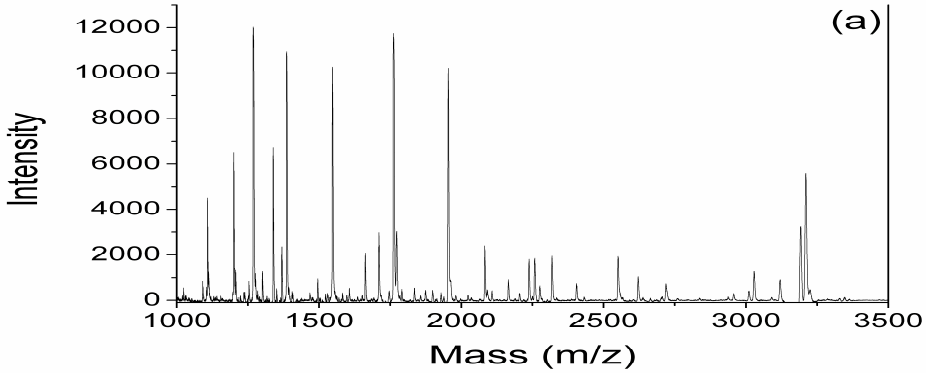
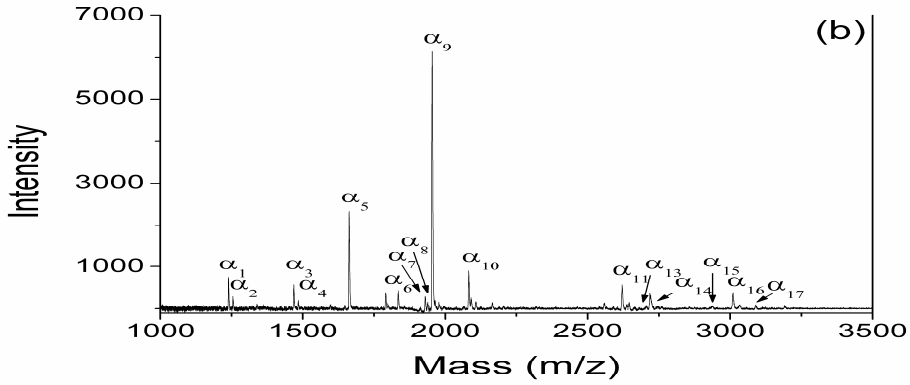
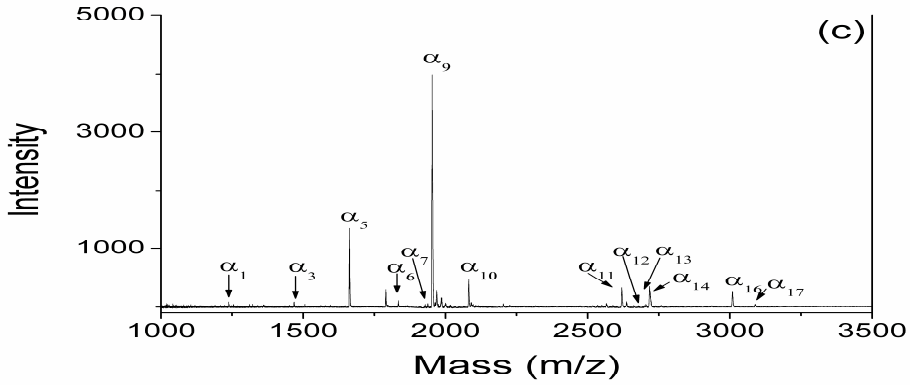


Figure S2. TG curves of TiAPO-5H-*x* and TiAPO-5-0.05.







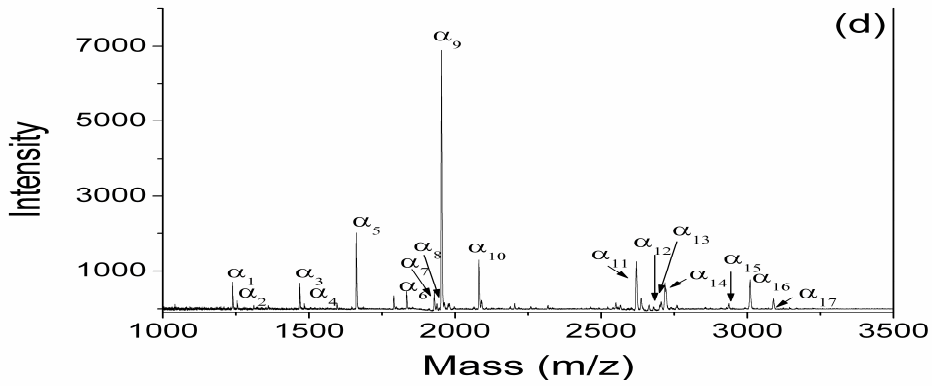


Figure S3. MALDI-TOF mass spectra of tryptic digest of α-casein (1 pmol) obtained (a) by direct analysis, (b) after treated by TiAPO-5H-0.05 with loading buffer containing 50% ACN (v/v)/0.1% TFA, (c) after treated by TiO_2 with loading buffer containing 65% ACN (v/v)/2% TFA/saturated by glutamic acid, [1]and (d) after treated by Ti^{4+}-IMAC with loading buffer containing 80% ACN (v/v)/6% TFA.[2] The detailed information of the phosphopeptides are listed in Table S1.

Table S1. Detailed information of the observed phosphopeptides obtained from tryptic digest of α-casein and β-casein.

Protein	No.	Amino acid sequence	Number of phosphorylation site	$[M+H]^+$
α-casein	α_1	TVDMEpSTEVF	1	1237.08
	α_2	TVD[Mo]MEpSTEVF	1	1253.11
	α_3	TVDMEpSTEVFTK	1	1466.97
	α_4	TVD[Mo]EpSTEVFTK	1	1482.61
	α_5	VPQLEIVPNpSAEER	1	1660.15
	α_6	YLGEYLIVPNpSAEER	1	1832.70
	α_7	DIGpSEpSTEDQAMEDIK	2	1927.89
	α_8	DIGpSEpSTEDQA[Mo]EDIK	2	1943.89
	α_9	YKVPQLEIVPNpSAEER	1	1951.09
	α_{10}	KKYKVPQLEIVPNpSAEERL	1	2080.00
	α_{11}	NTMEHVpSpSpSEESIIpSQETYK	4	2618.93
	α_{12}	VNELpSKDIGpSEpSTEDQAMEDIK	3	2678.02
	α_{13}	Q*MEAEpSIpSpSpSEEIVPNpSVEAQK	5	2703.75
	α_{14}	QMEAEpSIpSpSpSEEIVPNPNpSVEQK	5	2720.05
	α_{15}	KEKVNELpSKDIGpSEpSTEDQAMEDIKQ	3	2935.87
	α_{16}	NANEEEYSIGpSpSpSEEpSAEVATEEVK	4	3008.22
	α_{17}	NANEEEYpSIGpSpSpSEEpSAEVATEEVK	5	3087.93
β-casein	β_1	FQpSEEQQQTEDELQDK	1	2061.94
	β_2	FQpSEEQQQTEDELQDKIHPF	1	2556.93
	β_3	RELEELNVPGEIVEpSLpSpSpSEESITR	4	3122.56

Footnotes: pS, phosphorylated sites; [Mo], oxidation on methionine; Q*, pyroglutamylation on the N-terminal.

References:

[1] Q. R. Li, Z. B. Ning, J. S. Tang, S. Nie, R. Zeng, *J. Proteome Res.* 2009, *8*, 5375-5381.

[2] H. J. Zhou, M. L. Ye, J. Dong, G. H. Han, X. N. Jiang, R. N. Wu, H. F. Zou, *J. Proteome Res.* 2008, *7*, 3957-3967.