Supporting Information

The Synthesis of Ti-Hexagonal Mesoporous Silica for Selective Capture of Phosphopeptides

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Experimental details

Chemicals and reagents. Tetraethyl orthosilicate (TEOS, $Si(OEt)_4$, 99%), ethanol (C_2H_6O, 99.5%), isopropanol (C_3H_8O, 99.7%), and mesitylene (C_9H_{12}, 98%) were obtained from Tianjin Kermel Chemical Reagent Development Center (Tianjin, China). Tetra-n-butyl titanate (TBT, $C_{16}H_{36}O_4Ti$, 98%) was obtained from Shanghai 3S Reagent Co. LTD (Shanghai, China). Hexadecylamine (HDA, $C_{16}H_{35}N$, 90%) was obtained from Alfa Aesar (Ward Hill, MA, USA). α- and β-caseins (from bovine milk), trypsin, bovine serum albumin (BSA), 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 purchased from BioRad (Hercules, CA, USA). Acetonitrile and trifluoroacetic acid (TFA) were purchased from Merck (Darmstadt, Germany). Water used in all experiments was doubly distilled and purified by a Milli-Q water purification system (Millipore, Milford, MA, USA).

Synthesis of Ti-HMS. The Ti-HMS with different Ti content was synthesized by using HDA as template, TEOS as silicon source, and TBT as Ti source, which was similar to the literature [16]. Briefly, a certain amount of ethanol, isopropanol, de-ionized water, and hexadecylamine were stirred for 20 min at room temperature. To this gel, the solution (TEO, TBT, and isopropanol) was added under vigorous stirring. After 5 min, mesitylene was added as swelling agent. The stirring was continued for 24 h. The molar composition of the gel was as follows: 0.02 (TBT): 1 (TEOS): 0.27 (HDA): 4.5 (C_2H_6O): 4.7 (C_3H_8O): 72(H_2O). It was then filtered, washed with de-ionized water and dried at 393 K for 6 h. Organic template was removed from the as-synthesized material by calcination at 823 K for 6 h, and the heating rate is 20 ^oC/m. The synthesized material was denoted as Ti-HMS-002. In addition, higher Ti content material Ti-HMS-008 was prepared with the following molar composition of the gel: 0.08 (TBT): 1 (TEOS): 0.27 (HDA): 4.5 (C_2H_6O): 4.7 (C_3H_8O): 72(H_2O).

Characterizations. The X-ray powder diffraction (XRD) patterns were obtained using Rigaku D/Max 2500 powder diffraction system (Rigaku, Tokyo, Japan) with Cu Kα radiation. N_2 adsorption-desorption measurements were carried out at 77 K on Quantachrome Autosorb-1 instrument (Quantachrome, Boynton Beach, FL, USA). And the materials were outgassed at 120 °C for 5 h before the measurements. Pore sizes were estimated from desorption branch using Berrett–Joyner–Halenda (BJH) method. The microstructures of the material were examined by transmission electron microscopy (TEM) on a JEOL JEM-2000EX electron microscope (JEOL, Tokyo, Japan) at an acceleration voltage of 120 kV. Ultraviolet-visible diffuse reflectance spectra (UV-VIS DRS) were collected on Shimadzu UV-2550 spectrophotometer (Shimadzu, Kyoto, Japan) equipped with a diffuse reflectance attachment. UV resonance Raman spectra were collected at room temperature with a Jobin–Yvon T6400 triple-stage spectrograph with a spectral

resolution of 2 cm^{-1}. The 244 nm line from a Coherent Innova 300 Fred laser was used as an excitation source in the deep UV region. Material acidities were determined by NH_3-TPD with a Micromeritics ASAP 2920 Autochem II system (Micromeritics, Norcross, GA USA).

Tryptic digestion of proteins. α- and β-Casein (1 mg) were respectively dissolved in a 1 ml of ammonium bicarbonate buffer (50 mM, pH 8.2), and digested at 37 °C for 16 h with trypsin at the ratio of enzyme-to-substrate of 1:40 (w/w). BSA (6.6 mg) and ovalbumin (4 mg) were respectively dissolved in 1 ml denaturing buffer containing 8 M urea in 50 mM ammonium bicarbonate; after the addition of 20 μl of DTT (50 mM), the mixtures were incubated at 60 °C for 1 h to reduce the disulfide bonds of proteins; subsequently, 40 μl of IAA (50 mM) were added and the mixtures were then incubated at room temperature in dark for 30 min; finally, the mixtures were diluted 10-fold with 50 mM ammonium bicarbonate buffer (pH 8.2) and digested at 37 °C for 16 h with trypsin at the ratio of enzyme-to-substrate of 1:40 (w/w).

The capture of phosphopeptides by Ti-HMS. Protein digests were diluted with loading buffer containing 6% TFA in 50% (v/v) ACN (pH 0.85). A protein digest solution (1 pmol, 1 μl) was added into a 50 μl suspension of Ti-HMS (10 mg/ml) in loading buffer, and incubated at room temperature for 30 min. The supernatant was removed after centrifugation at 13 500g for 10 min and the Ti-HMS with captured phosphopeptides were rinsed with 150 μl of the loading buffer solutions containing 500 mM NaCl, 150 μl of buffer solutions containing 0.1% TFA in 50% (v/v) ACN, respectively. The bound phosphopeptides were then eluted with 25 μL of 10% $NH_3.H_2O$ under sonication for 10 min. After centrifugation at 13 500 g for 10 min, the supernatant was collected and lyophilized to dryness. 5 μL of DHB solution (25 mg/mL in 70% ACN) containing 1% H_3PO_4 (v/v) was added to dissolve the dried residue and 0.5 μL of resulting solution was deposited on MALDI target for MALDI-TOF MS analysis.

Mass spectrometry. All MALDI-TOF mass spectra were acquired by a BRUKER Autoflex™ time-of-flight mass spectrometer (Bruker, Bremen, Germany) equipped with a delayed ion-extraction device and a 337-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 MS spectrum was acquired by the accumulation of 30 laser shots.

Table S1. Acidity of Ti-HMS and TiO_2

Materials	Acidity amount (μmol/g)
TiO_2	71.6
Ti-HMS-008	~0
Ti-HMS-002	~0

Material acidities were determined by NH_3-TPD with a Micromeritics ASAP 2920 Autochem II system (Micromeritics, Norcross, GA USA). The materials were treated at 550 ^oC for 0.5 h in Ar flow. Adsorption of ammonia was performed at 150 ^oC. After saturation, materials were heated from 150 to 600 ^oC with a rate of 15 C/min under Ar with a constant flow of 25 ml/min. The amounts of ammonia desorbed were detected by thermal conductivity detector (TCD).

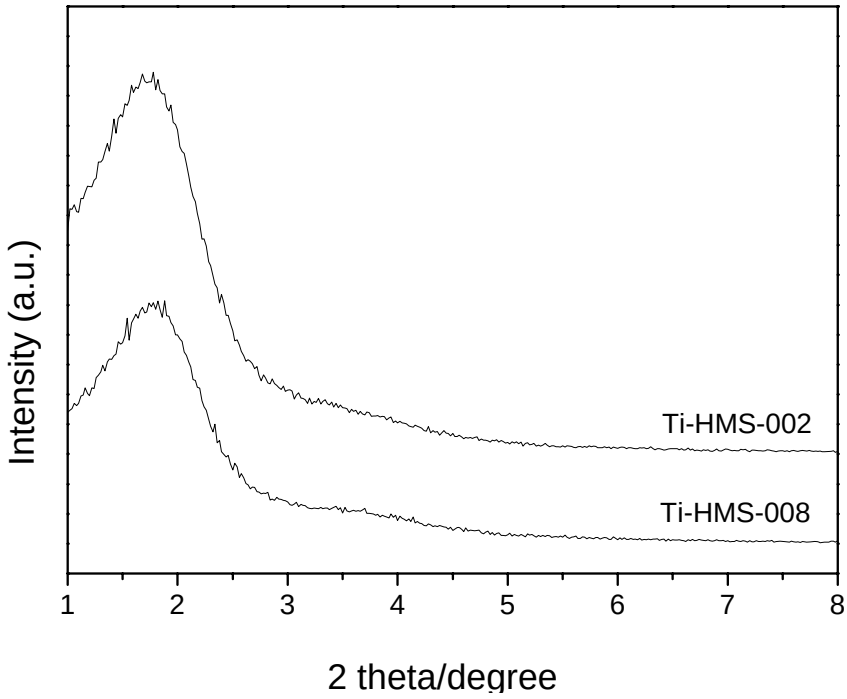
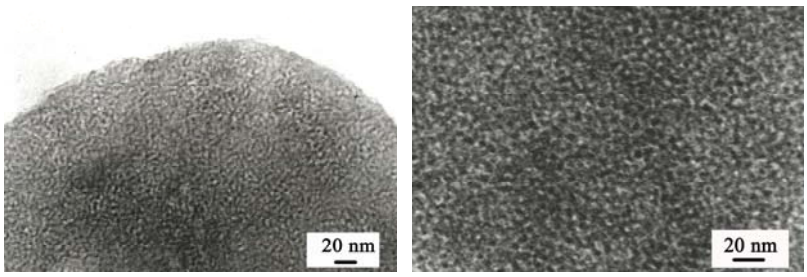


Figure S1. Small-angle XRD patterns of Ti-HMS-008 and Ti-HMS-002. The X-ray powder diffraction (XRD) patterns were obtained using Rigaku D/Max 2500 powder diffraction system (Rigaku, Tokyo, Japan) with Cu Kα radiation (λ=0.1542 nm), operating at 40 kV and 30 mA. Scanning rate is 2^{o}/min.



(a) (b)

Figure S2. TEM images of Ti-HMS-002 (a) and Ti-HMS-008 (b). The microstructures of the material were examined by transmission electron microscopy (TEM) on a JEOL JEM-2000EX electron microscope (JEOL, Tokyo, Japan) at an acceleration voltage of 120 kV.

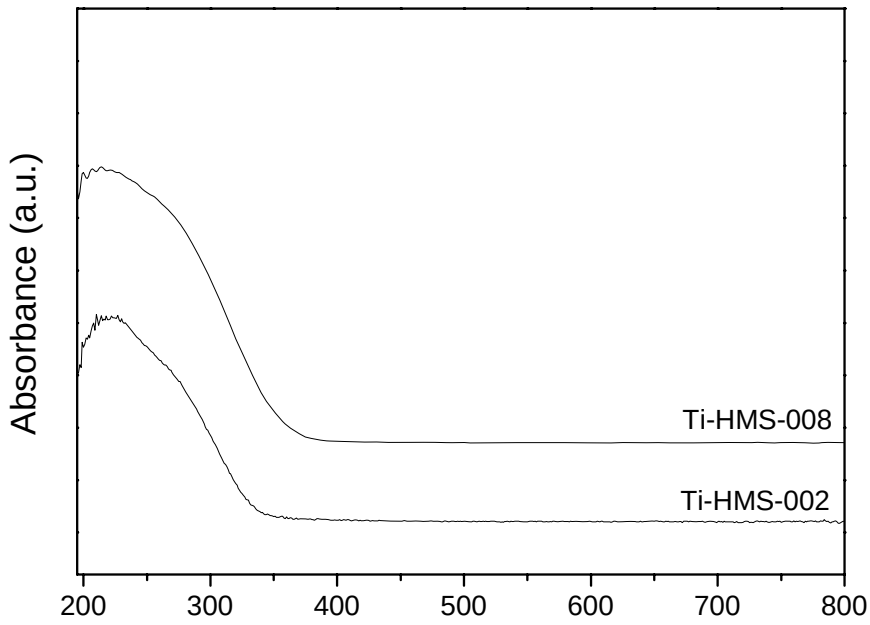


Figure S3. Ultraviolet-visible diffuse reflectance spectra (UV-VIS DRS) of Ti-HMS-008 and Ti-HMS-002. UV-VIS DRS were collected on Shimadzu UV-2550 spectrophotometer (Shimadzu, Kyoto, Japan) equipped with an integrating sphere, using $BaSO_4$ as reference.

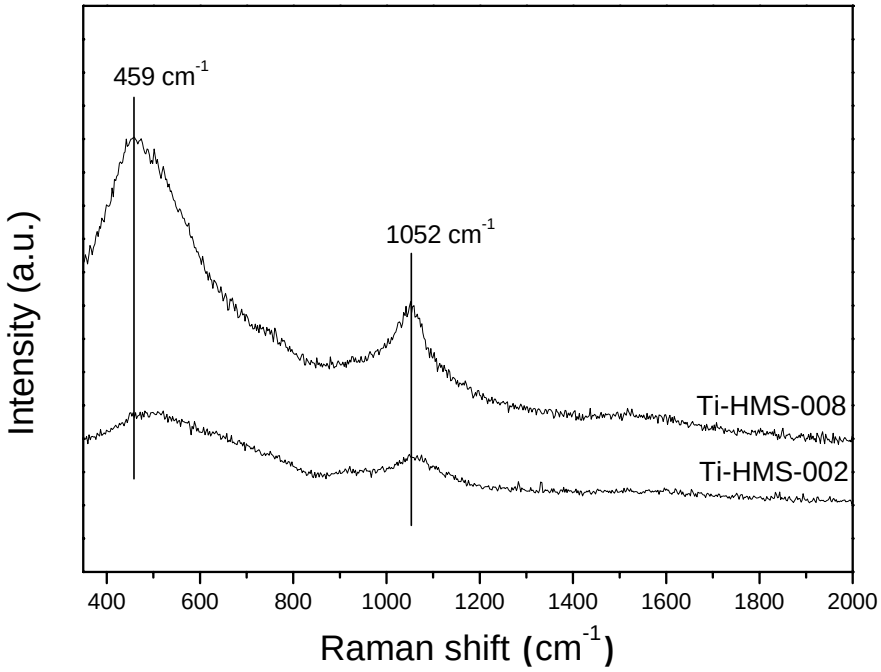
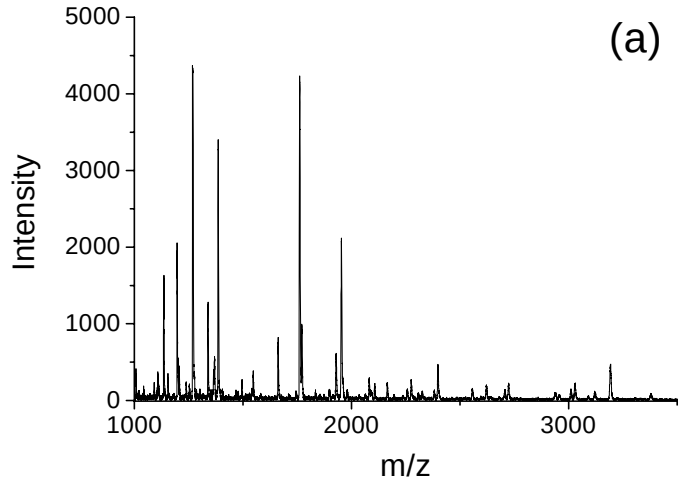


Figure S4. UV resonance Raman spectra of Ti-HMS-008 and Ti-HMS-002. UV resonance Raman spectra were collected at room temperature with a Jobin–Yvon T6400 triple-stage spectrograph with a spectral resolution of 2 cm^{-1}. The 244 nm line from a Coherent Innova 300 Fred laser was used as an excitation source in the deep UV region.



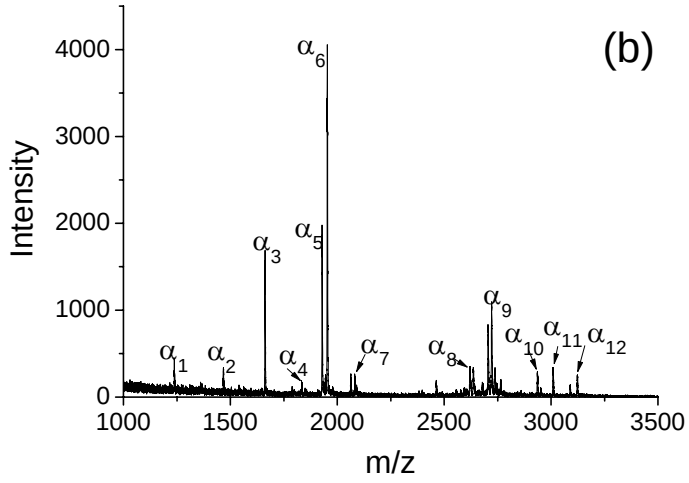


Figure S5. MALDI-TOF mass spectra of tryptic digest of α-casein (1 pmol) obtained (a) by direct analysis and (b) after treated by Ti-HMS-008. α_1, α_2, α_3, α_4, α_5, α_6, α_7, α_8, α_9, α_{10}, α_{11} and α_{12} at *m*/*z* of 1237.87, 1467.43, 1661.75, 1833.68, 1929.06, 1952.94, 2081.61, 2620.43, 2722.35, 2935.29, 3009.25 and 3089.82 represent the phosphopeptides of TVDME[pS]TEVF, TVDME[pS]TEVFTK, VPQLEIVPN[pS]AEER, YLGEYLIVPN[pS]AEER, DIG[pS]E[pS]TEDQAMEDIK, YKVPQLEIVPN[pS]AEER, KKYKVPQLEIVPN[pS]AEERL, NTMEHV[pS][pS][pS]EESII[pS]QETYK, QMEAE[pS]I[pS][pS][pS]EEIVPNPN[pS]VEQK, KEKVNEL[pS]KDIG[pS]E[pS]TEDQAMEDIKQ, NANEEEYSIG[pS][pS][pS]EE[pS]AEVATEEVK, and NANEEEY[pS]IG[pS][pS][pS]EE[pS]AEVATEEVK, respectively.

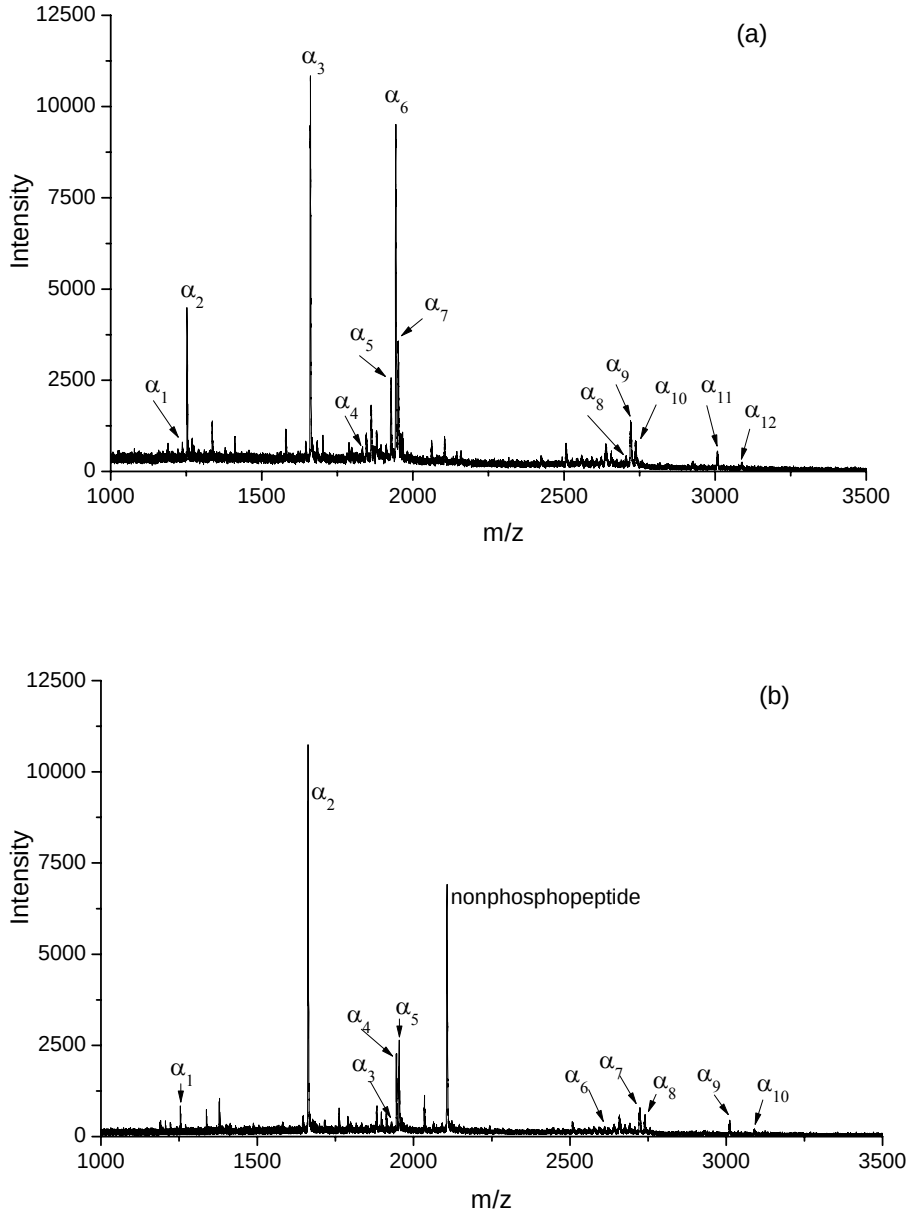


Figure S6. MALDI-TOF mass spectra of the selective capture of phosphopeptides from α-casein by (a) Ti-HMS-008 and (b) TiO_2 with loading buffer containing 0.1% TFA in 50% (v/v) ACN.