Supporting Information

Experimental

Materials

Ammonium dihydrogen phosphate ($NH_4H_2PO_4$) and Ammonium hydroxide ($NH_3 \cdot H_2O$) were obtained from Beijing Chemical Regent Co., Ltd. (Beijing, China). Lanthanum nitrate hexahydrate ($La(NO_3)_3 \cdot 6H_2O$), Neodymium nitrate hexahydrate ($Nd(NO_3)_3 \cdot 6H_2O$), Terbium nitrate hexahydrate ($EuNO_3)_3 \cdot 6H_2O$)**,** Trifluoroacetic acid (TFA), Acetonitrile (ACN), Ammonium bicarbonate (NH_4HCO_3), 2,5-Dihydroxybenzoic acid (2,5-DHB) and Dithiothreitol (DTT) were purchased from Aladdin (Shanghai, China). Bovine β-casein, Bovine serum albumin (BSA), and Trypsin (from bovine pancreas, TPCK treated) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Iodoacetamide (IAA) was provided by Alfa Aesar (USA). All the chemical agents were used without further purification. Human and serum samples were offered by a local hospital from a healthy volunteer.

Preparation of modified affinity MALDI target plate

The $REPO_4$ nanorods were first prepared via a facile precipitation method. Typically, 0.5 mmol of $RE(NO_3)_3 \cdot 6H_2O$ (RE=La, Nd or Eu) was completely dissolved in 10 mL of deionized water and 1.0 mmol of $NH_4H_2PO_4$ was dissolved in 20 mL of deionized water, respectively. The obtained homogeneous solution of $RE(NO_3)_3$ was slowly dropped into the $NH_4H_2PO_4$ solution under vigorous magnetic stir. The obtained white suspension was stirred constantly for 12 h at room temperature. The products were obtained after separated by centrifugation, washed with deionized water and ethanol for three times and lyophilized to dryness.

For preparation of affinity MALDI target plate modified with $REPO_4$ (RE=La, Nd and Eu) nanorods, MALDI target plate was first purified with water and ethanol, and then dried naturally at room temperature. The prepared $REPO_4$ nanorods (15 mg) were subsequently redispersed into 1 mL of ethanol/aqueous solution (1:4, v/v) via ultrasonication to obtain the homogeneous suspension, and the pH of the suspension was adjusted to 2 using diluted hydrochloric acid and ammonia. The homogeneous

suspension of $REPO_4$ nanorods was dropped onto the purified MALDI target plate to form an array of spots (1 μL for each spot) and the MALDI target plate was first kept at room temperature for 5 min, and then heated at 350 °C for 5 h. The MALDI target plate modified with $REPO_4$ (RE=La, Nd and Eu) nanorods was finally obtained after it was cooled to room temperature naturally. To further inspect possible morphologies of the samples on the MALDI target plate, we conducted the same modification process on the small steel plates because the MALDI target plate is too big to be placed into the SEM sample room for direct observation.

Preparation of tryptic digests of proteins

1.0 mg protein (β-casein or BSA) was dissolved in 1mL of 50 mM NH_4HCO_3 (pH=7.8) solution without additional additive, and trypsin was then added into the solution with a molar ratio of 50:1 (protein/trypsin). The resulting solution was subsequently incubated for 16 h at 37 °C to produce a tryptic digest solution. The obtained tryptic digest solution was finally diluted to the target concentration.

The nonfat milk digest was prepared according to the previous report with modification.[1] Briefly, 0.25 mL milk sample was mixed with the 0.25 mL of 50mM NH_4HCO_3 (pH=7.8) solution, followed by vortex-mixing at 37℃ for 30 min. Then, 50 μL of 100 mM DTT was added into the solution and mixed for an additional 1 h at 50℃. After it was cooled to room temperature, 20 μL of 0.5M IAA was added into the mixture, followed by shaken for another 2.5 h at room temperature in the dark. Subsequently, the resulting solution was incubated with 20 μg trypsin in 50 mM aqueous ammonium bicarbonate at 37℃ for 16 h. The obtained tryptic digest solution was finally diluted to the target concentration.

Selective capture of phosphopeptides

Direct capture of phosphopeptides using affinity MALDI target plate is described as follows. 1 μL of 0.5% TFA solution (in 50% ACN/water, v/v) was first dripped onto the nanorod functionalized spot on target plate. Subsequently, 1 μL of digests was dripped onto the target spot and incubated for 5 min at room temperature. Then, the residual solution was removed and the target spots on plate were purified using 20 μL of buffer solution by dripping and removing. Finally, 1 μL of 20 mg mL^{-1} DHB (in 50%

ACN/water containing 1% H_3PO_4, v/v) was dropped on the MALDI target plate with the trapped phosphopeptides, and then dried at room temperature. The phosphorylated peptides captured by the affinity MALDI target plate were directly analyzed by MALDI-TOF MS. To capture phosphopeptides from the nonfat milk digestion, the nonfat milk tryptic digest (5 μL) was diluted to 1 mL with an aqueous solution containing 50% acetonitrile and 0.5% TFA (v/v). 1 μL of the diluted nonfat milk digestion without pretreatment and 1 μL of 0.5% TFA solution (in 50% ACN/water, v/v) were dripped onto the modified target plate as the real sample, and then treated according to the same procedure as that of the standard protein digests. For binding and isolating the phosphopeptides from pristine human serum, 0.5 μL of the human serum without pretreatment and 1 μL of 0.5% TFA solution (in 50% ACN/water, v/v) were dripped onto the modified target plate as the real sample, and then treated according to the same procedure as the standard protein digests. For regeneration of the affinity MALDI target plate, it was immersed in an aqueous solution containing 30% ACN and TFA 6% (v/v) with gentle shake for 20 min. Subsequently, the affinity target plate was washed with 0.5% TFA solution (in 50% ACN/water, v/v) and water, respectively. The purified affinity target plate was dried at 50 $^{\circ}$C for 6 h.

For comparison, 1 μL of digests were directly dripped onto the normal MALDI target plate without any treatment, and then 1 μL of 20 mg mL^{-1} DHB (in 50% ACN/water containing 1% H_3PO_4, v/v) was dropped on the MALDI target plate and dried at room temperature, and then analyzed by MALDI-TOF MS. Enrichment of the phosphopeptides using commercial TiO_2 microspheres is described as follows. 5μL of commercial TiO_2 microspheres (20 mg mL^{-1}) was mixed with 100 μL of mixture of β-casein (1 pmol/μL) and BSA (1:25, molar ratio) digestion and then shaken for 2 min. Subsequently, the TiO_2 microspheres captured phosphopeptides were collected and isolated by centrifugation at a speed of 10000 rpm. The obtained microspheres were washed with 50 μL 0.5% TFA solution in acetonitrile/deionized water (1/1, v/v) three times. After that, the trapped peptides were eluted with ammonium hydroxide (10%, 30 μL) to form a supernatant, and the supernatant was collected and lyophilized to dryness. The lyophilized samples were mixed with 2 μL of 20 mg mL^{-1} DHB (in 50% ACN/water containing 1% H_3PO_4, v/v) and 1 μL of the mixture was then deposited on

the normal MALDI target for MS analysis. To enrich phosphopeptides from the nonfat milk digestion, the nonfat milk tryptic digest (5 μL) was diluted to 1 mL with an aqueous solution containing 50% acetonitrile and 0.5% TFA (v/v). The peptide solution (100 μL) was mixed with the suspension of TiO_2 microspheres (20 mg mL^{-1}, 5 μ L). The rest of the enrichment process was similar to that of β-casein digest

Measurements and characterizations

A field emission scanning electron microscope (FESEM, S4800, Hitachi) equipped with an energy-dispersive X-ray spectrum (EDX, JEOLJXA-840) was applied to determine morphologies and compositions of the as-prepared samples. Transmission electron microscopy (TEM) and high-resolution TEM (HRTEM) images were taken with a FEI Tecnai G2 S-Twin transmission electron microscope operated at 200 kV. Powder X-ray diffraction (XRD) patterns were collected on a Bruker D8 Focus X-ray diffractometer using Cu Kα radiation (λ=1.5405 Å). Fourier-transform infrared spectroscopy (FTIR) analyses were carried out on a Perkin-Elmer 580B infrared spectrophotometer using the KBr pellet technique. Zeta-potential was measured using a ZetaPal (BIC, USA)**.** MALDI-TOF MS experiments were performed on AB SCIEX MALDI-TOF/TOF 5800 mass spectrometer (Foster City, CA, USA) equipped with a 349-nm neodymium: yttrium-aluminum-garnet laser. MS spectra were acquired as an average of 400 laser shots accumulation in positive ion reflection mode, which was obtained and analyzed by AB SCIEX Analyst Software and Data Explorer.

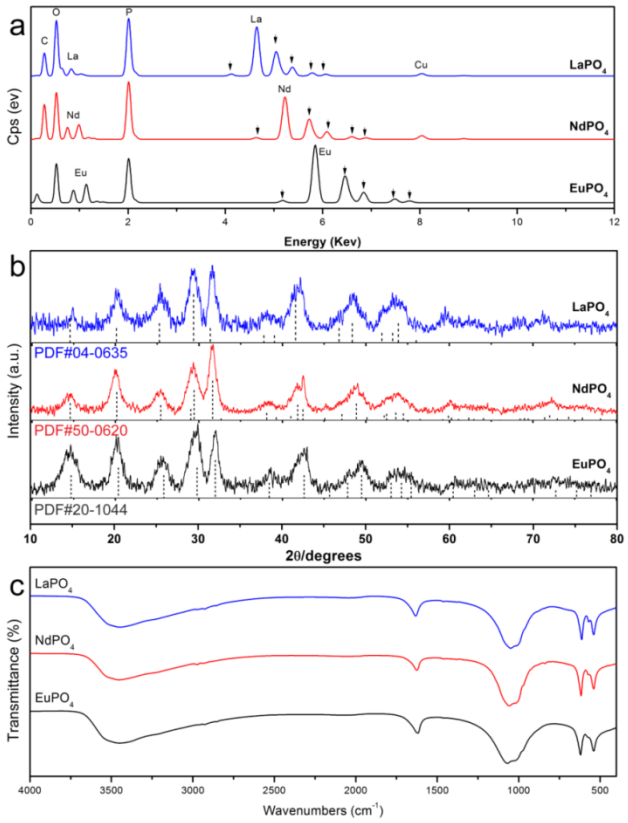

Fig. S1 EDX spectra (a), XRD patterns (b) and FTIR spectra (c) of the prepared $LaPO_4$, $NdPO_4$ and $EuPO_4$ nanorods

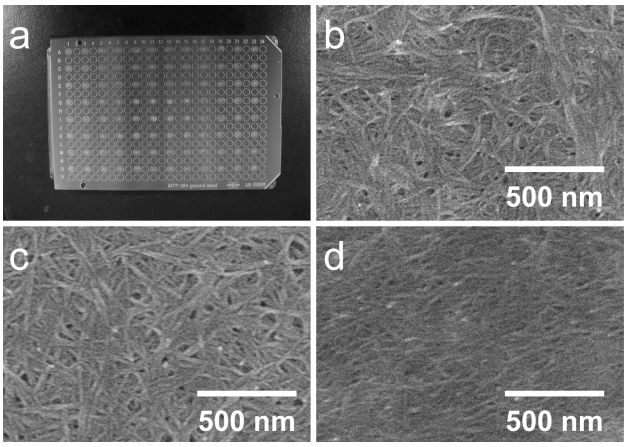

Fig. S2 The photo of the affinity MALDI target plate (a), and SEM images of the steel plates modified with $LaPO_4$ (b), $NdPO_4$ (c) and $EuPO_4$ (d) nanorods

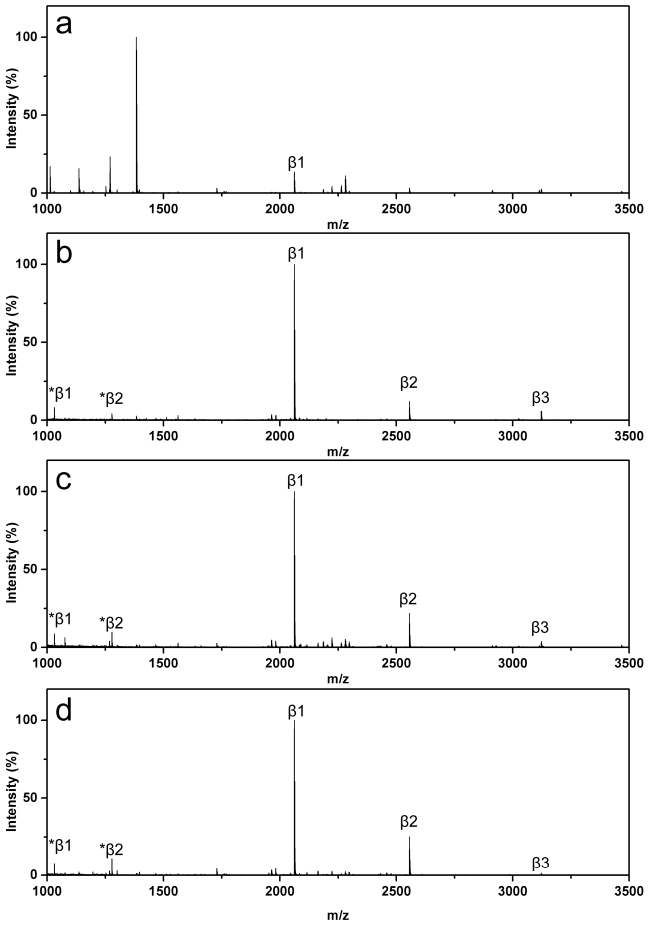

Fig. S3 MALDI mass spectra of 1 μL tryptic digest of β-casein (1pmol) on a normal MALDI plate (a) without enrichment and using an affinity plate modified with (b) $LaPO_4$, (c) $NdPO_4$ and (d) $EuPO_4$ nanorods.

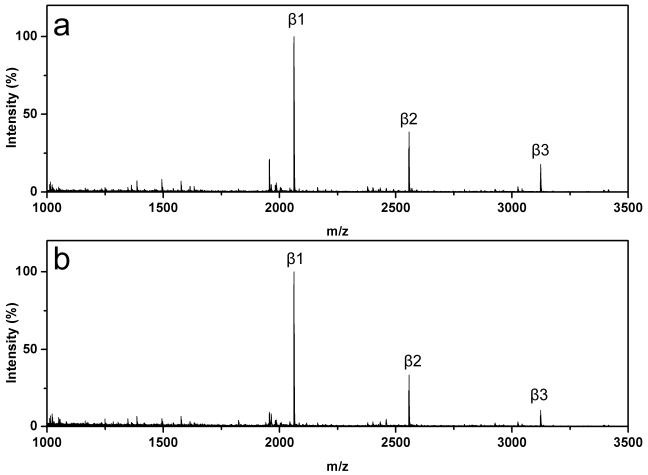

Fig. S4 MALDI mass spectra of 1 μL tryptic digest mixture of β-casein (1 pmol) and BSA (25 pmol) after enrichment using an affinity plate modified with (a) $NdPO_4$ and (b) $EuPO_4$ nanorods.

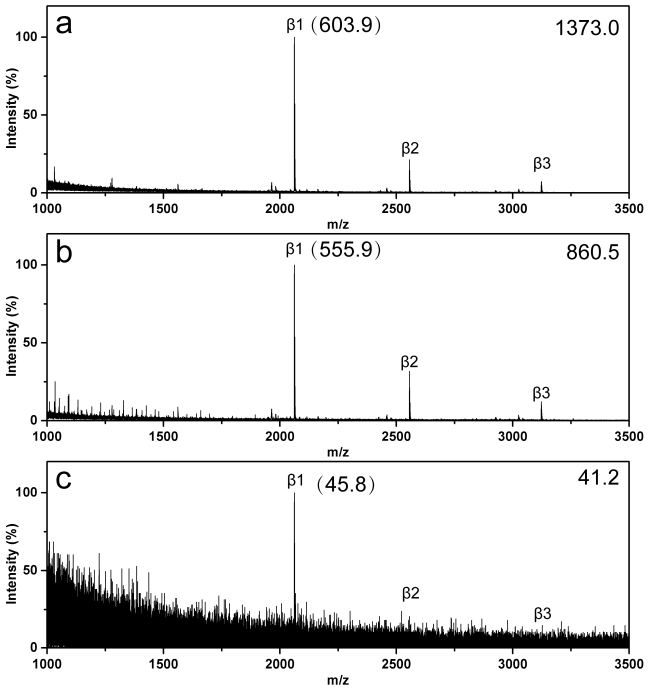

Fig. S5 MALDI-TOF mass spectra of 1 μL β-casein digest with diffirent concentrations after enrichment using the affinity MALDI target plate modified with $LaPO_4$ nanorods: (a) 10^{-7} M, (b) 5×10^{-8} M and (c) 10^{-8} M. The number in the top right corner is the highest peak intensity, while the number in the brackets is the S/N ratio of the target peak.

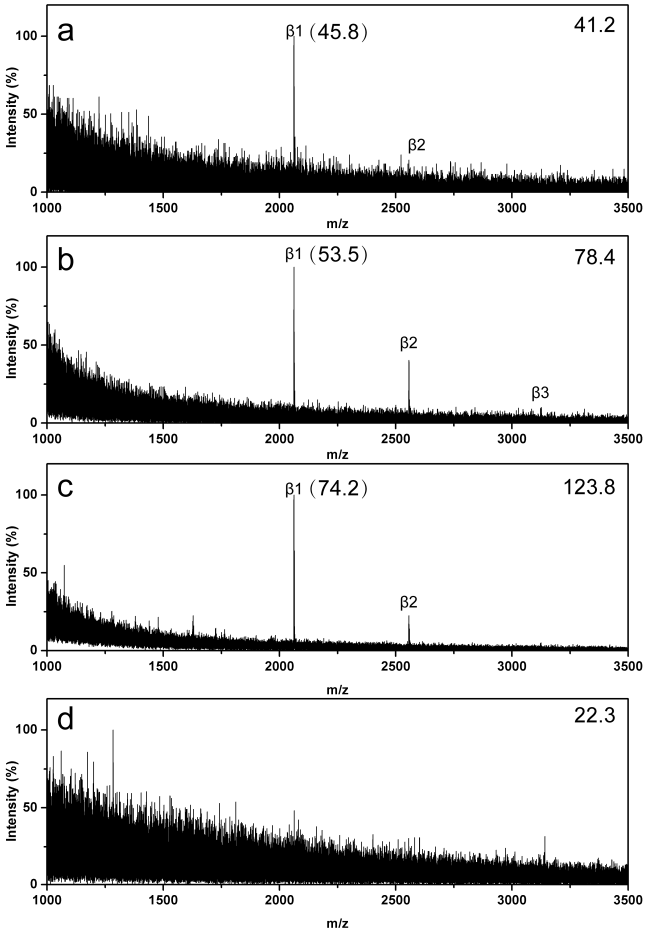

Fig. S6 MALDI-TOF mass spectra of 1 μL β-casein (10 fmol) digest after treatment using the affinity MALDI target plate modified with (a) $LaPO_4$, (b) $NdPO_4$ and (c) $EuPO_4$ nanorods and (d) commercial TiO_2 microspheres. The number in the top right corner is the highest peak intensity, while the number in the brackets is the S/N ratio of the target peak.

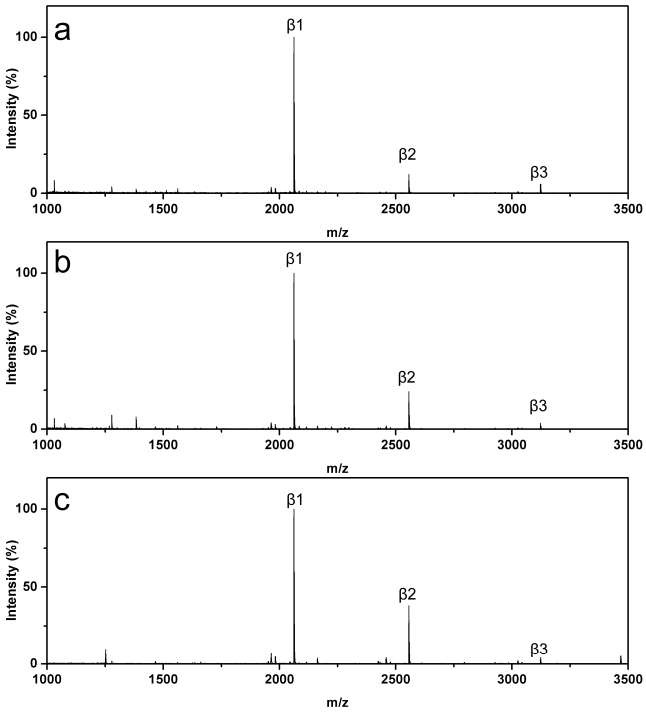

Fig. S7 MALDI-TOF mass spectra of 1 μL of β-casein (1 pmol) digest after treatment using the fourth regenerated affinity MALDI target plate modified with (a) $LaPO_4$, (b) $NdPO_4$ and (c) $EuPO_4$ nanorods.

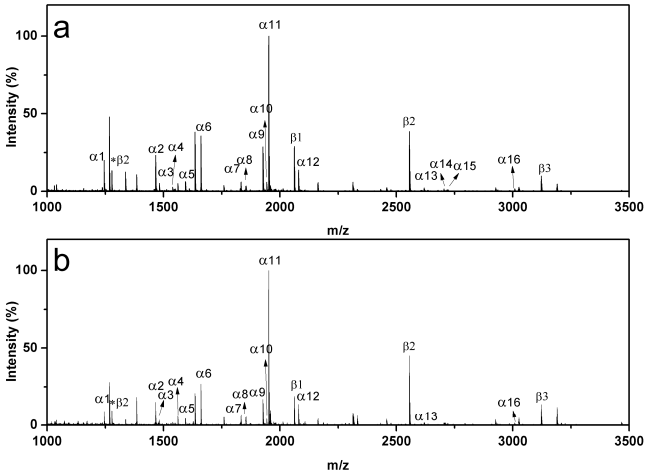

Fig. S8 MALDI-TOF mass spectra of 1 μL nonfat milk digest after treatment using the affinity MALDI target plate modified with the (a) $NdPO_4$ and (b) $EuPO_4$ nanorods.

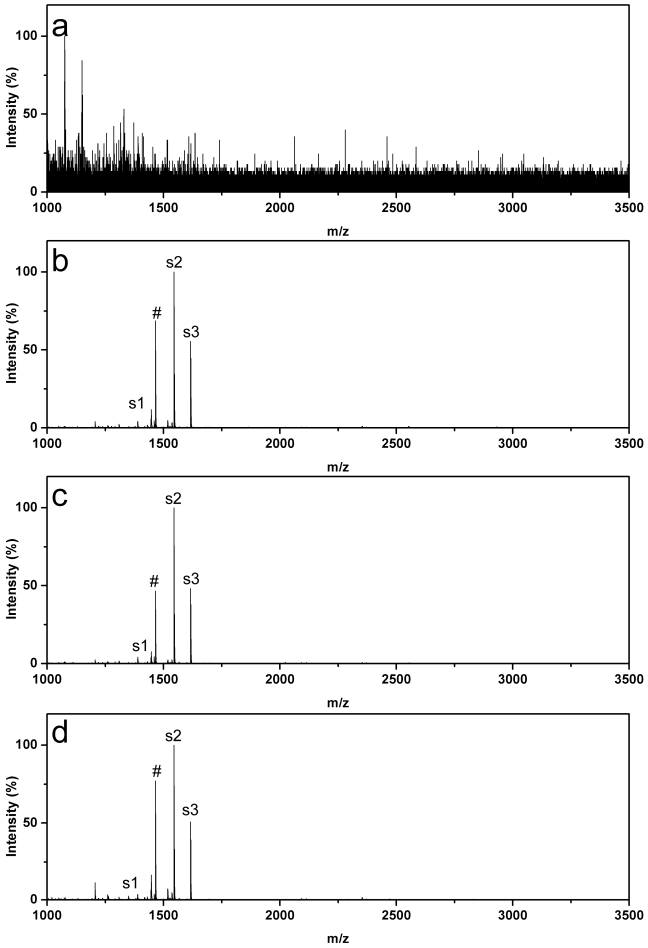

Fig. S9 MALDI-TOF mass spectra of 0.5 μL human serum: (a) before enrichment, after enrichment with the (b) $LaPO_4$, (c) $NdPO_4$ and (d) $EuPO_4$ nanorods modified on the MALDI target plate. '#' indicates the corresponding dephosphorylated peptides**.**

Table S1 Zeta potential of $REPO_4$ nanorods under different pH values (mV)

pH	$LaPO_4$	$NdPO_4$	$EuPO_4$	TiO_2[2]
2	38.97	36.47	39.44	41.0
4	31.67	36.83	29.07	22.5

The zeta potentials of the $REPO_4$ nanorods were investigated under different pH values. (Table S1). The zeta potentials for the three different $REPO_4$ nanorods in acidic solution (pH=2, 4) all have positive values, which is similar to that of commercial TiO_2 beads. Notably, the $REPO_4$ nanorods can selectively capture the phosphopeptides in acidic solution (e.g. pH=4) because the negatively charged carboxyl groups (pKa=4) in peptides can be protonated when pH is lower than 4, while the phosphate groups (pKa=2) in phosphopeptides are still negative at pH=4, thereby being efficiently enriched due to the Lewis acid and ion exchange properties of the affinity materials,[3] which are always introduced for explanation of selective phosphopeptide enrichment. The metal cations in the affinity materials, as the Lewis acid, coordinate preferentially with phosphate group (Lewis base) to form mononuclear, dinuclear, or multinuclear metal-oxygen complexes.[4]

Table S2 The phosphopeptides captured from the digest of nonfat milk

No.	Peptide sequences	MW	Phosphorylation site
β1	FQ[pS]EEQQQTEDELQDK	2061.8	1
β2	FQ[pS]EEQQQTEDELQDKIHPF	2556.0	1
β3	RELEELNVPGEIVE[pS]L[pS][pS][pS]EESITR	3122.2	4

Table S3 The phosphopeptides captured from the digest of nonfat milk

No.	Peptide sequence	MW	Phosphorylation site	$LaPO_4$	$NdPO_4$	$EuPO_4$	TiO_2
α1	TVDMEpSTEVF	1237.4	1	√	√	√	-
α2	TVDMEpSTEVFTK	1466.6	1	√	√	√	-
α3	TVDoMEpSTEVFTK	1482.2	1	√	√	√	√
α4	EQLpSTpSEENSKK	1539.2	2	-	√	√	-
α5	TVDMEpSTEVFTKK	1594.7	1	√	-	√	√
α6	VPQLEIVPNpSAEER	1660.8	1	√	√	√	√

α7	YLGEYLIVPNpSAEER	1832.8	1	√	√	√	√
α8	DIGSEpSTEDQAMEDIK	1847.7	1	√	√	√	√
α9	DIGpSEpSTEDQAMEDIK	1927.7	2	√	√	√	-
α10	DIGpSEpSTEDQAM(ox)EDIK	1943.7	2	√	√	√	-
α11	YKVPQLEIVPNpSAEER	1951.9	1	√	√	√	√
α12	KKYKVPQLEIVPNpSAEERL	2079.9	1	√	√	√	√
α13	NTMEHVpSpSpSEESIIpSQETYK	2618.1	4	√	√	√	√
α14	pyroEMEAEpSIpSpSpSGEIVPNpSVEQK	2703.5	5	-	√	-	-
α15	QMEAEpSIpSpSpSEEIVPNPNpSVEQK	2720.9	5	-	√	-	-
α16	NANEEEYSIGpSpSpSEEpSAEVATEEVK	3008.0	4	√	√	√	-
β1	FQpSEEQQQTEDELQK	2061.8	1	√	√	√	√
β2	FQpSEEQQQTEDELQDKIHPF	2555.9	1	√	√	√	√
β3	RELEELNVPGEIVEpSLpSpSpSEESITR	3122.2	4	√	√	√	-
Number of identified phosphopeptides				16	18	17	10

Table S4 The phosphopeptides captured from the human serum solution

No.	Peptide sequences	MW	Phosphorylation site
1	D[pS]GEGDFLAEGGGV	1389.5	1
2	D[pS]GEGDFLAEGGGVR	1545.7	1
3	AD[pS]GEGDFLAEGGGVR	1616.7	1

References:

1. C. T. Chen, W. Y. Chen, P. J. Tsai, K. Y. Chien, J. S. Yu and Y. C. Chen, *J Proteome Res*, 2007, **6**, 316-325.
2. L. A. L. Tang, J. Wang, T. K. Lim, X. Bi, W. C. Lee, Q. Lin, Y.-T. Chang, C. T. Lim and K. P. Loh, *Anal Chem*, 2012, **84**, 6693-6700.
3. S. R. Schmidt, F. Schweikart and M. E. Andersson, *J Chromatogr B*, 2007, **849**, 154-162.
4. (a) G. Han, M. Ye and H. Zou, *Analyst*, 2008, **133**, 1128-1138; (b) A. Leitner, M. Sturm and W. Lindner, *Anal Chim Acta*, 2011, **703**, 19-30; (c) A. Leitner, *Trac-Trend Anal. Chem.*, 2010, **29**, 177-185.