Electronic Supplementary Material (ESI) for Analytical Methods.

A Novel IMAC Platform-Adenosine Coupled Functional Magnetic Microspheres for Phosphoproteome Research

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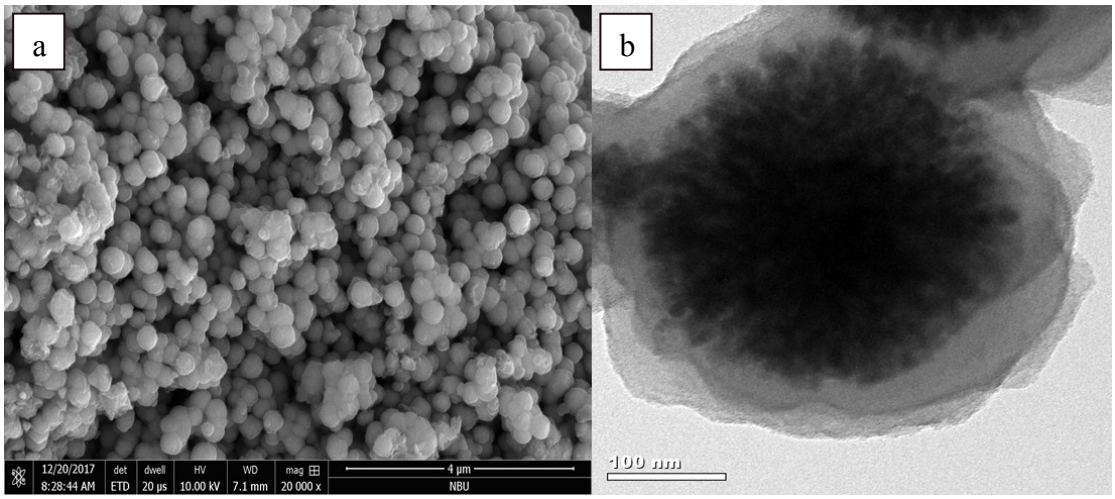

Fig. S1 Images of (a) SEM and (b) TEM of Fe_3O_4@PDA microspheres.

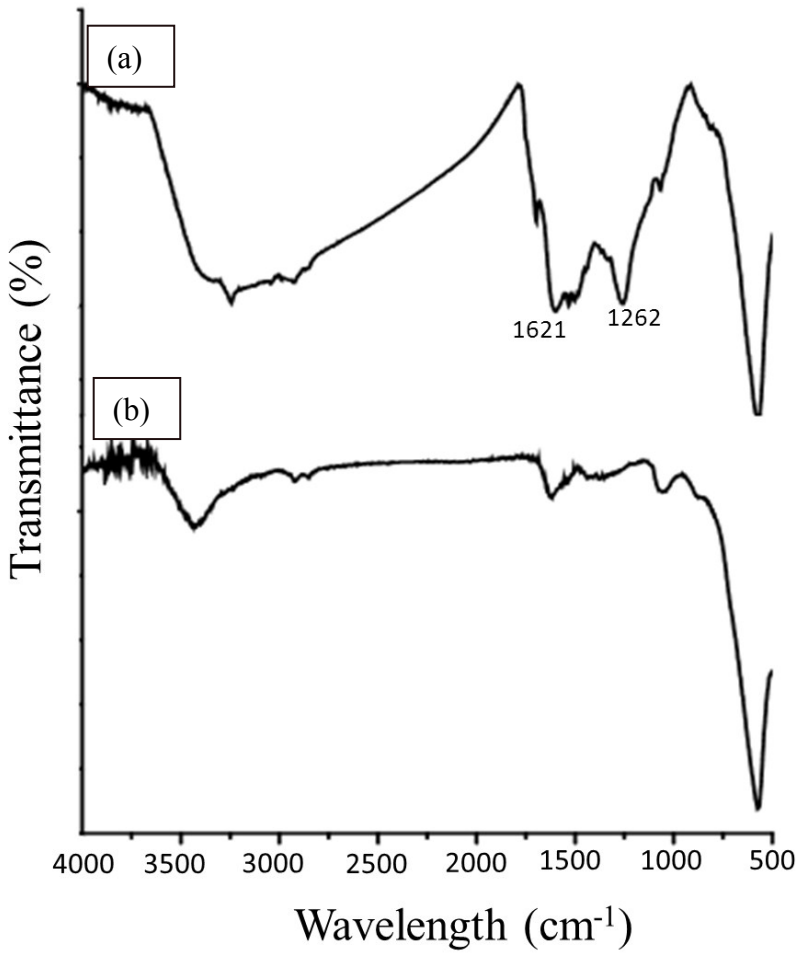

Fig. S2 FTIR spectrum of (a) Fe_3O_4@PDA@ATP-Ti^{4+} and (b) Fe_3O_4 microspheres.

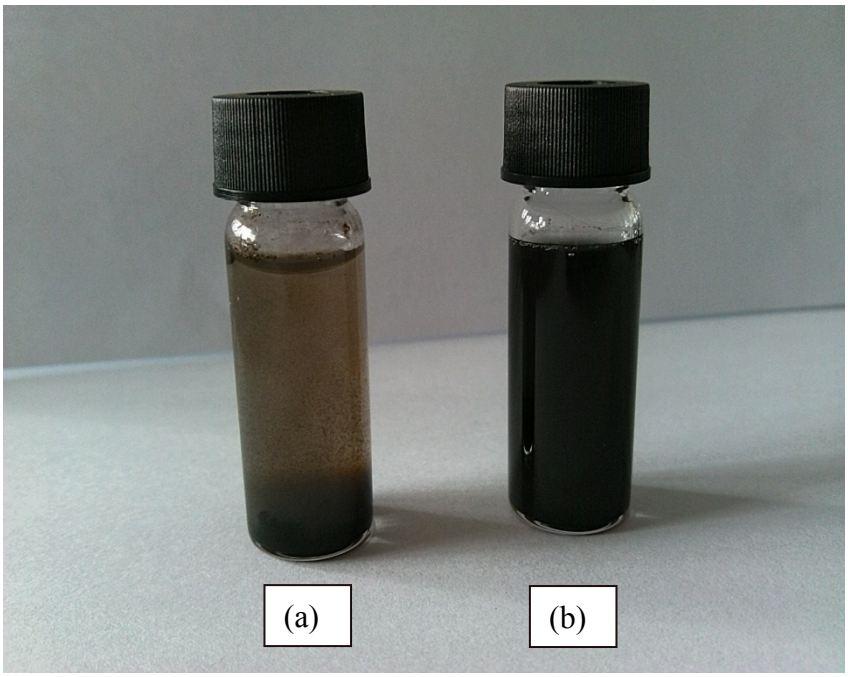

Fig. S3 Photographs of sample solutions after standing for 30 min (a) Fe_3O_4 and (b) Fe_3O_4@PDA@ATP-Ti^{4+} microspheres.

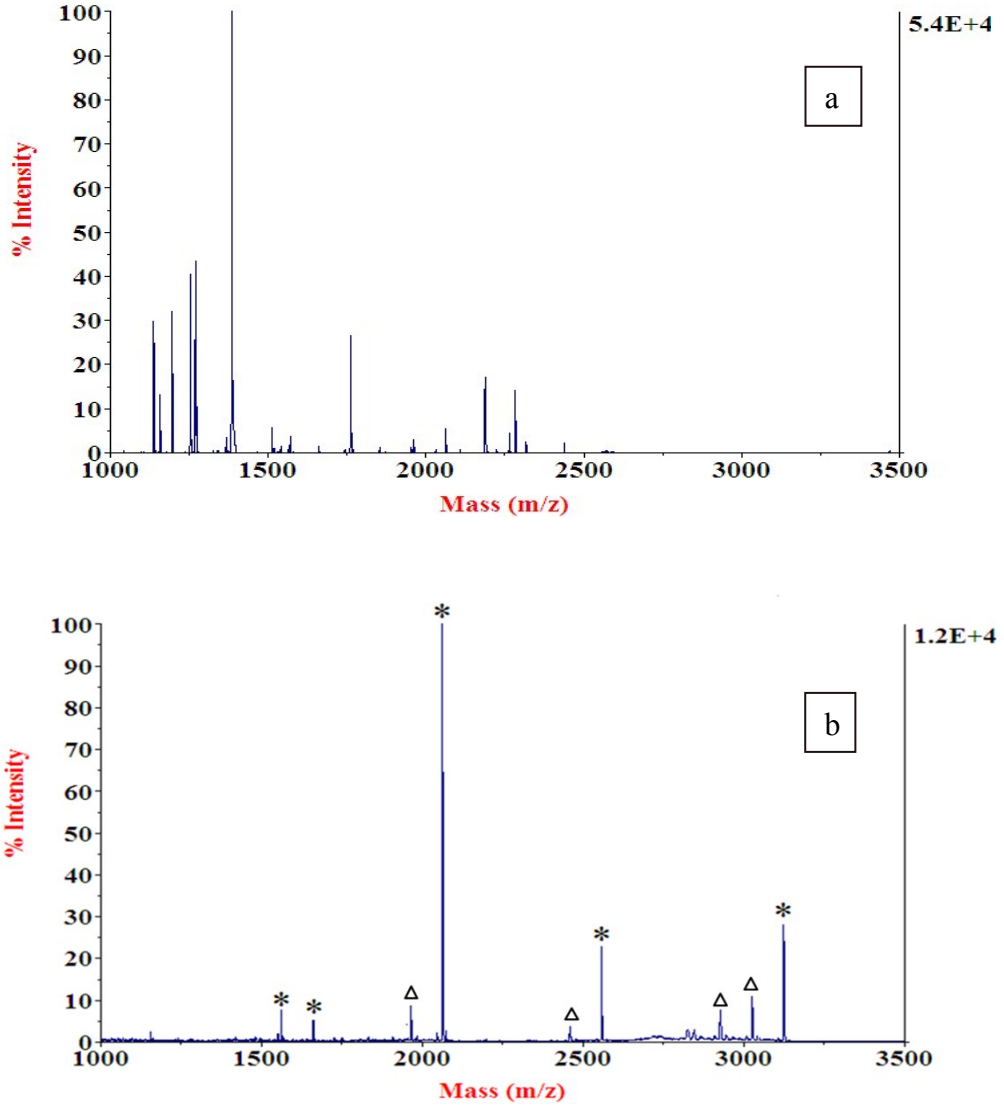

Fig. S4 MALDI mass spectrum of peptides derived from β-casein (a) before enrichment and (b) after enrichment by Fe_3O_4@PDA@ATP-Ti^{4+} microspheres, where phosphopeptides were marked with *, and Δ indicates the losses of phosphoric acid.

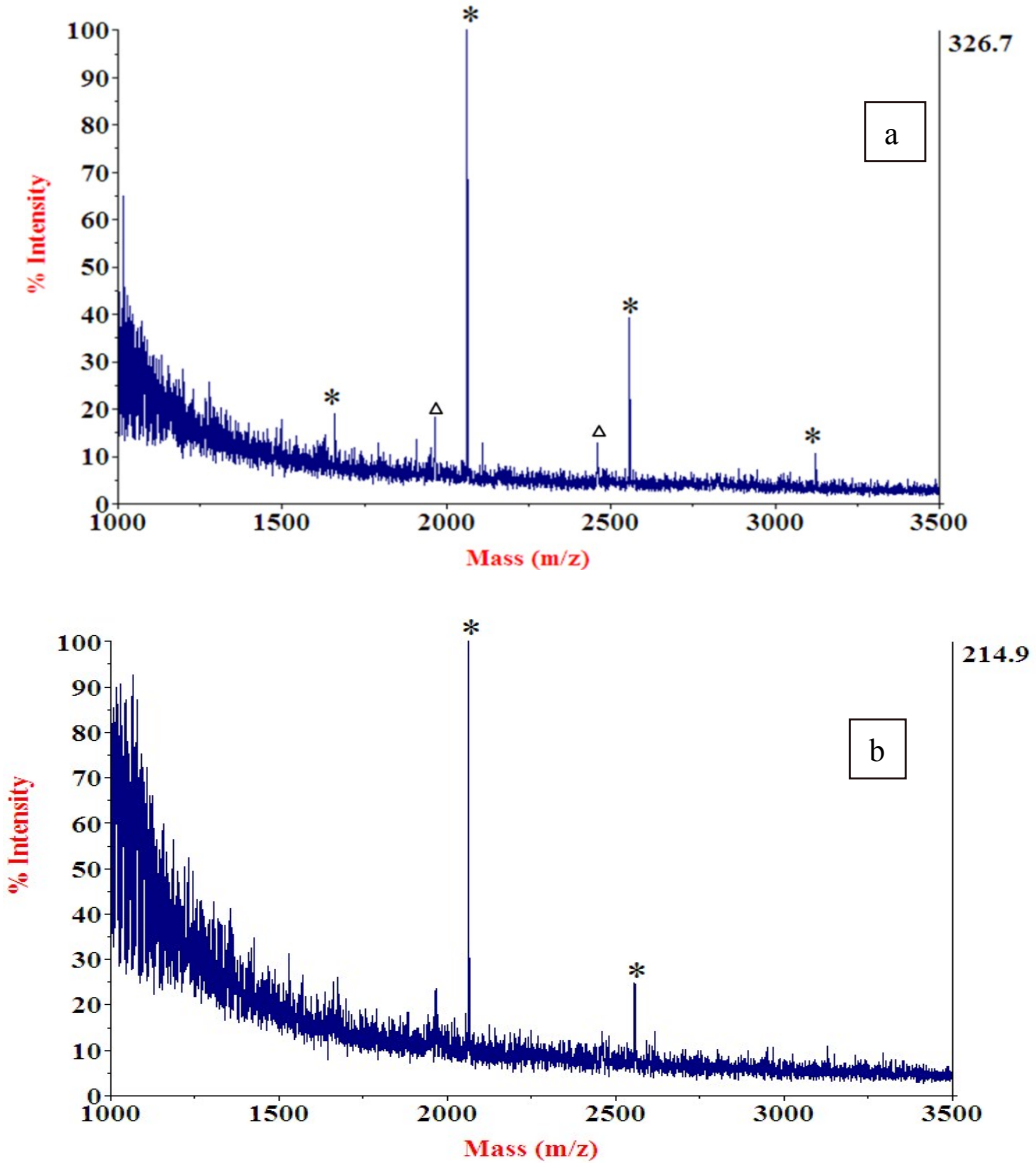

Fig. S5 MALDI mass spectra of phosphopeptides enrichment by Fe_3O_4@PDA@ATP-Ti^{4+} microspheres, with the tryptic digests of β-casein amount as (a) 20 fmol and (b) 2 fmol, where phosphopeptides were marked with *, and Δ indicates the losses of phosphoric acid.

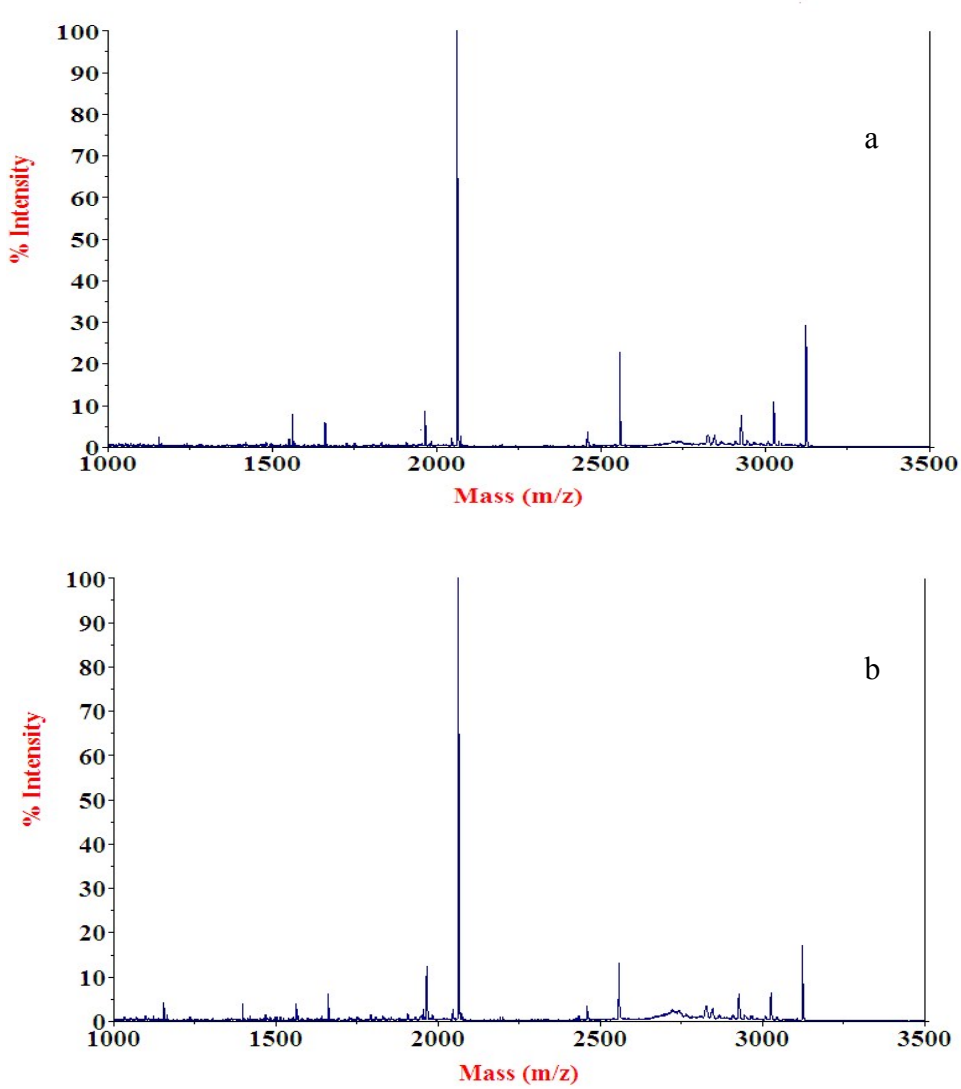

Fig. S6 MALDI mass spectrum of phosphopeptides enrichment from β-casein using Fe_3O_4@PDA@ATP-Ti^{4+} microspheres, (a) for the first time and (b) for the fifth time.

Table S1 Detailed information of phosphopeptides identified from β-casein digest.

m/z	Protein	Phosphopeptide Sequence
1561.6424	β/1-25	RELEELNVPGEIVEpSLpSpSpSEESITR
1660.5761	α-S1/106-119	VPQLEIVPNpSAEER
2061.8446	β/33-48	FQpSEEQQQTEDELQDK
2556.4561	β/33-52	FQpSEEQQQTEDELQDKIHPF
3122.2587	β/1-25	RELEELNVPGEIVEpSLpSpSpSEESITR

Table S2 Comparison between Fe_3O_4@PDA@ATP-Ti^{4+} and the other magnetic materials for phosphopeptide enrichment reported before.

Material	Selectivity (the molar ratio of β-casein and BSA)	Detection limit	Ref.
Fe_3O_4@Au-GSH-Gd^{3+}	1:1000	10 fmol	*Talanta* 2018, 180, 368–375
Fe_3O_4@PDA-Fe^{3+}	1:100	200 fmol	*Talanta* 2018, 178, 600–607
ZnMMs	1:100	25 fmol	*Anal. Sci.* 2017, 33, 1381-1386
Fe_3O_4@PD-Ti^{4+}	1:500	2 fmol	*Chem. Commun.* 2013, 49, 5055−5057
Fe_3O_4@PDA@ATP-Ti^{4+}	1:1000	2 fmol	This work

Table S3 Detailed information of the observed phosphopeptides obtained from human serum.

m/z(Da)	peptides	Peptide sequence
1389.6	1	ADpSGEGDFLAEGGGV
1460.6	2	DpSGEGDFLAEGGGV
1545.3	3	DpSGEGDFLAEGGGVR
1616.5	4	ADpSGEGDFLAEGGGVR