Supporting Information

A poly(ethylene glycol)-brush decorated magnetic polymer for highly specific enrichment of phosphopeptides

Liang Zhao, Hongqiang Qin, Zhengyan Hu, Yi Zhang, Ren'an Wu and Hanfa Zou**

National Chromatographic R&A Center, CAS Key Laboratory of Separation Sciences for Analytical Chemistry, Dalian Institute of Chemical Physics, Chinese Academy of Sciences (CAS), Dalian 116023, China

*To whom correspondence should be addressed:

Prof. Dr. Ren'an Wu

Tel: +86-411-84379576, Fax: +86-411-84379620, Email: wurenan@dicp.ac.cn

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Prof. Dr. Hanfa Zou

Tel: +86-411-84379610, Fax: +86-411-84379620, E-mail: hanfazou@dicp.ac.cn

Table S1. Zeta-potential results of Fe_3O_4@SiO_2@PEG-Br^{-1}, amine functionalized Fe_3O_4@SiO_2@PEG nanoparticles (Fe_3O_4@SiO_2@PEG-NH_2), phosphonate-modified Fe_3O_4@SiO_2@PEG nanoparticles (Fe_3O_4@SiO_2@PEG-PO_3^{2-}), and Fe_3O_4@SiO_2@PEG-Ti^{4+} nanoparticles for the subsequent modification reactions.

Functional nanoparticles	Zeta potential(mV)
Fe_3O_4@SiO_2@PEG-Br^{-1}	-3.73
Fe_3O_4@SiO_2@PEG-NH_2	+32.7
Fe_3O_4@SiO_2@PEG-PO_3^{2-}	-18.4
Fe_3O_4@SiO_2@PEG-Ti^{4+}	+17.2

Table S2. The surface compositions of functionalized PEG-decorated magnetic nanoparticle were determined by X-ray photoelectron spectroscopy (XPS) analysis. The C/O ratios for the poly(ethylene glycol) methacrylate brushes of Fe_3O_4@SiO_2@PEG nanoparticles (great than the sampling depth of XPS) was 2.3, which is reasonably consistent with the stoichiometry value 2.1 of poly(ethylene glycol) methacrylate. The change of chemical compositions of functionalized PEG-decorated magnetic nanoparticles and the appearance of P and Ti element indicated the Fe_3O_4@SiO_2@PEG-Ti^{4+} nanoparticles were successfully synthesized through multiply reactions.

Functional nanoparticles	XPS chemical compositions (%)						
	C	O	N	Si	Fe	P	Ti
Fe_3O_4@SiO_2@PEG	67.71	29.28	1.94	0.90	0.17		
Fe_3O_4@SiO_2@PEG-NH_2	71.27	25.40	2.16	1.04	0.13		
Fe_3O_4@SiO_2@PEG-PO_3^{2-}	61.12	32.26	2.68	0.81	0.11	2.02	
Fe_3O_4@SiO_2@PEG-Ti^{4+}	44.04	43.43	1.66	0.88	0.11	0.92	8.95

Table S3. Sequences of four standard phosphopeptides and identified phosphopeptides from α-casein, β-casein and ovalbumin tryptic digestMALDI-TOF MS analysis.

Protein	No.	Amino acid sequence	No. of phosphosite	$[M+H]^+$
α-Casein	α_1	TVDME[pS]TEVF	1	1237.08
	α_2	TVD[Mo]ME[pS]TEVF[a]	1	1253.11
	α_3	EQL[pS]T[pS]EENSK	2	1411.90
	α_4	VPQLEIVPN[pS]AEER	1	1660.15
	α_5	YLGEYLIVPN [pS]AEER	1	1832.70
	α_6	DIGSE[pS]TEDQAMEDIK	1	1847.95
	α_7	DIG[pS]E[pS]TEDQAMEDIK	2	1927.89
	α_8	DIG[pS]E[pS]TEDQA[Mo]EDIK[a]	2	1943.89
	α_9	YKVPQLEIVPN[pS]AEER	1	1951.09
	α_{10}	KKYKVPQLEIVPN[pS]AEERL	1	2080.00
	α_{11}	NTMEHV[pS][pS][pS]EESII[pS]QETYK	4	2618.93
	α_{12}	VNEL[pS]KDIG[pS]E[pS]TEDQAMEDIK	3	2678.02
	α_{13}	Q*MEAE[pS]I[pS] [pS] [pS]EEIVPN[pS]VEAQK[b]	5	2703.75
	α_{14}	QMEAE[pS]I[pS][pS][pS]EEIVPNPN[pS]VEQK	5	2720.05
	α_{15}	Q[Mo]EAE[pS]I[pS][pS][pS]EEIVPNPN[pS]VEQK	5	2736.05
	α_{16}	KEKVNEL[pS]KDIG[pS]E[pS]TEDQAMEDIKQ	3	2935.87
	α_{17}	NANEEEYSIG[pS][pS][pS]EE[pS]AEVATEEVK	4	3008.22
	α_{18}	NANEEEY[pS]IG[pS][pS][pS]EE[pS]AEVATEEVK	5	3087.93
β-Casein	β_1	FQ[pS]EEQQQTEDELQDK	1	2061.94
	β_2	RELEELNVPGEIVE[pS]L[pS][pS][pS]EESITR	4	3122.56
Ovalbumin	O_1	EVVG[pS]AEAGVDAASVSEEFR	1	2090.62
	O_2	FDKLPGFGD[pS]IEAQCGTSVNVHSSLR	1	2903.46
---	S_1	NVPL[pY]K	1	813.39
	S_2	HLADL[pS]K	1	863.40
	S_3	VNQIGTL[pS]E[pS]IK	2	1448.64
	S_4	VNQIG[pT]LSESIK	1	1368.68

Notes: Superscripts: a) Oxidation on methionine; b) Pyroglutamylation on the N-terminal Q*.
Subscript: [pS] phosphorylated site.

Fig. S1 TGA curves of (a) Fe_3O_4@SiO_2; (b) Fe_3O_4@SiO_2-Br; (c) Fe_3O_4@SiO_2@PEG, respectively. TGA was carried out in nitrogen at a heating rate of 35°C/10 min. For the relative mass increase of Fe_3O_4@SiO_2@PEG nanoparticles and Fe_3O_4@SiO_2-Br nanoparticles were calculated from formula: [(1 - *W*pp)/*W*pp - (1 - *W*ip)/*W*ip]× 100%, where *W*pp and *W*ip represent the percent weight retention at 600 °C of Fe_3O_4@SiO_2@PEG nanoparticles and Fe_3O_4@SiO_2-Br nanoparticles or Fe_3O_4@SiO_2-Br nanoparticles and Fe_3O_4@SiO_2 nanoparticles, respectively. Assuming the magnetic nanoparticles were monodisperse with a spherical shape and considering the density of Fe_3O_4@SiO_2 was 5.18 g/cm^3 [1], the grafting density of PEG brushes and initiator bromide were calculated according to a literature[2].

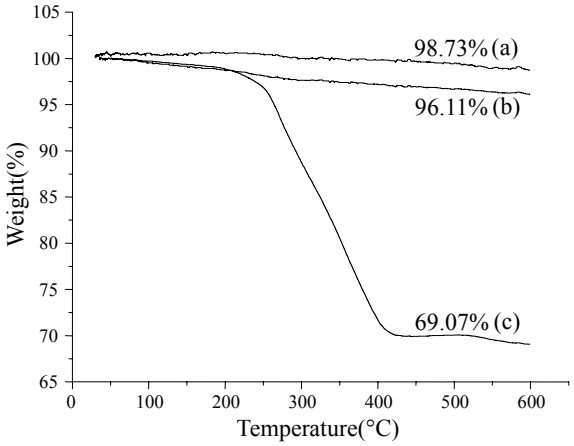


Fig. S2 FT-IR spectra of (a) Fe_3O_4@SiO_2-Br (b) Fe_3O_4@@SiO_2PEG, respectively. Fig.S3a showed that the magnetite particles exhibit strong bands in the low-frequency region (440 and 578 cm^{-1}) due to the iron oxide skeleton, and the peaks of Si-O-Si stretching vibrations center at 1081cm^{-1}; the stretching vibrations of –C=O from amide occur at 1650 cm^{-1}, which confirms the attachment of initiator Br on the Fe_3O_4@SiO_2 nanoparticles. After large quantities of PEG brushes are grown on the surface of Fe_3O_4@SiO_2-Br nanoparticles, the C-H stretching bands emerge at 2950, 2870 cm^{-1} and the C=O stretching bands show a strong bands at 1720 cm^{-1} in the spectrum. The presence of C=O and CH_2 adsorption bands provide strong evidence that the PEG brushes were successfully coated on the Fe_3O_4@SiO_2-Br nanoparticles.

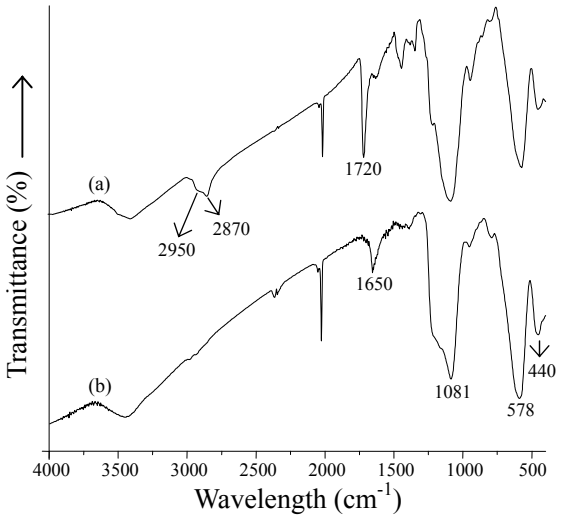


Figure S3. Wide-scan XPS spectra of the functionalized magnetic nanoparticles: (a) Fe_3O_4@SiO_2@PEG; (b) Fe_3O_4@SiO_2@PEG-NH_2; (c) Fe_3O_4@SiO_2@PEG-PO_3^{2-}; (d) Fe_3O_4@SiO_2@PEG-Ti^{4+} magnetic nanoparticles.

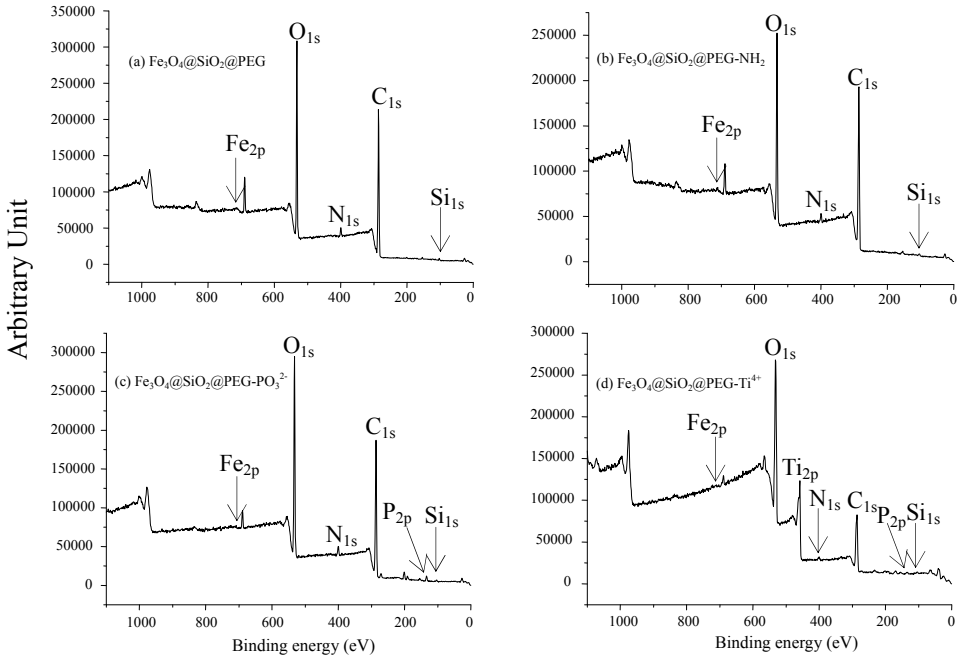


Fig. S4. The EDX spectrum data of Fe_3O_4@SiO_2@PEG-Ti^{4+} magnetic nanoparticles.

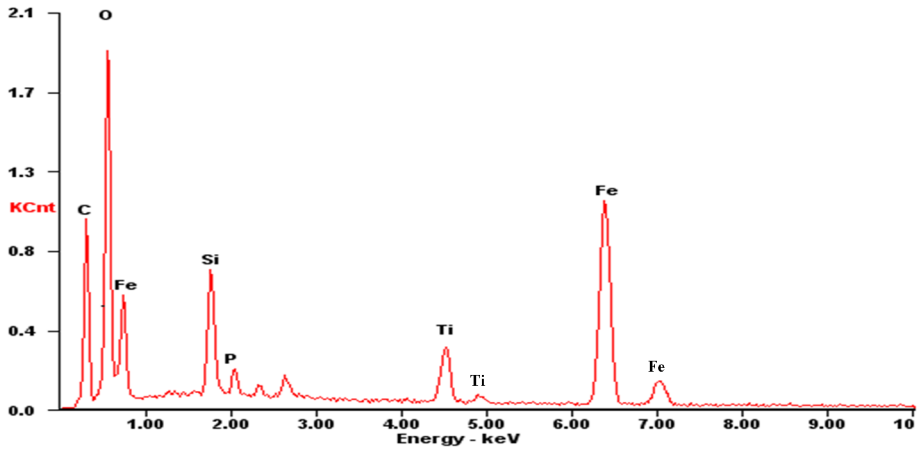


Fig. S5 The hysteresis loops of Fe_3O_4@SiO_2@PEG-Ti^{4+} magnetic nanoparticles.

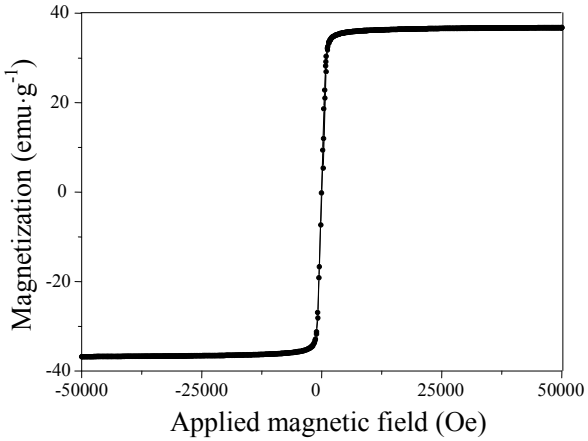


Fig. S6 MALDI-TOF MS analysis of tryptic digests of β-casein (200 μL) enriched by Fe_3O_4@SiO_2@PEG-Ti^{4+} (a,b,c) and Fe_3O_4@SiO_2-Ti^{4+} (d,e,f) IMAC nanoparticles at different concentrations of (i) 1×10^{-10} M, (ii) 5×10^{-11} M and (iii) 2.5×10^{-11} M, respectively.

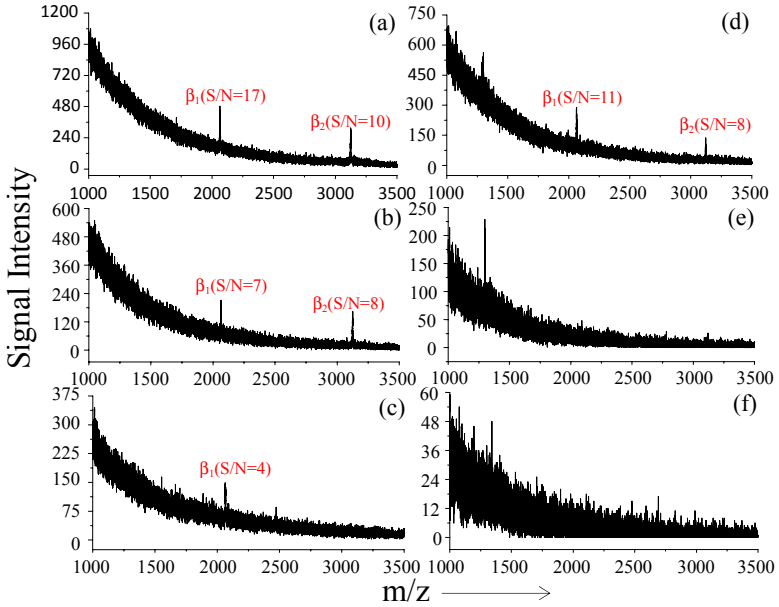
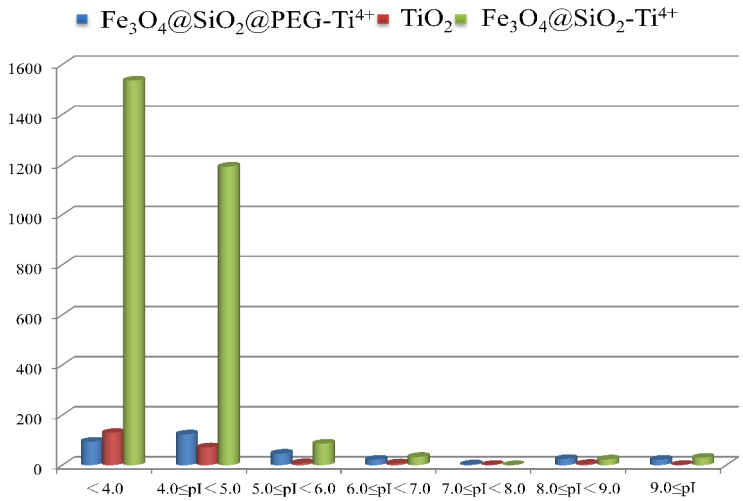


Fig. S7 The distribution of isoelectric points of nonphosphopeptides obtained by using Fe_3O_4@SiO_2@PEG-Ti^{4+}, Fe_3O_4@SiO_2-Ti^{4+} IMAC nanoparticles and TiO_2 microspheres.



Literature：

1. R. Matsuno, K. Yamamoto, H. Otsuka and A. Takahara, *Macromolecules*, 2004, **37**, 2203-2209.
2. A. P. Majewski, A. Schallon, V. Jerome, R. Freitag, A. H. Muller and H. Schmalz, *Biomacromolecules*, 2012, **13**, 857-866.