Electronic Supplementary Information

Development of magnetic $LuPO_4$ microspheres for highly selective enrichment and identification of phosphopeptides for MALDI-TOF MS analysis

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Part 1. Supporting figures

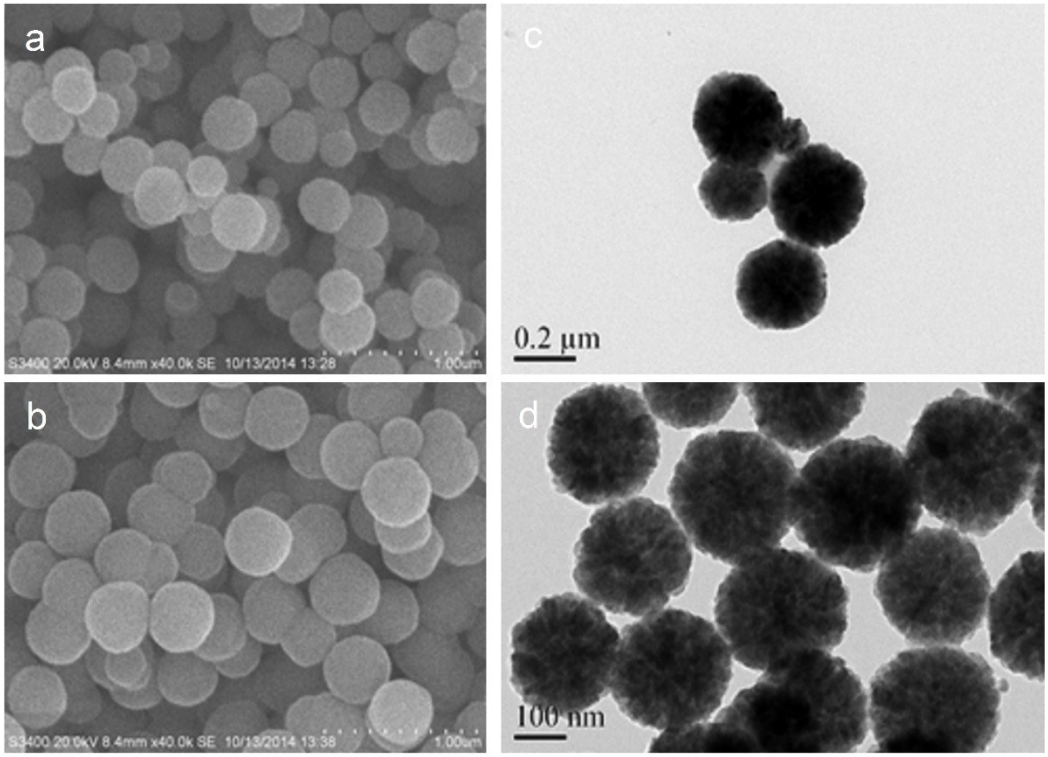


Fig. S1. SEM (left) and TEM (right) images of Fe_3O_4 (a, c), Fe_3O_4@SiO_2 (b, d), respectively.

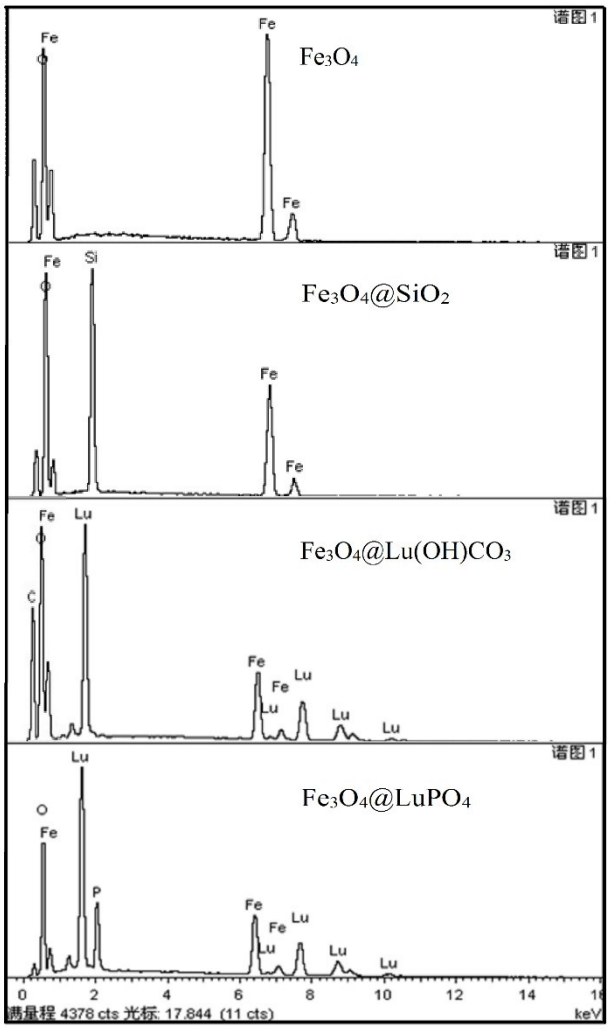


Fig. S2. EDX spectra of the prepared Fe_3O_4, $Fe_3O_4@SiO_2$, $Fe_3O_4@Lu(OH)CO_3$ and $Fe_3O_4@LuPO_4$ microspheres (From top to down).

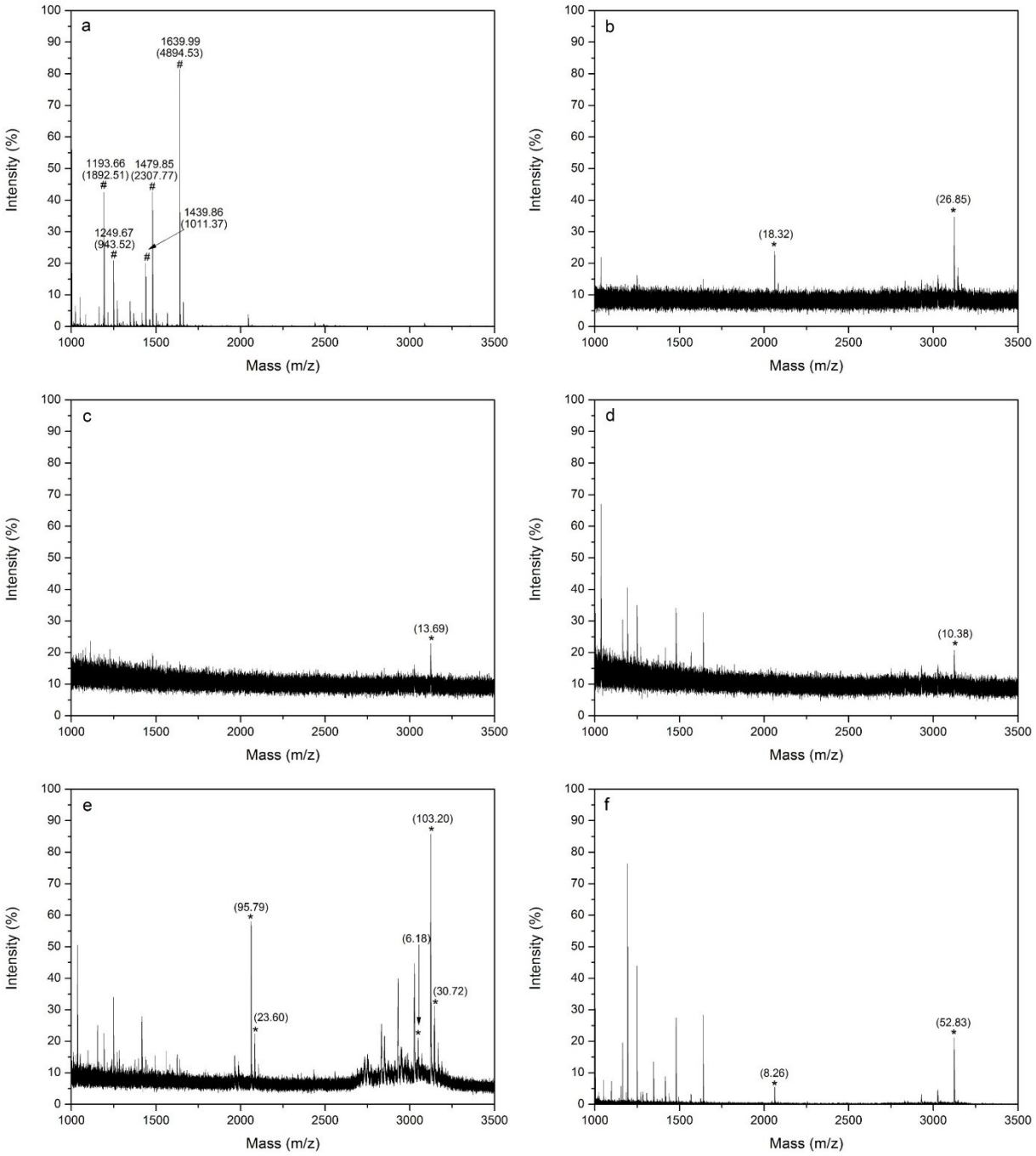


Fig. S3. MALDI-TOF MS spectra of phosphopeptides from tryptic digests of bovine *β*-casein and BSA (1:50 molar ratio) with different affinity materials. a. Without enrichment; b. Fe_3O_4; c. Fe_3O_4@SiO_2; d. Fe_3O_4@$Lu(OH)CO_3$; e. Fe_3O_4@$LuPO_4$; f. Commercial TiO_2. The data in parentheses represent *S*/*N* ratios.

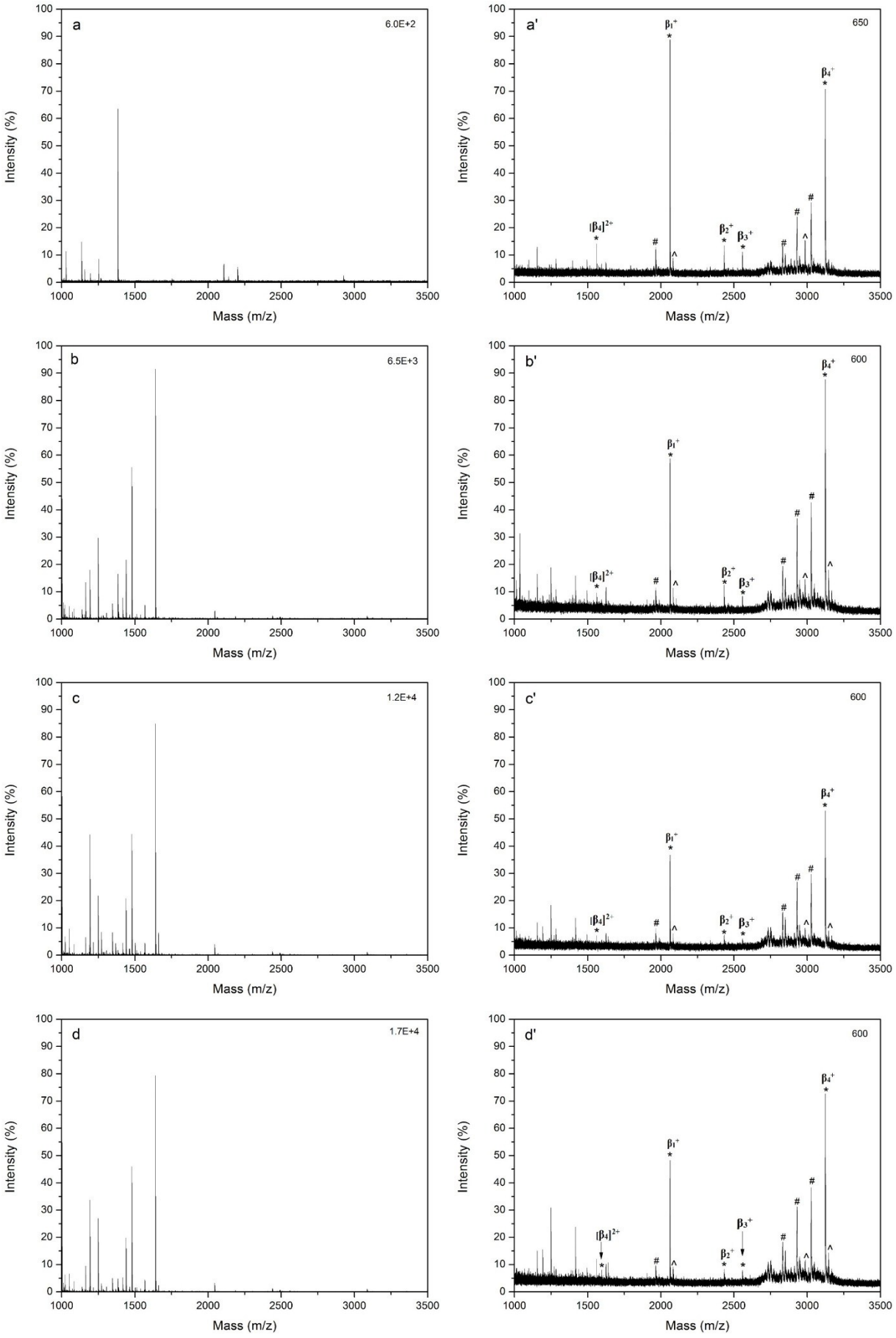

a
6.0E+2
a'
650
b
6.5E+3
b'
600
c
1.2E+4
c'
600
d
1.7E+4
d'
600
Intensity (%)
Mass (m/z)
β1+
β2+
β3+
β4+
[β4]2+
#
^

Fig. S4. MALDI-TOF MS spectra of phosphopeptides from tryptic digests of bovine *β*-casein and BSA with Fe_3O_4@$LuPO_4$ microspheres affinity materials. Left: Without enrichment; Right: After enrichment. a. 1:0; b. 1:10; c. 1:20; d. 1:50. "*" β_1, β_2, β_3, β_4: Phosphopeptides; $[\beta_4]^{2+}$: Doubly charged; "#": Metastable ions; "^": Na^+ adducted ions.

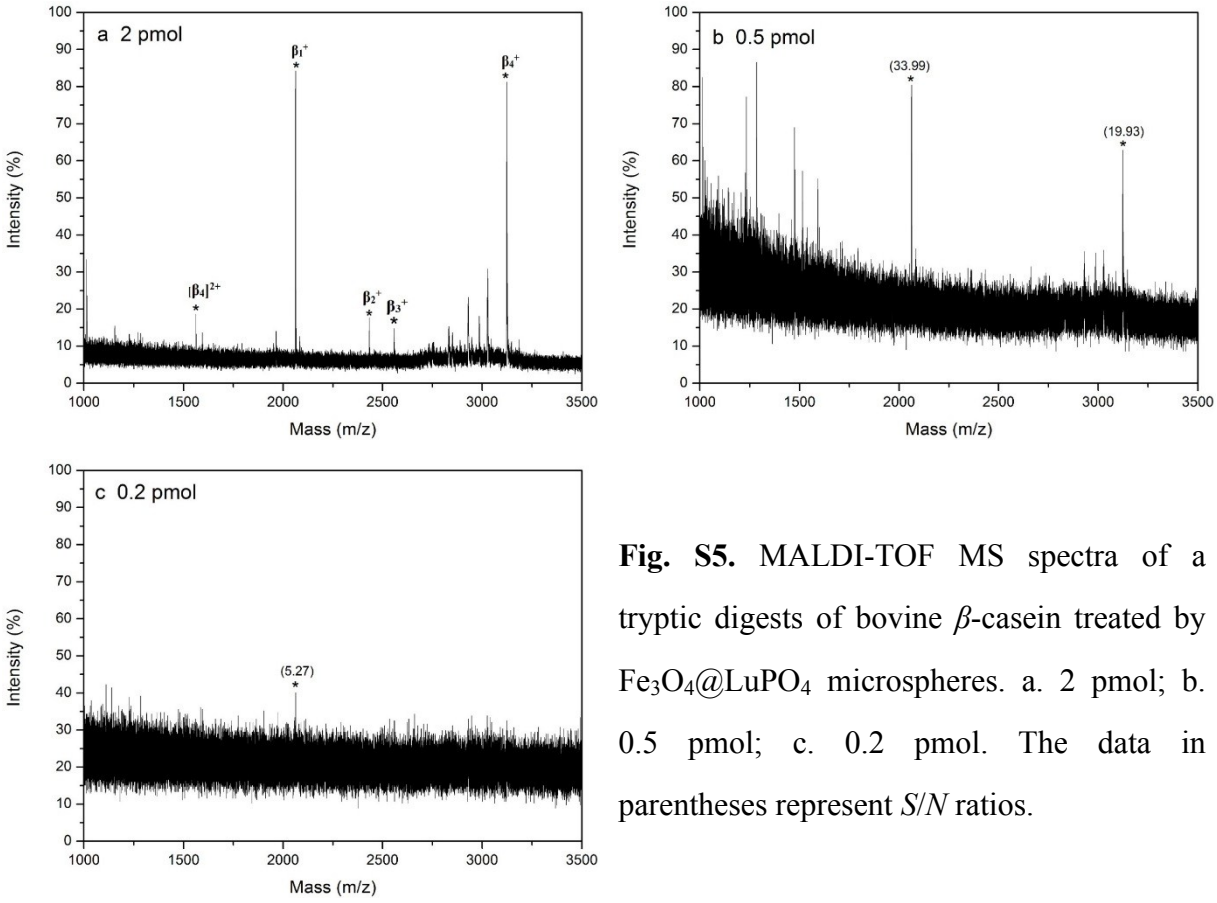


Fig. S5. MALDI-TOF MS spectra of a tryptic digests of bovine *β*-casein treated by Fe_3O_4@$LuPO_4$ microspheres. a. 2 pmol; b. 0.5 pmol; c. 0.2 pmol. The data in parentheses represent *S*/*N* ratios.

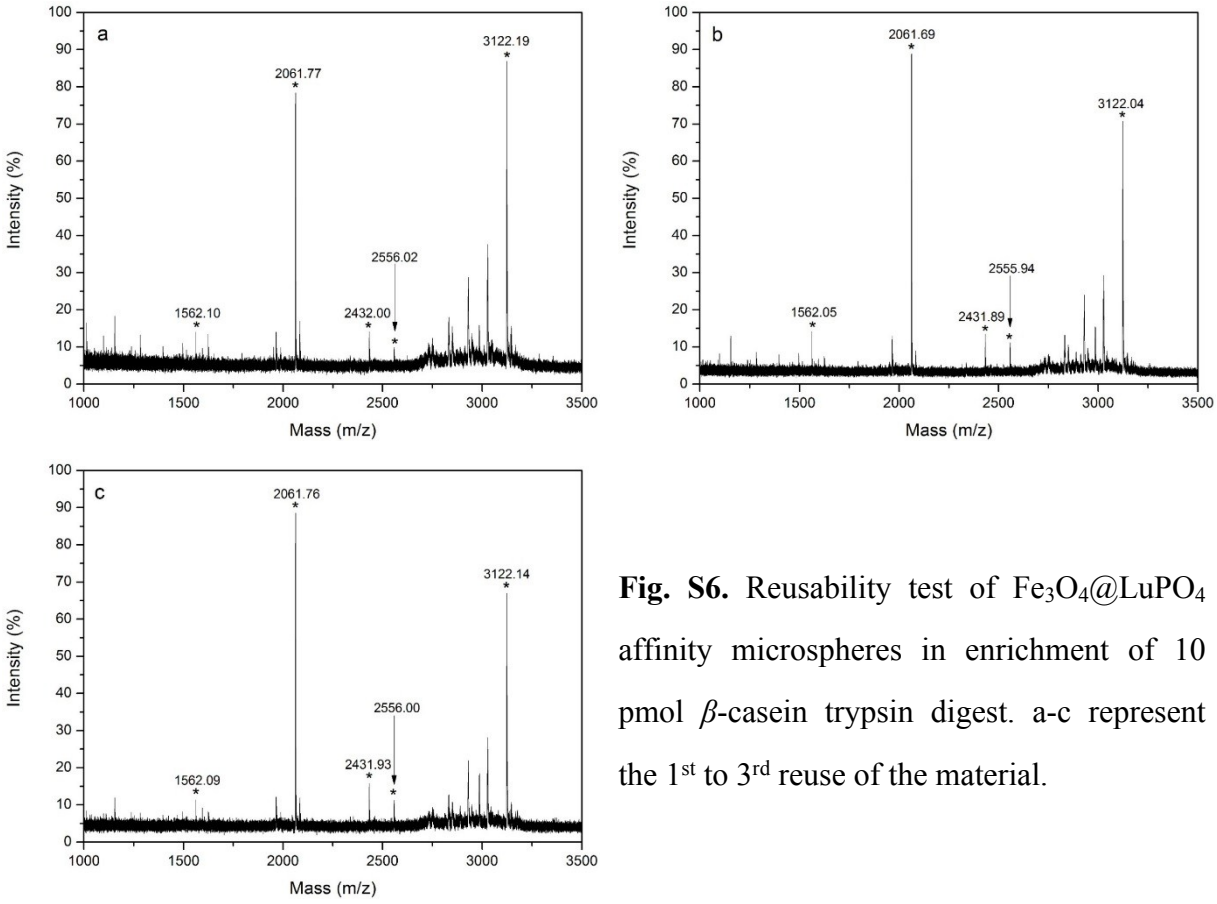
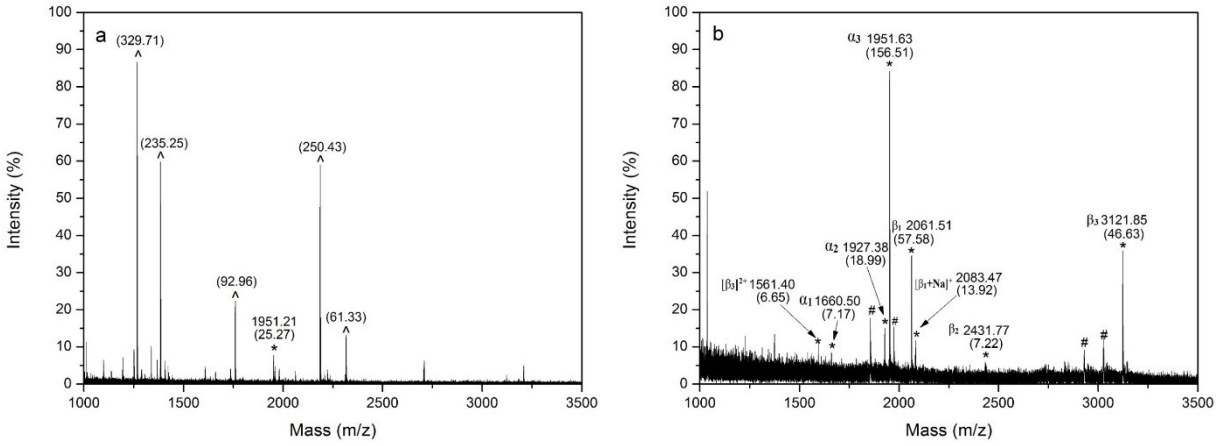


Fig. S6. Reusability test of Fe_3O_4@$LuPO_4$ affinity microspheres in enrichment of 10 pmol *β*-casein trypsin digest. a-c represent the 1st to 3rd reuse of the material.



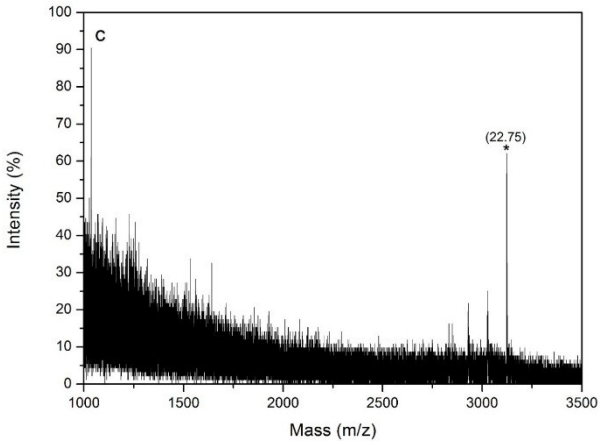


Fig. S7. MALDI-TOF MS spectra of phosphopeptides from tryptic digests of fresh pure milk treated with different affinity materials. a. Without enrichment; b. Fe_3O_4@$LuPO_4$; c. Fe_3O_4@$Lu(OH)CO_3$. "*" β_1, β_2, β_3: Phosphopeptides; $[\beta_3]^{2+}$: Doubly charged; "#": Metastable ions or Na^+ adducted ions; "^": Non-phosphopeptides. The data in parentheses represent *S*/*N* ratios.

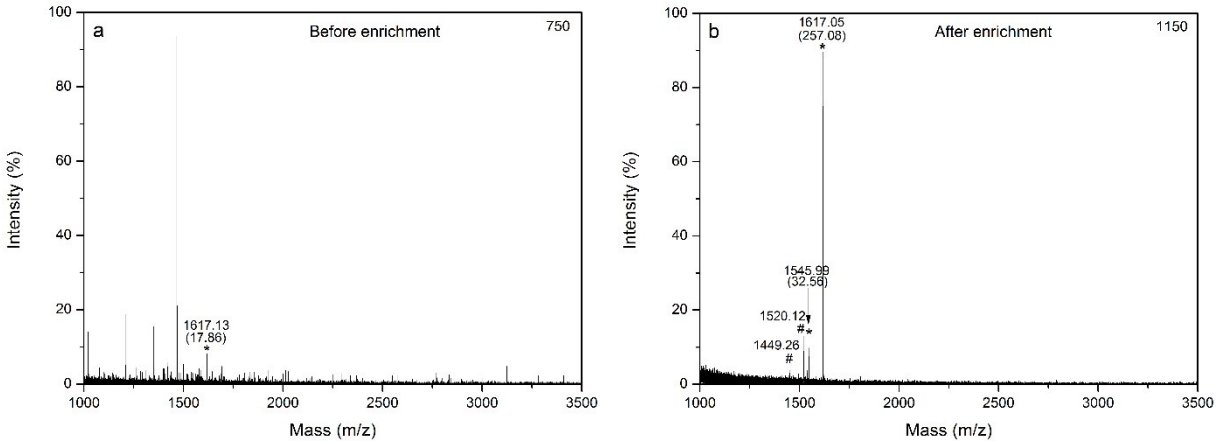


Fig. S8. MALDI-TOF MS spectra of human serum treated with Fe_3O_4@$LuPO_4$ affinity microspheres. a. Without enrichment; b. After enrichment. "*": Phosphopeptides; "#": Metastable ions phosphopeptides. The data in parentheses represent *S*/*N* ratio.

Part 2. Supporting tables

Table S1. Identified phosphopeptides from proteolytic digests of *β*-casein and fresh pure milk, and human serum sample.

m/z	*S/N*	Position	Amino acid sequence (sites)
1545.99	32.56	HS1	D[pS]GEGDFLAEGGGVR (1P)
1562.05	22.46	*β*-(16-40)*	RELEELNVPGEIVE[pS]L[pS][pS][pS]EESITR (4P)
1617.05	257.08	HS2	AD[pS]GEGDFLAEGGGVR (1P)
1660.41	14.22	*α*-S1(121-134)	VPQLEIVPN[pS]AEER (1P)
1927.23	184.18	*α*-S1(58-73)	DIG[pS]E[pS]TEDQAMEDIK (2P)
1951.49	140.96	*α*-S1(119-134)	YKVPQLEIVPN[pS]AEER (1P)
2061.69	305.65	*β*-(48-63)	FQ[pS]EEQQQTEDELQDK (1P)
2083.69	11.16	*β*-(48-63)^	FQ[pS]EEQQQTEDELQDK-Na (1P)
2431.89	36.39	*β*-(45-63)	IEKFQ[pS]EEQQQTEDELQDK (1P)
2555.94	21.43	*β*-(48-67)	FQ[pS]EEQQQTEDELQDKIHPF (1P)
2935.50	10.69	*α*-S1(50-73)	EKVNEL[pS]KDIG[pS]E[pS]TEDQAMEDIK (3P)
2984.09	27.98	*β*-(16-40)^	RELEELNVPGEIVE[pS]L[pS][pS][pS]EESITR-Na (2P)
3042.06	13.68	*β*-(16-40)	RELEELNVPGEIVE[pS]L[pS][pS][pS]EESITR (3P)
3122.04	162.86	*β*-(16-40)	RELEELNVPGEIVE[pS]L[pS][pS][pS]EESITR (4P)
3144.01	10.31	*β*-(16-40)^	RELEELNVPGEIVE[pS]L[pS][pS][pS]EESITR-Na (4P)

"[pS]/[pS]" shows phosphorylation on serine or probable; "Mo" indicates oxidation on methionine; "*" denotes doubly charged peak; "^" represents Na^+ adducted ions peak.

Table S2. Apparent and true *m*/*z* of metastable ions of phosphopeptides*

m/*z*					*r*	$\bar{r}$
M_a	M_b	M_x	M_c (Observed)	M_c' (Calculated)		
2061.76	1963.76	1870.42	1965.32	1965.44	0.992	
3122.21	3024.21	2929.29	3027.05	3025.17	0.988	
3122.21	2926.21	2742.51	2929.62	2930.30	0.996	0.992
1617.05	1519.05	1426.99	1520.96	1520.96	0.991	
1545.99	1447.99	1356.20	1449.20	1449.88	0.993	

* Calculated by Eq. 1 (Harvey derivation formula). M_a: Precursor ion; M_b: Product ion; M_c: Metastable ion.

$$M_x = \frac{M_b^2}{M_a} \qquad r = \frac{M_b - M_c + \sqrt{M_c(M_a - 2M_b + M_x)}}{(M_c - M_x)}$$

$$M_c^{'} = M_a\left[\frac{1 + \frac{M_b}{M_a}r}{(1+r)}\right]^2 \quad (1)$$

Table S3. High-abundant nonphosphopeptides from proteolytic digests of *β*-casein and BSA at molar ratio of 1:50.

m/*z*	*S*/*N*	Position	Amino acid sequence
1037.46	6.45	BSA(310-318)^	SHCIAEVEK-Na
1154.58	24.08	*β*-(113-122)	VKEA[Mo]APKHK
1249.59	29.89	BSA(35-44)	FKDLGEEHFK
1416.68	27.95	BSA(569-580)	TV[Mo]ENFVAFVDK
1495.77	11.65	BSA(387-399)	DDPHACYSTVFDK

"Mo" indicates oxidation on methionine; "^" represents Na^+ adducted ions peak.

Table S4. High-abundant non-phosphopeptides from proteolytic digest of fresh pure milk (*S*/*N* > 60)

m/*z*	*S*/*N*	Position	Amino acid sequence
1267.22	329.71	*α*-S1 (106-115)	YLGYLEQLLR
1384.19	235.25	*α*-S1(38-49)	FFVAPFPEVFGK
1759.30	92.96	*α*-S1(23-37)	HQGLPQEVLNENLLR
2185.37	250.43	*α*-S2(18-36)	TMEHVSSSEESIISQETYK
2315.30	61.33	*α*-S1 (148-166)	EPMIGVNQELAYFYPELFR