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PAMAM-PMAA brushes-functionalized magnetic composite nanospheres: A smart nanoprobe with tunable selectivity for effective enrichment of mono-, multi-, or global phosphopeptides

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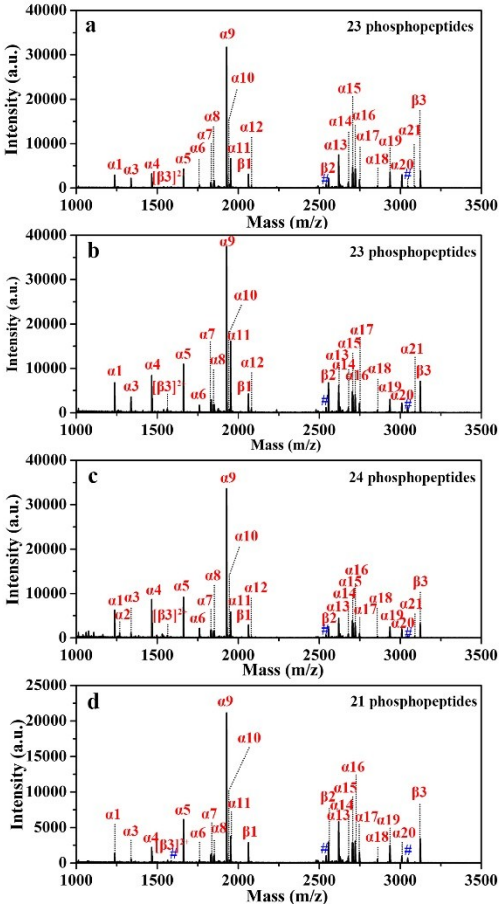

Figure S1 MALDI-TOF mass spectra of α-casein (10^{-6} M) after enrichment by Fe_3O_4@PDA@PMAA@PAMAM nanospheres with different PAMAM dendrimers amounts in 70% ACN-H_2O, 0.1 M HAC: (a) 25 μL, (b) 75 μL, (c) 150 μL and (d) 300 μL. (#, dephosphorylated peptide)

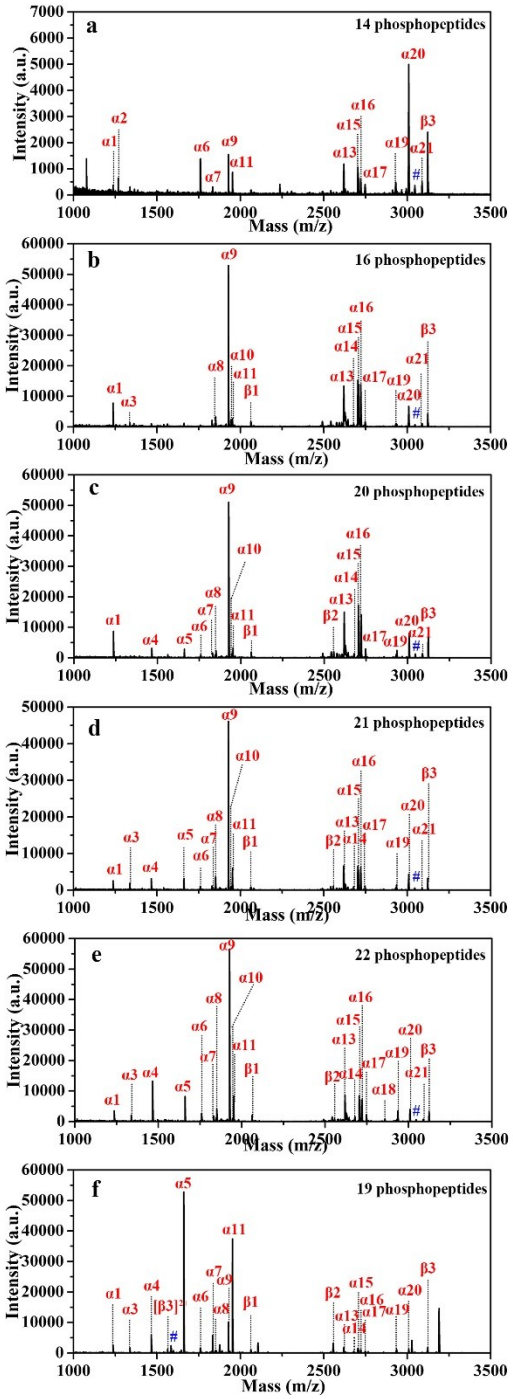

Figure S2 MALDI-TOF mass spectra of α-casein (10^{-6} M) after enrichment by Fe_3O_4@PDA@PMAA@PAMAM nanospheres with different loading buffer solution (a) 30% ACN-H_2O, 0.1 M HAC, (b) 40% ACN-H_2O, 0.1 M HAC, (c) 50% ACN-H_2O, 0.1 M HAC, (d) 60% ACN-H_2O, 0.1 M HAC, (e) 70% ACN-H_2O, 0.1 M HAC and (f) 80% ACN-H_2O, 0.1 M HAC. (#, dephosphorylated peptide)

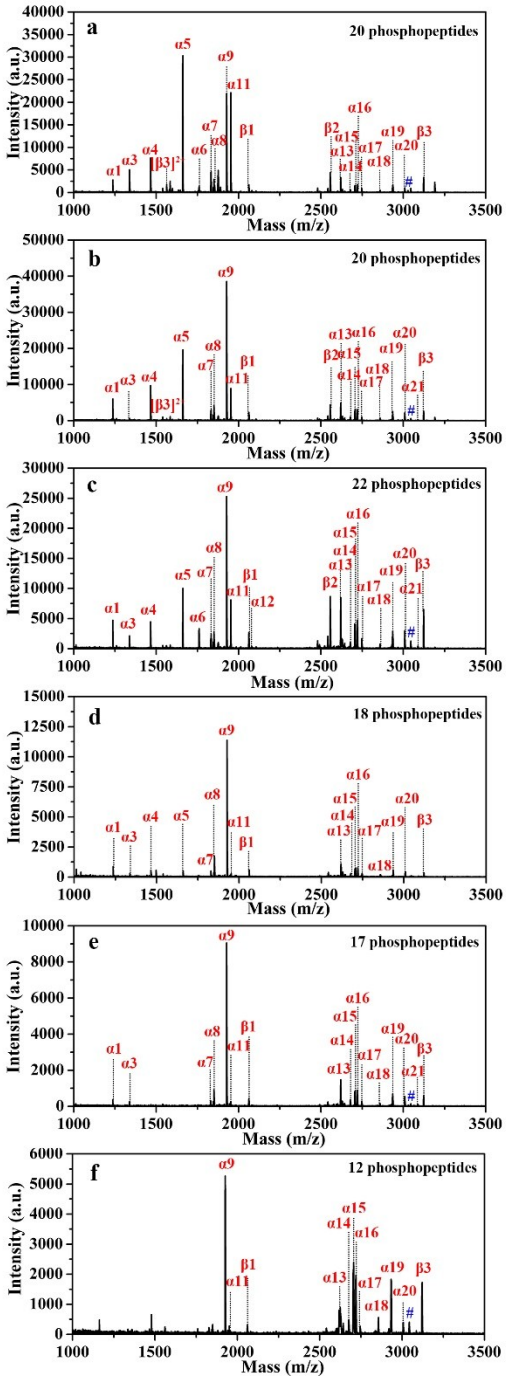

Figure S3 MALDI-TOF mass spectra of α-casein (10^{-6} M) after enrichment by Fe_3O_4@PDA@PMAA@PAMAM nanospheres with different loading buffer solution (a) 70% ACN-H_2O, 0.01 M HAC, (b) 70% ACN-H_2O, 0.05 M HAC, (c) 70% ACN-H_2O, 0.1 M HAC, (d) 70% ACN-H_2O, 0.2 M HAC, (e) 70% ACN-H_2O, 0.4 M HAC and (f) 70% ACN-H_2O, 0.8 M HAC. (#, dephosphorylated peptide)

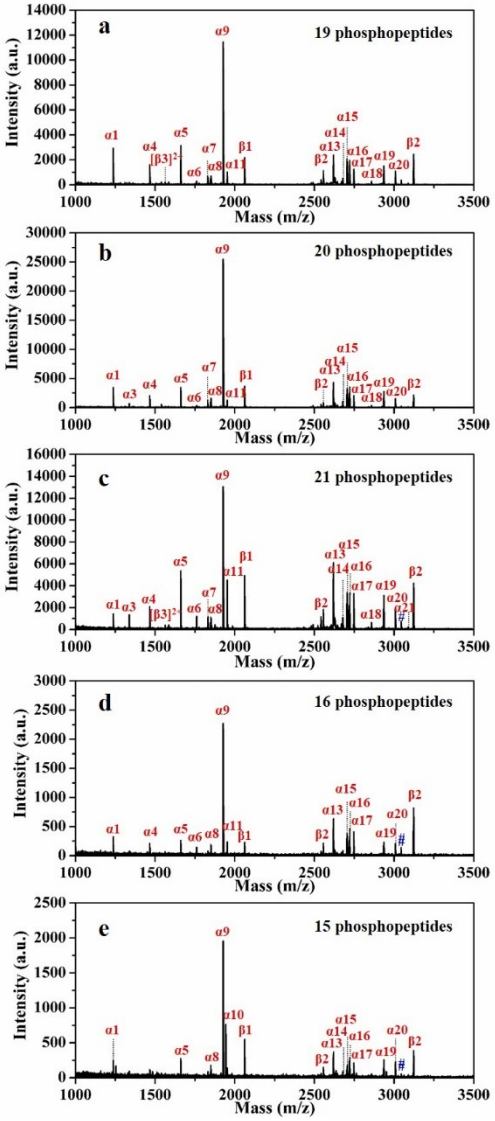

Figure S4 MALDI-TOF mass spectra of α-casein (10^{-6} M) after enrichment by Fe_3O_4@PDA@PMAA@PAMAM nanospheres with different elution buffer solution (a) 30% ACN-H_2O, 2% TFA, (b) 40% ACN-H_2O, 2% TFA, (c) 50% ACN-H_2O, 2% TFA, (d) 60% ACN-H_2O, 2% TFA and (e) 70% ACN-H_2O, 2% TFA. (#, dephosphorylated peptide)

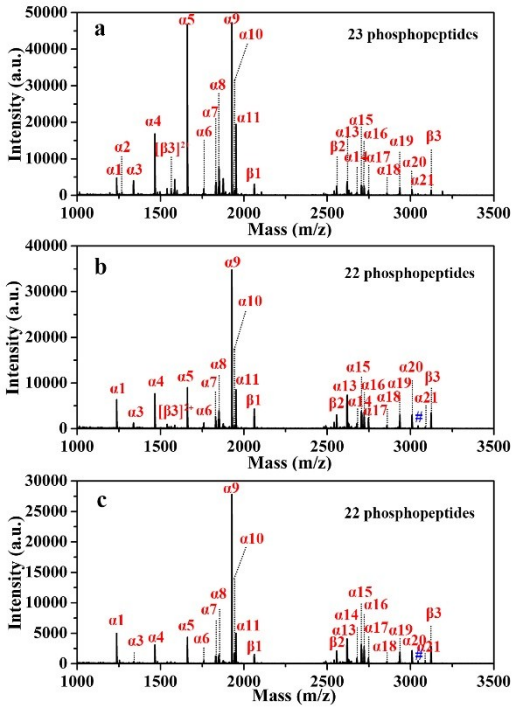

Figure S5 MALDI-TOF mass spectra of α-casein (10^{-6} M) after enrichment by Fe_3O_4@PDA@PMAA@PAMAM nanospheres with different elution buffer solution (a) 50% ACN-H_2O, 2% TFA, (b) 50% ACN-H_2O, 4% TFA and (c) 50% ACN-H_2O, 6% TFA. (#, dephosphorylated peptide)

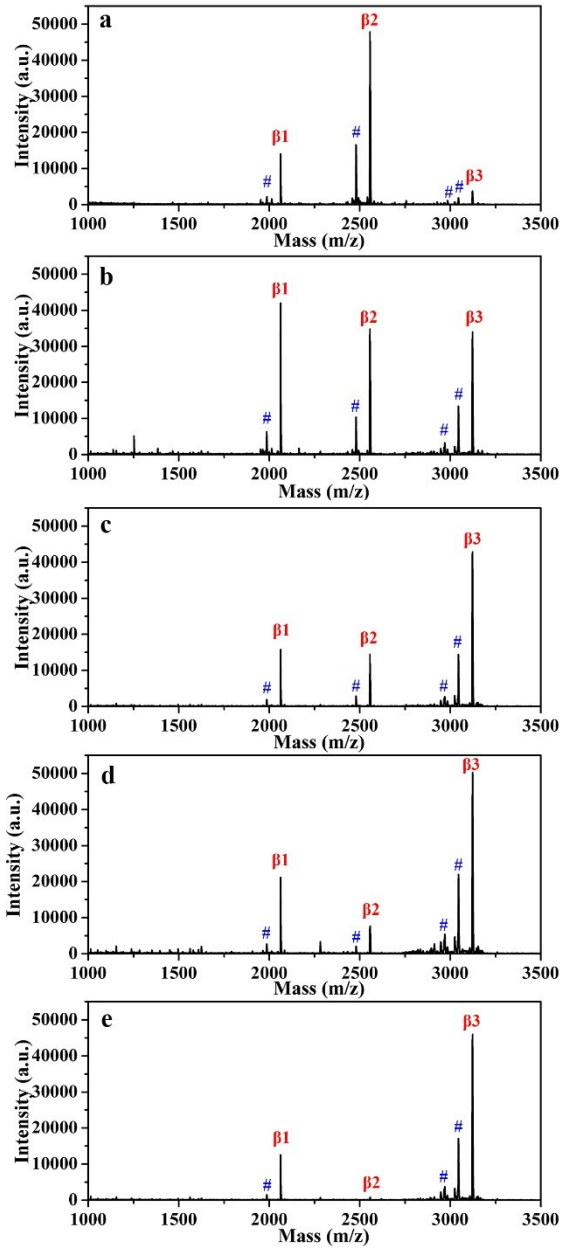

Figure S6 MALDI mass spectra of β-casein digest (10^{-6} M) after enrichment by Fe_3O_4@PDA@PMAA@PAMAM nanospheres in 70% ACN-H_2O, 0.1 M HAC. Cycling 1st (a), cycling 2nd (b), cycling 3rd (c), cycling 4th (d), and cycling 5th (e). (#, dephosphorylated peptide)

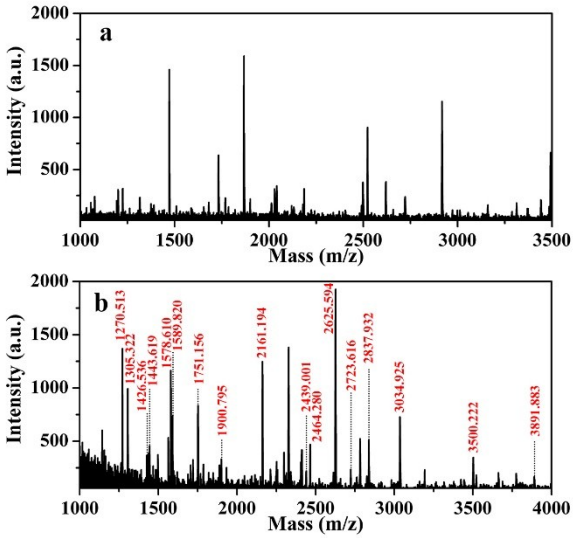

Figure S7 MALDI-TOF mass spectra of non-digest human saliva (a) direct analysis and (b) after enrichment by Fe_3O_4@PDA@PMAA@PAMAM nanospheres in 70% ACN-H_2O, 0.1 M HAC.

Table 1 Detail information of the observed phosphopeptides obtained from tryptic digest of α-casein after enrichment by Fe_3O_4@PDA@PMAA@PAMAM nanospheres in MALDI-TOF MS analysis

No.	Observed m/z	Peptide sequence	Number of phosphoryl groups	Reported reference
α1	1237.427	TVDME[pS]TEVF	1	1, 2
α2	1267.628	YLGYLEQLLR		5
α3	1337.682	HIQKEDV[pS]ER	1	1
α4	1466.566	TVDME[pS]TEVFIK	1	1, 2, 5, 6
α5	1660.767	VPQLEIVPN[pS]AEER	1	1, 5, 6
α6	1759.906	HQGLPQEVLNENLLR		5
α7	1832.802	YLGEYLIVPN[pS]AEER	1	1, 2, 6
α8	1847.647	DIGSE[pS]TEDQAMEDIK	1	1, 2, 5, 6
α9	1927.631	DIG[pS]E[pS]TEDQAMEDIK	2	1, 2, 5, 6
α10	1943.589	DIG[pS]E[pS]TEDQA[Mo]EDIK	2	1, 2, 6
α11	1951.930	YKVPQLEIVPN[pS]AEER	1	1, 2, 5, 6
α12	2080.000	KYKVPQLEIVPN[pS]AEER	1	2, 3, 6
α13	2618.850	NTMEHV[pS][pS][pS]EESII[pS]QETYK	4	1, 2
α14	2677.897	VNEL[pS]KDIG[pS]E[pS]TEDQAMEDIK	3	1, 2, 6
α15	2703.789	QMEAE[pS]I[pS][pS][pS]EEIVPN[pS]VEA	5	1, 2, 6

α16	2720.900	QMEAE[pS]I[pS][pS][pS]EEIVPNPN[pS]VE	5	1, 2, 6
α17	2746.960	NTMEHV[pS][pS][pS]EE[pS]IISQETYKQ	4	2, 6
α18	2857.324	VNELSKDIG[pS]E[pS]TEDQAMEDIKQM	2	4, 6
α19	2935.087	EKVNEL[pS]KDIG[pS]E[pS]TEDQAMEDI	3	1, 2, 6
α20	3007.954	NANEEEYSIG[pS][pS][pS]EE[pS]AEVATE	4	1, 2, 6
α21	3087.818	NANEEEY[PS]IG[PS][PS][PS]EE[PS]AEVA	5	2, 6
β1	2061.780	FQ[pS]EEQQQTEDELQDK	1	1, 2, 6
β2	2555.975	FQ[pS]EEQQQTEDELQDKIHPF	1	1, 2
β3	3122.138	RELEELNVPGEIVE[pS]L[pS][pS][pS]EESI	4	1, 2, 5, 6

Table 2 Detail information of the observed phosphopeptides from tryptic digest of proteins extracted from non-fat milk after enrichment by Fe_3O_4@PDA@PMAA@PAMAM nanospheres in MALDI-TOF MS analysis

No.	Observed m/z	Peptide sequence	Number of phosphoryl groups	Reported reference
1	1121.519	KEKVNEL[pS]KDIG[pS]E[pS]TEDQA	3	1, 3
2	1237.660	TVDME[pS]TEVF	1	1, 2
3	1252.759	TVD[Mo]E[pS]TEVF	1	1, 2
4	1282.662	KKIEKFQ[pS]EEQQQTEDELQDKIHPFAQ	1	3
5	1454.696	LSKDIG[pS]E[pS]TEDQA	2	3, 4
6	1494.724	RFFVAPFPEVFGKEKVNELSKDIG[pS]E[p	2	4
7	1660.571	VPQLEIVPN[pS]AEER	1	1, 2, 3, 5, 6
8	1760.139	HQGLPQEVLNENLLR		5
9	1847.742	DIGSE[pS]TEDQAMEDIK	1	1, 2, 5, 6
10	1926.350	DIG[pS]E[pS]TEDQAMEDIK	2	1, 2, 3, 5, 6
11	1981.787	NMAINP[pS]KENLCSTFCK	1	2, 6
12	2060.446	FQ[pS]EEQQQTEDELQDK	1	1, 2, 3, 6
13	2351.396	NVPGEIVESL[pS][pS][pS]EE[pS]ITR	4	2
14	2422.602	KYKVPQLEIVPN [pS]AEERLHSMK	1	4
15	2554.502	FQ[pS]EEQQQTEDELQDKIHPF	1	1, 2, 3, 5
16	2807.452	KVNEL[pS]KDIG[pS]E[pS]TEDQAMEDIK	3	1
17	2865.600	KVNELSKDIG[pS]E[pS]TEDQAMEDIKQ	2	5

18	2933.641	EKVNEL[pS]KDIG[pS]E[pS]TEDQAMEDI	3	1, 2, 6
19	2950.581	KEKVNEL[pS]KDIG[pS]E[pS]TEDQA[Mo]	3	2
20	2963.424	ELEELNVPGEIVE[pS]L[pS][pS][pS]EESIT	4	1, 2, 3, 5
21	3022.490	RELEELNVPGEIVESL[pS] [pS]	2	6
22	3120.430	RELEELNVPGEIVE[pS]L[pS][pS][pS]EESI	4	1, 2, 3, 5, 6

Table 3 Enrichment performance of different materials to endogenous phosphopeptides from human saliva

Number	Type of materials	The number of identified phosphopeptides	Reference
1	TiO_2-NH_2	11	7
2	magG@PDA-Sn^{4+}	20	8
3	Ti^{4+}-MGMSs	14	9
4	MG@ $mSiO_2$-ATP-Ti^{4+}	19	10
5	Fe_3O_4 MNCs	11	11
6	MNPs-(POM_4/$CYECS_4$)	16	12
7	Fe_3O_4-LDH_2	9	13
8	Fe_3O_4@TiO_2-ZrO_2@$mSiO_2$	14	14
9	2-D Hf-BTB	17	15
10	Fe_3O_4@PDA@PMAA@PAMAM	17	This work

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