Electronic Supplementary Material (ESI) for Lab on a Chip

Tailoring of TiO_2 Nanotube Array-Integrated Portable Microdevice for Efficient on-Chip Enrichment and Isotope Labeling of Serum Phosphopeptides

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Supplementary Information

Table S1 The phosphopeptides identified from tryptic digests of β-casein after on-chip enrichment by using TNA-integrated microdevice with linear channel followed by MALDI-TOF MS analysis.

No.	Peptide sequence	Number of phosphoryl groups	Observed mass
β1	FQ[pS]EEQQQTEDELQDK	1	2062.202
β2	IEKFQ[pS]EEQQQTEDELQDK	1	2432.693
β3	FQ[pS]EEQQQTEDELQDKIHPF	1	2556.708
β4	RELEELNVPGEIVE[pS]L[pS][pS][pS]EESITR	4	3122.911

[pS]: phosphorylated site.

Table S2 The phosphopeptides identified from tryptic digests of α-casein after on-chip enrichment by using S-shaped microdevice followed by MALDI-TOF MS analysis.

No.	Peptide sequence	Number of phosphoryl groups	Observed mass
α1	TVDME[pS]TEVFTK	1	1466.754
α2	TVD[Mo]E[pS]TEVFTK	1	1482.601
α3	KTVDME[pS]TEVFTK	1	1594.579
α4	KTVD[Mo]E[pS]TEVFTK	1	1610.554
α5	VPQLEIVPN[pS]AEER	1	1660.666
α6	YLGEYLIVPN [pS]AEER	1	1833.003
α7	DIG[pS]E[pS]TEDQAMEDIK	2	1927.456
α8	DIG[pS]E[pS]TEDQA[Mo]EDIK	2	1943.376
α9	YKVPQLEIVPN[pS]AEER	1	1951.892
α10	KKYKVPQLEIVPN[pS]AEERL	1	2080.933
α11	NTMEHV[pS][pS][pS]EESII[pS]QETYK	4	2619.099
α12	NT[Mo]EHV[pS][pS][pS]EESII[pS]QETYK	4	2635.232

α13	VNEL[pS]KDIG[pS]E[pS]TEDQAMEDIK	3	2678.808
α14	Q*MEAE[pS]I[pS][pS] [pS]EEIVPN[pS]VEAQK	5	2703.554
α15	QMEAE[pS]I[pS][pS][pS]EEIVPNPN[pS]VEQK	5	2720.479
α16	KEKVNEL[pS]KDIG[pS]E[pS]TEDQAMEDIKQ	3	2936.012
α17	KEKVNEL[pS]KDIG[pS]E[pS]TEDQA[Mo]EDIKQ	3	2952.091
α18	NANEEEYSIG[pS][pS][pS]EE[pS]AEVATEEVK	4	3008.452
α19	NANEEEY[pS]IG[pS][pS][pS]EE[pS]AEVATEEVK	5	3088.108

[pS]: phosphorylated site;

[Mo]: oxidation on methionine;

*Pyroglutamylation on the N-terminal Q.

Table S3 The phosphopeptides identified from human serum after on-chip enrichment and isotope dimethyl labeling by using TNA-integrated microdevice followed by MALDI-TOF MS analysis.

No.	Peptide sequence	Number of phosphoryl groups	Original mass (m/z)	Labeled by CH_2O (m/z)	Labeled by CD_2O(m/z)
HS1	D[pS]GEGDFLAEGGGV	1	1389.520	1417.680	1421.704
HS2	AD[pS]GEGDFLAEGGGV	1	1460.557	1488.718	1492.756
HS3	D[pS]GEGDFLAEGGGVR	1	1545.635	1573.801	1577.811
HS4	AD[pS]GEGDFLAEGGGVR	1	1616.670	1644.834	1648.868

[pS]: phosphorylated site.

Table S4 The intensity ratios of the four serum phosphopeptides enriched from normal sera to control sera.

Intensity ratio (H/D)	HS1	HS2	HS3	HS4
Sample1	1.166	0.551	1.265	0.614
Sample2	1.105	0.634	1.072	0.595
Sample3	0.690	0.670	1.076	0.776
Sample4	0.639	0.799	1.044	1.067
Sample5	0.952	0.727	0.985	0.967
Sample6	1.134	1.157	1.018	0.931
Sample7	1.045	0.939	0.993	1.467
Average	0.961	0.783	1.065	0.917
Standard Deviation	0.215	0.207	0.095	0.301

Table S5 The intensity ratios of the four serum phosphopeptides enriched from patient sera to control sera.

Intensity ratio (H/D)	**HS1**	**HS2**	**HS3**	**HS4**
Sample1	1.648	0.864	0.891	0.184
Sample2	2.105	0.715	0.858	0.215
Sample3	1.862	0.697	0.993	0.316
Sample4	3.134	0.609	1.148	0.171
Sample5	2.629	0.835	0.877	0.181
Sample6	2.658	1.172	1.060	0.289
Sample7	2.222	0.815	0.856	0.243
Average	2.322	0.815	0.955	0.229
Standard Deviation	0.515	0.181	0.115	0.057

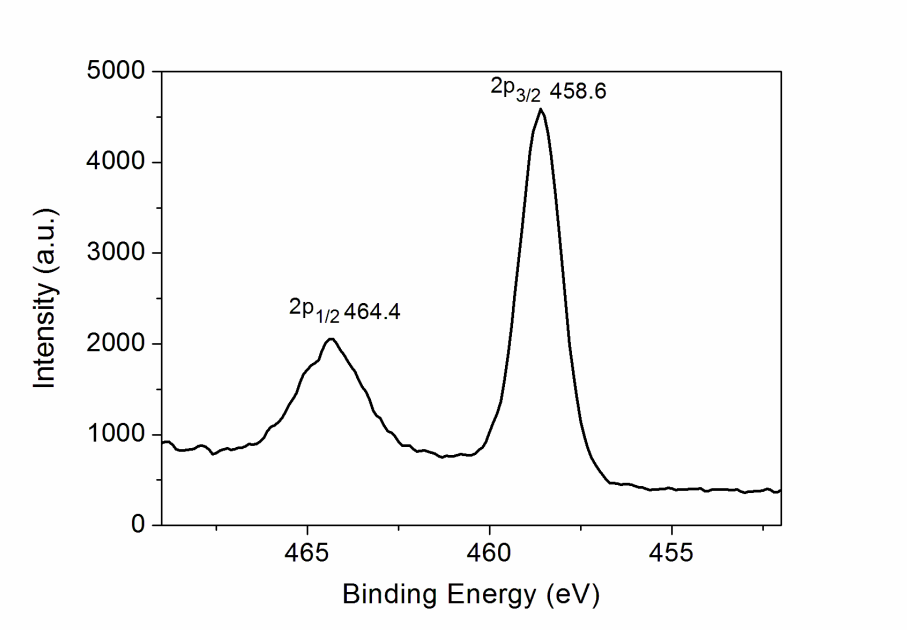

Fig. S1 XPS spectrum of Ti 2p in the TNAs.

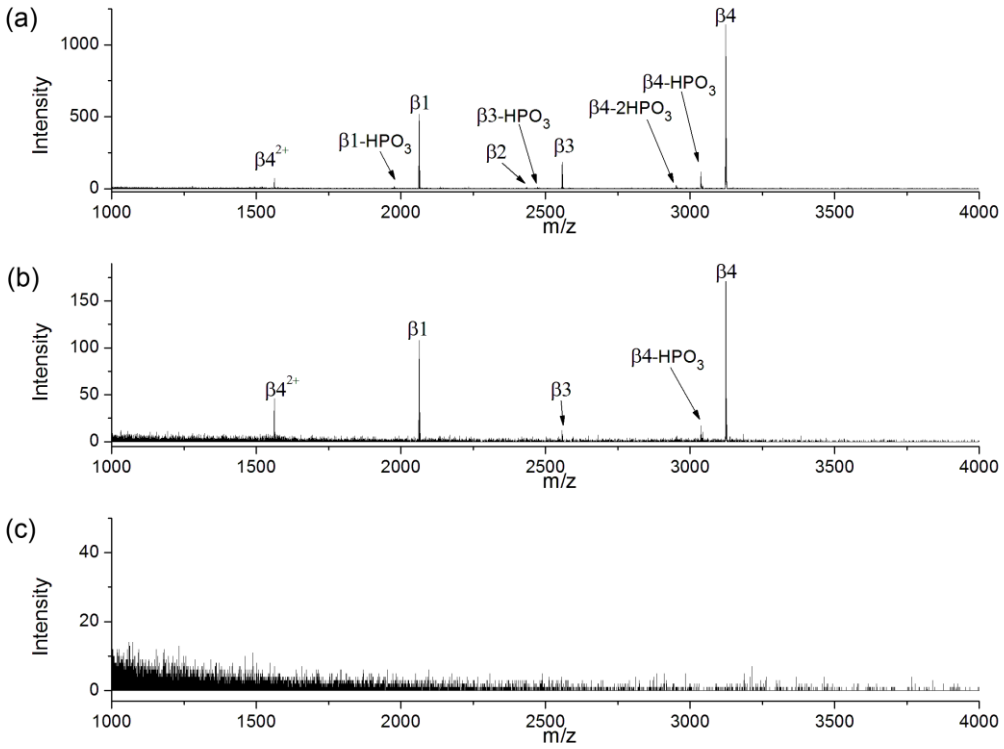

Fig. S2 MALDI-TOF MS spectra of (a) the first fraction (0~5 μL), (b) the second fraction (5~10 μL) and (c) the third fraction (10~15 μL) eluted from TNA-integrated microdevice after enrichment of phosphopeptides from tryptic digests of β-casein.

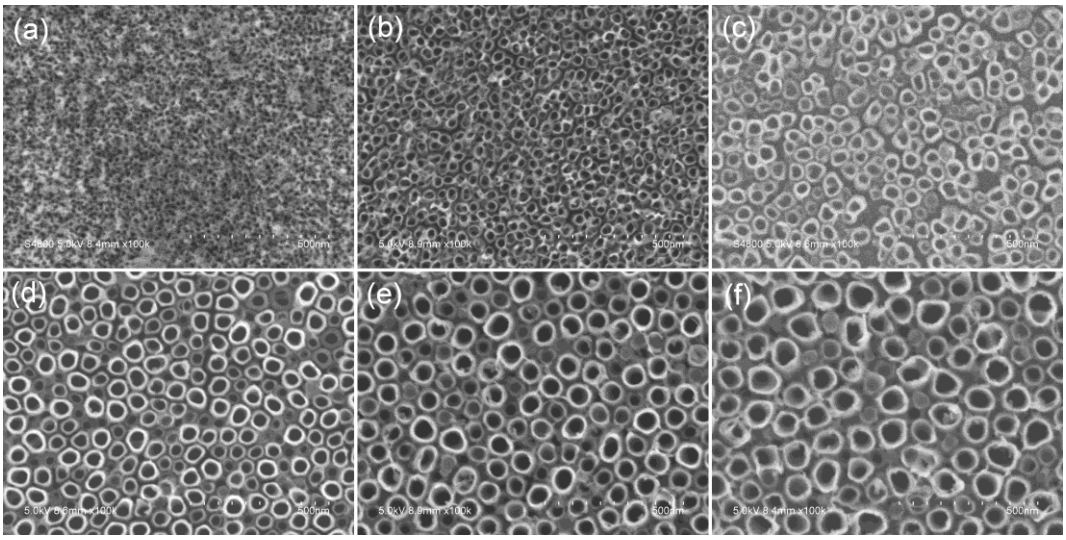

Fig. S3 SEM images of TNAs anodized at the voltage of (a) 5 V, (b) 10 V, (c) 15 V, (d) 20 V, (e) 25 V and (f) 30 V.

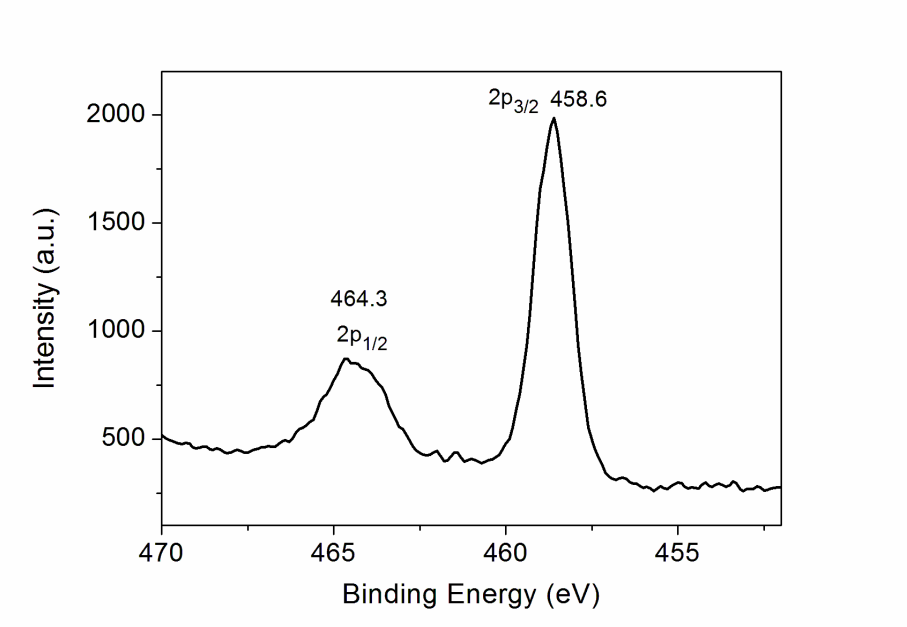

Fig. S4 XPS spectrum of Ti 2p in the calcined Ti film without anodization.

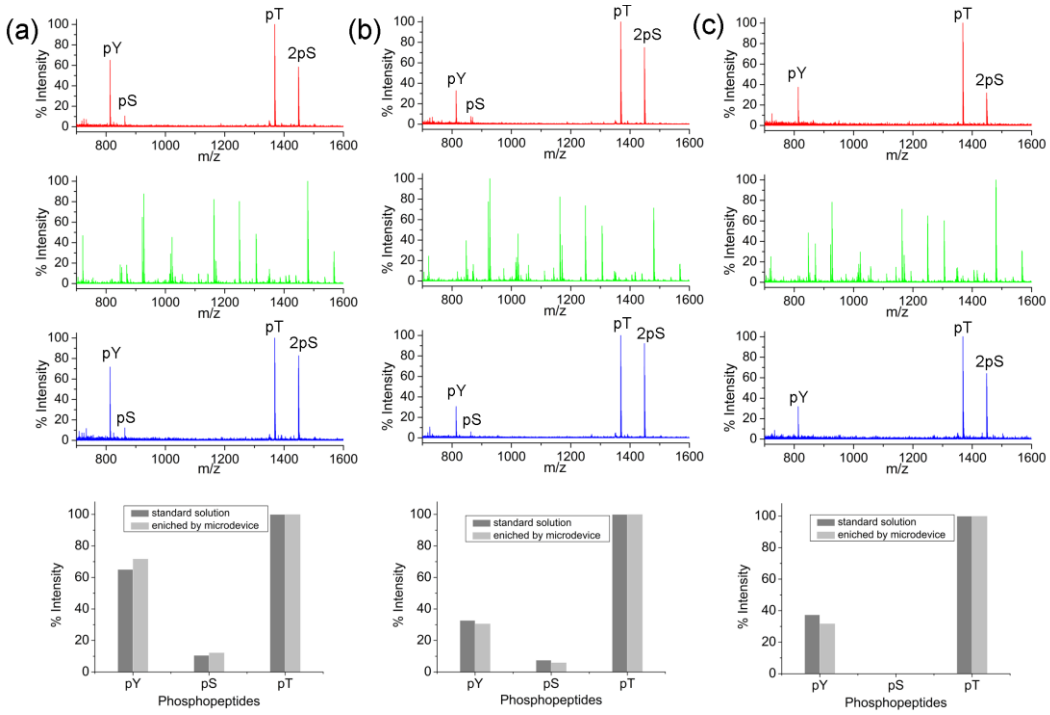

Fig. S5 Enrichment selectivity of phosphopeptides with different phosphorylation sites. 10 μl (a) 0.8 pmol/μl, (b) 0.2 pmol/μl and (c) 0.08 pmol/μl standard phosphopeptide mixtures containing equivalent pY, pS, pT and 2pS were used for the investigation. Red spectra: MS spectra of standard phosphopeptide mixture; green spectra: MS spectra of digests of BSA spiked with standard phosphopeptide mixture; blue spectra: phosphopeptides enriched by microdevice and eluted by the first 10 μl $NH_3 \cdot H_2O$. The bar charts show the comparison of relative intensities of three phosphopeptides before and after enrichment.

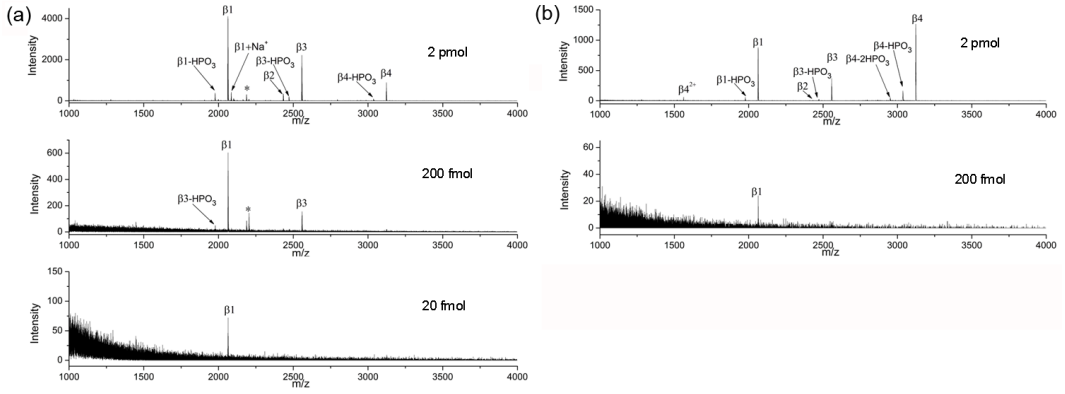

Fig. S6 MALDI-TOF MS spectra of phosphopeptides enriched from the tryptic digests of β-casein by TNA-integrated microdevice with a) S-shaped and b) linear channel.

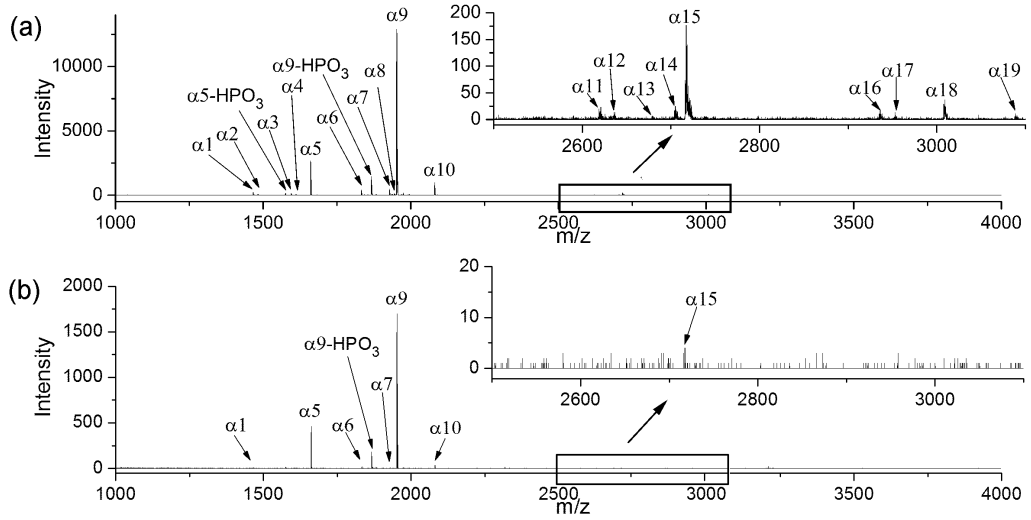

Fig. S7 MALDI-TOF MS spectra of phosphopeptides enriched from the tryptic digests of α-casein by TNA-integrated microdevice with (a) S-shaped and (b) linear channel.

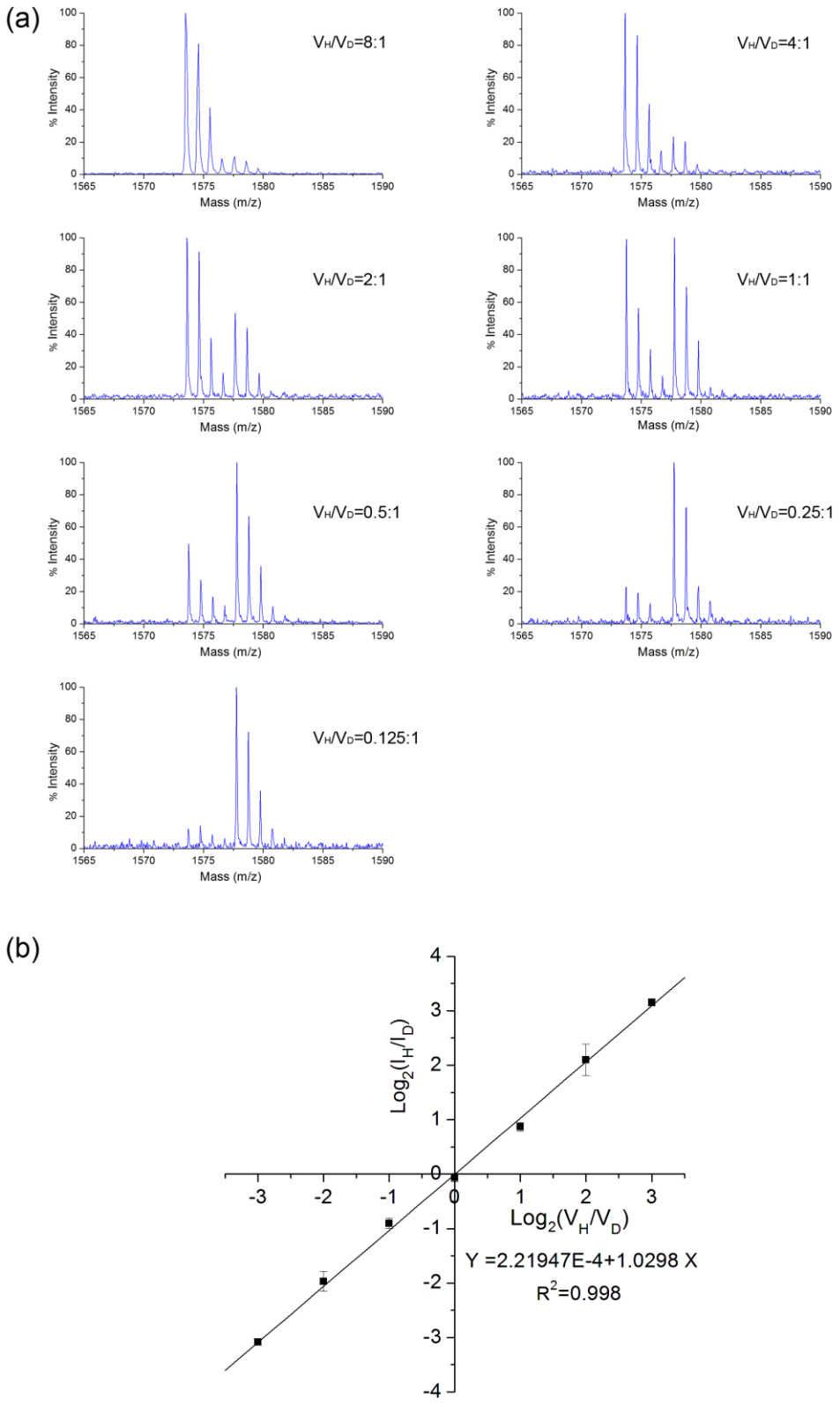

Fig. S8 (a) MS peak intensity ratios (I_H/I_D) of light-isotope-labeled to heavy-isotope-labeled phosphopeptide HS3. The initial volume ratio (V_H/V_D) injected for enrichment and isotope dimethyl labeling was set as 8:1, 4:1, 2:1, 1:1, 1:0.5, 1:0.25 and 1:0.125, respectively. (b) Linear relationship between the logarithms of I_H/I_D and V_H/V_D.

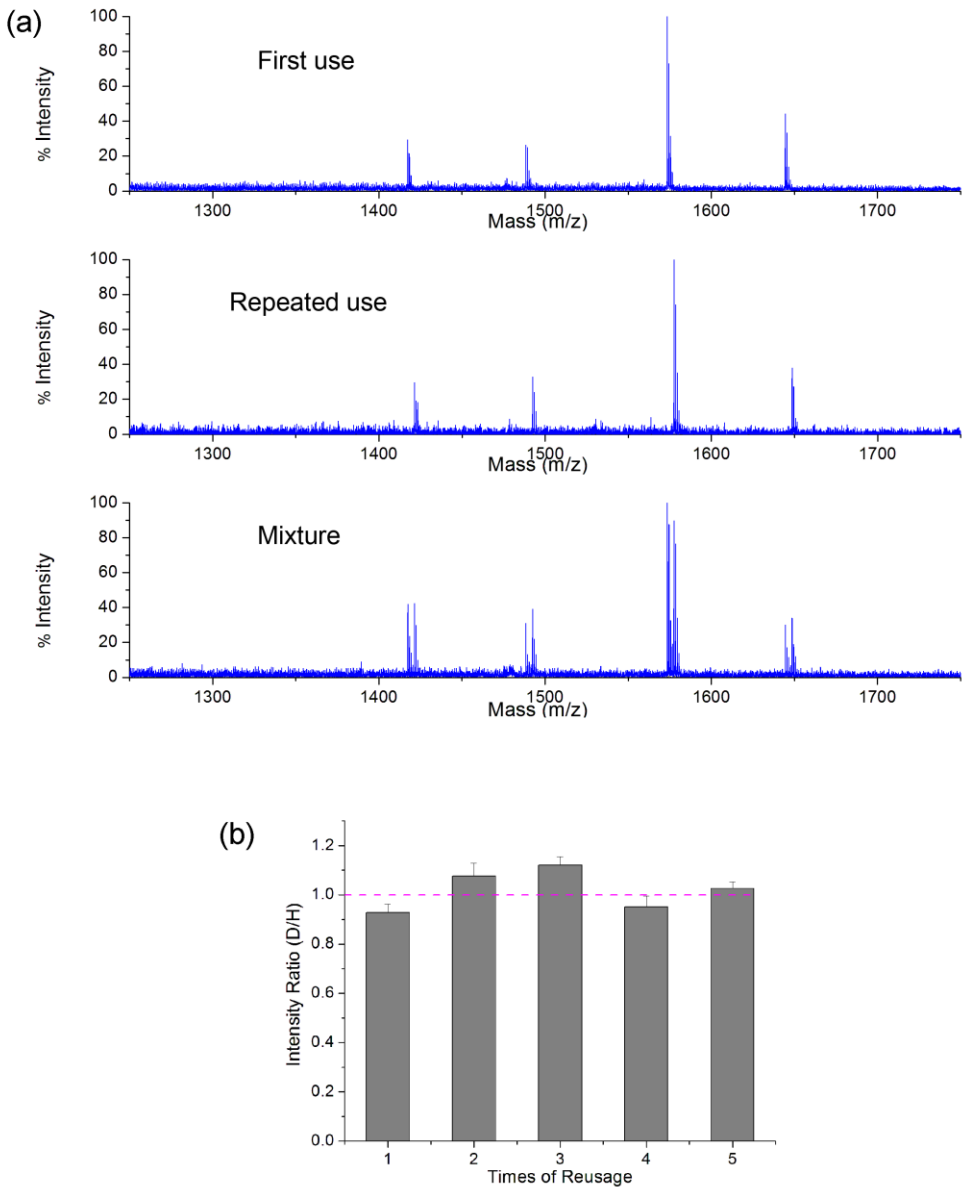

Fig. S9 (a) MALDI-TOF MS spectra of the four serum phosphopeptides from control sample treated by on-chip enrichment tandem isotope labeling. Light (CH_2O) and heavy (CD_2O) isotope labeling were conducted for the first and repeated uses, respectively. (b) MS peak intensity ratios of the heavy-isotope-labeled phosphopeptide HS3 after five consecutive reuses to the light-isotope-labeled phosphopeptide HS3 after the first use.