Supporting Information

Table and figures

Table S1. List of phosphopeptide obtained from tryptic digest of β-casein.

no.	observed m/z	no. of phosphorylation	protein	sequence
1	2063.288	1	β-casein	FQ[pS]EEQQQTEDELQDK
2	2558.577	1	β-casein	FQ[pS]EEQQQTEDELQDKIHPE
3	3124.466	4	β-casein	RELEELNVPGEIVE[pS]L[pS][pS][pS]EESITR

Footnote: [pS] represents phosphorylation on serine.

Table S2. The obtained signal-to-noise of phosphopeptides in Figure 3.

material / no.	B T-TS-1	C TS-1	D Ti^{4+}-IMAC	E TiO_2	F Ti-HMS-0.08	G TiAPO-5H-0.05
1	13.0	-	28.1	21.2	3.2	6.6
2	5.0	-	8.2	13.5	-	-
3	7.3	-	33.1	17.0	-	10.8
4	-	-	6.4	10.3	-	2.1
5	38.2	147.8	121.7	94.6	41.5	23.4
6	6.5	-	30.7	5.2	3.6	8.9
7	14.6	59.3	23.5	17.8	7.3	1.9
8	-	5.0	-	8.0	-	-
9	74.1	55.9	165.0	121.4	10.2	80.7
10	19.7	-	45.3	11.8	-	15.9
11	16.5	17.5	19.8	7.4	2.1	9.3
12	2.0	-	5.9	3.7	1.4	-
13	3.4	36.4	11.4	4.6	7.4	1.2
14	6.1	6.0	11.0	15.2	10.6	5.4
15	3.4	-	7.3	2.7	-	1.3
16	11.0	2.6	26.8	10.5	2.9	2.5
17	4.6	-	6.3	5.5	1.7	-

Footnote: phosphopeptides with S/N>3 are considered to be reliable identification.

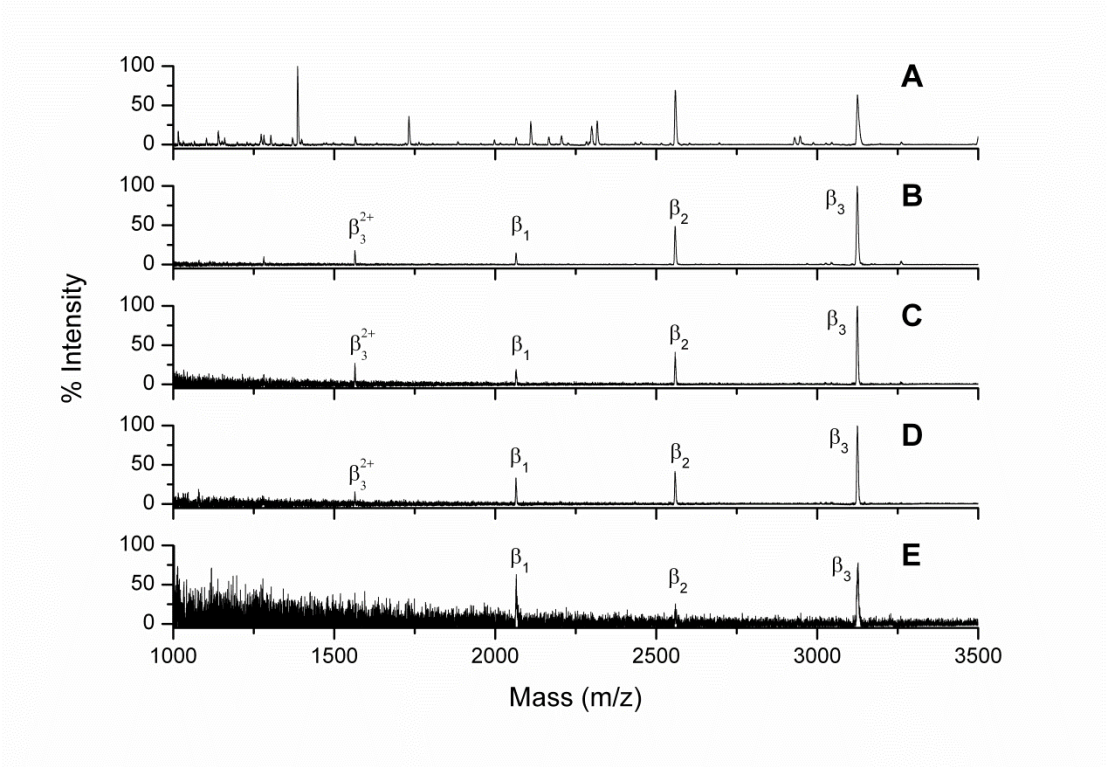

Fig.S1 MALDI-TOF mass spectra for analysis of the enriched phosphopeptides by T-TS-1 material from tryptic digest of β-casein. (A) 100 fmol, direct analysis; (B) 100 fmol; (C) 50 fmol; (D) 25 fmol and (E) 10 fmol.

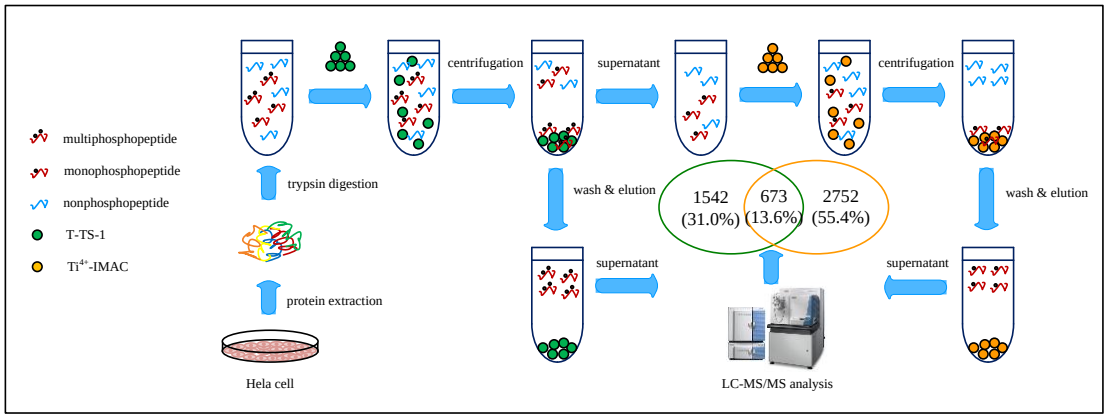

Fig.S2 A method using two materials (T-TS-1 and Ti^{4+}-IMAC) for successive phosphopeptide enrichment from real biological sample. T-TS-1 material was applied as a first adsorbent for the enrichment of multi-phosphopeptides and the resulted flowthrough was further purified with Ti^{4+}-IMAC material, which showed better performance of mono-phosphopeptides enrichment.

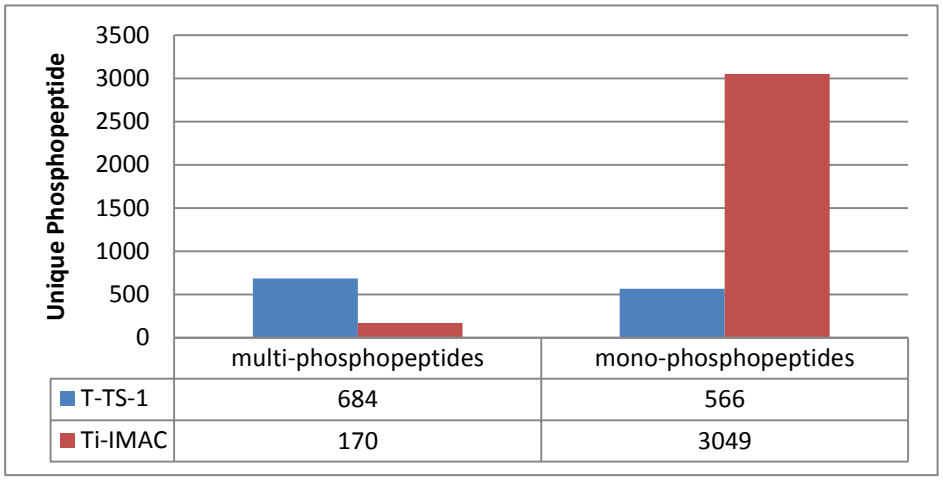

Fig.S3 The distribution of multi- and mono-phosphopeptides in each step of successive enrichment strategy. The first T-TS-1 enrichment step contributed 80.1% of the obtained multi-phosphopeptides while the second Ti^{4+}-IMAC enrichment step contributed 84.3% of the obtained mono-phosphopeptides. By utilized this strategy, efficient separation of multi-phosphopeptides from mono-phosphopeptides from cell lysates of high protein complexity can be feasibly achieved.

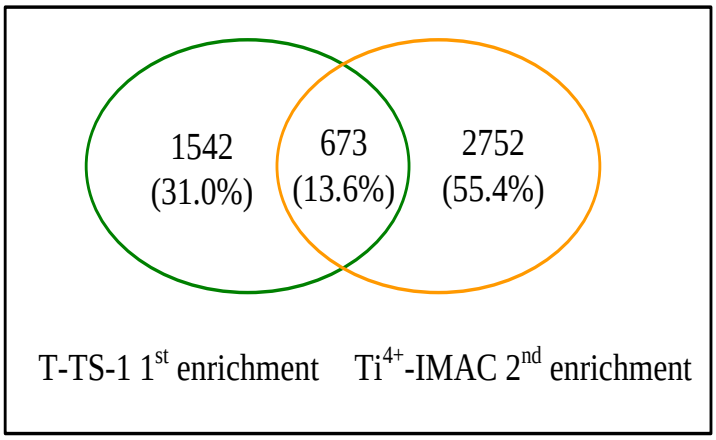

Fig.S4 The Number of phosphorylation sites identified in each enrichment step and the overlap between the two data sets. This successive enrichment strategy indicates good complementary and adequate recovery of phosphopeptides enrichment.