Supporting Information

Experimental Section

1. Materials

Formic acid (FA), trifluoroacetic acid (TFA), 2,5-dihydroxybenzoic acid (2,5-DHB), dithiothreitol (DTT), iodoracetamide (IAA), formaldehyde (CH_2O, 37% vol/vol), formaldehyde (CD_2O, 20 wt%, 98% D), sodium cyanoborohydride ($NaBH_3CN$), bovine serum albumin (BSA), α-casein, β-casein (bovine) and trypsin (TPCK-treated) were obtained from Sigma (St. Louis, MO); Acetonitrile (ACN) was chromatographic grade from Merck (Darmstadt, Germany). Deionized water used for all experiments was purified with a Milli-Q water system (Millipore, Milford, MA). All other chemicals, including acetic acid (HAc), sodium acetate (NaAc) and ethanol were analytical grade.

2. Preparation of tryptic digests of proteins

β-casein and α-casein were dissolved in ammonium bicarbonate buffer (100mM, pH 8.0) with a final concentration of 1 mg/ml (40 pmol/μL), and digested at 37 °C for 16 h with trypsin at the ratio of enzyme-to-substrate of 1: 40 (wt/wt). The phosphopeptides were enriched and labeled as previous reported.[1] Firstly, 10 μL suspension of the nanoparticles were added into certain of the casein digest and 50 μL 50% ACN/6% TFA was added and incubated for 30 min. The supernatant was removed after centrifugation at 20, 000 g for 3 min. And the resulting IMAC material was respectively rinsed with 100 μL solutions containing 50% (vol/vol) ACN/0.1% TFA with 200 mM NaCl and 30% (vol/vol) ACN/ 0.1% TFA respectively. Then the phosphopeptides were labeled with 2 μL of 4% (vol/vol) formaldehyde (CH_2O or CD_2O) and 2 μL of 0.6 M sodium cyanoborohydride ($NaBH_3CN$) in different acidic strength buffer for 40 min.

Bel-7402 Cell proteins (or BSA, 100 μg) were reduced by DTT at 60℃ for 1 h. After that, the proteins were alkylated by iodoacetamide at room temperature for 45 min in dark, followed by dilution with 100 mM NH_4HCO_3 (pH 8.1). Then, trypsin (or Lys C for BSA) was added with a weight ratio of trypsin/protein at 1:25 (1:100 for

LysC) and incubated at 37□ overnight. For the evaluation of the highly selective labeling, the tryptic peptides were divided into two equations, desalted and labeled by dimethylation in the medium solution (10 mg $NaBH_3CN$ and 30 μL CH_2O (37%, vol/vol) or 50 μL CD_2O (20%, vol/vol) dissolved in 4 ml 1% HAc, pH 2.8) on a homemade C18 SPE. Then the samples were eluted, dried in vacuum concentrator and resolved in 0.1% FA for LC-MS/MS analysis. For proteome quantification, the proteolytic peptides by Lys C were divided into two equations, desalted and labeled by dimethylation in the medium solution (10 mg $NaBH_3CN$ and 30 μL CH_2O (37%, vol/vol) or 50 μL CD_2O (20%, vol/vol) dissolved in 4 ml 1% HAc, pH 2.8) and in weak acidic buffer (the equivalents of labeling reagents as before only with modification of the opposite isotope labeling regents in the solution using 50 mM NaAc, pH 6.2) on a homemade C18 SPE, sequentially. Then the samples were eluted, dried in vacuum concentrator and mixed for analysis.

3. Mass spectrometry analysis

The results of MALDI-TOF MS were obtained by using BRUKER UltraflexTM time-of-flight mass spectrometer (Bruker Daltoniccs Ultraflex, Germany). And the measurements were carried out in reflex positive-ion mode with delayed ion extraction. DHB (25 mg/mL, in 50% ACN/ H_2O (v/v) solution containing 1% H_3PO_4) was used as the matrix for the analysis of labeled phosphopeptides. 0.5 μL of sample aliquots were first placed onto MALDI plate, dried at room temperature, and then 0.5 μL of the DHB matrix was added and dried prior to MS analysis.

4. Nano-LC/MS/MS analysis

A nano-RPLC-MS/MS system consisting of a quaternary Surveyor pump and LTQ spectrometer (Thermo Electron Finnigan, San Jose, CA) was used to evaluate the efficiency and selectivity of the labeling reaction. All MS and MS/MS spectra were acquired in the data dependent mode with the six most intense ions were fragmented by CID for MS/MS. A capillary column was first manually pulled to a fine point as spray tip, and then packed with C18 AQ beads (3 μm, 120 Å) from Michrom Bio Resources (Auburn, CA, USA). 0.1% (v/v) formic acid in H_2O (A) and 0.1% (v/v) formic acid in ACN (B) were applied as the mobile phase. Gradient elution from 5%

to 35% (v/v) of the mobile phases B in 60 min was selected to elute each sample.

The labeled peptides were analyzed by a nano-RPLC-MS/MS system consisting of a quaternary Surveyor pump and LTQ-Orbitrap XL mass spectrometer (Thermo, San Jose, CA). And the MS with resolution of 30, 000 at m/z 400 and MS/MS with resolution of 7, 500 were acquired in the data dependent mode with the three most intense ions were fragmented by HCD. The gradient elution was the same as above.

5. Data processing and analysis

The peak lists of MS spectra were generated from the raw data by Bioworks v3.2 (Thermo-electron Finnigan). And the data were searched against the International Protein Index (IPI) human database 3.80 using SEQUEST with the following parameters: proteins were fully tryptic cleavage constraints and up to two missed cleavages sites; precursor-ion mass tolerance, 2.0 Da; fragment-ion mass tolerance, 1.0 Da; Cysteine residues were searched as static modification of +57.0215 Da; variable modification for oxidized Met with +15.9949 Da, and +28.0313 Da for the dimethyl N-terminus and ε-amino group of lysine, respectively. In this study, to achieve FDR <1%, the following filter criteria was optimized with the homemade software in our previous work.[2]

Reference

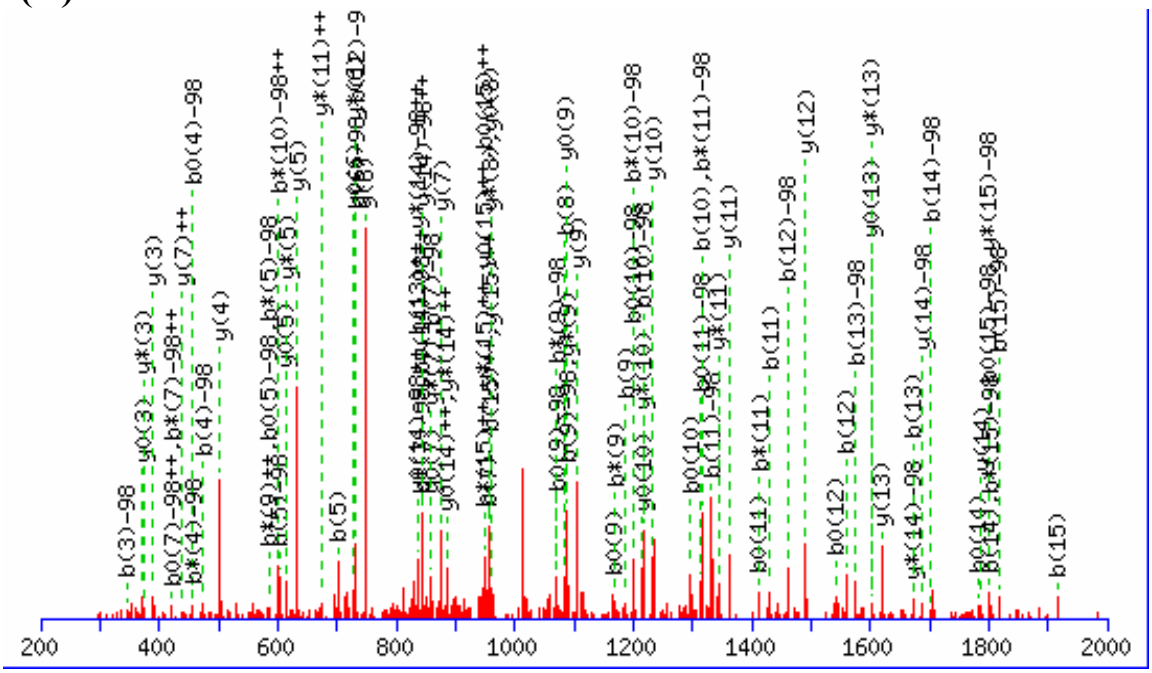
[1] H. Qin, F. Wang, P. Wang, L. Zhao, J. Zhu, Q. Yang, R. Wu, M. Ye, H. Zou, *Chem. Commun.* **2012,** *48*, 961-963.

[2] X. N. Jiang, X. L. Dong, M. L. Ye, H. F. Zou, *Anal. Chem.,* **2008**, *80*, 9326-9335.

Table S1. The percentage of the peptide (β1) from β-casein tryptic digests (1 μg/μL, 0.5 μL) labeled by dimethyl groups in different reaction buffers: 50 mM NaAc (pH 6.2), 50 mM NaAc (pH 4.2), 1% HAc (pH 2.8), 5% FA (pH 1.9), 5% TFA (pH ≤ 0.5) and direct analysis. (R"-K, QpSEEQQQTEDELQDK, 2061.5 Da). (None means no labeling, and M stands for the number of methyl labeled onto the peptides).

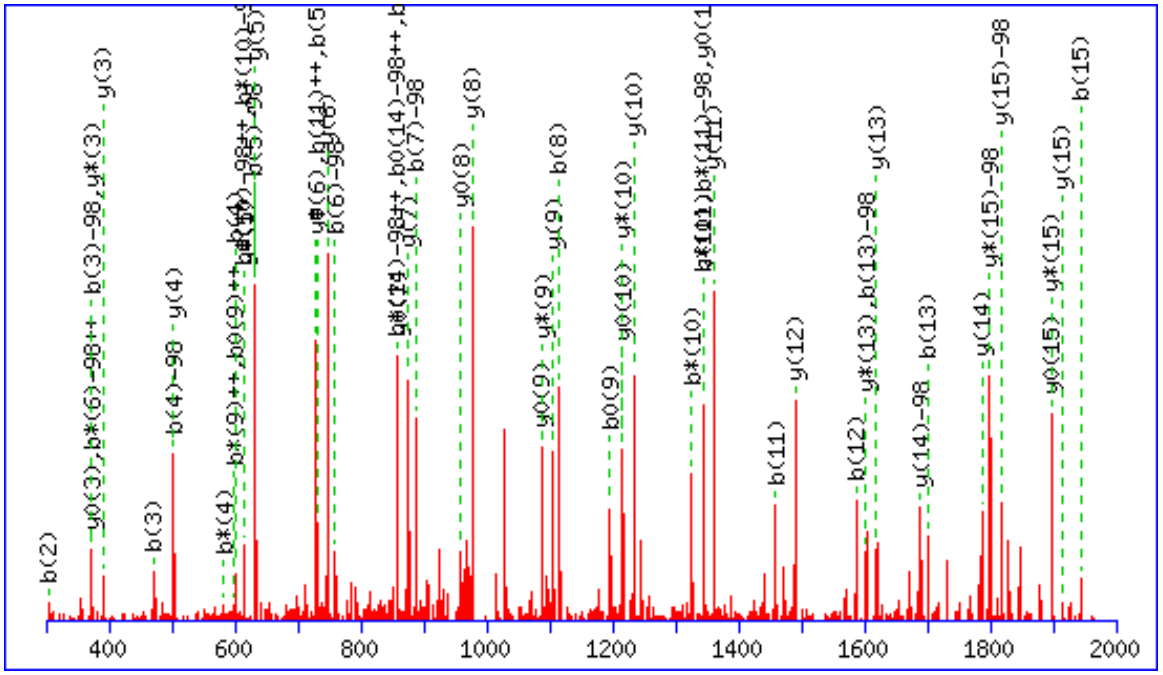
	Ratio (%)				
	None	1M	2M	3M	4M
pH6.2	--	2	2	--	96
pH4.2	--	2	37	30	30
1% HAc	--	3	87	10	--
5% FA	50	25	23	2	--
5% TFA	94	6	--	--	--
Direct	100	--	--	--	--

(A)



#	b	b^{++}	b*	b^{*++}	b^{0}	b^{0++}	Seq.	y	y^{++}	y*	y^{*++}	y^{0}	y^{0++}	#
1	148.1	74.5					F							16
2	276.1	138.6	259.1	130.1			Q	1816.8	908.9	1799.8	900.4	1798.8	899.9	15
3	345.2	173.1	328.1	164.6	327.1	164.1	S	1688.7	844.9	1671.7	836.4	1670.7	835.9	14
4	474.2	237.6	457.2	229.1	456.2	228.6	E	1619.7	810.4	1602.7	801.8	1601.7	801.3	13
5	603.2	302.1	586.2	293.6	585.2	293.1	E	1490.7	745.8	1473.6	737.3	1472.7	736.8	12
6	731.3	366.2	714.3	357.6	713.3	357.1	Q	1361.6	681.3	1344.6	672.8	1343.6	672.3	11
7	859.4	430.2	842.3	421.7	841.3	421.2	Q	1233.6	617.3	1216.5	608.8	1215.5	608.3	10
8	987.4	494.2	970.4	485.7	969.4	485.2	Q	1105.5	553.3	1088.5	544.7	1087.5	544.2	9
9	1088.5	544.7	1071.4	536.2	1070.5	535.7	T	977.4	489.2	960.4	480.7	959.4	480.2	8
10	1217.5	609.3	1200.5	600.7	1199.5	600.3	E	876.4	438.7	859.4	430.2	858.4	429.7	7
11	1332.5	666.8	1315.5	658.3	1314.5	657.8	D	747.4	374.2	730.3	365.7	729.3	365.2	6
12	1461.6	731.3	1444.5	722.8	1443.6	722.3	E	632.3	316.7	615.3	308.2	614.3	307.7	5
13	1574.7	787.8	1557.6	779.3	1556.7	778.8	L	503.3	252.1	486.3	243.6	485.3	243.1	4
14	1702.7	851.9	1685.7	843.3	1684.7	842.9	Q	390.2	195.6	373.2	187.1	372.2	186.6	3
15	1817.7	909.4	1800.7	900.9	1799.7	900.4	D	262.1	131.6	245.1	123.1	244.1	122.6	2
16							K	147.1	74.1	130.1	65.5			1

(B)



#	b	b^{++}	b*	b^{*++}	b^0	b^{0++}	Seq.	y	y^{++}	y*	y^{*++}	y^0	y^{0++}	#
1	176.1	88.6					F							16
2	304.2	152.6	287.1	144.1			Q	*1816.8*	908.9	1799.8	900.4	1798.8	899.9	15
3	373.2	187.1	356.2	178.6	355.2	178.1	S	*1688.7*	844.9	1671.7	836.4	1670.7	835.9	14
4	502.2	251.6	485.2	243.1	484.2	242.6	E	*1619.7*	810.4	1602.7	801.8	1601.7	801.3	13
5	631.3	316.1	614.2	307.6	613.3	307.1	E	*1490.7*	745.8	1473.6	737.3	1472.7	736.8	12
6	759.3	380.2	742.3	371.7	741.3	371.2	Q	*1361.6*	681.3	1344.6	672.8	1343.6	672.3	11
7	887.4	444.2	870.4	435.7	869.4	435.2	Q	*1233.6*	617.3	1216.5	608.8	1215.5	608.3	10
8	1015.4	508.2	998.4	499.7	997.4	499.2	Q	*1105.5*	553.3	1088.5	544.7	1087.5	544.2	9
9	1116.5	558.8	1099.5	550.2	1098.5	549.7	T	*977.4*	489.2	960.4	480.7	959.4	480.2	8
10	1245.5	623.3	1228.5	614.8	1227.5	614.3	E	*876.4*	438.7	859.4	430.2	858.4	429.7	7
11	1360.6	680.8	1343.5	672.3	1342.6	671.8	D	*747.4*	374.2	730.3	365.7	729.3	365.2	6
12	1489.6	745.3	1472.6	736.8	1471.6	736.3	E	*632.3*	316.7	615.3	308.2	614.3	307.7	5
13	1602.7	801.8	1585.7	793.3	1584.7	792.8	L	*503.3*	252.1	486.3	243.6	485.3	243.1	4
14	1730.8	865.9	1713.7	857.4	1712.7	856.9	Q	*390.2*	195.6	373.2	187.1	372.2	186.6	3
15	1845.8	923.4	1828.8	914.9	1827.8	914.4	D	262.1	131.6	245.1	123.1	244.1	122.6	2
16							K	147.1	74.1	130.1	65.5			1

Fig. S1. MS/MS of peptide FQpSEEQQQTEDELQDK before (A) and after (B) labeling in the medium acidic buffer (1% HAc, pH 2.8) with a mass shift of 28.0 Da for all the $(M+H)^+$ and b^{+1}.

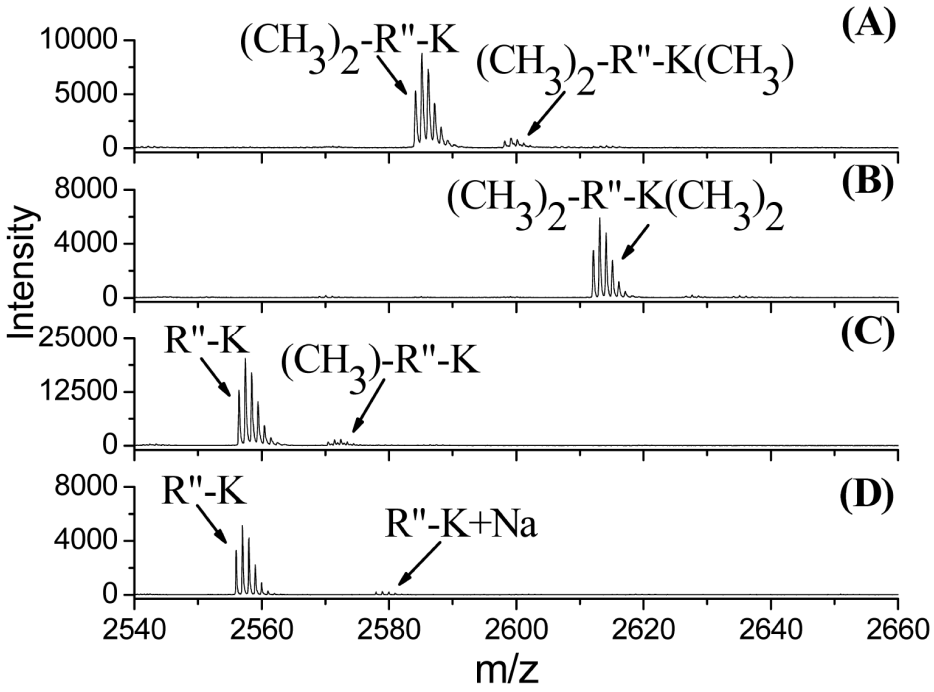


Fig. S2. MALDI mass spectra of (R"-K, FQpSEEQQQTEDELQDKIHPF, 2556.1 Da) from β-casein tryptic digests (1 μg/L, 0.5 μL) labeled by dimethyl regents at different buffer: (A) 1% HAc, (B) 50 mM NaAc (pH 6.2), (C) 5% TFA and (D) direct analysis.

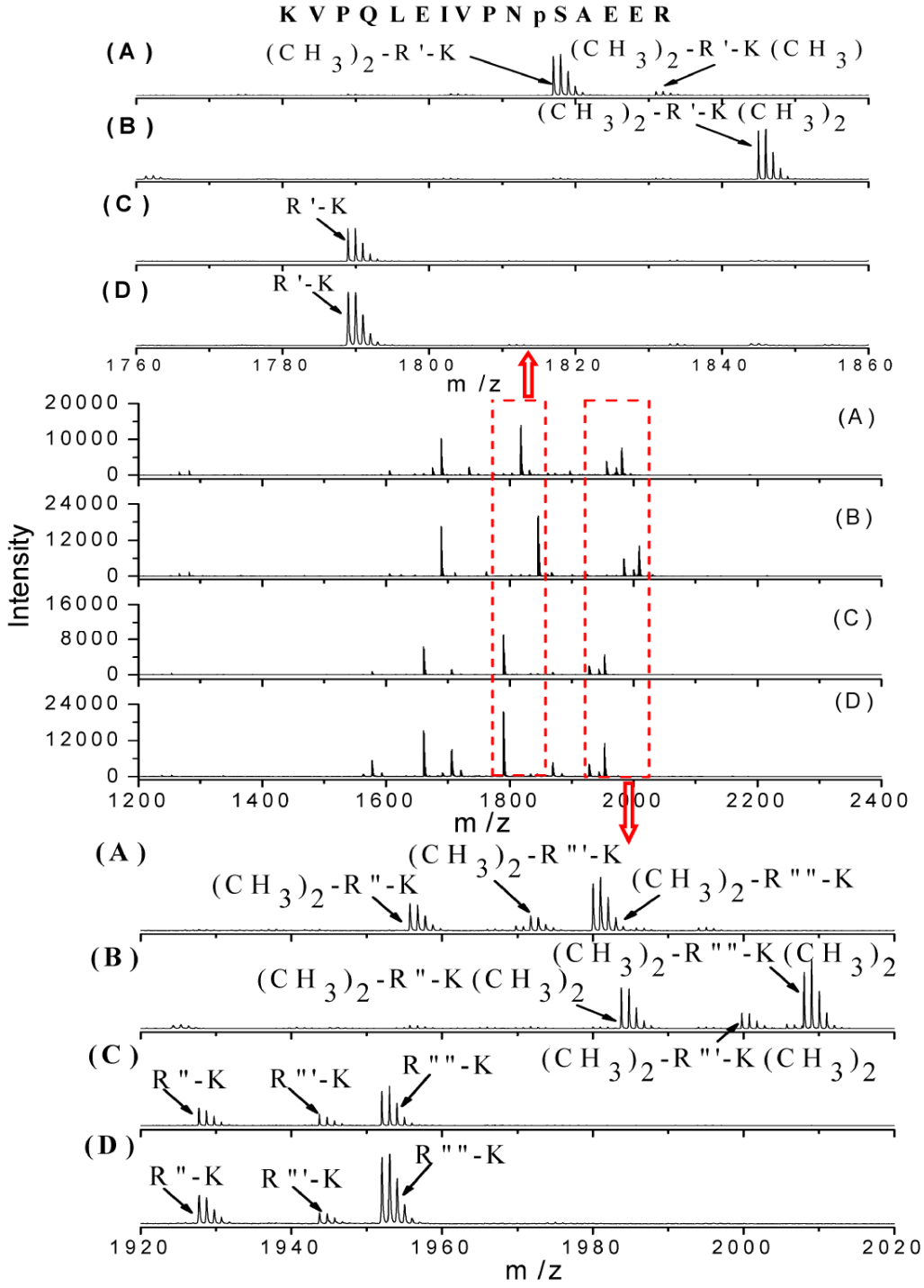


Fig. S3. MALDI mass spectra of phosphopeptides from α-casein tryptic digests (1 μg/μL, 1 μL) labeled by dimethyl regents at different buffer: (A) 1% HAc, (B) 50 mM NaAc (pH 6.2), (C) 5% TFA and (D) direct. (R'-K, KVPQLEIVPNpSAEER; R"-K, DIGpSEpSTEDQAMEDIK; R"'-K, DIGpSEpSTEDQA*MEDIK, * oxidized; R""-K, YKVPQLEIVPNpSAEER).

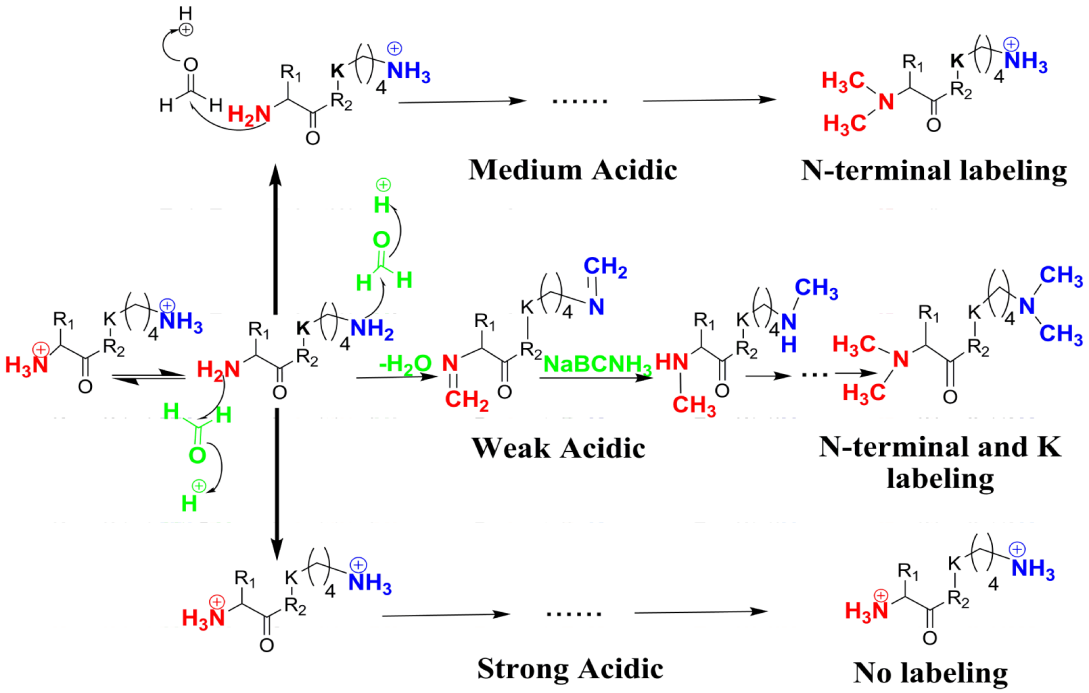


Fig. S4. The reaction mechanism for the site-selective labeling of N-terminus and ε-amino group of lysine in different acidic strength buffer. (R_1, the side chain of the acids; R_2, peptide sequence; K, lysine).

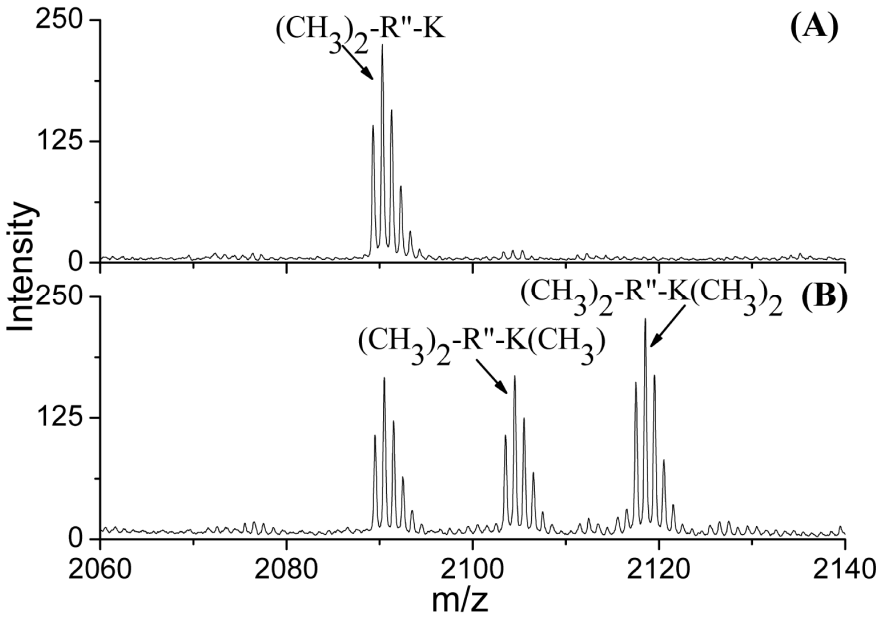


Fig. S5 MALDI mass spectra of the peptide (β1) from β-casein tryptic digests (1 μg/μL, 0.5 μL) labeled by different ratios of labeling reagents in the medium acidic solution (1% HAc, pH 2.8): (A) 4% CH_2O (2 μL, 2.6 μmol) and 0.6 M $NaBH_3CN$ (2 μL, 1.2 μmol); (B) 37% CH_2O (2 μL, 25 μmol) and 6 M $NaBH_3CN$ (2 μL, 12 μmol). (R"-K, QpSEEQQQTEDELQDK, 2061.5 Da).

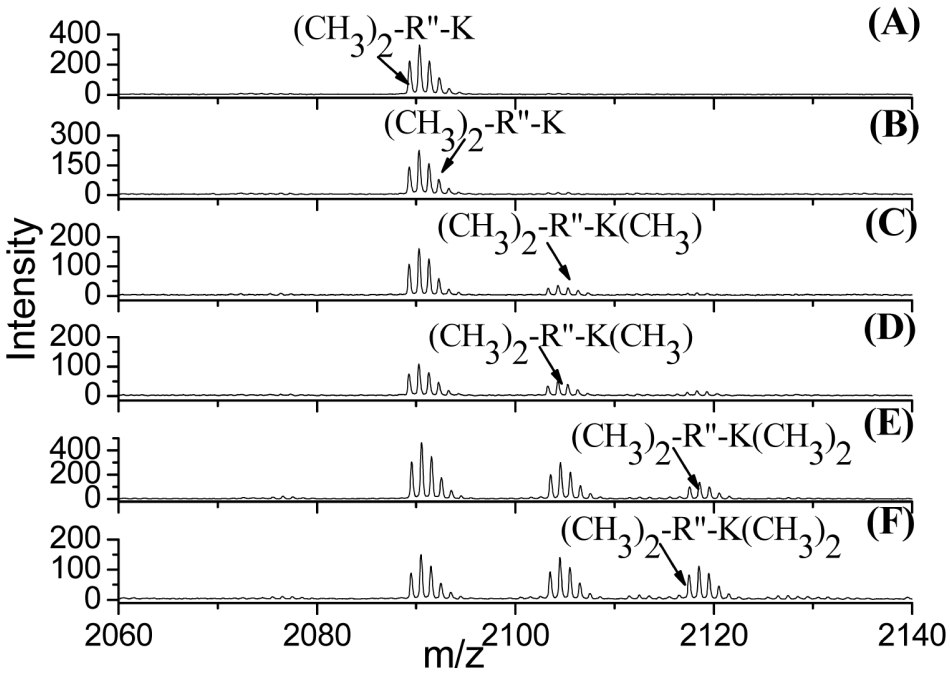


Fig. S6 MALDI mass spectra of the peptide (β1) from β-casein tryptic digests (1 μg/μL, 0.5 μL) labeled by labeling reagents 4% CH_2O (2 μL, 2.6 μmol) and 0.6 M $NaBH_3CN$ (2 μL, 1.2 μmol) in the medium acidic solution (1% HAc, pH 2.8) for different time: (A) 10 min, (B) 40 min, (C) 90 min, (D) 150 min, (E) 240 min and (F) 24 h. (R"-K, QpSEEQQQTEDELQDK, 2061.5 Da).