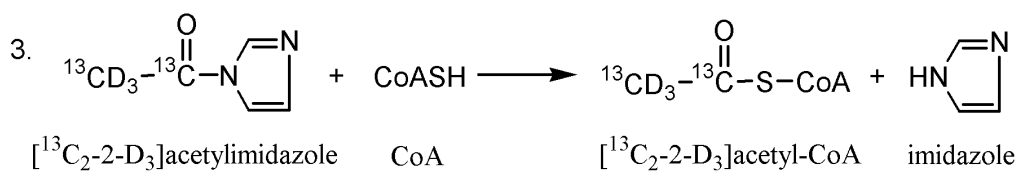
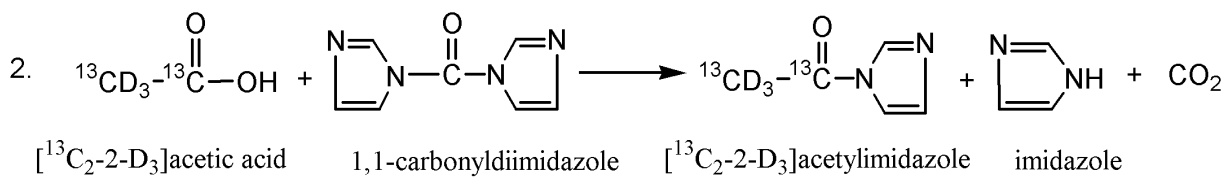
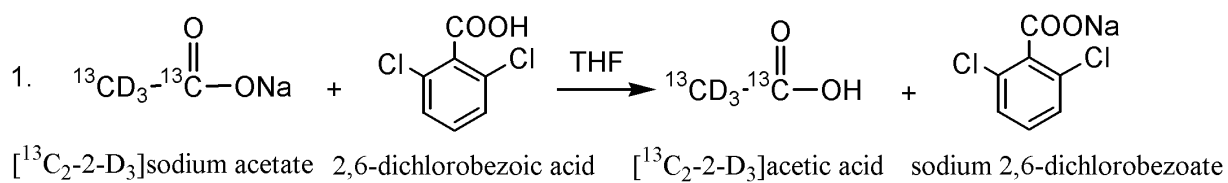


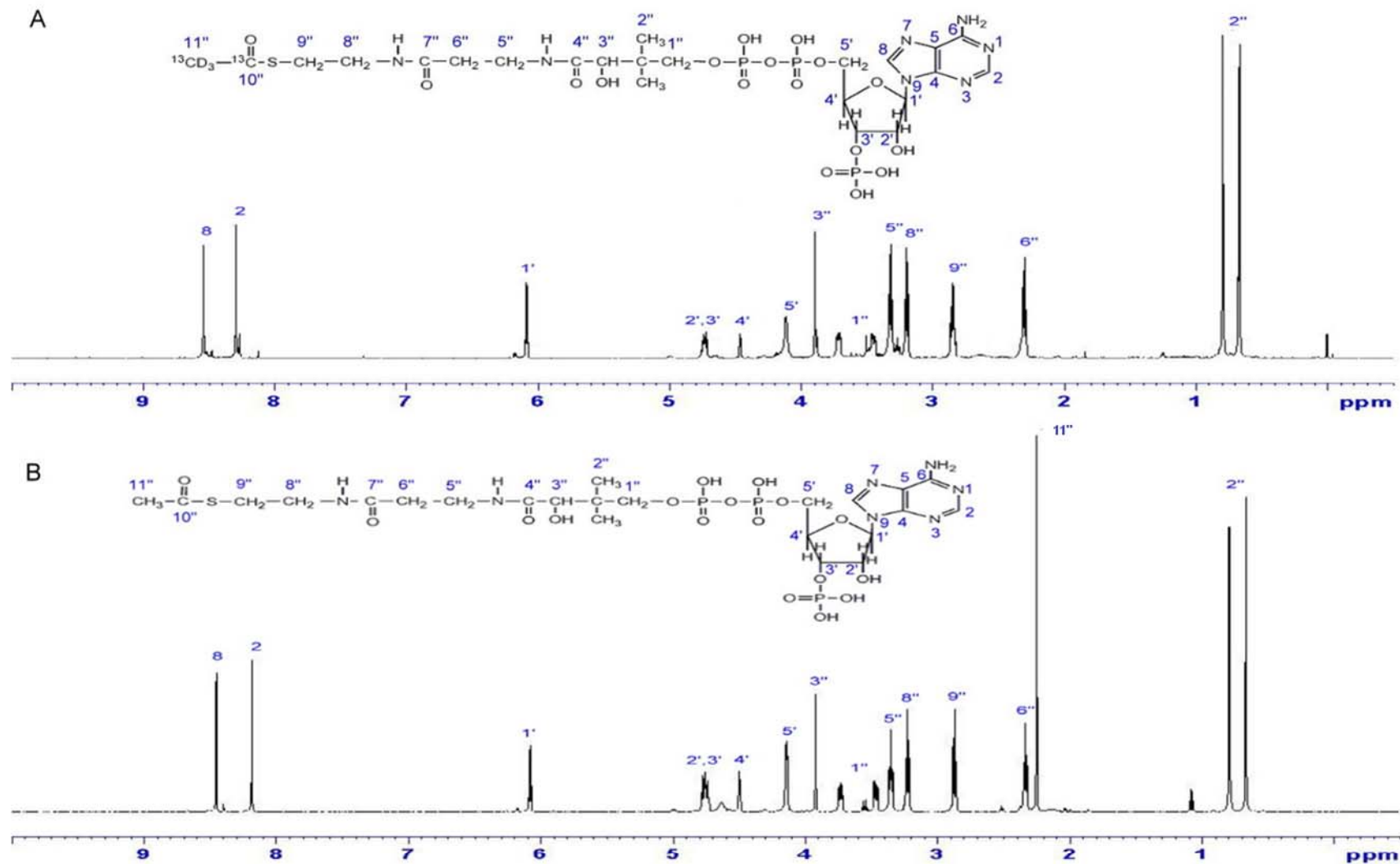
Supplemental Table A. [$^{13}\text{C}_2\text{-2-D}_3$]acetyl-CoA and acetyl-CoA ^1H -NMR chemical shift assignments and coupling constants. Multiplicity was indicated as follows: s (singlet); d (doublet); t (triplet); m (multiplet).

$^{13}\text{C}_2\text{-2-D}_3$ acetyl-CoA				Acetyl-CoA			
^1H chemical shift	Proton position	Integration	Multiplicity	^1H chemical shift	Proton position	Integration	Multiplicity
8.54	8	1	s	8.45	8	1	s
8.29	2	1	s	8.18	2	1	s
6.08	1'	1	d	6.08	1'	1	d
4.73	2',3'	2	m	4.76	2',3'	2	m
4.46	4'	1	m	4.49	4'	1	m
4.18	5'	2	d	4.15	5'	2	d
3.88	3"	1	s	3.92	3"	1	s
3.70	1"	1	dd	3.74	1"	1	dd
3.43	1"	1	dd	3.53	1"	1	dd
3.30	5"	2	t	3.35	5'	2	t
3.18	8"	2	t	3.22	8"	2	t
2.83	9"	2	t	2.87	9"	2	t
2.29	6"	2	t	2.34	6"	2	t
—	—	—	—	2.25	11"	3	s
0.79	2"	3	s	0.80	2"	3	s
0.67	2"	3	s	0.67	2"	3	s

Supplemental Figure A. Scheme of [$^{13}\text{C}_2$ -2- D_3] acetyl-CoA synthesis.

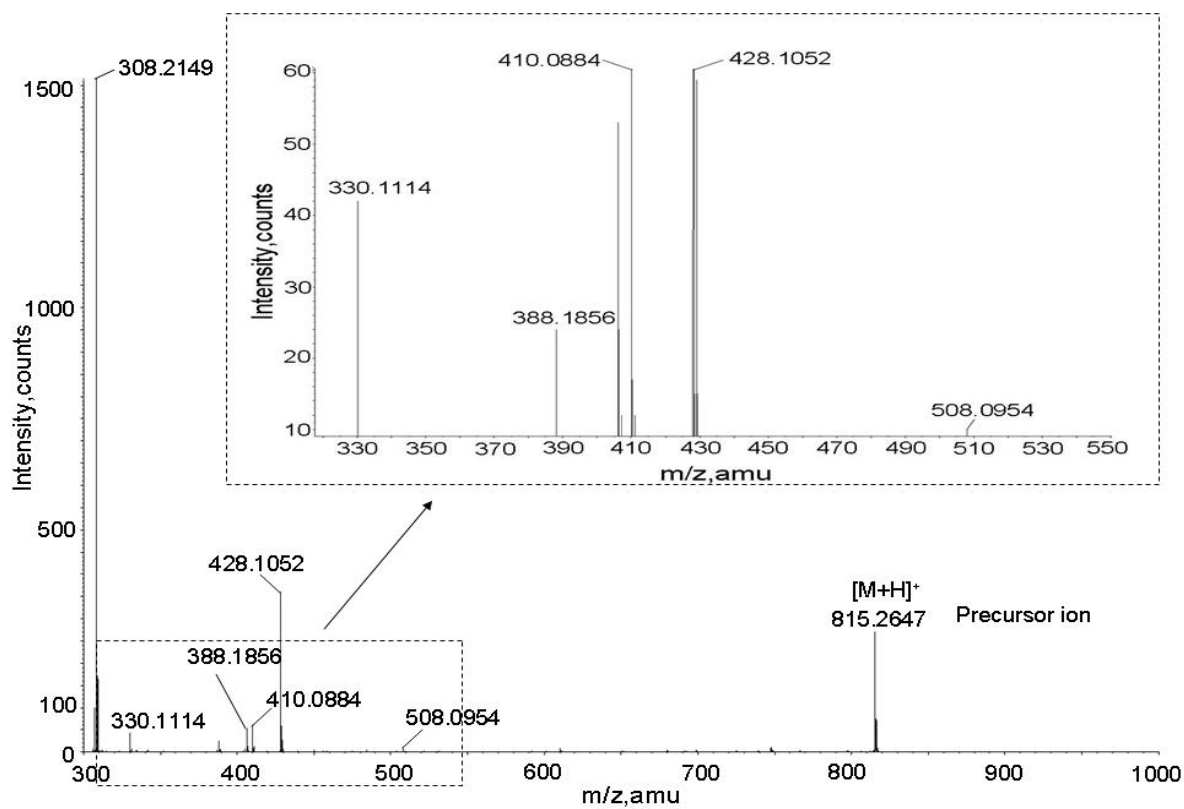


Supplemental Figure B. ^1H NMR spectra of (A) $[\text{}^{13}\text{C}_2\text{-2-D}_3]$ acetyl-CoA and (B) unlabeled acetyl-CoA. Signal of 11"-proton was detected at 2.25 ppm in the NMR spectrum of the unlabeled acetyl-CoA whereas it was absent from the spectrum of the $[\text{}^{13}\text{C}_2\text{-2-D}_3]$ acetyl-CoA.

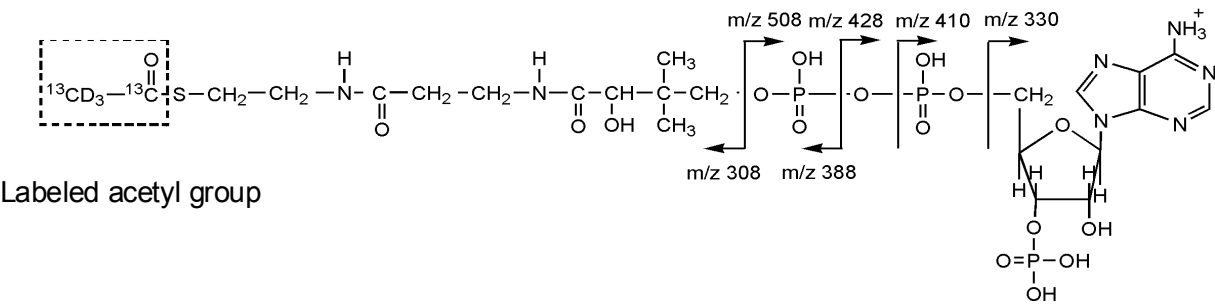


Supplemental Figure C. (A) MS/MS analysis of [$^{13}\text{C}_2$ -2- D_3] acetyl-CoA and (B) its chemical structure with fragment masses indicated. Mass range m/z 320 to 620 was enlarged to display more fragment ions.

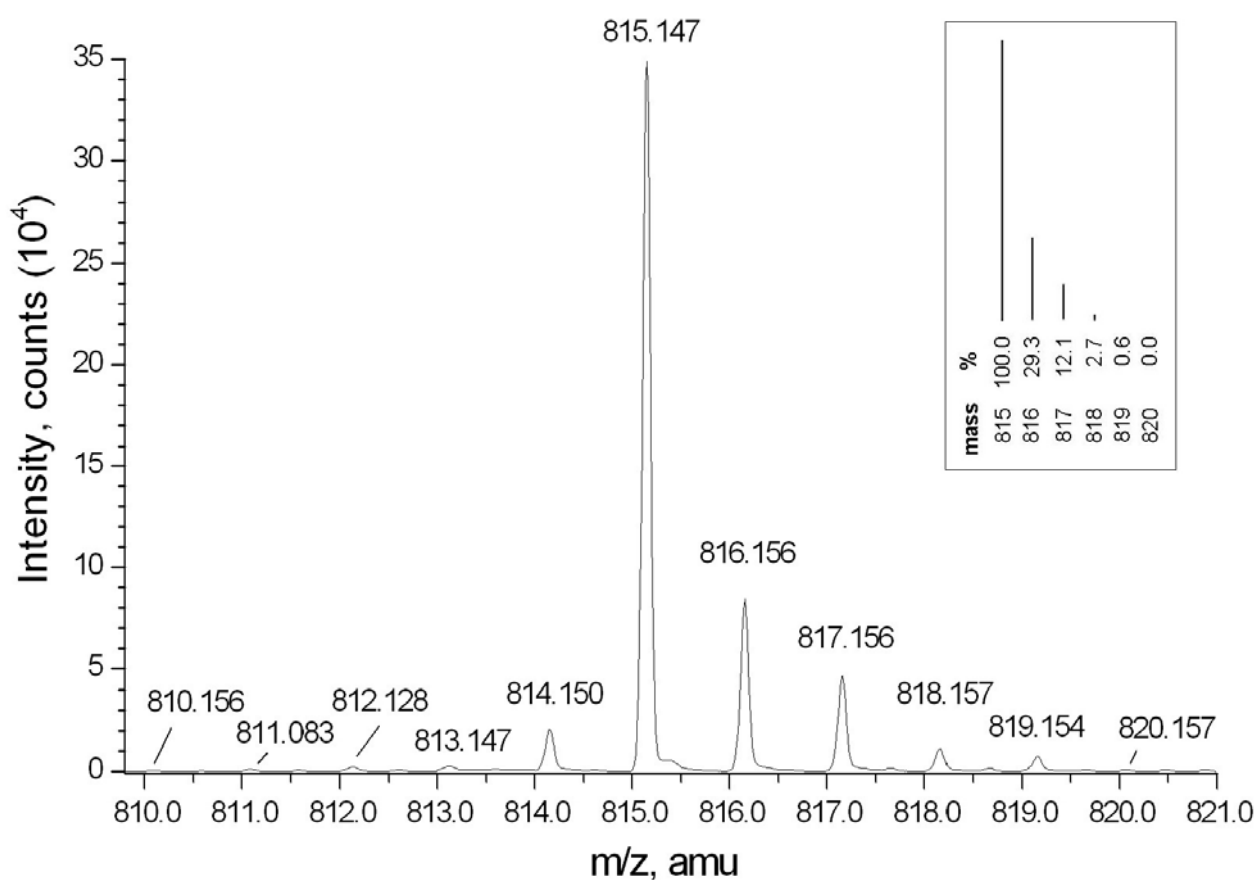
A.



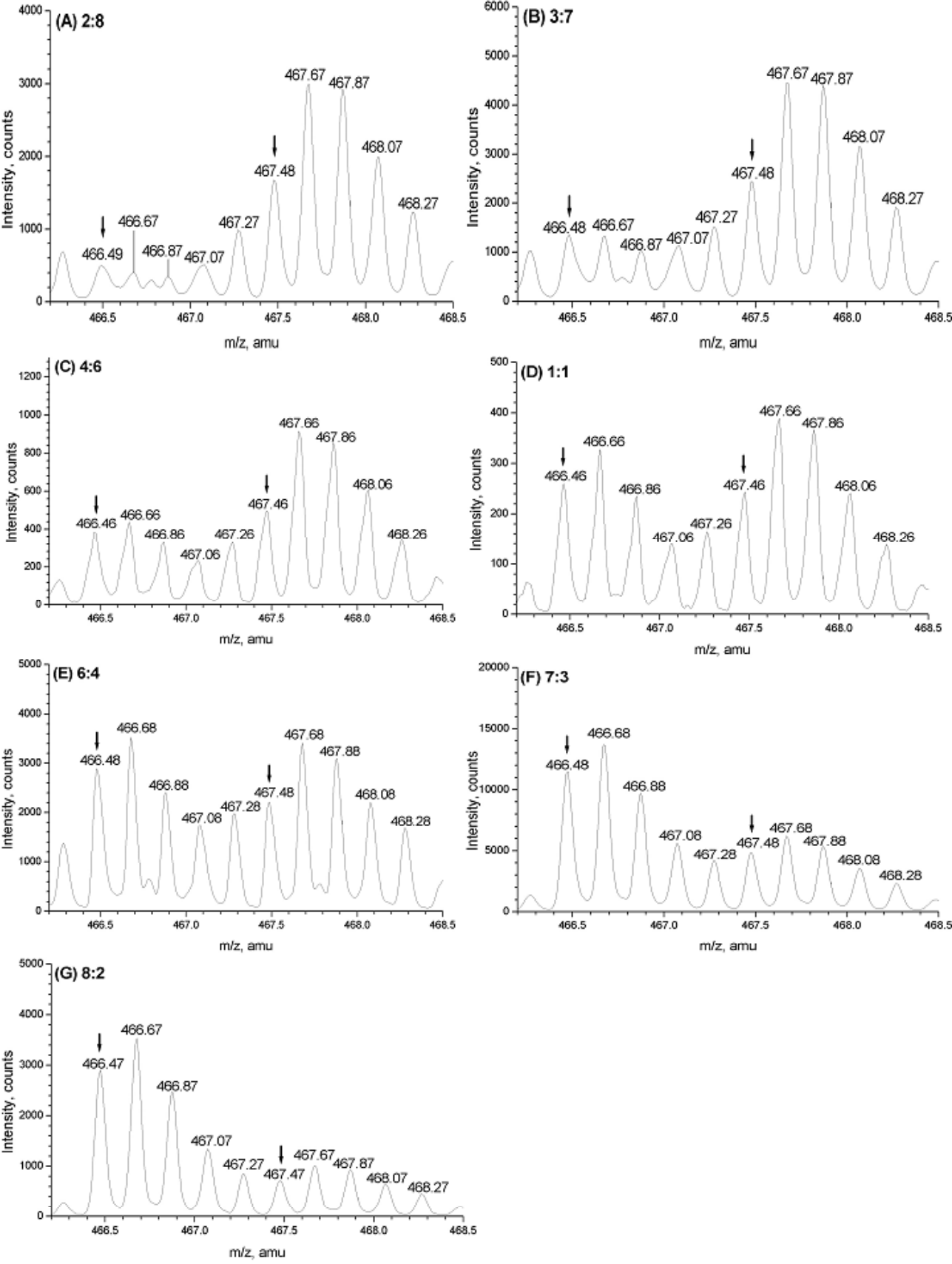
B.



Supplemental Figure D. MS analysis and purity evaluation of [$^{13}\text{C}_2\text{-2-D}_3$] acetyl-CoA. Peak at m/z 815.2 represented the fully labeled acetyl-CoA while signals at m/z 810 to m/z 814 were considered as unlabeled or partially labeled products. Signals at m/z 815 to m/z 820 were isotope peak cluster of [$^{13}\text{C}_2\text{-2-D}_3$] acetyl-CoA. The theoretical isotope pattern was displayed as inset.



Supplemental Figure E. Different mix ratio of unlabeled to labeled AcCoA.

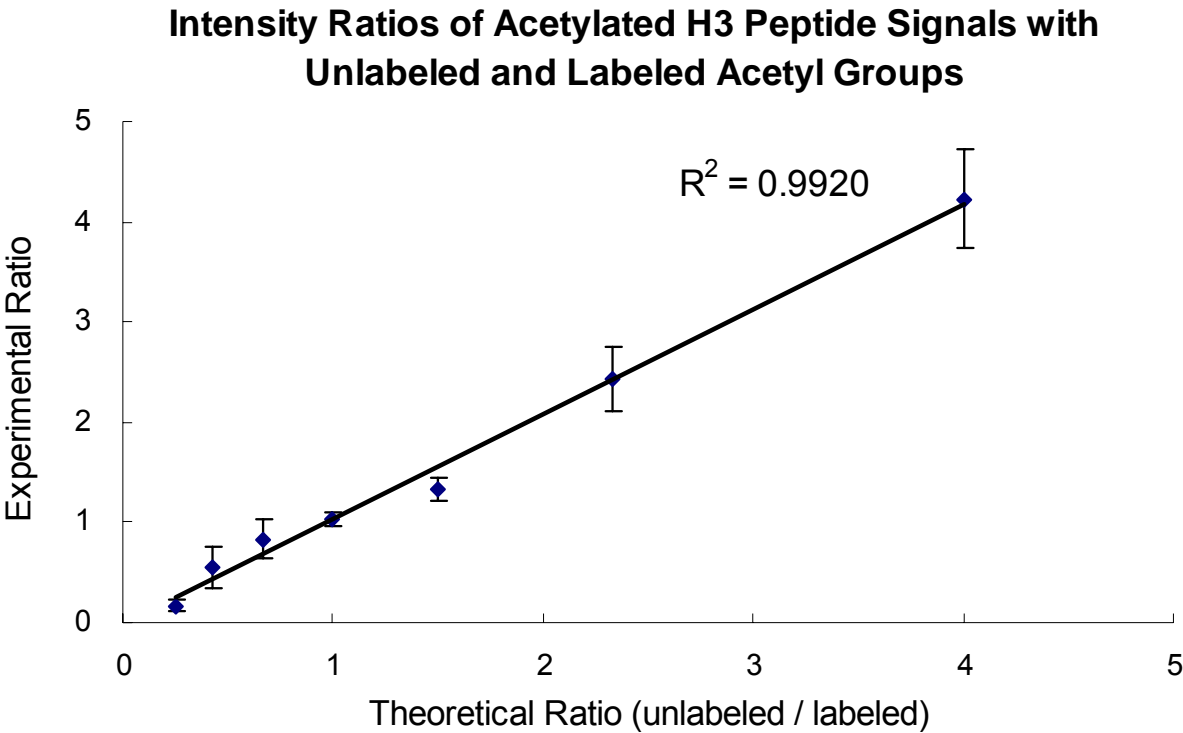


Supplemental Table A.

Intensity (area) output from MES

Theoretical Ratio		Measured Ratio		
unlabeled:labeled	Ratio	Average	SD	Offset (%)
2:8	0.25	0.17	0.06	33.33
3:7	0.43	0.55	0.21	29.11
4:6	0.67	0.83	0.20	24.00
5:5	1.00	1.04	0.08	3.67
6:4	1.50	1.34	0.11	10.89
7:3	2.33	2.44	0.32	4.43
8:2	4.00	4.23	0.50	5.67

Supplemental Figure F.



Supplemental Figure G. MALDI spectrum of the acetylation reaction mixture containing histone H3 peptide (aa 1-20). MALDI MS was performed using 4700 Proteomics Analyzer (TOF/TOF) (Applied Biosystems, USA) equipped with a 355 nm Nd/YAG (yttrium aluminum garnet) laser. Roughly 0.3 μ L (300 fmole) of the sample solution was mixed with the same volume of a saturated matrix solution (CHCA; Aldrich, Milwaukee, WI), 10 mg/mL in 50% ACN/0.1% TFA). The MS instrument was operated in the positive ion reflector mode with accelerating voltage of 20 kV. Typically, 1600 shots were accumulated. Between the signal doublets appeared at m/z 2328.29 and m/z 2333.31, a mass difference of 5 Da can be observed. Besides, 47.02-Da and 42.00-Da mass differences between the doublet and m/z 2286.28 corresponded to the unreacted histone H3 peptide (aa 1-20) imply that it was a monoacetylated peptide.

