

**Supplementary material**  
to  
**EpiProfile 2.0: A Computational Platform for Processing Epi-Proteomics Mass  
Spectrometry Data**

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Mary R. Szurgot<sup>1</sup>, Benjamin A. Garcia<sup>1\*</sup>

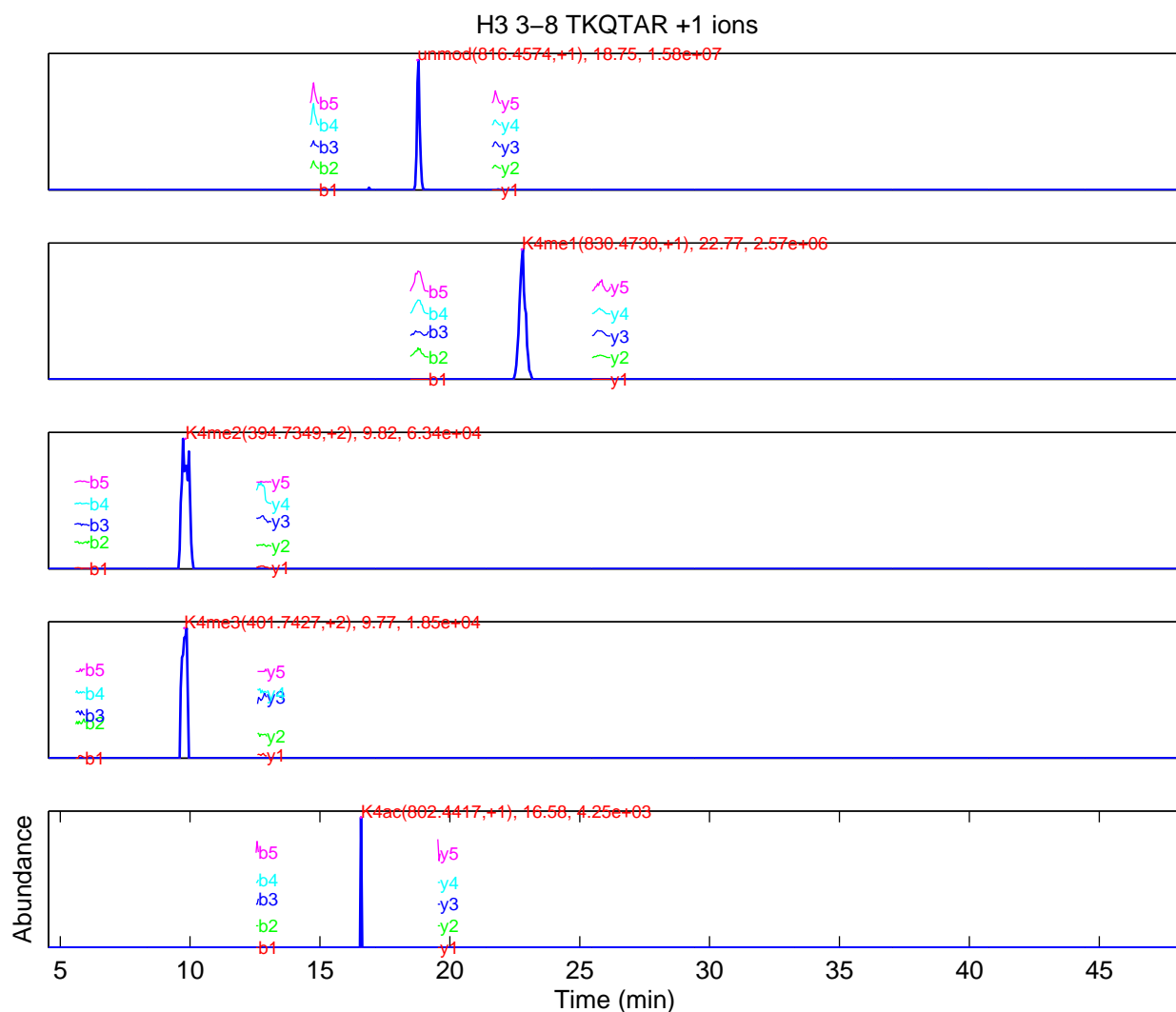
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<sup>2</sup>Department of Systems Pharmacology and Translational Therapeutics, Perelman School of Medicine University of Pennsylvania, Philadelphia, PA 19104, USA.

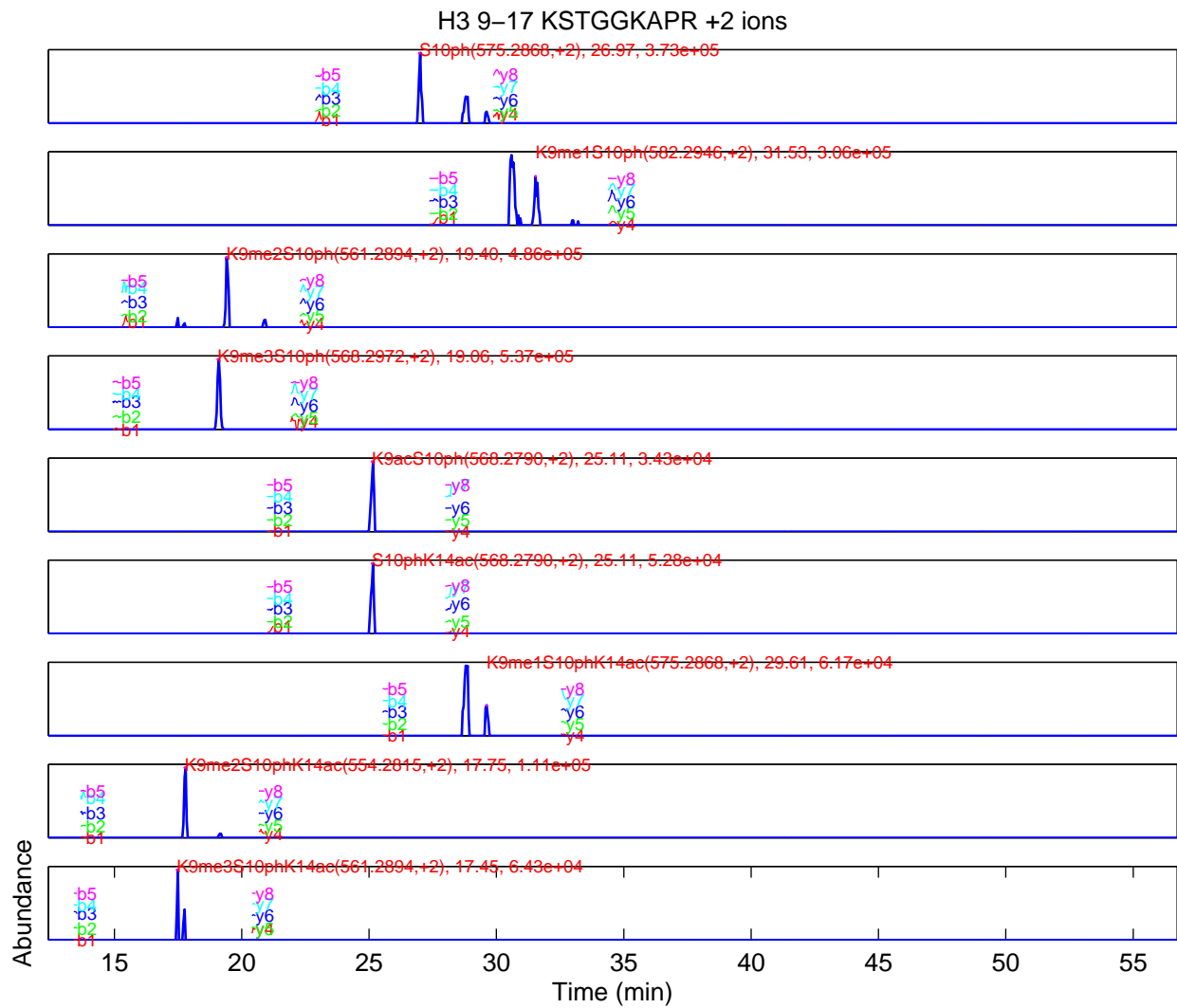
Correspondence should be addressed to B.A.G. (bgarci@mail.med.upenn.edu).

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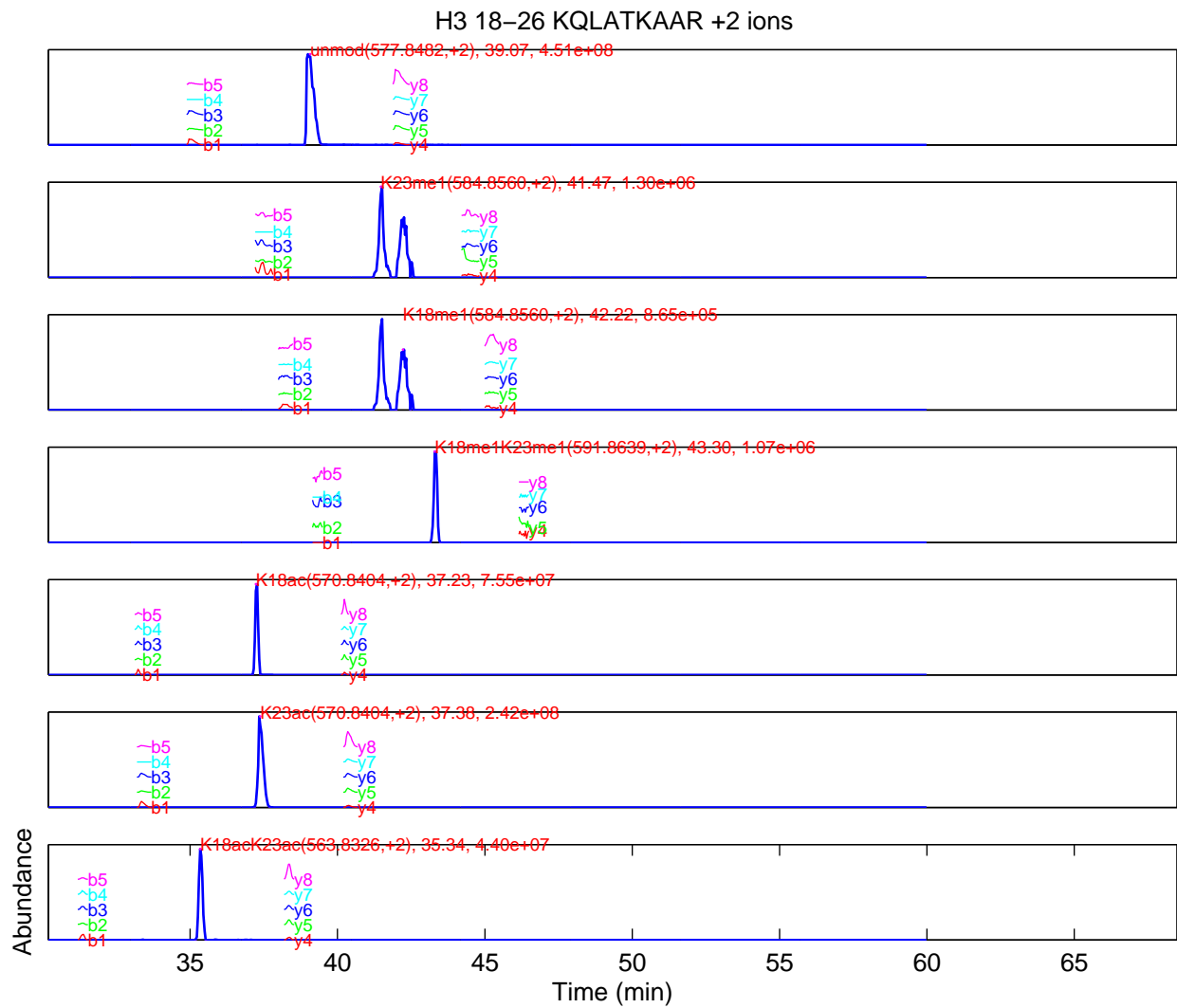
<b>Figure S1</b>	<b>Layouts of histone peptides.</b> (A) H3 3–8 TKQTAR. (B) H3 9–17 KSTGGKAPR S10ph. (C) H3 18–26 KQLATKAAR. (D) Layout of H3.1/2 27–40 KSAPATGGVKKPHR. (E) Layout of H3.3 27–40 KSAPSTGGVKKPHR. (F) Layout of H4 4–17 GKGGKGLGKGGAKR. (G) Layout of H4 20–23 KVLRL. (H) Layout of H2A.V 1–19 AGGKAGKDSGKAKAKAVSR. (I) Layout of H2A.Z 1–19 AGGKAGKDSGKAKTKAVSR. (J) Layout of H2B 1–29 variants.
<b>Figure S2</b>	<b>Isobaric histone peptides.</b> (A) H3 9–17 K9ac/K14ac. (B) H3 18–26 K18ac/K23ac. (C) H4 4–17 1ac. (D) H4 4–17 2ac. (E) H4 4–17 3ac. (F) H3 9–17 K9acS10ph/S10phK14ac. (G) H3.1/2 27–40 K36me3/K27me2K36me1. (H) H3.3 27–40 K36me3/K27me2K36me1. (I) H2AV 1–19 1ac. (J) H2AV 1–19 2ac. (K) H2AV 1–19 3ac. (L) H2AZ 1–19 1ac. (M) H2AZ 1–19 2ac. (N) H2AZ 1–19 3ac. (O) H2A 4–11 K5ac/K9ac. (P) H2A 12–17 K13ac/K15ac.
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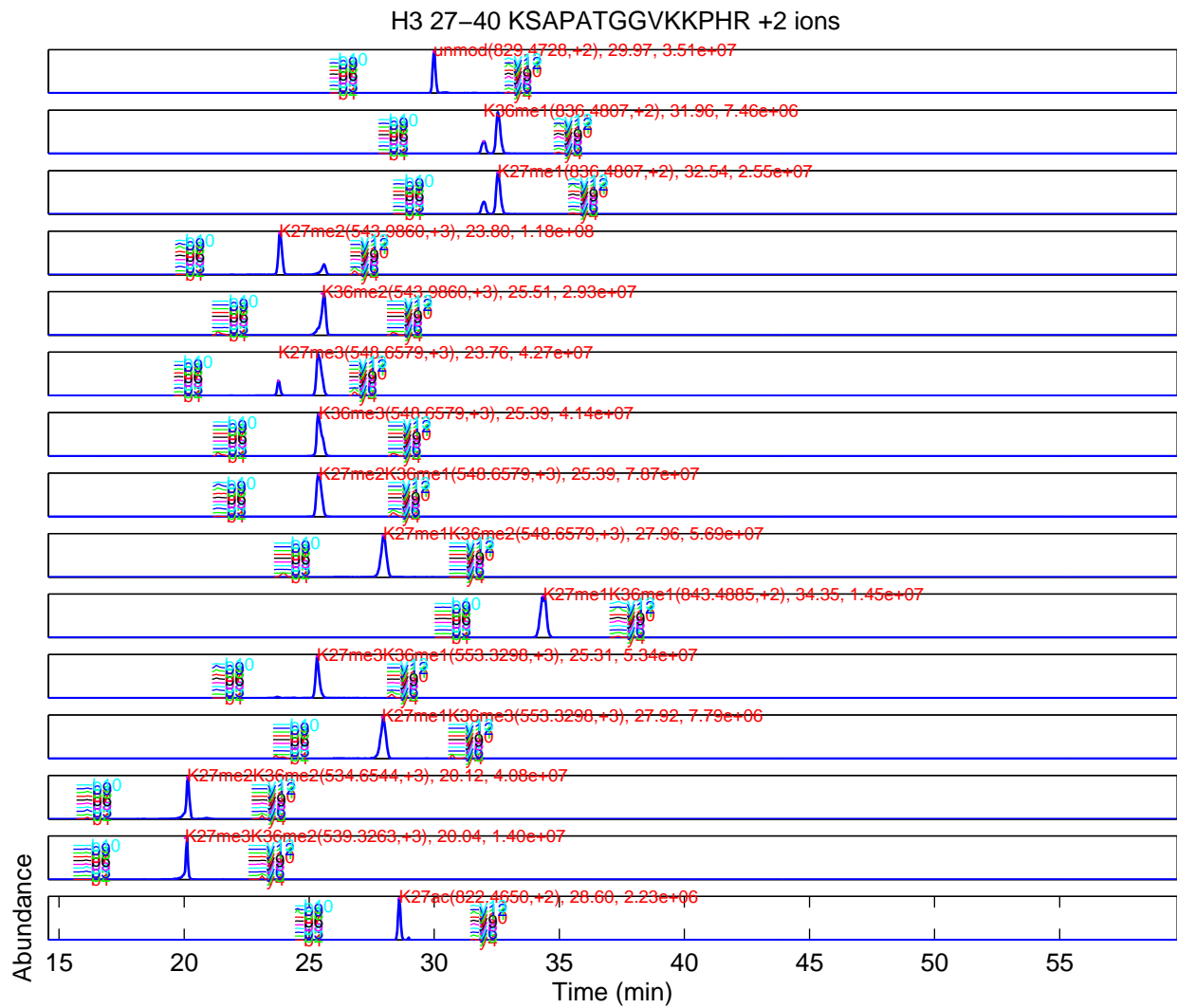
**Figure S1A. Layout of H3 3–8 TKQTAR.** The layout is K4me3 < K4me2 < K4ac < unmodified < K4me1. The subplots show the extracted ion chromatography (XIC) for each peptide based on the precursor m/z (i.e. x-axis is the retention time, y-axis is the intensity). The type and retention time of each peptide are above the corresponding chromatographic peak. Fragment ions have the same retention time as the precursor ion. Because fragment ions are usually much lower than the precursor ion, they are aside the precursor ion rather than under the precursor ion profile.



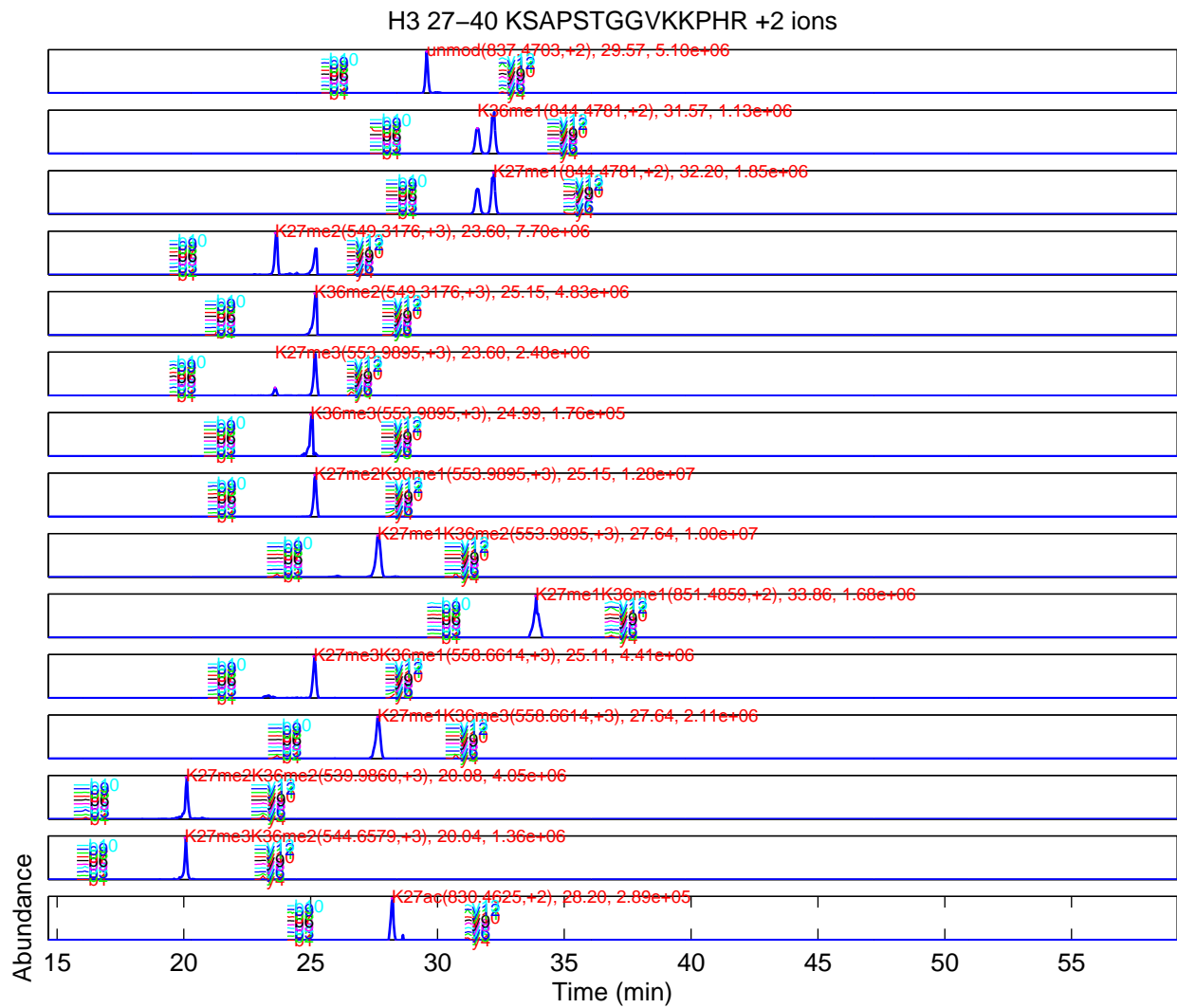
**Figure S1B. Layout of H3 9–17 KSTGGKAPR S10ph.**



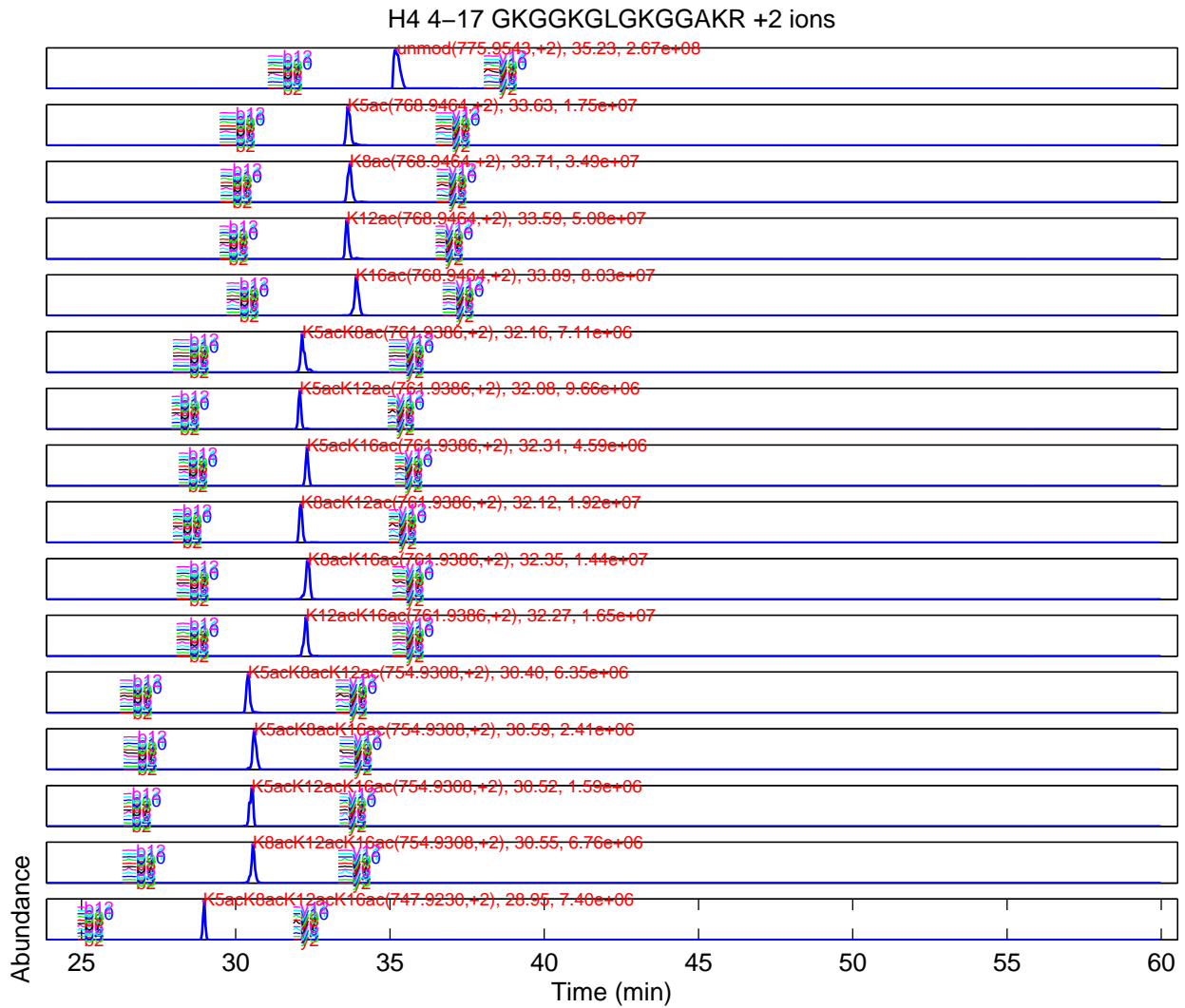
**Figure S1C. Layout of H3 18–26 KQLATKAAR.**



**Figure S1D. Layout of H3.1/2 27-40 KSAPATGGVKKPHR.**

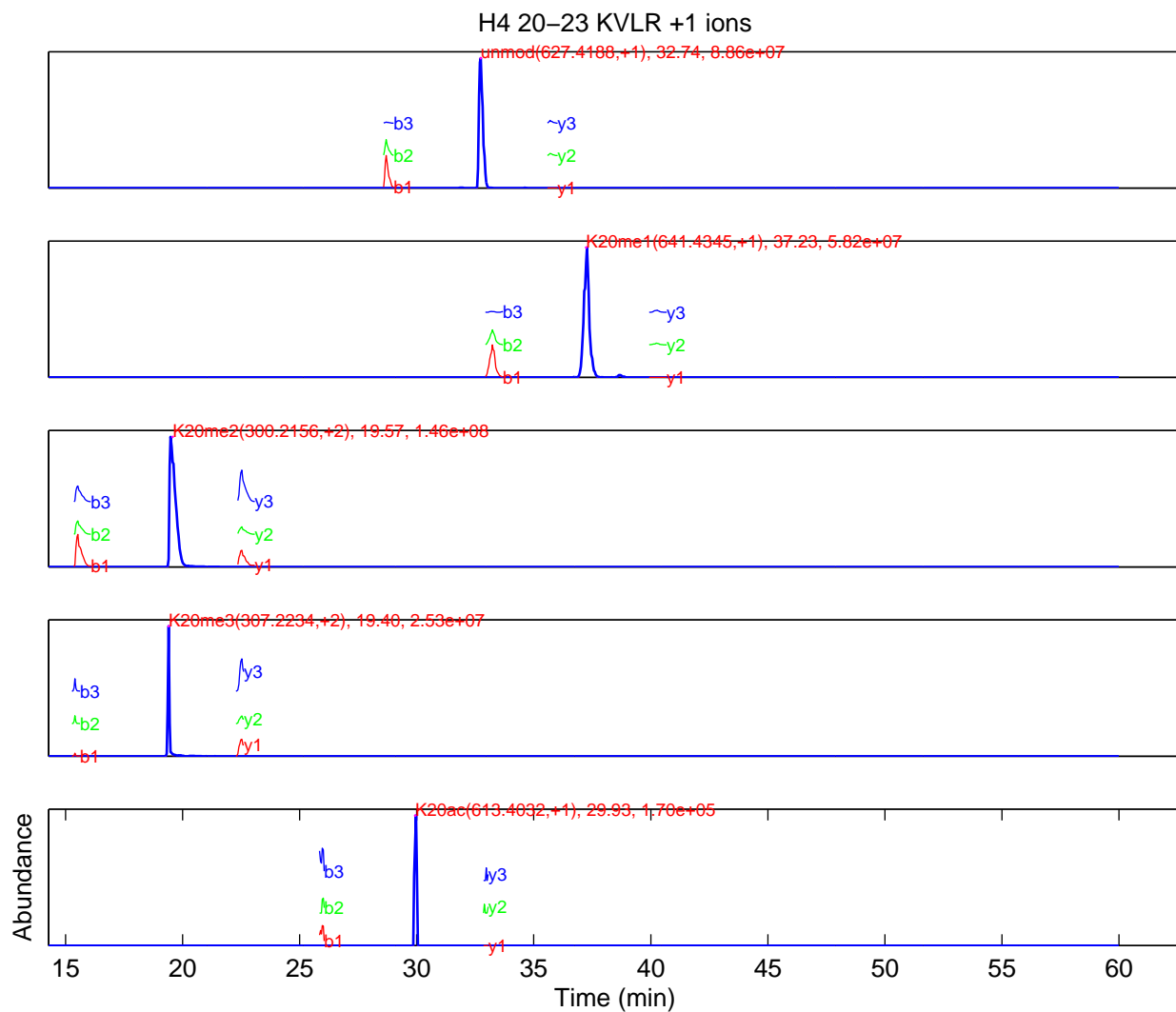


**Figure S1E. Layout of H3.3 27–40 KSAPSTGGVKKPHR.**

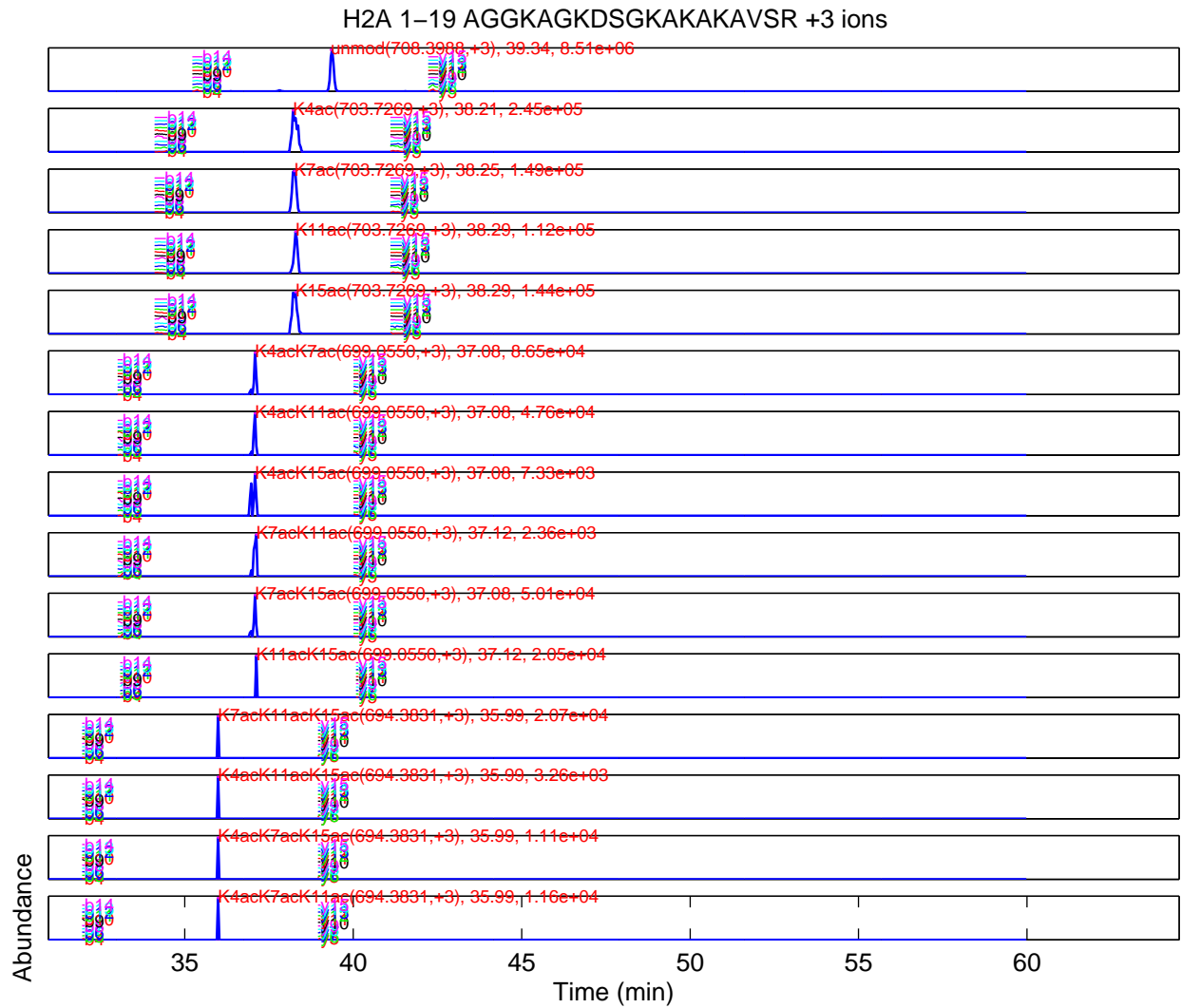


**Figure S1F. Layout of H4 4–17 GKGGKGLGKGGAKR.**

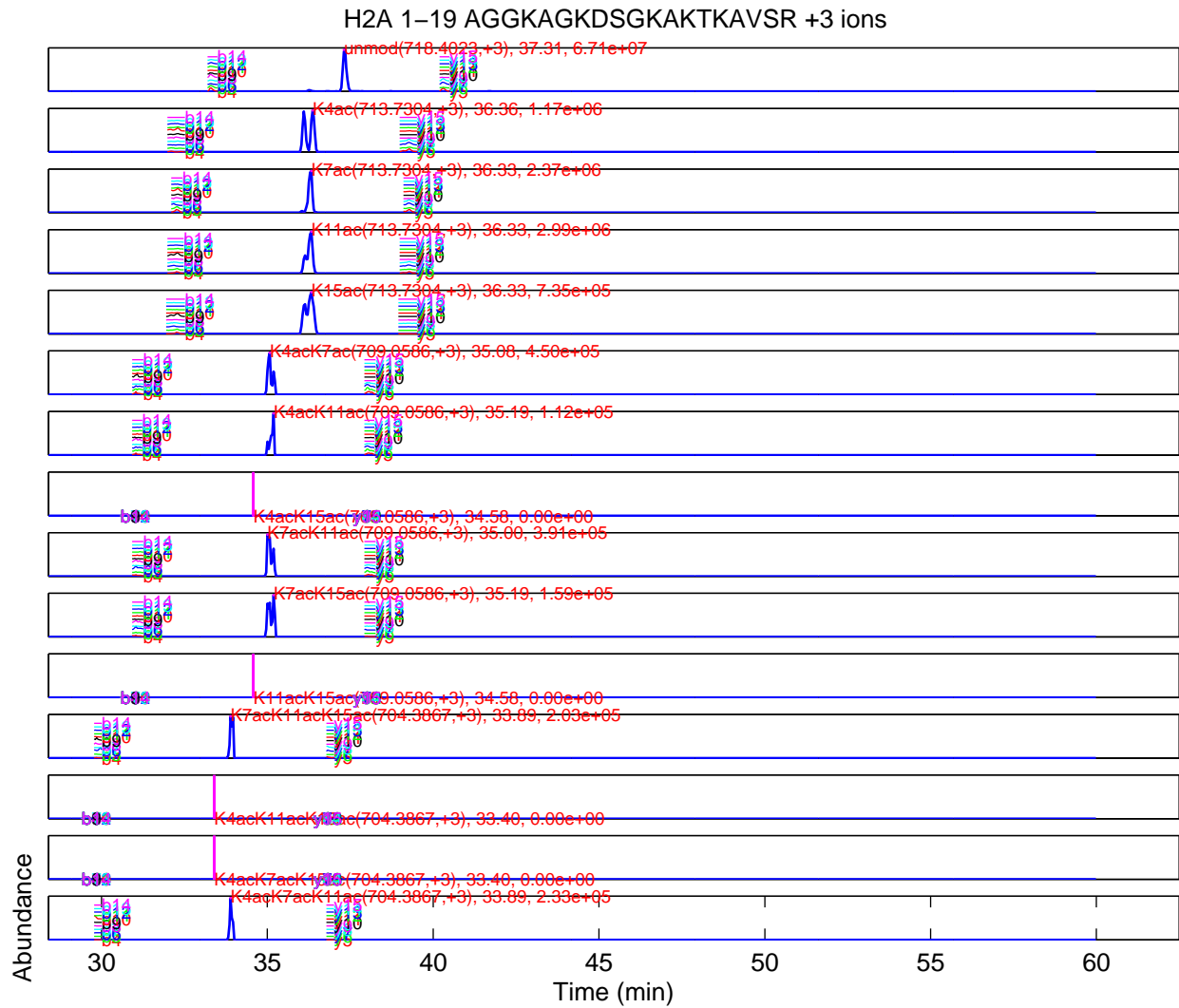




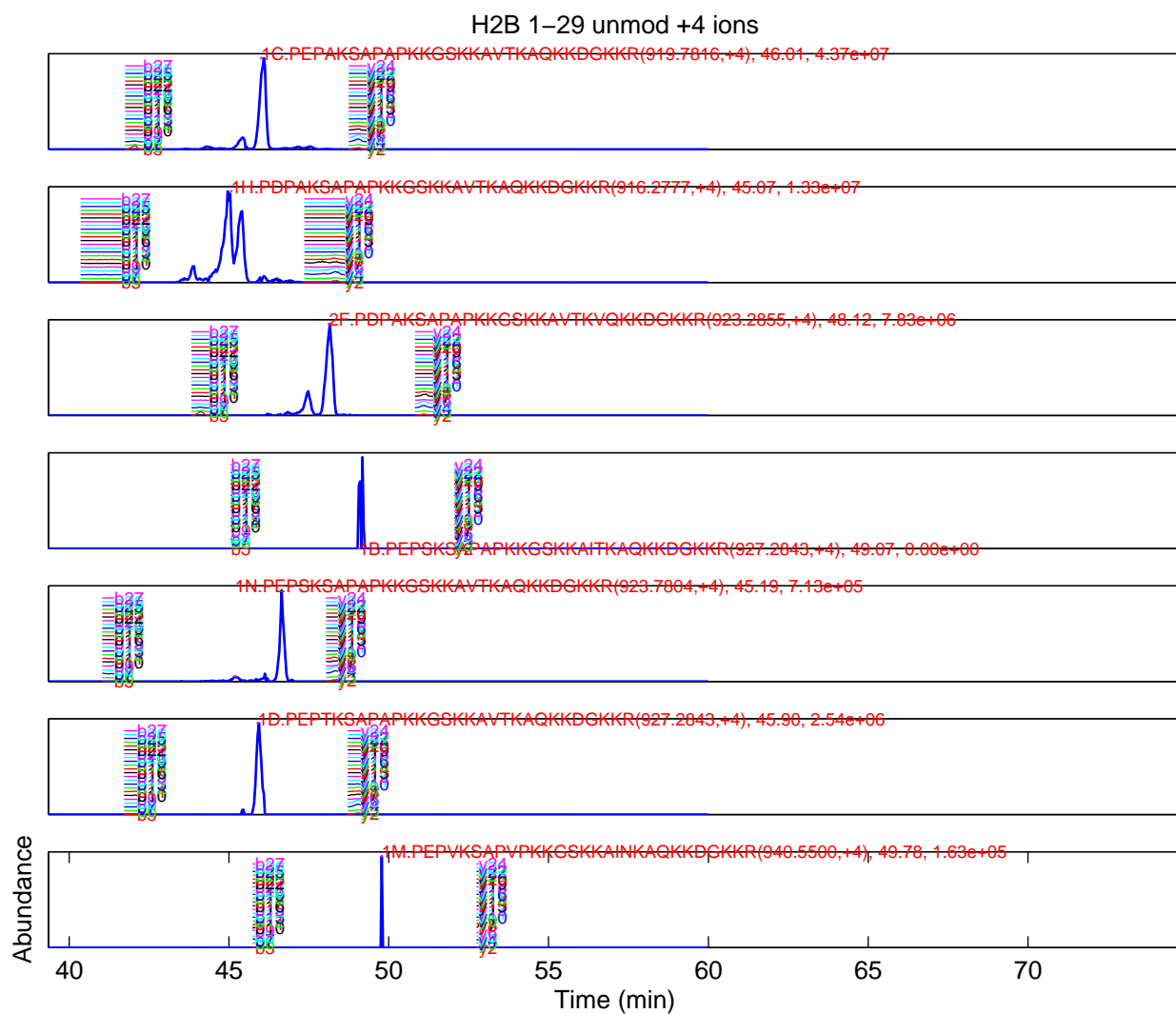
**Figure S1G. Layout of H4 20–23 KVL R.**



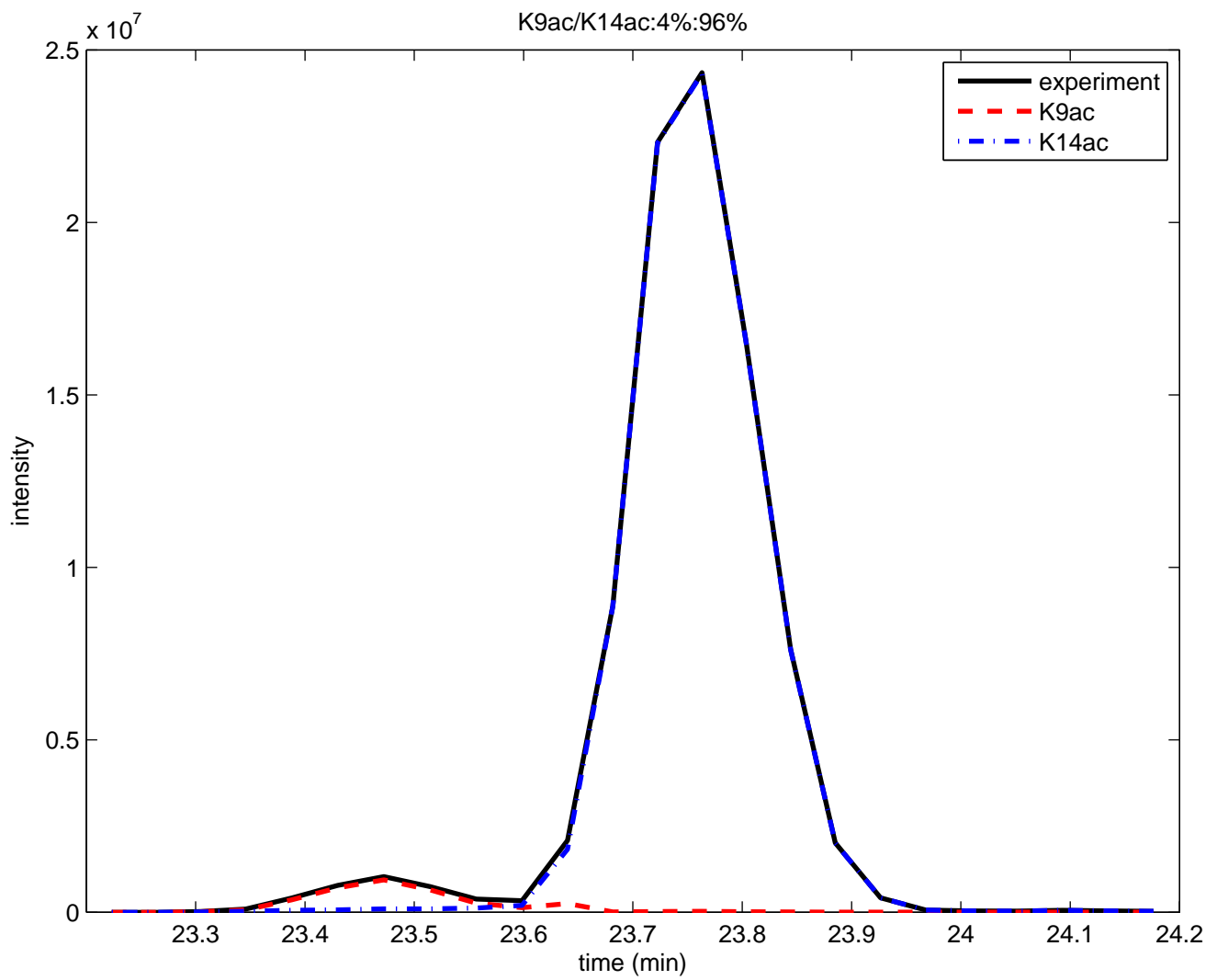
**Figure S1H. Layout of H2A.V 1-19 AGGKAGKDSGKAKAKAVSR.**



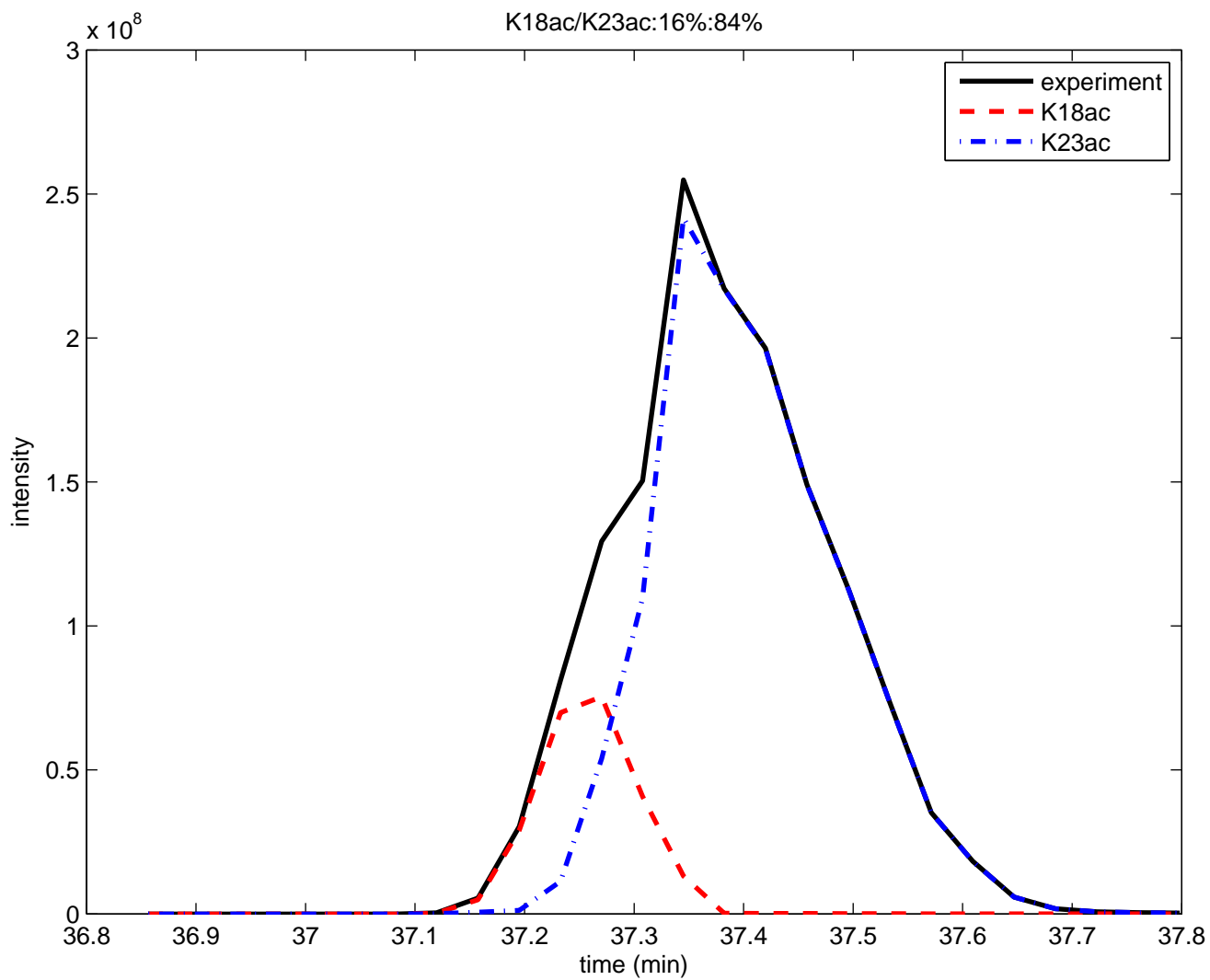
**Figure S1I. Layout of H2A.Z 1-19 AGGKAGKDSGKAKTKAVSR.**



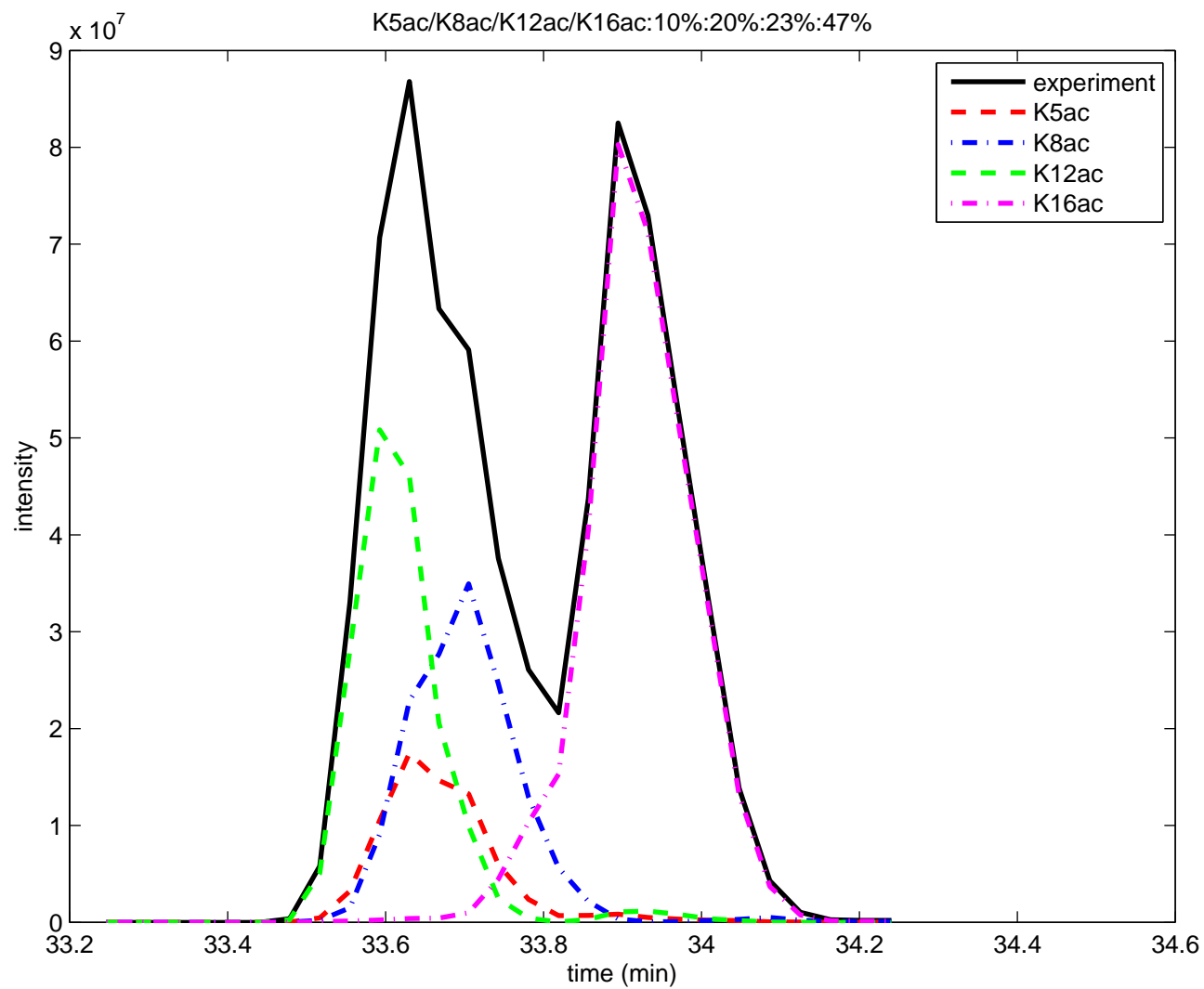
**Figure S1J. Layout of H2B 1–29 variants.**



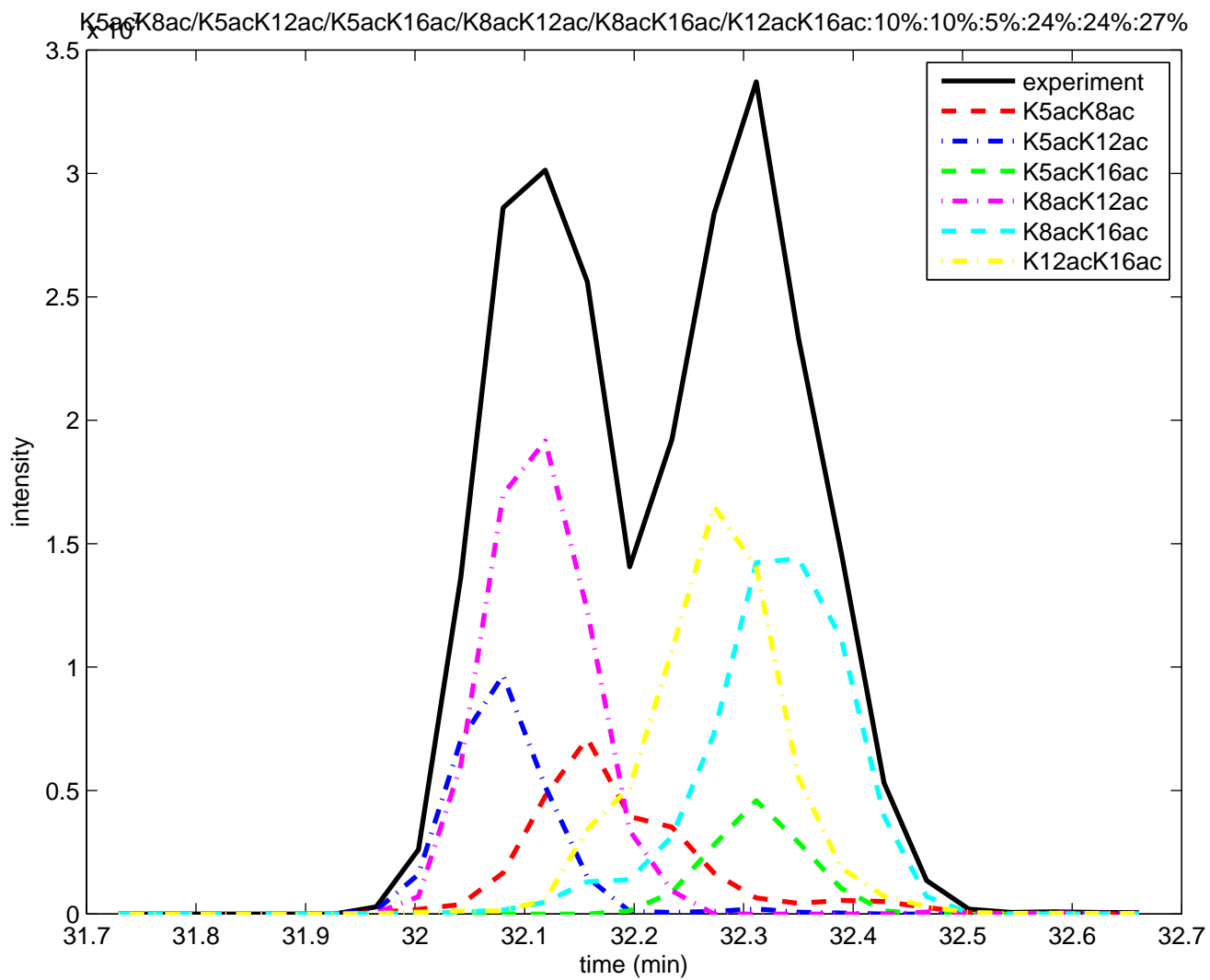
**Figure S2A. Isobaric peptides of H3 9–17 K9ac/K14ac.**



**Figure S2B. Isobaric peptides of H3 18–26 K18ac/K23ac.**

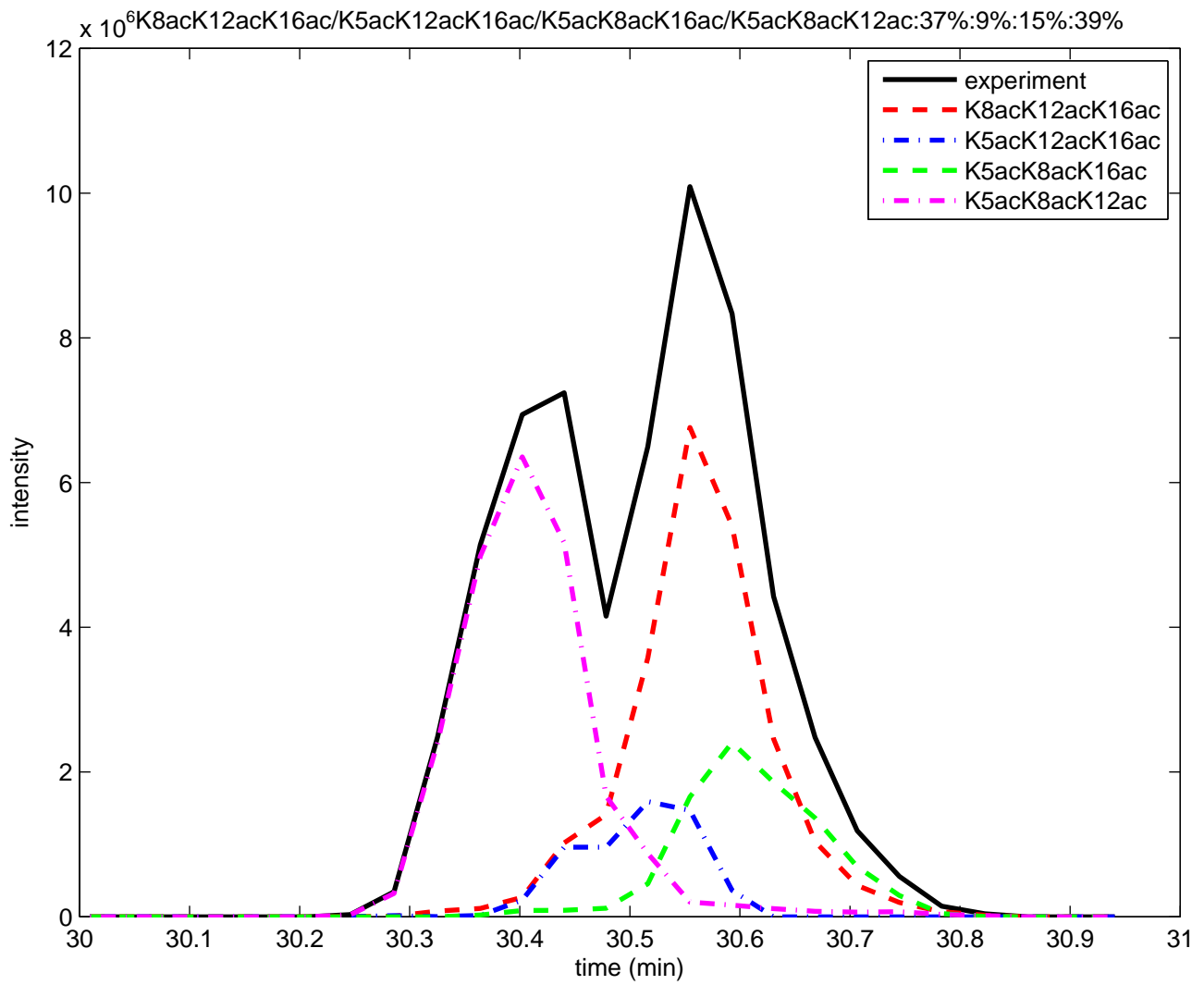


**Figure S2C. Isobaric peptides of H4 4–17 1ac.**

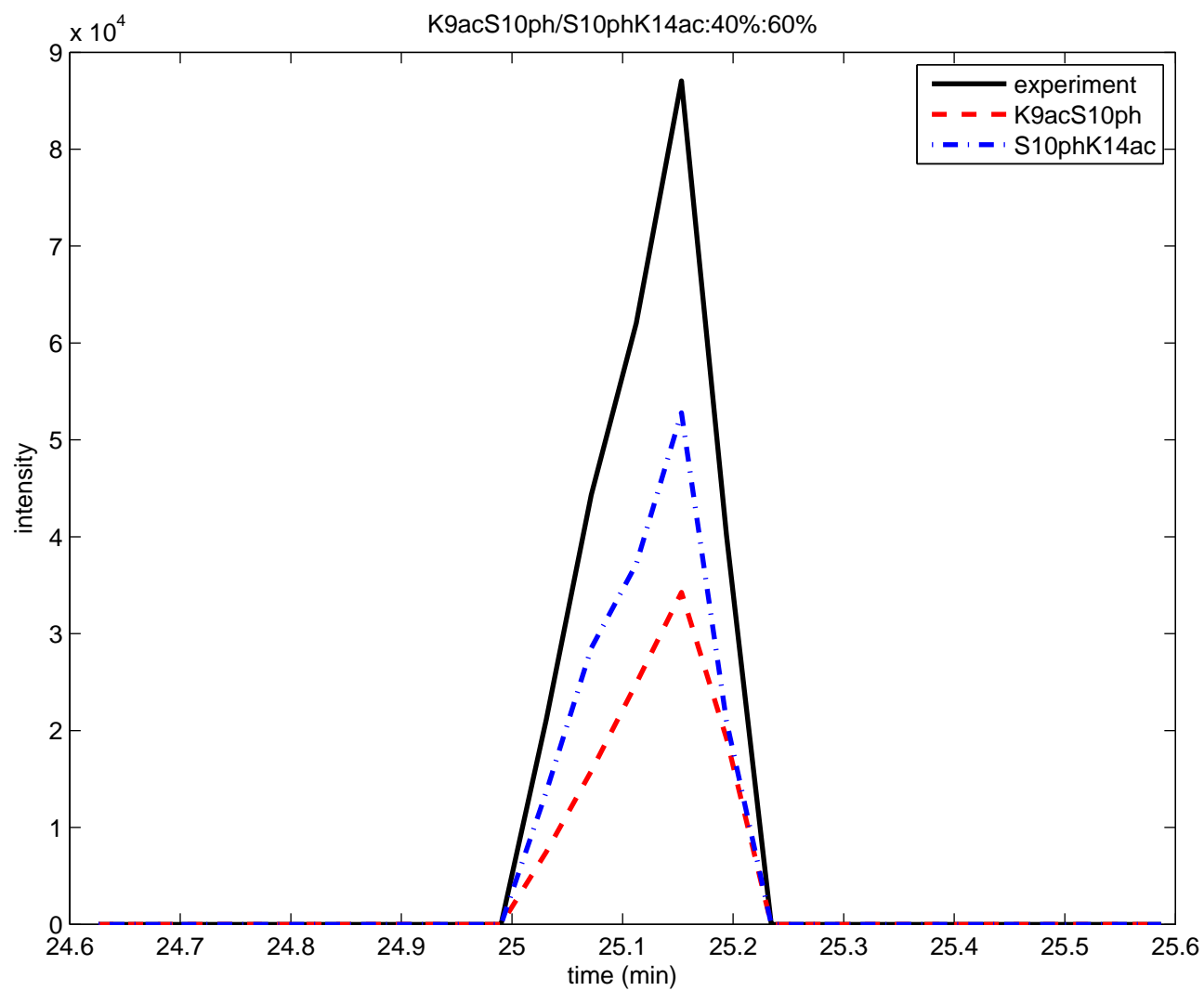


**Figure S2D. Isobaric peptides of H4 4–17 2ac.**

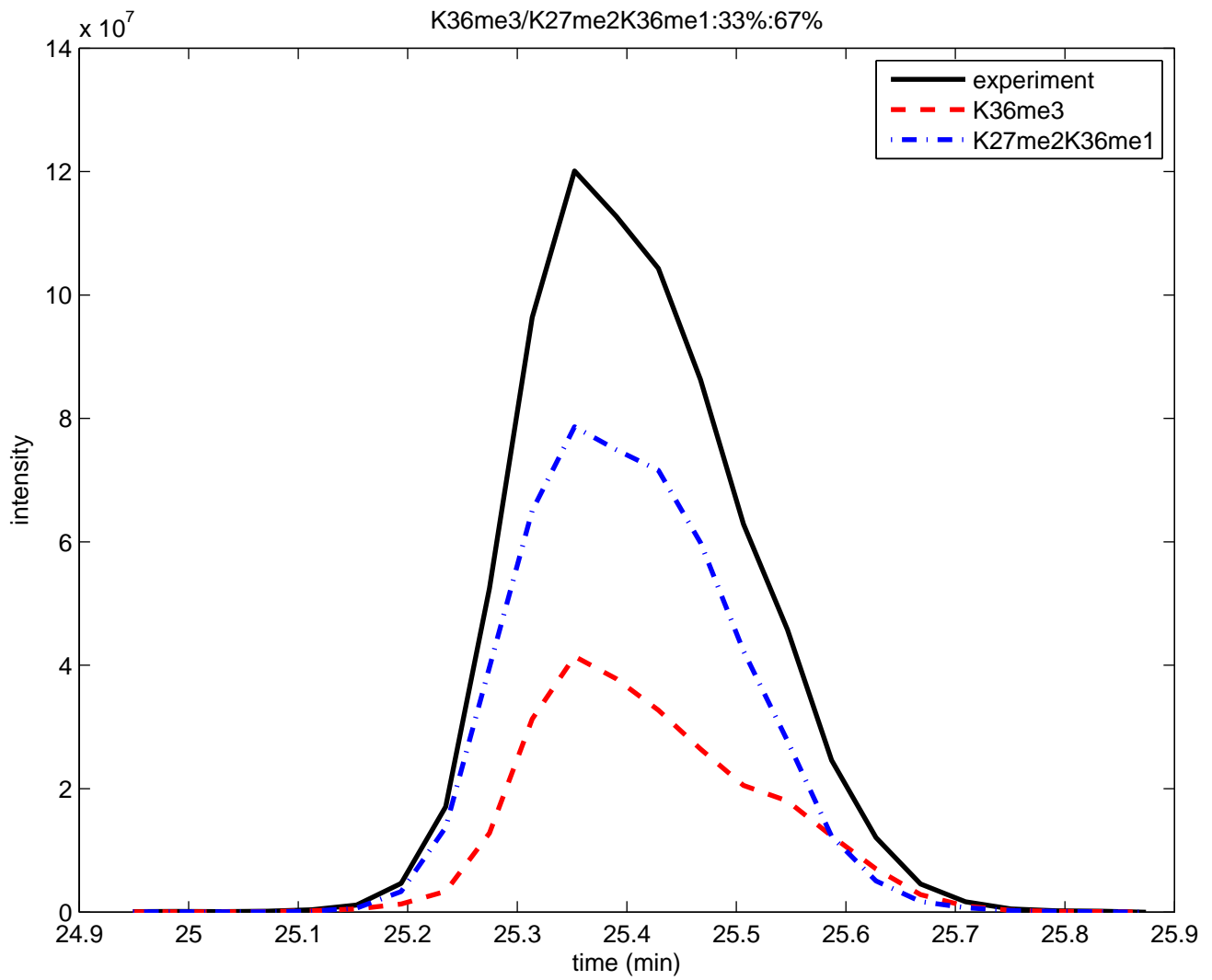




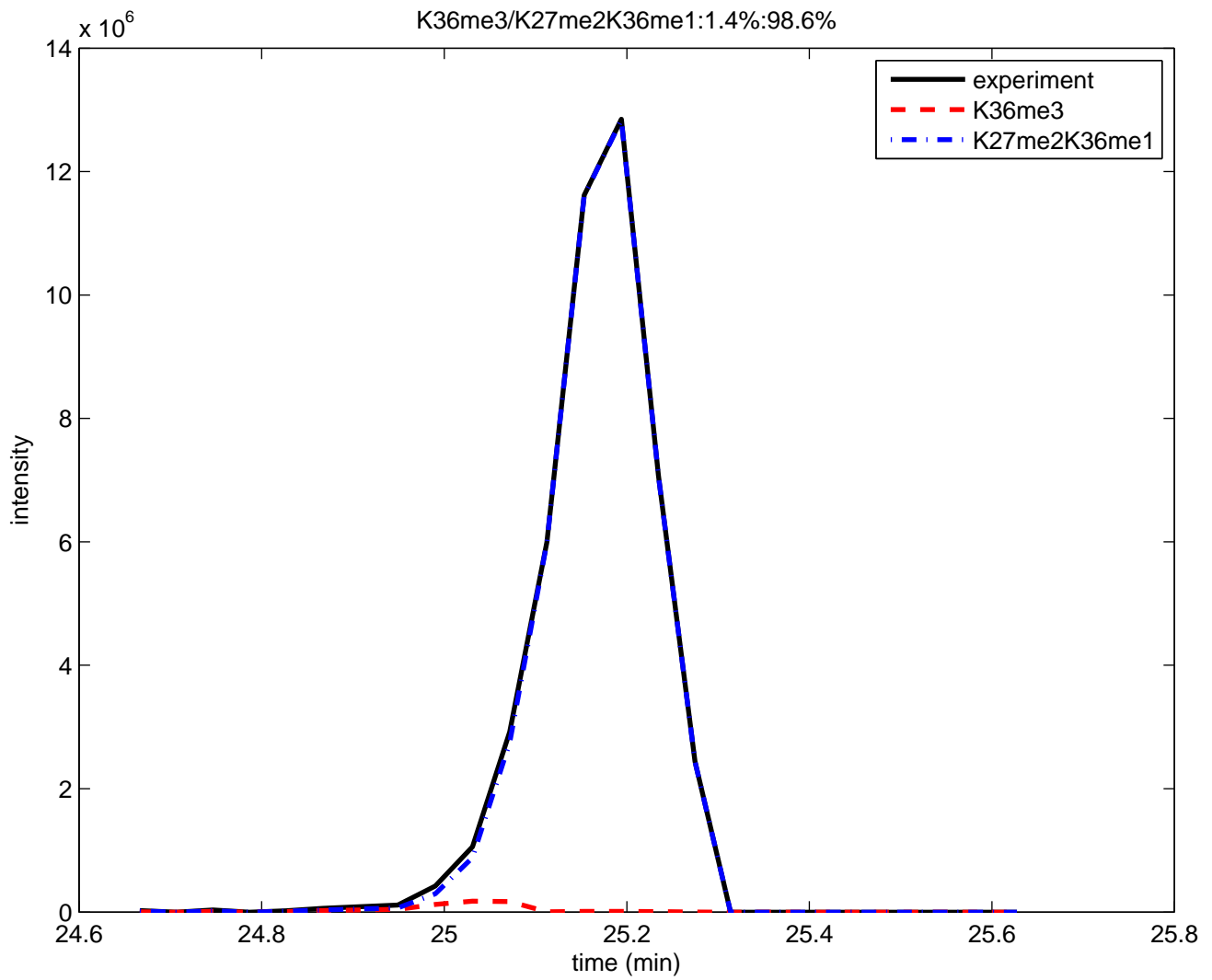
**Figure S2E. Isobaric peptides of H4 4–17 3ac.**



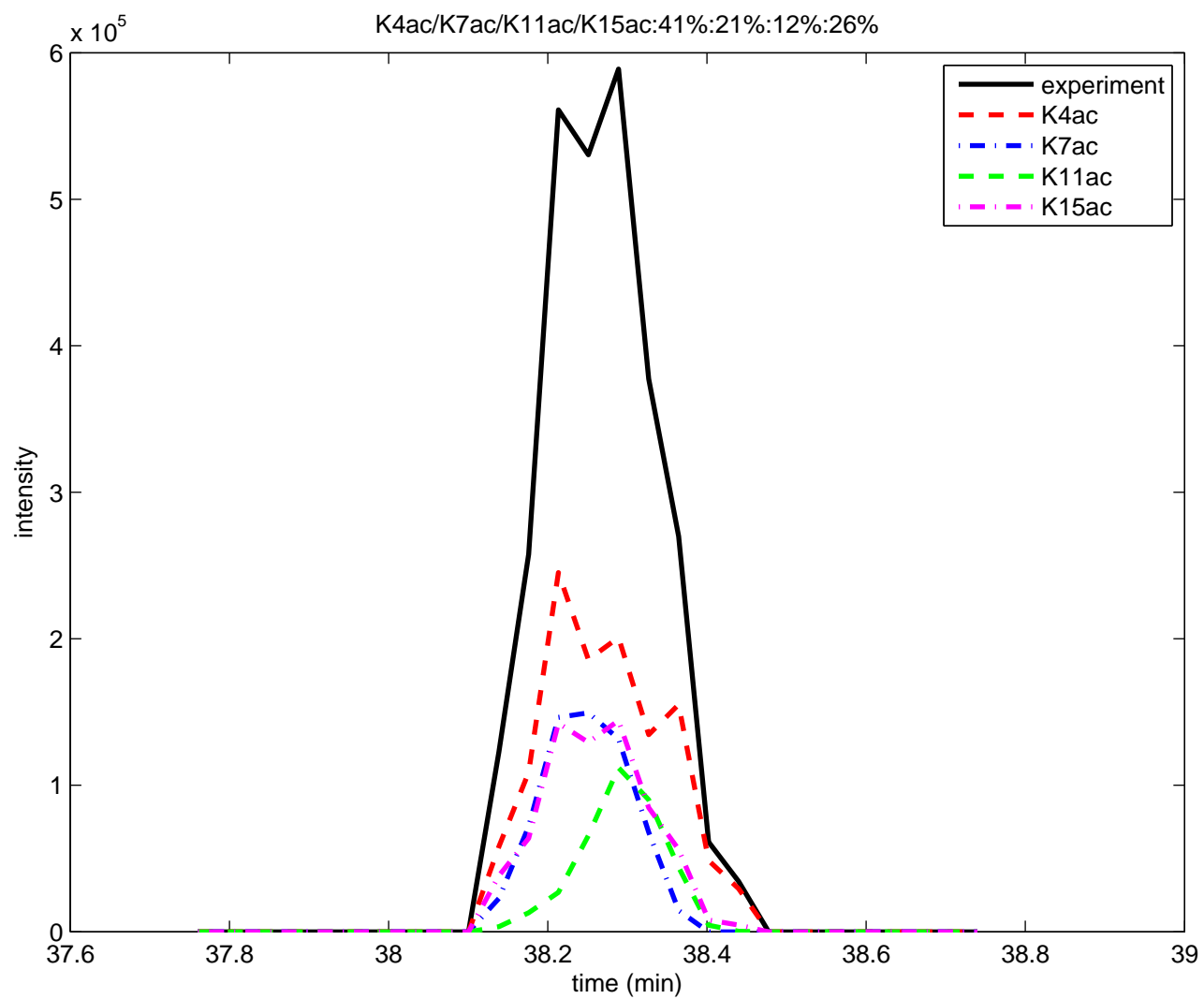
**Figure S2F. Isobaric peptides of H3 9–17 K9acS10ph/S10phK14ac.**



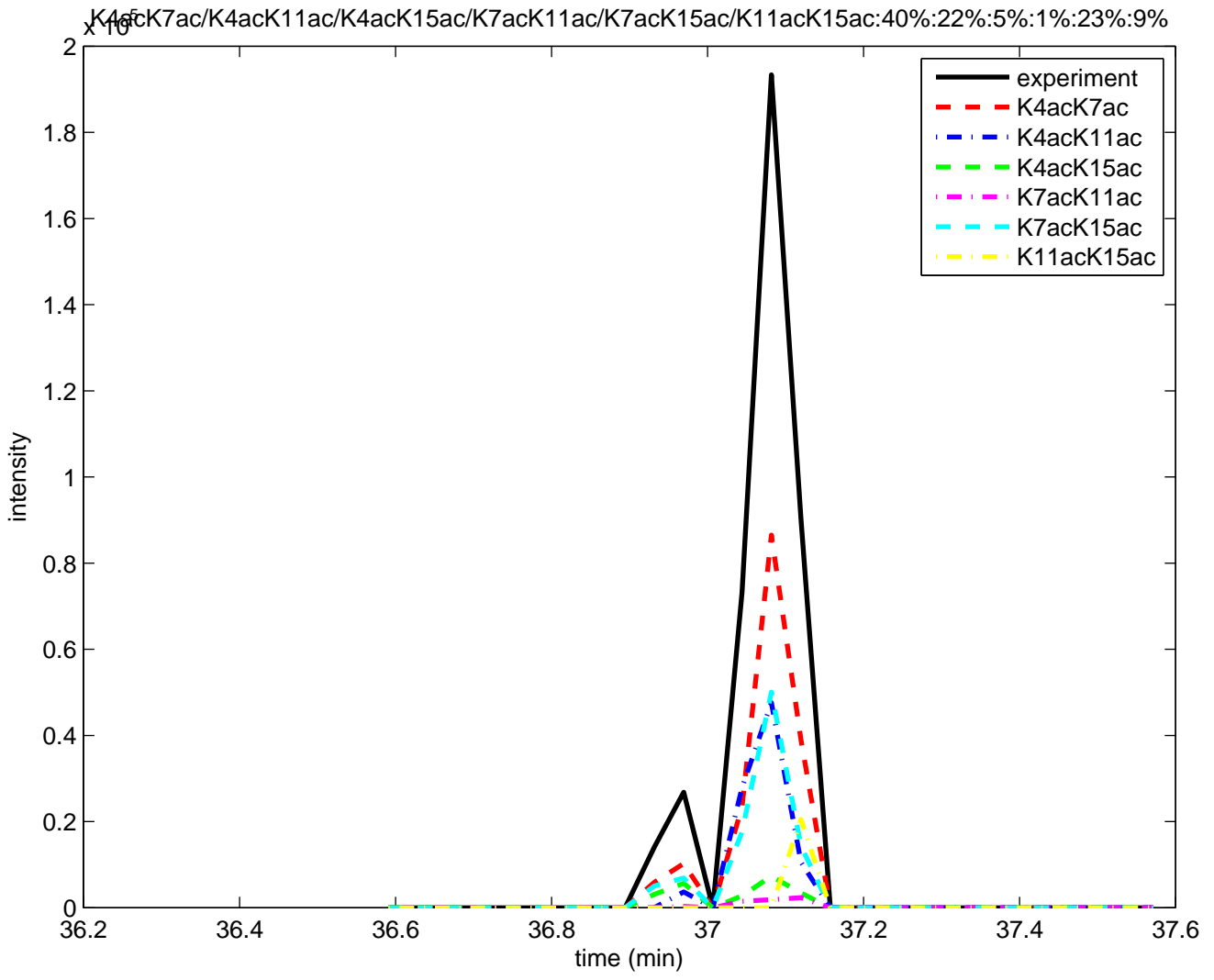
**Figure S2G. Isobaric peptides of H3.1/2 27–40 K36me3/K27me2K36me1.**



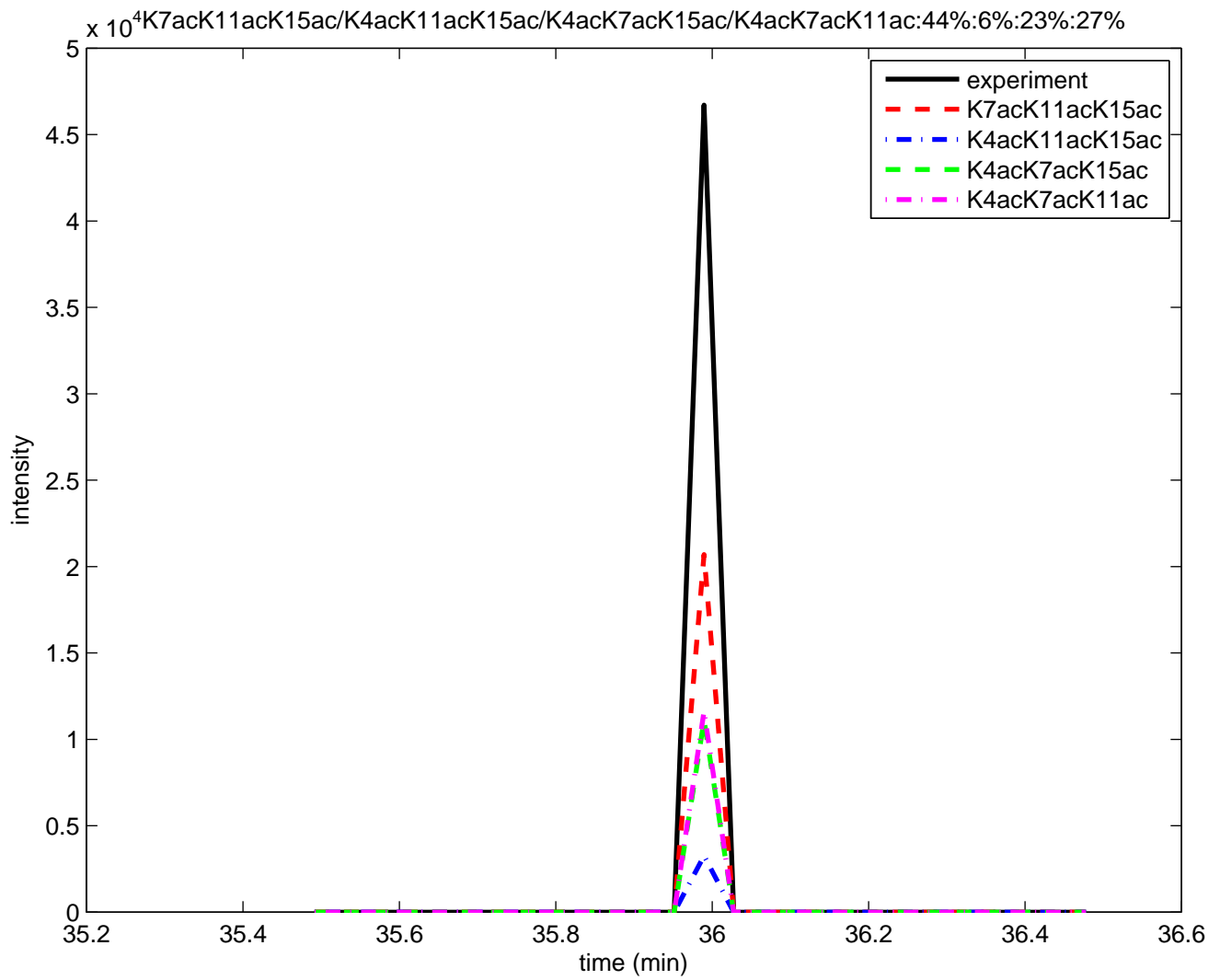
**Figure S2H. Isobaric peptides of H3.3 27--40 K36me3/K27me2K36me1.**



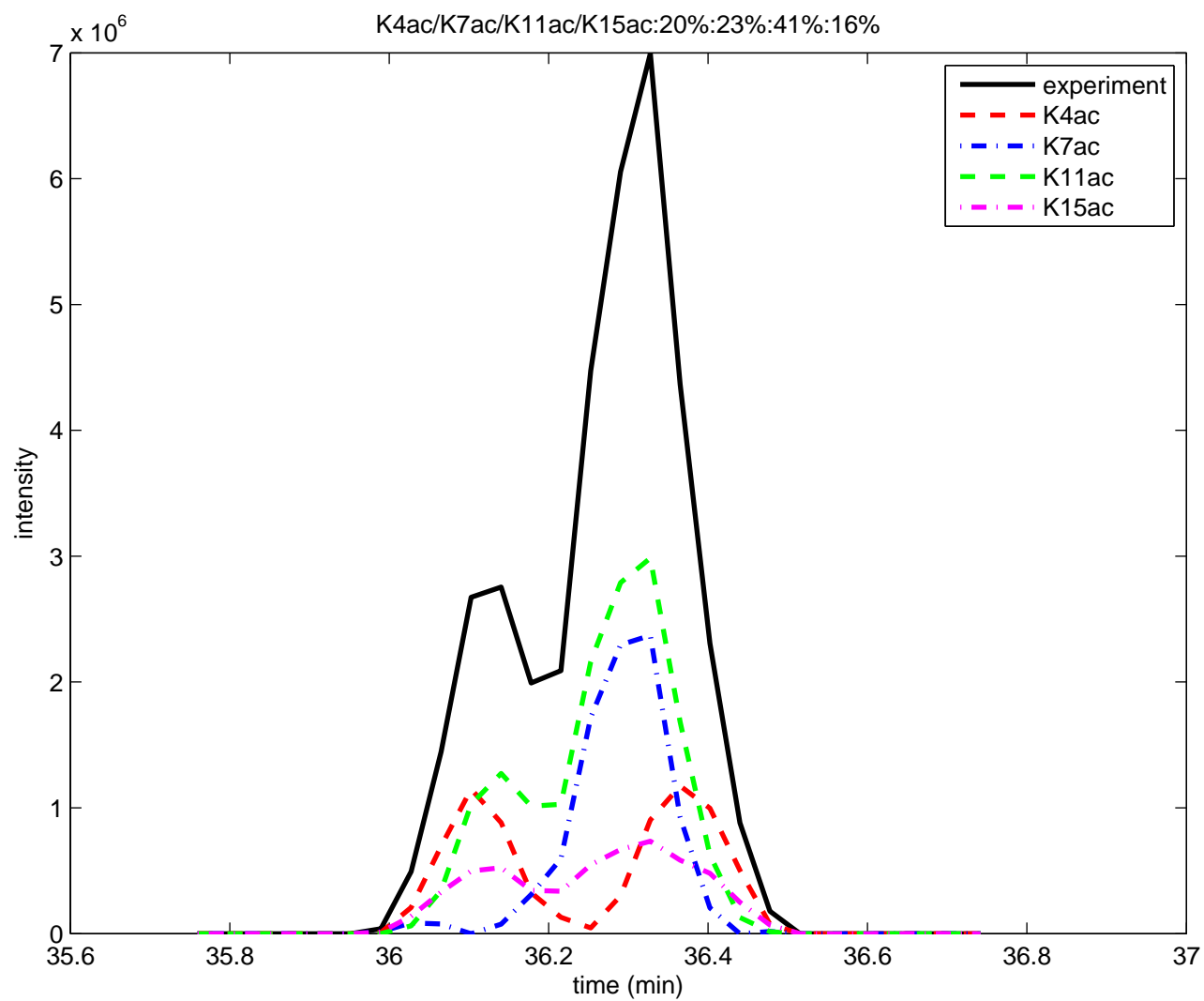
**Figure S2I. Isobaric peptides of H2AV 1--19 1ac.**



**Figure S2J. Isobaric peptides of H2AV 1–19 2ac.**

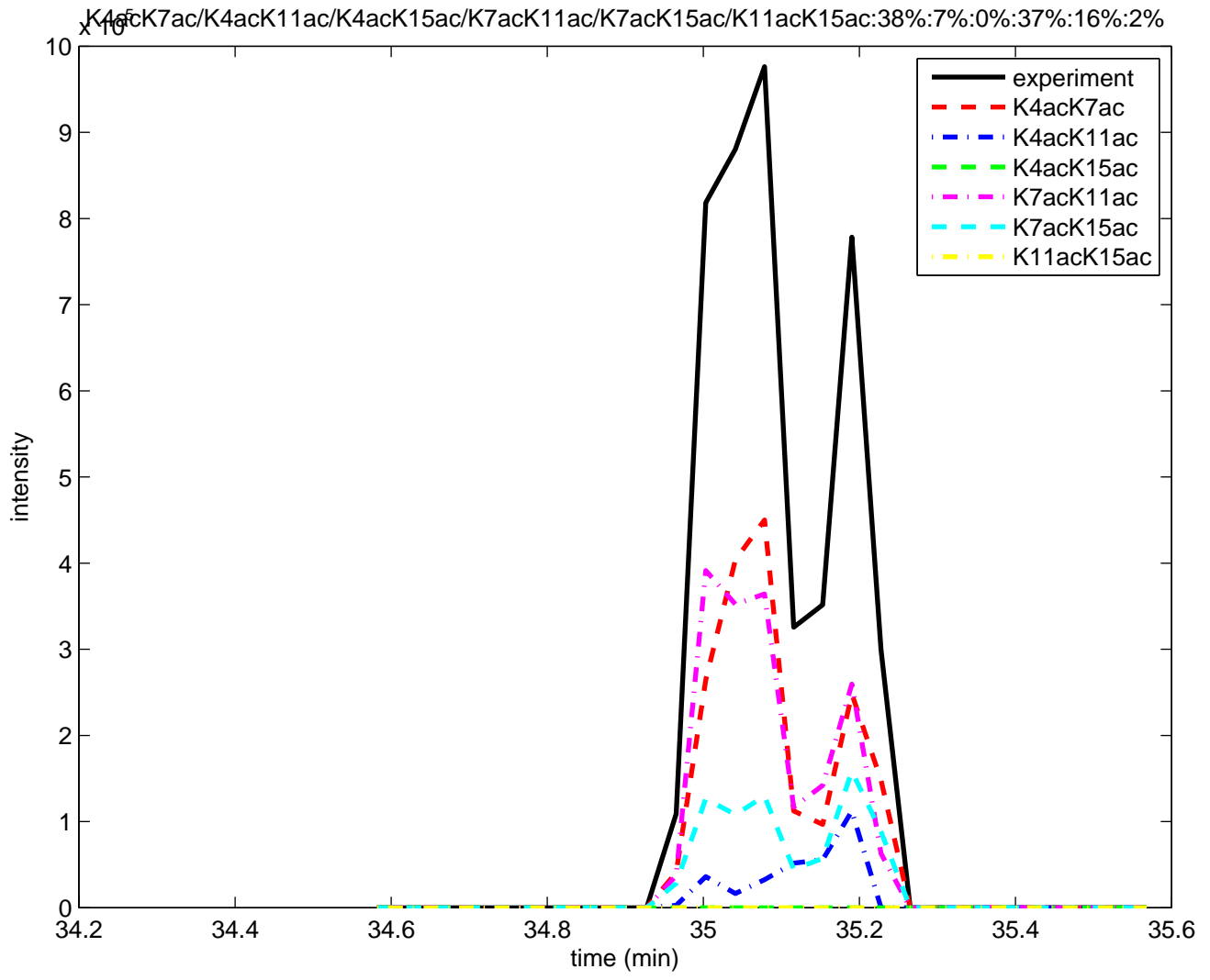


**Figure S2K. Isobaric peptides of H2AV 1–19 3ac.**

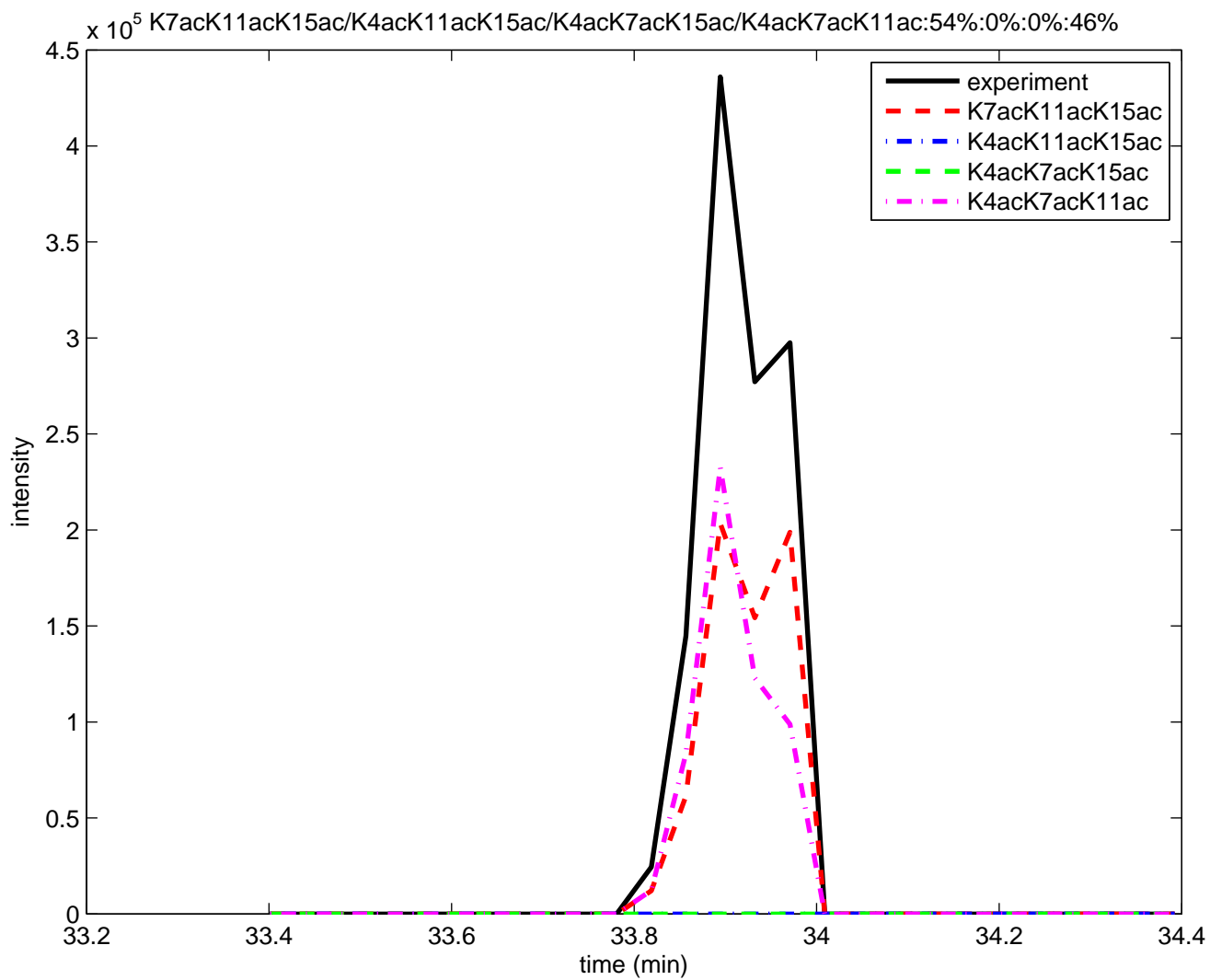


**Figure S2L. Isobaric peptides of H2AZ 1--19 1ac.**

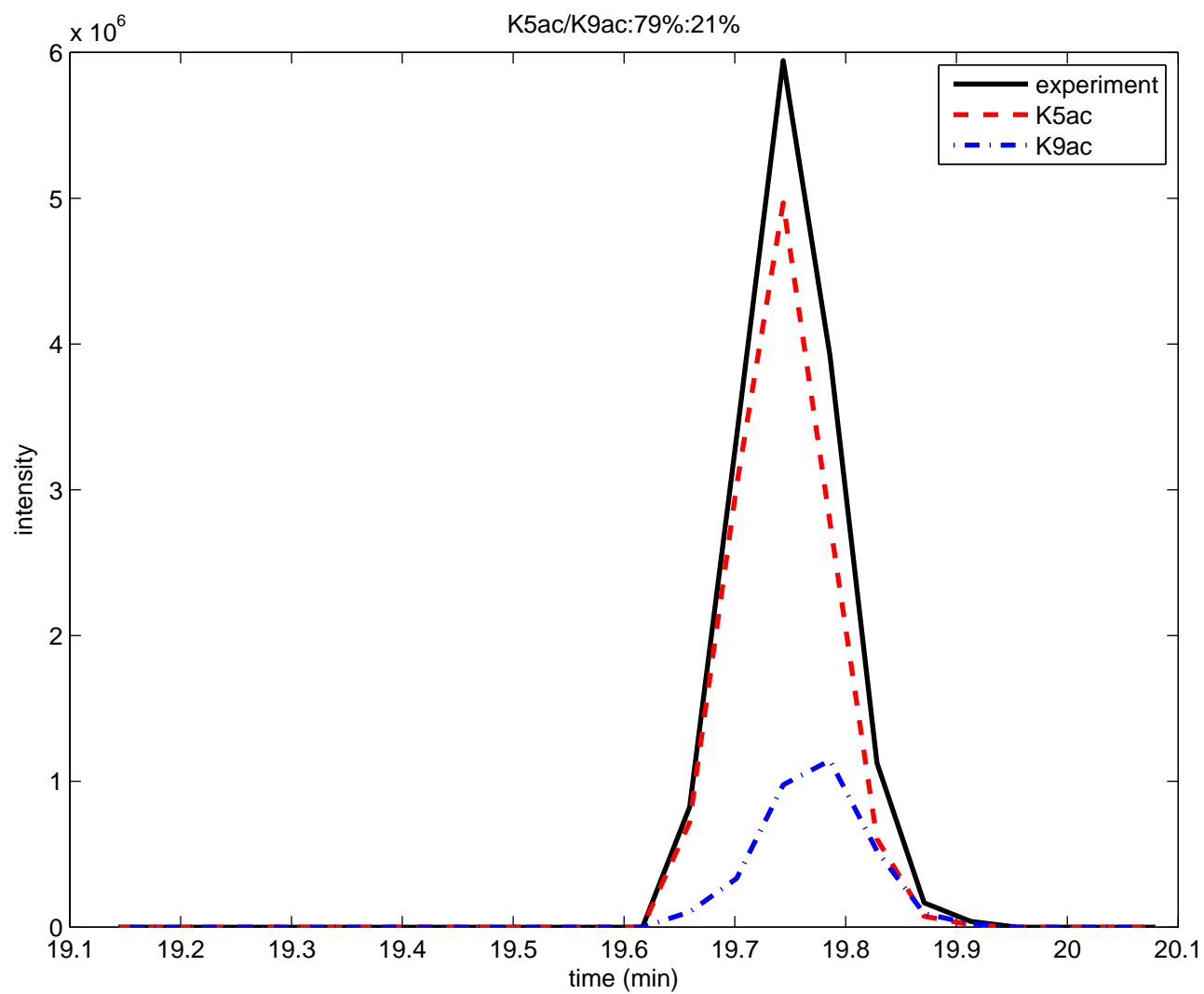




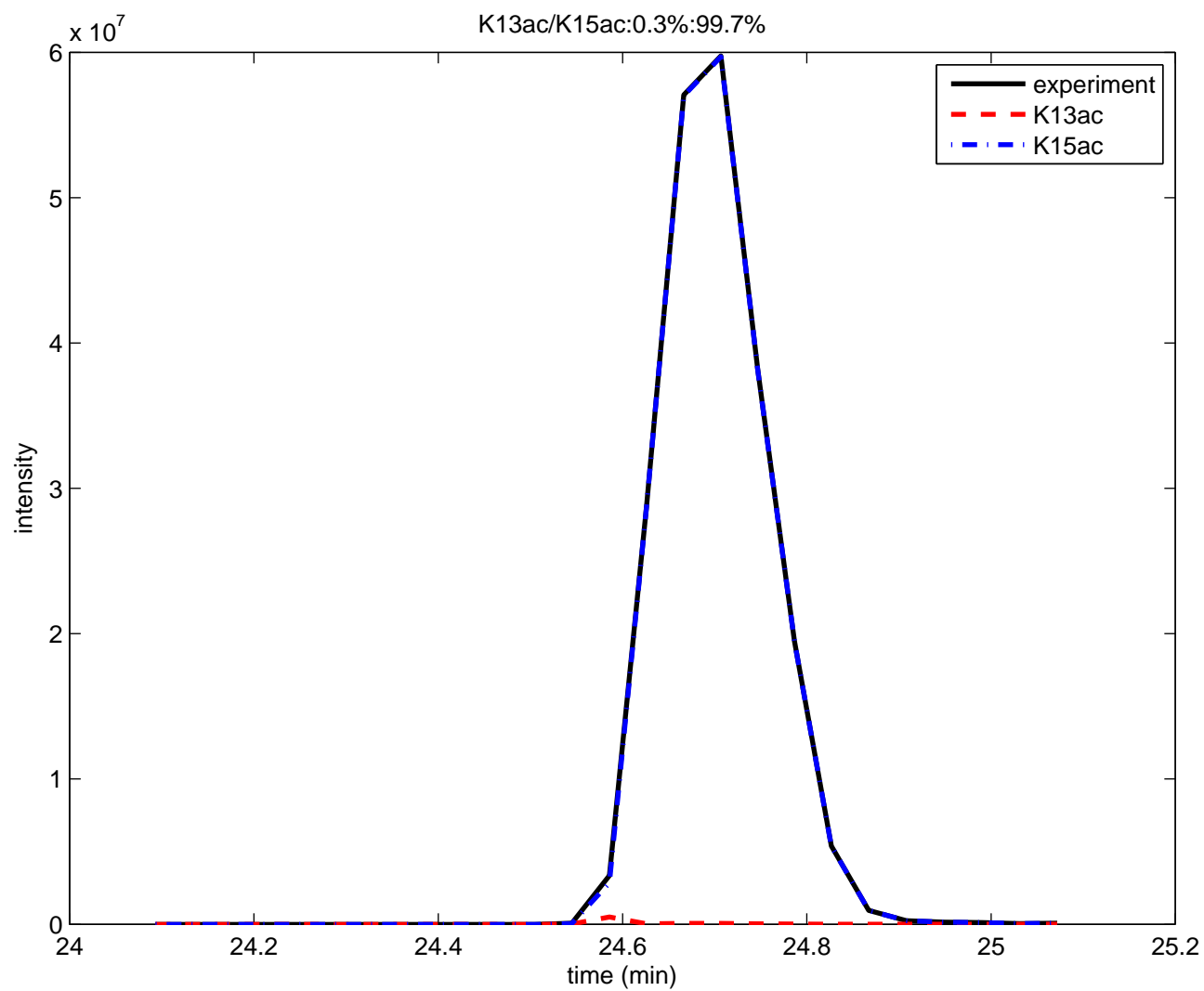
**Figure S2M. Isobaric peptides of H2AZ 1--19 2ac.**



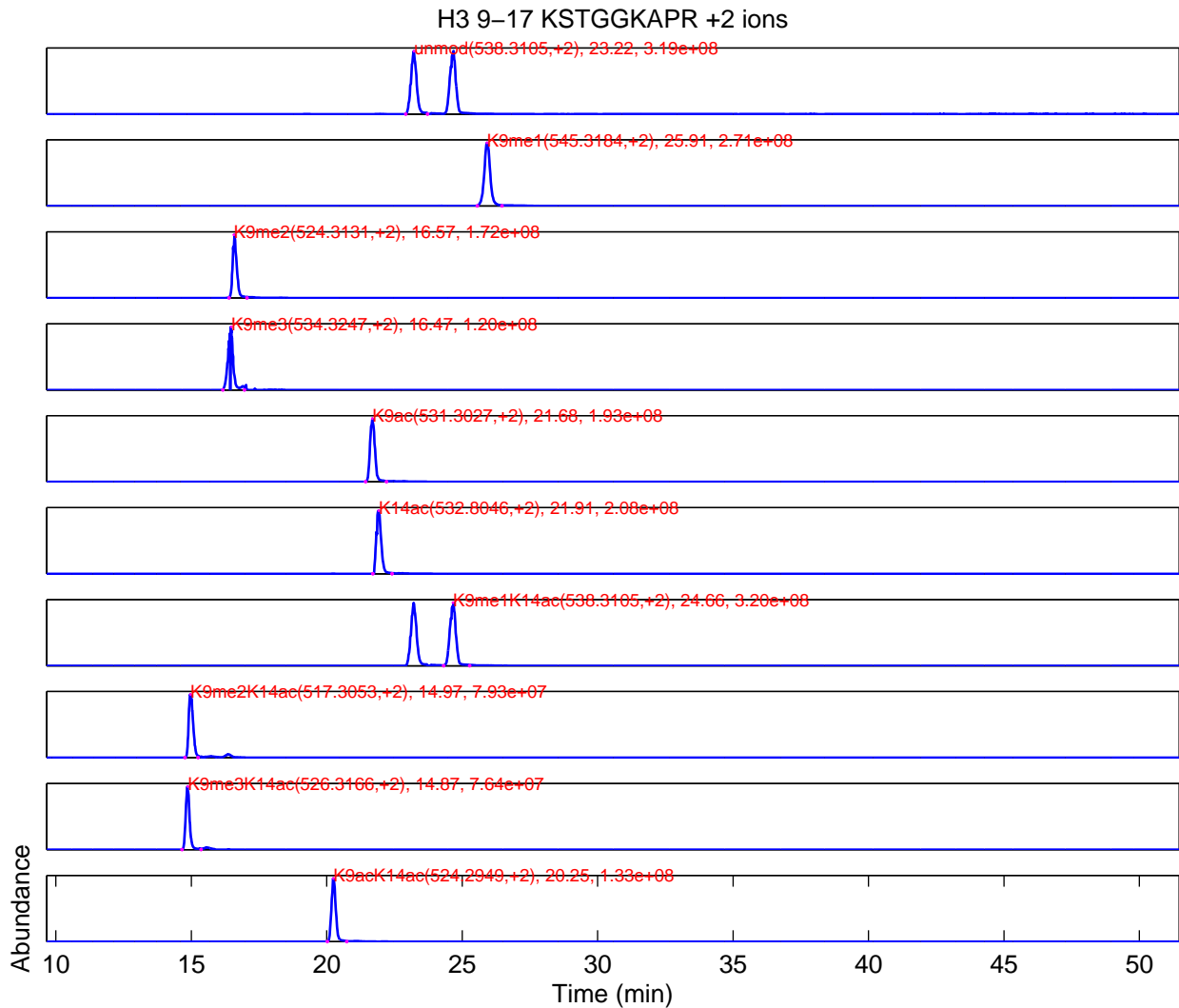
**Figure S2N. Isobaric peptides of H2AZ 1--19 3ac.**



**Figure S2O. Isobaric peptides of H2A 4--11 K5ac/K9ac.**



**Figure S2P. Isobaric peptides of H2A 12--17 K13ac/K15ac.**



**Figure S3. Layouts on H3 9–17 KSTGGKAPR in the synthetic samples.** The layouts of synthetic peptides show similar pattern to the layouts of endogenous peptides, which can be used to validate the relative retention time relationship between endogenous peptides as shown in Figure 1. For example, K9ac is a little earlier than K14ac.

**▲ EpiProfile2.0\_Family**

▶ **EpiProfile2.0\_1Basic**

1: Human, 2: Mouse

1: histone\_normal, 2: histone\_SILAC, 3: histone\_C13, 4: histone\_N15, 5: histone\_13CD3

▶ **EpiProfile2.0\_2Organisms**

EpiProfile2.0\_Bovine

EpiProfile2.0\_Celegans

EpiProfile2.0\_Hsaltator

EpiProfile2.0\_NakedMoleRat

EpiProfile2.0\_Neurospora

EpiProfile2.0\_Oxytricha

EpiProfile2.0\_Piroplasma

EpiProfile2.0\_PlasmodiumFalciparum

EpiProfile2.0\_Sugarcane

EpiProfile2.0\_XenopusLaevis

EpiProfile2.0\_Yeast

▶ **EpiProfile2.0\_3Mutations**

EpiProfile2.0\_Mutation\_00H33A29V\_T32I

EpiProfile2.0\_Mutation\_01H33A15G

EpiProfile2.0\_Mutation\_02H33R17G

EpiProfile2.0\_Mutation\_03H33A29P

EpiProfile2.0\_Mutation\_04H33P121R

EpiProfile2.0\_Mutation\_05H33K27M

EpiProfile2.0\_Mutation\_06H33G34R

EpiProfile2.0\_Mutation\_07H33G34V

EpiProfile2.0\_Mutation\_08H33G34W

EpiProfile2.0\_Mutation\_09H33K36M

EpiProfile2.0\_Mutation\_10H31K27M

EpiProfile2.0\_Mutation\_11H33T45I

EpiProfile2.0\_Mutation\_12H33G90R

EpiProfile2.0\_Mutation\_13H33G33E

EpiProfile2.0\_Mutation\_14H33G34A

EpiProfile2.0\_Mutation\_15H33V35L

EpiProfile2.0\_Mutation\_16H33K36A

EpiProfile2.0\_Mutation\_17H33K36I

EpiProfile2.0\_Mutation\_18H33K36R

EpiProfile2.0\_Mutation\_19H33K36T

EpiProfile2.0\_Mutation\_20H33K36Q

EpiProfile2.0\_Mutation\_21H33K36E

EpiProfile2.0\_Mutation\_22H33K36Nle

EpiProfile2.0\_Mutation\_23H33K37E

EpiProfile2.0\_Mutation\_24H33K37Q

EpiProfile2.0\_Mutation\_25H33K37T

EpiProfile2.0\_Mutation\_26H33K37N

EpiProfile2.0\_Mutation\_27H33K37R

EpiProfile2.0\_Mutation\_28H31G34W

EpiProfile2.0\_Mutation\_29H33K27R\_G34R

▶ **EpiProfile2.0\_4Anhydrides**

EpiProfile2.0\_pr\_pic

▶ **EpiProfile2.0\_5Low-abundancePTMs**

EpiProfile2.0\_51SpecialPTMs

EpiProfile2.0\_H2AK13K15ub

EpiProfile2.0\_H3K27acK36me

EpiProfile2.0\_52CoA

EpiProfile2.0\_CoA\_0single

EpiProfile2.0\_CoA\_1labeled

EpiProfile2.0\_CoA\_2combination

EpiProfile2.0\_CoA\_3D5

EpiProfile2.0\_CoA\_4multi

EpiProfile2.0\_H3R17meR42me

EpiProfile2.0\_H3T3ph

Ac, Bu, Cr, Gl, He, Hi, Hm, Ma, Pr, Su

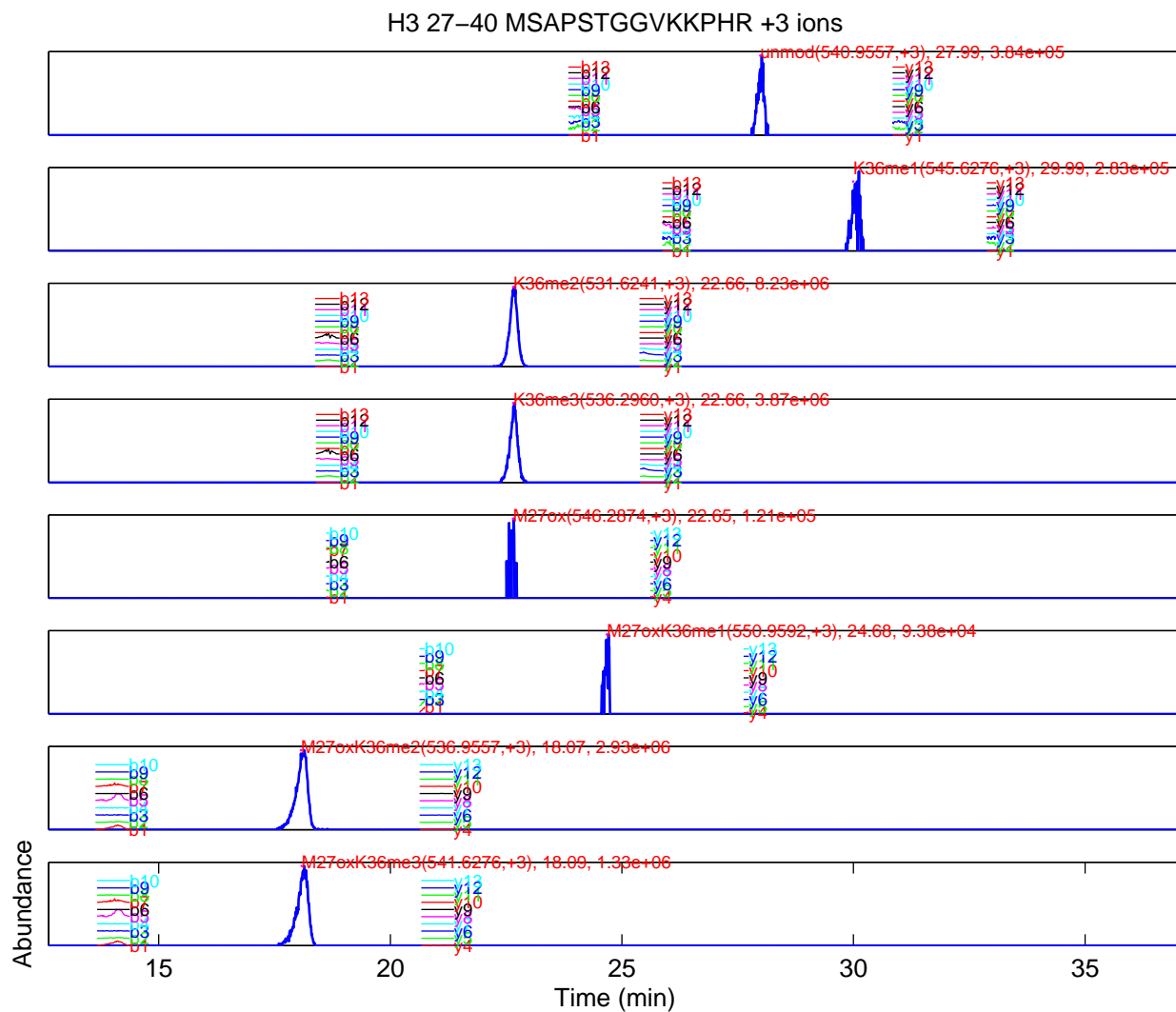
Bu, Cr, Gl, Ma, Pr, Su

AcBu, AcCr, AcGl, AcHi, AcMa, AcPr, AcSu

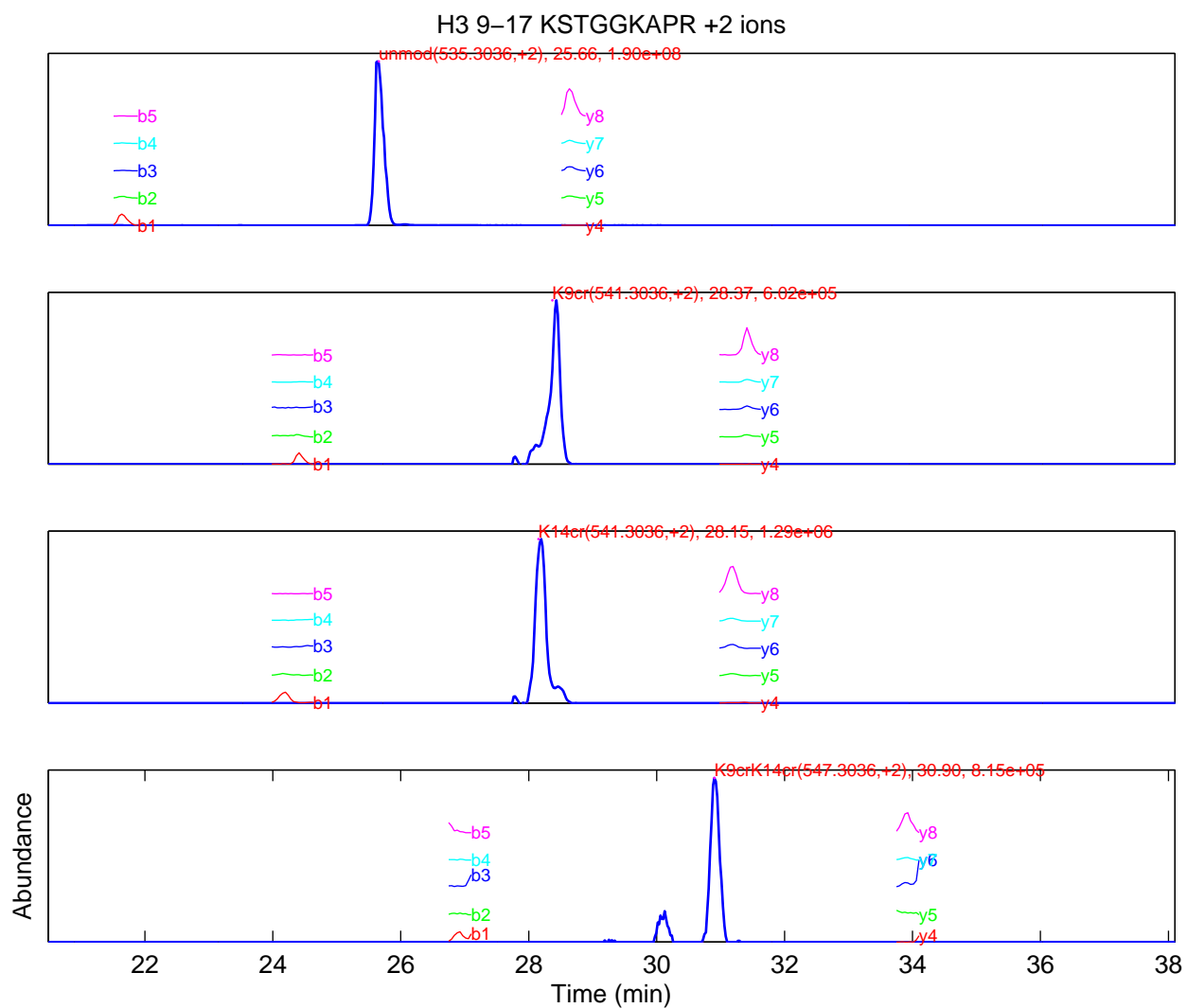
Bu, Pr

D0, D5

**Figure S4. EpiProfile 2.0 family contains the basic version (human and mouse), different organisms, mutations, anhydrides, special PTMs and CoAs.**



**Figure S5. Layout of the mutant peptide H3.3 K27M MSAPSTGGVKKPHR.** It has 8 different forms (i.e. unmodified, K36me1, K36me2, K36me3, M27ox, M27oxK36me1, M27oxK36me2, M27oxK36me3). The oxidative peptides elute earlier than the corresponding un-oxidative ones (e.g. M27ox elutes earlier than unmodified).



**Figure S6. Layout on H3 9–17 KSTGGKAPR by Cr-CoA.** It has unmodified, K9cr, K14cr, K9crK14cr. K9cr and K14cr are discriminated by unique fragment ions acquired from DIA. K9cr elutes a little later than K14cr. Cr elutes later than Pr.