Export annotation of EpiProfile

EpiProfile 2.0: A Computational Platform for Processing Epi-Proteomics Mass Spectrometry Data

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Details of quantifying endogenous histone PTMs by EpiProfile 2.0

EpiProfile 2.0 can quantify PTMs for all histones H3, H4, H1, H2A, and H2B by the following results: layouts (i.e. retention time relationship between peptides, identifying PTMs), ratios (quantifying PTMs), snapshots (a global view of PTM sites and types), spectral matches (PTM fragmentation), identification list (benchmark for search engines), and heat maps (statistic features).

Layouts — Layout is the retention time relationship between histone variants or PTMs on the same peptide sequence. To determine correct PTMs, we can use two kinds of information: retention time and MS/MS. Layouts focus on the retention time. Firstly we take four layouts on H3 9−17 KSTGGKAPR as examples (the first two layouts are shown in Figure 1, and the last two layouts are shown in Figure S1B). The first layout of five peptides is K9me3 < K9me2 < K9ac < unmodified < K9me1 (i.e. the unmodified layout), in which < means earlier. K9me3 and K9me2 are close in about 0.5 min. The retention time helps to distinguish K9me3 and K9ac though they have a close overall mass. Similarly, the second layout of five peptides is K9me3K14ac < K9me2K14ac < K9acK14ac < K14ac < K9me1K14ac (i.e. the K14ac layout); the third layout of five peptides is K9me3S10ph < K9me2S10ph < K9acS10ph < S10ph < K9me1S10ph (i.e. the S10ph layout); the fourth layout of five peptides is K9me3S10phK14ac < K9me2S10phK14ac < K9acS10phK14ac < S10phK14ac < K9me1S10phK14ac (i.e. the S10phK14ac layout). The K14ac layout is earlier than the unmodified layout, and the S10ph layout is later than the unmodified layout, and the S10phK14ac layout is between the unmodified layout and the S10ph layout. These results show that the unmodified layout is a common layout (also as shown in Figure S1A and G).

The layout of seven peptides on H3 18−26 KQLATKAAR is K18acK23ac < K18ac < K23ac < unmodified < K23me1 < K18me1 < K18me1K23me1 (Figure S1C). K18ac and K23ac are isobaric peptides, since they have the same peptide sequence, but different sites of modification. K18me1 and K23me1 are also isobaric peptides. For acetylation, K18ac is a little earlier than K23ac. For methylation, K23me1 is a little earlier than K18me1. In the layouts of H3 9-17 peptides, K9ac and K14ac, K9acS10ph and S10phK14ac are also isobaric peptides. For acetylation, K9ac and K14ac, K9acS10ph and S10phK14ac, K18ac and K23ac, usually overlap.

For methylation, K18me1 and K23me1 overlap or separate. The unique fragment ions in MS/MS spectra can be used to discriminate the overlapping chromatographic peaks (e.g. the discrimination of K9ac and K14ac is shown in Figure 2).

The layout of H3.1/3.2 27−40 KSAPATGGVKKPHR is the most complex. The layout is K27me3K36me2 < K27me2K36me2 < K27me3 < K27me2 < K27me3K36me1 < K27me2K36me1 < K36me3 < K36me2 < K27me1K36me3 < K27me1K36me2 < K27ac < unmodified < K36me1 < K27me1 < K27me1K36me1 (Figure S1D). When other sites are the same, me3 is a little earlier than me2. Isobaric peptides K27me1 and K36me1 overlap or separate. Isobaric peptides K36me3 and K27me2K36me1 overlap. K36me1 is a little earlier than K27me1. K27me2K36me1 is a little earlier than K36me3. K27me2 and K36me2 are two individual peaks. K27me3, K36me3 and K27me2K36me1, K27me1K36me2 are three individual peaks. K27me3K36me1 and K27me1K36me3 are two individual peaks. Overlapping peaks need be discriminated by the fragment ions. Individual peaks are already discriminated. The layout of H3.3 27−40 KSAPSTGGVKKPHR is the same as the layout of H3.1/3.2 27−40, but 0.3 min earlier (Figure S1E).

The layout of H4 4−17 GKGGKGLGKGGAKR is K5K8K12K16-4ac < K5K8K12K16-3ac < K5K8K12K16-2ac < K5K8K12K16-1ac < K5K8K12K16-0ac (Figure S1F). There are four isobaric peptides in K5K8K12K16-3ac and K5K8K12K16-1ac, respectively. There are six isobaric peptides in K5K8K12K16-2ac. The difficulty is how to discriminate four-component or six-component isobaric peptides. EpiProfile 2.0 extracts the fragment ions from the MS/MS spectra and obtains equations to get the relative abundance of each component. The discriminated results show that in K5K8K12K16-1ac, K12ac is the earliest, K5ac and K8ac are close and in the middle, K16ac is the latest; in K5K8K12K16-2ac, K5acK12ac and K8acK12ac are close and the earliest, K5acK8ac is in the middle, K5acK16ac and K8acK16ac and K12acK16ac are in the latest group where K12acK16ac is a little earlier; in K5K8K12K16-3ac, K5acK8acK12ac is the earliest, K5acK12acK16ac and K8acK12acK16ac are close and in the middle, K5acK8acK16ac is the latest.

On H2AV 1−19 AGGKAGKDSGKAKAKAVSR and H2AZ 1−19 AGGKAGKDSGKAKTKAVSR, if K13 is unmodified, the layouts of H2AV 1−19 and H2AZ 1−19 (K4K7K11K15) are the same as the layout of H4 4−17 (K5K8K12K16). In the DDA mode, H2AV 1−19 and H2AZ 1−19 are usually not set as the targets. Therefore, it is difficult to discriminate the isobaric peptides in H2AV 1−19 and H2AZ 1−19 in DDA. However, in the DIA mode they can be discriminated (Figure S1H and I). Generally, the peak height of each H2AZ 1−19 peptide (i.e. K4K7K11K15-0ac to 4ac) is higher than the peak height of the corresponding H2AV 1−19 peptide.

The variances of H2B can also be detected. H2B 1−29 has eight variances. The layout of the unmodified variances is 1H < 1N < 1D < 1C < 1B < 2F < 1M < 1L (Figure S1J). 1D and 1B are two individual isobaric peptides. 1D is earlier than 1B. 1N, 2F, and 1L have close masses. 1N is the earliest, 2F is in the middle, and 1L is the latest. All the layouts can be found in Supplementary Information (histone layouts in endogenous) and Figure S1A−J.

In DDA five isobaric peptides (i.e. H3 9−17 K9ac/K14ac, H3 18−26 K18ac/K23ac, H4 4−17 1ac, 2ac, 3ac) are usually set as targets to gather enough MS2 for the discrimination. In addition, there are more than ten isobaric peptides, e.g. H3 9−17 K9acS10ph/S10phK14ac, H3.1/3 27−40 K36me3/K27me2K36me1, H2AV/Z 1−19 1ac, 2ac, 3ac, H2A 4−11 K5ac/K9ac, H2A 12−17 K13ac/K15ac. As can be imagined, it is impractical to maintain a long target list in DDA. Based on the unique fragment ions, EpiProfile 2.0 can calculate the percentage of each form with each area divided by the total area from DIA data (as shown in Figure S2A−P). In summary, layout or retention time is valuable information to validate the correctness of PTMs, and unique fragment ions which are easily generated by DIA are the key to discriminate isobaric peptides.

Ratios − After the layouts are determined, the next step is to obtain the area under the curve (AUC) for each peptide and then the ratios. For example, AUCs for five peptides on H3 3−8 TKQTAR (i.e. K4un, K4me1, K4me2, K4me3, and K4ac) can be calculated. Then each AUC divided by the total AUC is the relative abundance (ratio) of each peptide (Table S1). The total AUC is a normalization factor. Therefore, the ratios of the same peptides in different samples are comparable. The ratios can be used to calculate the significance (e.g. p-value) between samples

with replicates. All ratios can be found in Supplementary Information (histone ratios in endogenous).

Snapshots – A snapshot of histone PTMs can show a global view of PTM sites and types. Because there are too many variances of H1, H2A, and H2B, EpiProfile 2.0 only provide the snapshots of H3 and H4. If the ratio is non-zero, the type and the site of the modification are recorded. In the list of the protein sequence, the type of the modification will be shown on the specific site (Table S2). On H3, K4, K9, K14, K18, K23, K27, K36, K56, K79, and K122 are the main modification sites. On H4, K5, K8, K12, K16, and K20 are the main modification sites. If phosphorylation enrichment has been done, then H3 S10 ph and S28 ph can be seen. Sometimes, H3 S10ac, T22ac, and S28ac can be detected. Snapshots can be found in Supplementary Information (histone snapshots in endogenous).

Spectral matches – MS/MS is the second important aspect to identify the PTMs. In the DDA mode, we can export all the MS/MS spectra under the corresponding peptide's chromatographic peak. For example, we can export the MS/MS spectra for H3 K9ac and K14ac (Figure S3). Most peaks are matched with the theoretical fragment ions. However, in the middle of the spectrum there are some high-abundance unmatched peaks. They are water-loss ions of the precursor ion. b1, y5-y8 are signed for H3 K9ac. On the right side of b1 and on the left side of y5-y8, there are unsigned peaks, which can be signed for H3 K14ac. Therefore, this spectrum is a mixture of H3 K9ac and K14ac. The unique fragment ions can be used to distinguish the two co-eluting peptides. The export of MS/MS spectra is a good way to study fragmentation of peptides. All the spectral matches can be found in Supplementary Information (spectral matches in endogenous). In the DIA mode, we draw the profile of fragment ions besides the profile of peptides as shown in the layouts (e.g. Figure S1A–J).

Identification list – In the DDA mode, it is easy to obtain the identification list. For each MS/MS spectrum under the corresponding peptide's chromatographic peak, all the identification information can be exported (Table S3). This identification list can be used as a benchmark (i.e. ground truth) for search engines, which means that it can be used to evaluate different database

search engines, e.g. pFind, Mascot (wiki2.org/en/List_of_mass_spectrometry_software). All the identification lists can be found in Supplementary Information (identification list in endogenous).

Heat maps ─ EpiProfile 2.0 can generate some statistic figures based on the ratios of histone PTMs. The bar plot represents the number of histone peptides quantified using EpiProfile 2.0 (Figure S4). The box plot displays the Log10 transformed total peptide intensity (Figure S5). Principal component analysis displays in two dimensions of the n-dimensional dataset, aiming to simplify the distance between samples into a spatial 2D graph (Figure S6). Heat map represents the relative abundance of single histone PTMs (Figure S7). For PTMs that belong into multiple combinations (e.g. K9me3 and K9me3K14ac) the relative abundance was obtained by summing the relative abundance of all peptides containing the given modification. When there are sample replicates, there is a need to calculate standard deviation of single PTMs. The standard deviation based on single PTMs after the sum of individuals is incorrect. In contrast, before the sum of individuals standard deviation is calculated based on each individual, and the root-sum square of individual standard deviation is the standard deviation of single PTMs (Table S4). In addition, histone PTM annotations are exported, which contain the histone sites and corresponding enzyme and function (Table S5).

Table S1. Ratios of histone peptides

Peptide	FT1_50 Da_01 Ratio	FT1_50 Da_02 Ratio	FT1_50 Da_03 Ratio	FT1_50 Da_01 Area	FT1_50 Da_02 Area	FT1_50 Da_03 Area	FT1_50 Da_01 RT (min)	FT1_50 Da_02 RT (min)	FT1_50 Da_03 RT (min)
TKQTAR (H3_3_8)									
unmod	0.659396	0.655326	0.649775	1.41E+09	1.41E+09	1.39E+09	18.75	18.81	18.88
K4me1	0.336878	0.340956	0.346551	7.21E+08	7.36E+08	7.39E+08	22.77	22.91	22.88
K4me2	0.002927	0.002988	0.002791	6.27E+06	6.45E+06	5.95E+06	9.82	9.83	10.08
K4me3	0.000707	0.00073	0.000814	1.51E+06	1.58E+06	1.74E+06	9.77	9.74	10.08
K4ac	0.000092	0	0.000069	1.97E+05	0.00E+00	1.48E+05	16.58	0	16.62

Table S2. Snapshot of histone H3

1	A				
2	R				
3	T				
4	K	me1	me2	me3	ac
5	Q				
6	T				
7	A				
8	R				
9	K	me1	me2	me3	ac
10	S	ph	ac		
11	T				
12	G				
13	G				
14	K	ac			
15	A				
16	P				
17	R				
18	K	me1	ac		
19	Q				
20	L				
21	A				
22	T	ac			
23	K	me1	ac		
24	A				
25	A				
26	R				
27	K	me1	me2	me3	ac
28	S	ph	ac		
29	A				
30	P				
31	A				
32	T				
33	G				
34	G				
35	V				
36	K	me1	me2	me3	
37	K				

Table S3. Identification list

MS2 Scan	measured m/z	calculated m/z	charge	ppm	sequence	modification1	modification2	Histone type	RT (min)
4587	408.7324	408.7323	2	0.07	TKQTAR	unmod	0,pr;2,pr;	H3	18.83
5639	415.7403	415.7402	2	0.26	TKQTAR	K4me1	0,pr;2,me1;	H3	23.03
5737	415.7401	415.7402	2	-0.18	TKQTAR	K4me1	0,pr;2,me1;	H3	23.03
2210	394.7349	394.7349	2	-0.03	TKQTAR	K4me2	0,pr;2,me2;	H3	9.72
2301	394.7349	394.7349	2	0.12	TKQTAR	K4me2	0,pr;2,me2;	H3	9.72
2389	394.7350	394.7349	2	0.36	TKQTAR	K4me2	0,pr;2,me2;	H3	9.72
2476	394.7352	394.7349	2	0.74	TKQTAR	K4me2	0,pr;2,me2;	H3	9.72
6386	535.3041	535.3036	2	0.81	KSTGGKAPR	unmod	0,pr;1,pr;6,pr;	H3	25.53
6496	535.3038	535.3036	2	0.24	KSTGGKAPR	unmod	0,pr;1,pr;6,pr;	H3	25.53
7388	542.3117	542.3115	2	0.44	KSTGGKAPR	K9me1	0,pr;1,me1;6,pr;	H3	29.14
7497	542.3109	542.3115	2	-1.14	KSTGGKAPR	K9me1	0,pr;1,me1;6,pr;	H3	29.14
7607	542.3116	542.3115	2	0.21	KSTGGKAPR	K9me1	0,pr;1,me1;6,pr;	H3	29.14
4192	347.8734	347.8732	3	0.56	KSTGGKAPR	K9me2	0,pr;1,me2;6,pr;	H3	17.18
4246	521.3057	521.3062	2	-1.00	KSTGGKAPR	K9me2	0,pr;1,me2;6,pr;	H3	17.18
4128	528.3139	528.3140	2	-0.21	KSTGGKAPR	K9me3	0,pr;1,me3;6,pr;	H3	17.01
4213	528.3129	528.3140	2	-2.05	KSTGGKAPR	K9me3	0,pr;1,me3;6,pr;	H3	17.01
5848	528.2952	528.2958	2	-1.12	KSTGGKAPR	K9ac	0,pr;1,ac;6,pr;	H3	23.58
5897	528.3000	528.2958	2	7.91	KSTGGKAPR	K9ac	0,pr;1,ac;6,pr;	H3	23.58
5908	528.3000	528.2958	2	7.91	KSTGGKAPR	K9ac	0,pr;1,ac;6,pr;	H3	23.58
5919	528.3000	528.2958	2	7.91	KSTGGKAPR	K9ac	0,pr;1,ac;6,pr;	H3	23.58
5897	528.3000	528.2958	2	7.91	KSTGGKAPR	K14ac	0,pr;1,pr;6,ac;	H3	23.85
5908	528.3000	528.2958	2	7.91	KSTGGKAPR	K14ac	0,pr;1,pr;6,ac;	H3	23.85
5919	528.3000	528.2958	2	7.91	KSTGGKAPR	K14ac	0,pr;1,pr;6,ac;	H3	23.85
5946	528.2963	528.2958	2	0.84	KSTGGKAPR	K14ac	0,pr;1,pr;6,ac;	H3	23.85
5985	528.3000	528.2958	2	7.91	KSTGGKAPR	K14ac	0,pr;1,pr;6,ac;	H3	23.85
6007	528.3000	528.2958	2	7.91	KSTGGKAPR	K14ac	0,pr;1,pr;6,ac;	H3	23.85

Table S4. Standard deviation of single PTMs

	1,FT1_50Da_01	2,FT1_50Da_02	3,FT1_50Da_03	AVG	STD	STD_incorrect(based on sum of peptides)
H3K4me1	0.336878	0.340956	0.34655	0.341461	0.004856	0.004856
H3K4me2	0.002927	0.002988	0.002791	0.002902	0.000101	0.000101
H3K4me3	0.000707	0.00073	0.000814	0.00075	0.000057	0.000057
H3K4ac	0.000092	0	0.000069	0.000054	0.000048	0.000048
H3K9me1	0.473197	0.474173	0.494601	0.480657	0.009769	0.012086
H3K9me2	0.168782	0.170589	0.163084	0.167485	0.004204	0.003917
H3K9me3	0.050974	0.049105	0.04895	0.049676	0.001065	0.001127
H3K9ac	0.004358	0.004212	0.004172	0.004247	0.000216	0.000098
H3S10ph	0.014837	0.015368	0.01311	0.014438	0.001872	0.001181
H3K14ac	0.203137	0.198477	0.20644	0.202685	0.00547	0.004001
H3K18me1	0.002173	0.002085	0.002169	0.002143	0.000059	0.00005
H3K18ac	0.081389	0.079647	0.077422	0.079486	0.001506	0.001988
H3K23me1	0.002138	0.002169	0.002385	0.002231	0.000106	0.000135
H3K23ac	0.279923	0.283525	0.28143	0.281626	0.002676	0.001809
H31K27me1	0.199899	0.199853	0.198051	0.199267	0.004219	0.001054
H31K27me2	0.454987	0.465089	0.46276	0.460945	0.00825	0.00529
H31K27me3	0.170958	0.169611	0.168276	0.169615	0.002645	0.001341
H31K27ac	0.002393	0.002372	0.002601	0.002455	0.000126	0.000126
H31K36me1	0.252601	0.25521	0.248377	0.252063	0.002745	0.003448
H31K36me2	0.396472	0.403939	0.40673	0.40238	0.008732	0.005304
H31K36me3	0.078867	0.07226	0.072608	0.074578	0.003554	0.003718
H33K27me1	0.286736	0.284679	0.286354	0.285923	0.002916	0.001094
H33K27me2	0.411315	0.406958	0.40005	0.406108	0.008503	0.005681
H33K27me3	0.152693	0.142006	0.147056	0.147252	0.003851	0.005346
H33K27ac	0.003212	0.0031	0.003145	0.003152	0.000056	0.000056
H33K36me1	0.246827	0.260938	0.24181	0.249858	0.014076	0.009918
H33K36me2	0.50646	0.503135	0.512405	0.507333	0.005431	0.004696
H33K36me3	0.036621	0.036163	0.041582	0.038122	0.0032	0.003005
H3K56me1	0.000106	0	0.00009	0.000066	0.000057	0.000057
H3K56me2	0.010825	0.000017	0.000244	0.003695	0.006175	0.006175
H3K56me3	0	0	0	0	0	0
H3K56ac	0.000463	0.000356	0.000723	0.000514	0.000189	0.000189
H3K79me1	0.098367	0.074992	0.102392	0.091917	0.014795	0.014795
H3K79me2	0.132966	0.084822	0.145403	0.121063	0.031996	0.031996
H3K79me3	0.01976	0.00146	0.019763	0.013661	0.010566	0.010566
H3K79ac	0.073326	0.083061	0.041431	0.06594	0.021776	0.021776
H3K122ac	0.004881	0.006323	0.003426	0.004877	0.001448	0.001448
H4K5ac	0.069305	0.069445	0.069669	0.069473	0.001013	0.000183
H4K8ac	0.132295	0.134197	0.136856	0.134449	0.001605	0.002291
H4K12ac	0.140443	0.139095	0.142988	0.140842	0.002232	0.001977
H4K16ac	0.192049	0.181255	0.18733	0.186878	0.00619	0.005411
H4K20me1	0.212375	0.21023	0.197496	0.206701	0.008043	0.008043
H4K20me2	0.540114	0.515671	0.541733	0.532506	0.014602	0.014602
H4K20me3	0.023399	0.026605	0.02812	0.026042	0.002411	0.002411
H4K20ac	0.000271	0.000253	0.000325	0.000283	0.000037	0.000037

Table S5. Histone PTM annotations

Real site	Enzyme	Function
H3 R2me1,me2a	CARM1	Gene expression
H3 R2me1,me2s	PRMT5	Gene expression
H3 R2me1,me2a	PRMT6	Gene expression
H3 R2me1,me2s	PRMT7	Gene expression
H3 T3ph	Haspin	Centromere mitotic spindle function
H3 K4ac	GCN5	Transcription activation at some promoters
H3 K4me3	Set1	rDNA/telomeric silencing (Sc)
H3 K4me1-3	Set-2	Germ cell maintenance
H3 K4me2-3	Set1	Transcriptional activation (All)
H3 K4me1-3	SETD1A	Transcriptional activation (All)
H3 K4me1-3	SETD1B	Transcriptional activation (All)
H3 K4me1-3	Trx	Trithorax activation
H3 K4me1-3	MLL	Gene activation
H3 K4me1-3	MLL2	Transcriptional activation (All)
H3 K4me1-3	Trr	Enhancer function
H3 K4me1-3	MLL3	Transcriptional activation (All)
H3 K4me1-3	MLL4	Transcriptional activation (All)
H3 K4me1-3	Ash-2	Germ cell specification
H3 K4me3	Ash1	Trithorax activation
H3 K4me1-3	ASH1L	Gene activation
H3 K4me1	SETD7	Transcriptional activation
H3 K4me2-3	SMYD3	Transcriptional activation
H3 K4me3	Meisetz	Meiotic prophase progression
H3 T6ph	PKCbeta	Inhibits AR-dependent transcription
H3 R8me1,me2s	PRMT5	Transcriptional repression
H3 K9ac	SAGA	Transcriptional activation
H3 K9ac	GCN5	Transcriptional activation
H3 K9ac	SRC1	Nuclear receptor coactivator
H3 K9me1,me2	Clr4	Centromeric and mating type silencing
H3 K9me3	Dim5	DNA methylation
H3 K9me3	Met-2	Germ cells
H3 K9me3	Mes-2	Germ cells
H3 K9me2-3	Su(var)3-9	Dominant PEV modifier
H3 K9me2	KRYPTONITE	DNA methylation
H3 K9me2-3	Suv39h1	Pericentric heterochromatin
H3 K9me2-3	Suv39h2	Pericentric heterochromatin
H3 K9me3	SUV39H1	Rb-mediated silencing
H3 K9me2-3	ESET	Transcriptional repression
H3 K9me2-3	SETDB1	Transcriptional repression
H3 K9me1-2	G9a	Transcriptional repression, Imprinting
H3 K9me1-2	EHMT1/GLP	Transcriptional repression
H3 K9me2	PRDM2/RIZ1	Tumor suppression and response to female sex hormones
H3 S10ph	Snf1	Transcriptional activation
H3 S10ph	Jil-1	Transcriptional upregulation of male X-chromosome
H3 S10ph	Rsk2	Transcriptional activation of immediate early genes (in concert with H3K14ac)
H3 S10ph	Msk1	Transcriptional activation of immediate early genes (in concert with H3K14ac)
H3 S10ph	Msk2	Transcriptional activation of immediate early genes (in concert with H3K14ac)
H3 S10ph	IKKα	Transcriptional upregulation
H3 S10ph	Ip11/AuroraB	Mitotic chromosome condensation
H3 S10ph	NIMA	Mitotic chromosome condensation
H3 S10ph	Fyn	UVB induced MAP kinase pathway

H3 T11ph	Dlk	Mitosis specific phosphorylation
H3 K14ac	Gcn5	Transcriptional activation
H3 K14ac	TAFII230	Transcriptional activation
H3 K14ac	TAFII250	Transcriptional activation
H3 K14ac	p300	Transcriptional activation
H3 K14ac	PCAF	Transcriptional activation
H3 K14ac	SRC1	Nuclear receptor coactivator
H3 R17me1,me2a	CARM1	Transcriptional activation (in concert with H3-K18/23 acetylation)
H3 K18ac	SAGA	Transcriptional activation
H3 K18ac	Ada	Transcriptional activation
H3 K18ac	GCN5	Transcriptional activation
H3 K18ac	p300	Transcriptional activation
H3 K18ac	CBP	Transcriptional activation (in concert with H3-R17 methylation)
H3 K23ac	SAGA	Transcriptional activation
H3 K23ac	CBP	Transcriptional activation (in concert with H3-R17 methylation)
H3 R26me1,me2a	CARM1	In vitro methylation site
H3 K27ac	CBP	Enhancer function, gene expression
H3 K27me3	E(z)/EZH2	Polycomb repression, Early B-cell development, X chromosome inactivation
H3 S28ph	Aurora-B	Mitotic chromosome condensation
H3 S28ph	MSK1	UVB induced phosphorylation
H3 K36me2	Set2	Gene repression
H3 K36me2	set2	Transcription activation
H3 K36me2	Set2	Transcription elongation
H3 K36me2	MES-4	Dosage compensation in germline
H3 K36me3	MET-1	Meiosis
H3 K36me3	MES4	Transcription elongation
H3 K36me3	SET2	Transcription elongation
H3 K36me1-3	SETD2	Transcription activation
H3 K36me1-2	NSD1-3	Transcription activation
H3 K36ac	GCN5	Promoter mark on active genes
H3 P38iso	Fpr4	Gene expression
H3 Y41ph	JAK2	Gene expression
H3 R43me	CARM1	Transcriptional activation
H3 T45ph	Cdc7	DNA replication; apoptosis
H3 K56ac	SPT10	Transcriptional activation; DNA damage
H3 K56me1	G9a	DNA replication
H3 K56me3	Suv39h	Heterochromatin
H3 K64ac	p300	Nucleosome dynamics and transcription
H3 K64me3		Pericentric heterochromatin
H3 K79me1-3	Dot1/DOT1L	Telomeric silencing, pachytene checkpoint, DNA damage response
H3 T80ph		Mitosis
H3.3 K4me1-3		Transcriptional activation
H3.3 K9me1-2		Transcriptional repression
H3.3 K9ac		Transcriptional activation
H3.3 K14ac		Transcriptional activation
H3.3 K18ac		Transcriptional activation
H3.3 K23ac		Transcriptional activation
H3.3 K27me1-3		Transcriptional repression
H3.3 S31ph		Mitosis specific phosphorylation
H3.3 K36me1-3		Transcriptional activation
H3.3 K79me1-2		Transcriptional activation

The contents of figures:

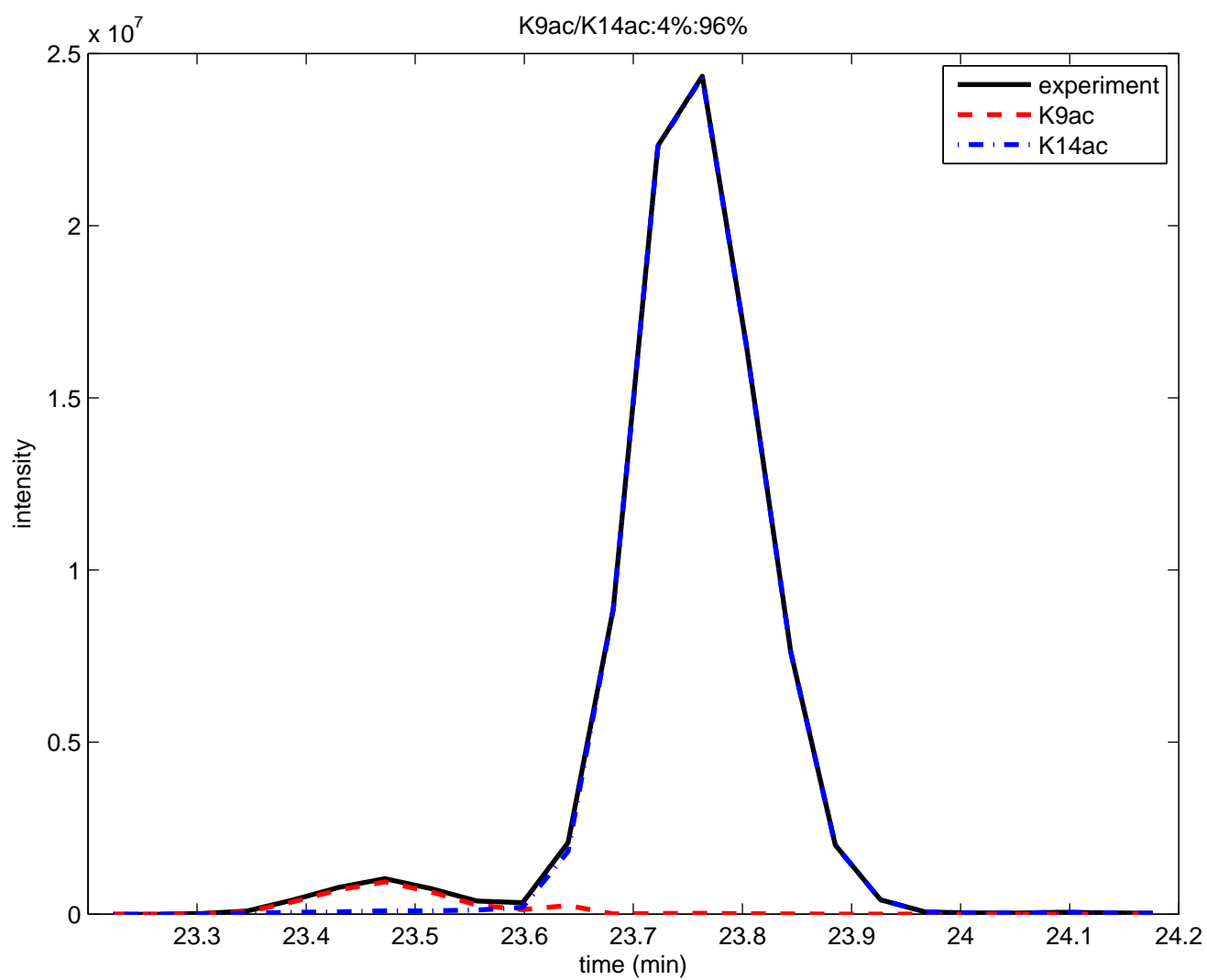|	
Figure 1	**Layouts on H3 9-17 KSTGGKAPR in the endogenous samples.**
Figure 2	**Discrimination of H3 K9ac and K14ac by unique fragment ions.**
Figure S1	**Layouts of histone peptides.** **(A) H3 3−8 TKQTAR.** **(B) H3 9−17 KSTGGKAPR S10ph.** **(C) H3 18−26 KQLATKAAR.** **(D) Layout of H3.1/2 27−40 KSAPATGGVKKPHR.** **(E) Layout of H3.3 27−40 KSAPSTGGVKKPHR.** **(F) Layout of H4 4−17 GKGGKGLGKGGAKR.** **(G) Layout of H4 20−23 KVLR.** **(H) Layout of H2A.V 1−19 AGGKAGKDSGKAKAKAVSR.** **(I) Layout of H2A.Z 1−19 AGGKAGKDSGKAKTKAVSR.** **(J) Layout of H2B 1−29 variants.**
Figure S2	**Isobaric histone peptides.** **(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.**
Figure S3	**MS/MS spectra for H3 K9ac and K14ac.**
Figure S4	**Bar plot represents the number of histone peptides quantified by EpiProfile.**
Figure S5	**Box plot displays the Log10 transformed total peptide intensity.**
Figure S6	**Principal component analysis (PCA) of all performed runs.**
Figure S7	**Heatmap represents the relative abundance of single histone PTMs.**

Figure 1. Layouts on H3 9−17 KSTGGKAPR in the endogenous samples. Two layouts are: K9me3 < K9me2 < K9ac < unmodified < K9me1, and K9me3K14ac < K9me2K14ac < K9acK14ac < K14ac < K9me1K14ac, where the later layout is earlier than the former layout. 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). PTM 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.
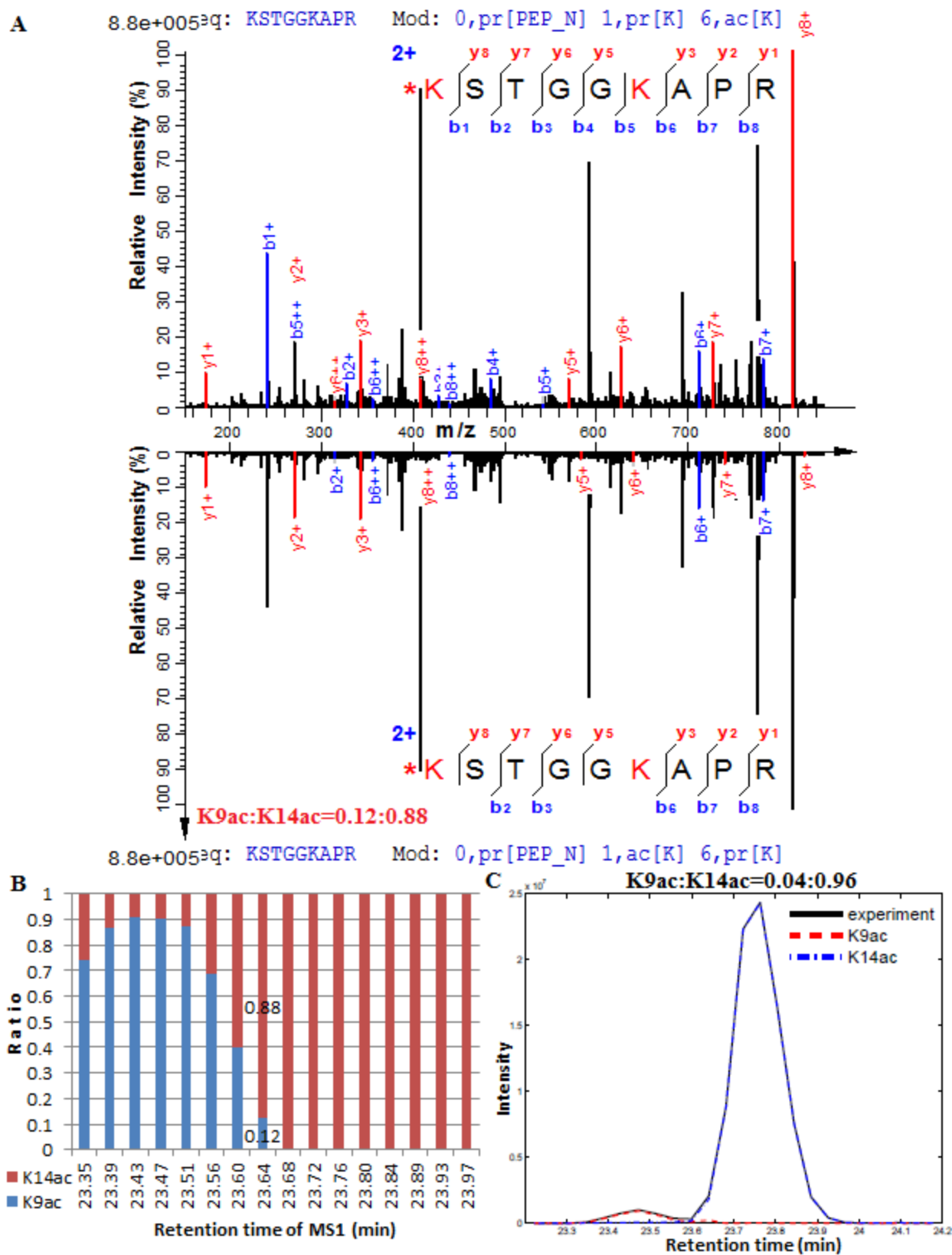
Figure 2. Discrimination of H3 K9ac and K14ac by unique fragment ions.
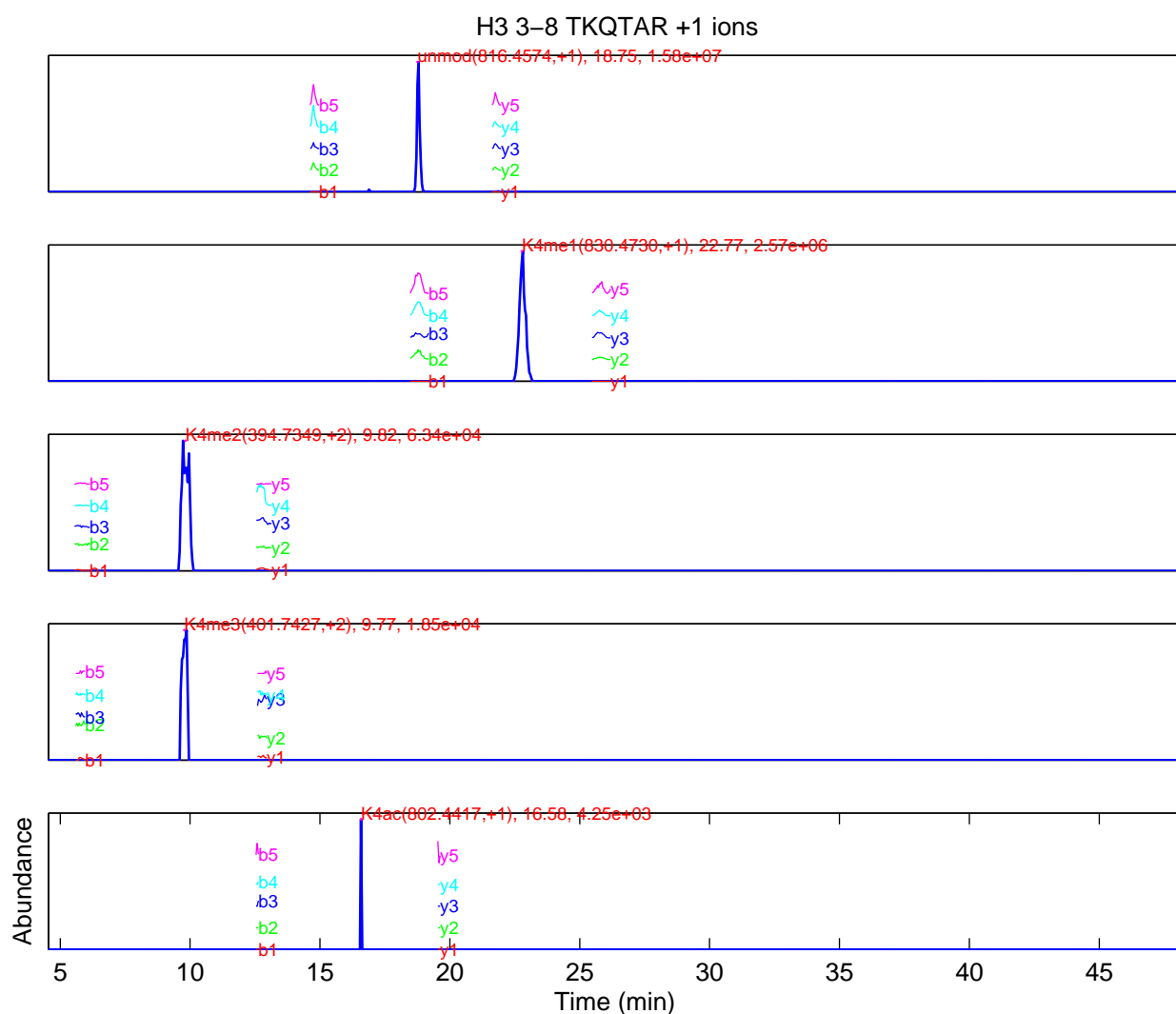
Figure S1A. Layout of H3 3−8 TKQTAR. The layout is K4me3 < K4me2 < K4ac < unmodified < K4me1. The subplots show the extracted ion chromatography (XIC) for each peptide based on the precursor m/z (i.e. x-axis is the retention time, y-axis is the intensity). The type and retention time of each peptide 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.
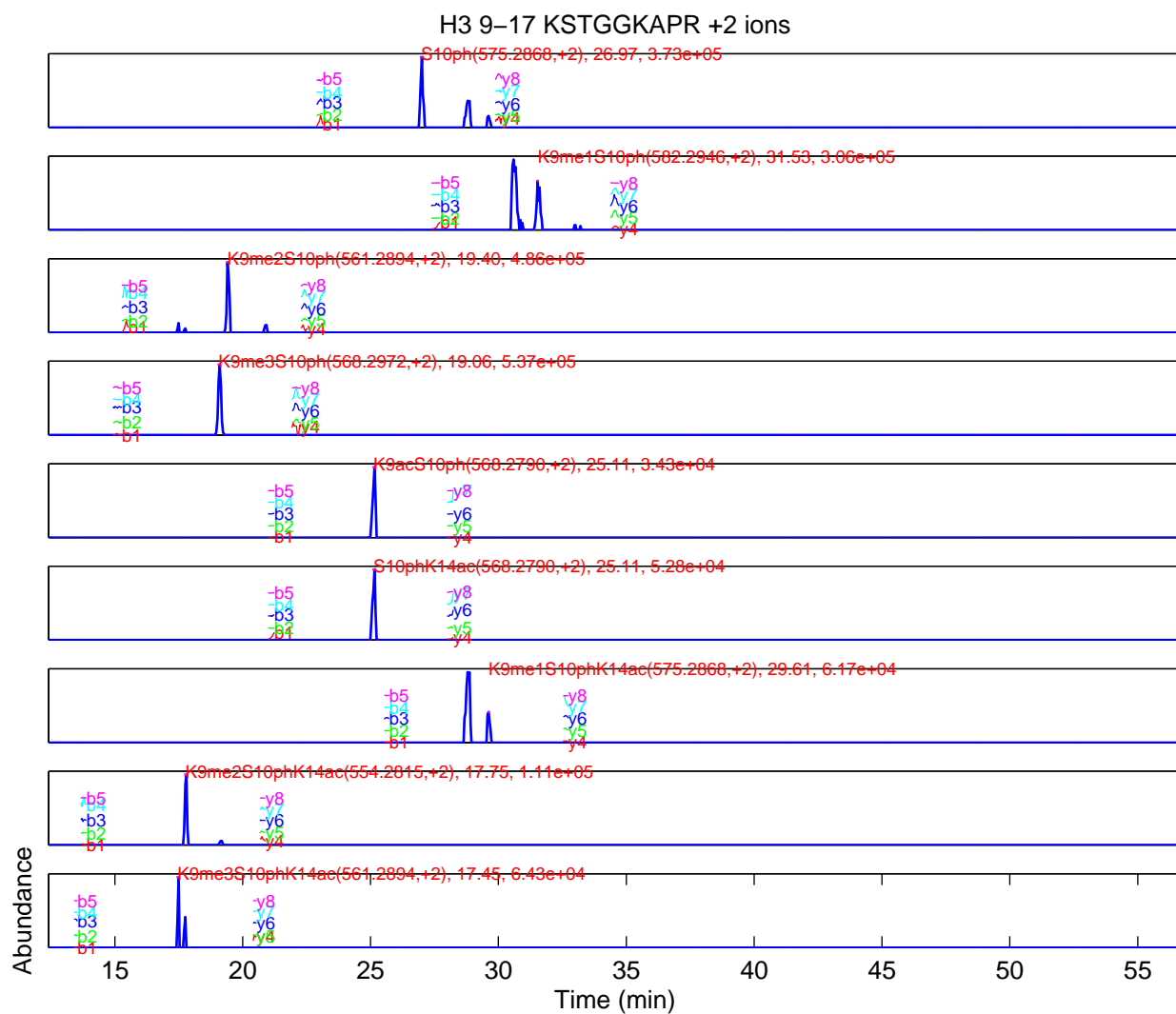
Figure S1B. Layout of H3 9−17 KSTGGKAPR S10ph.
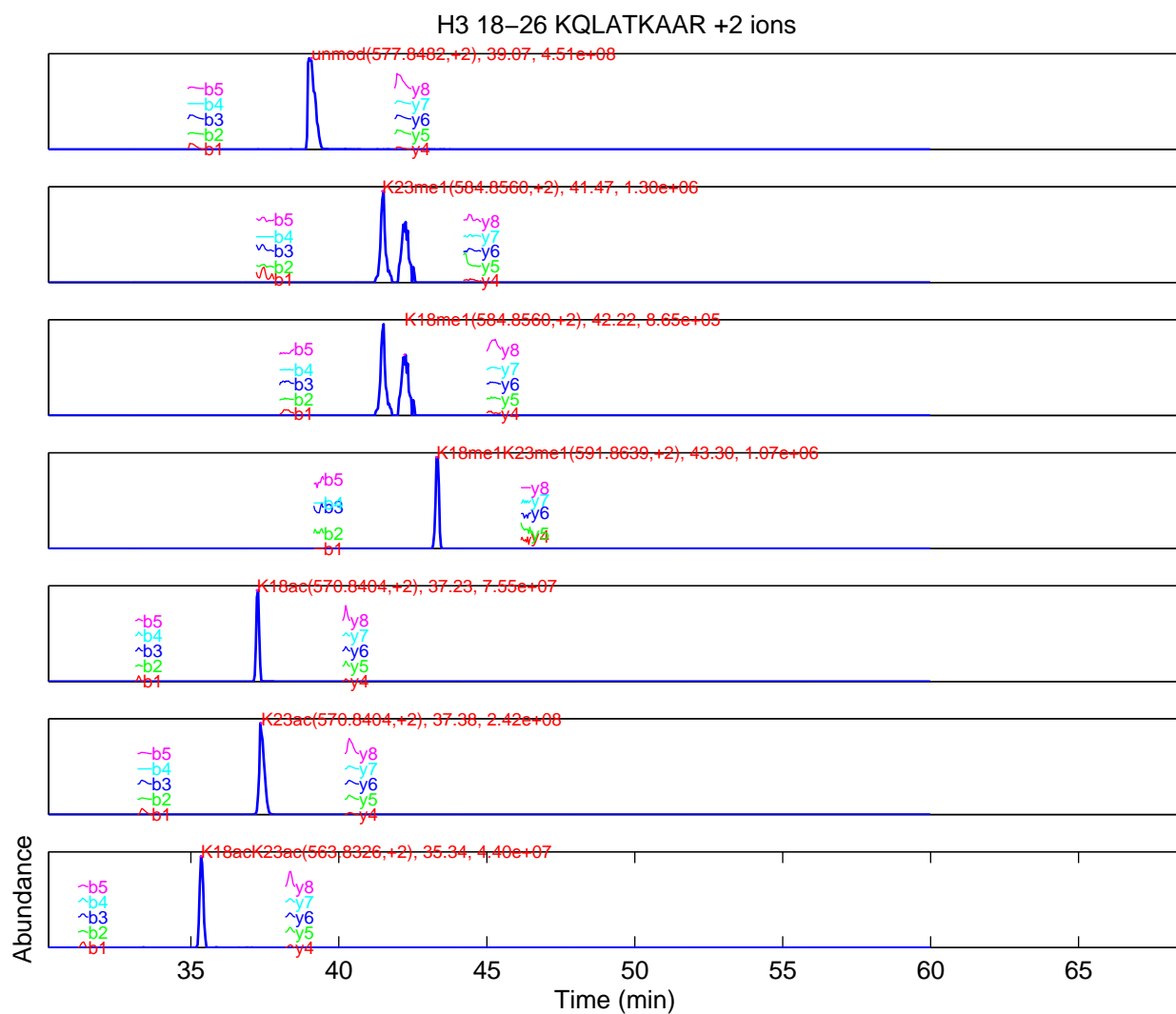
Figure S1C. Layout of H3 18−26 KQLATKAAR.
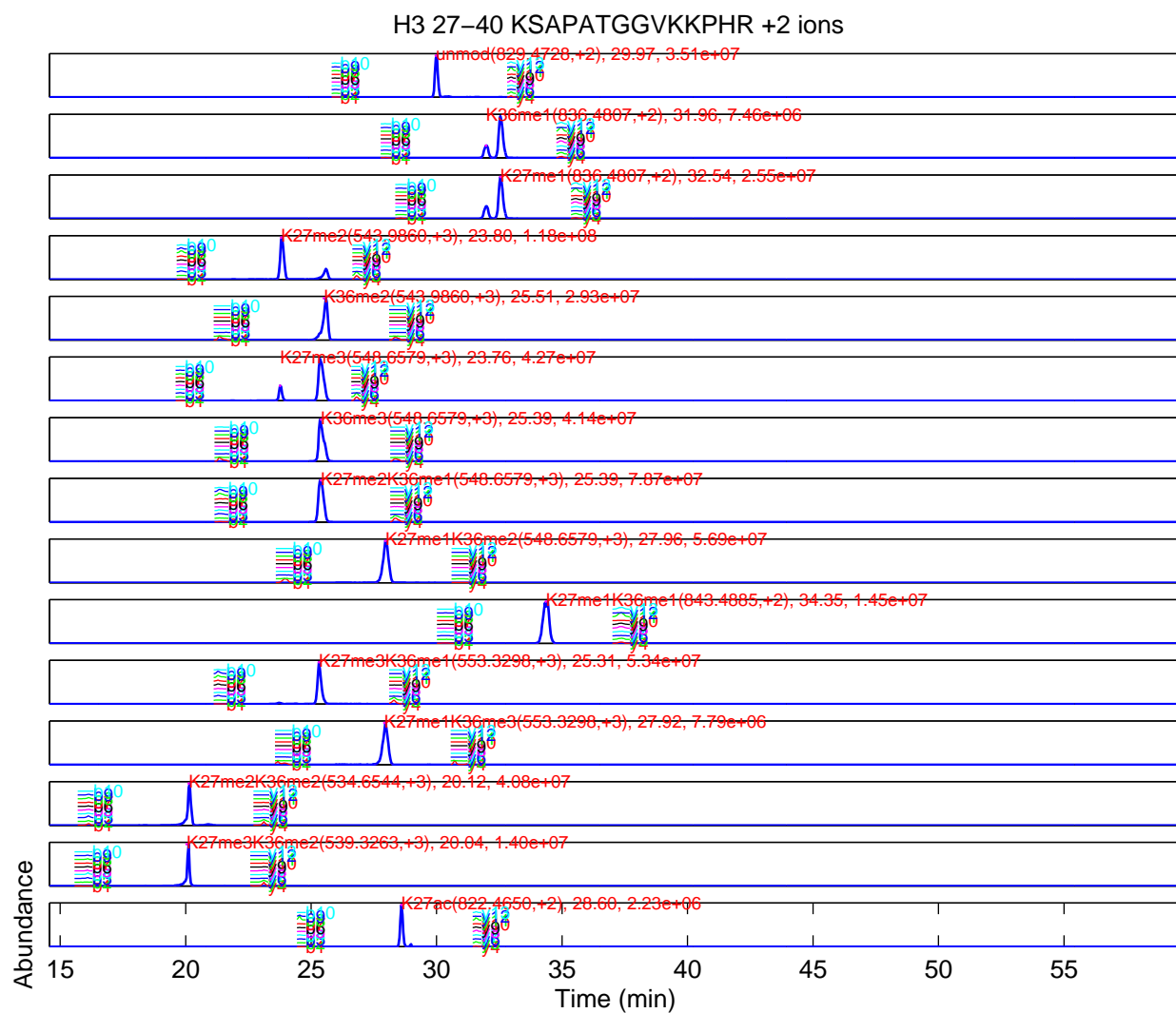
Figure S1D. Layout of H3.1/2 27−40 KSAPATGGVKKPHR.
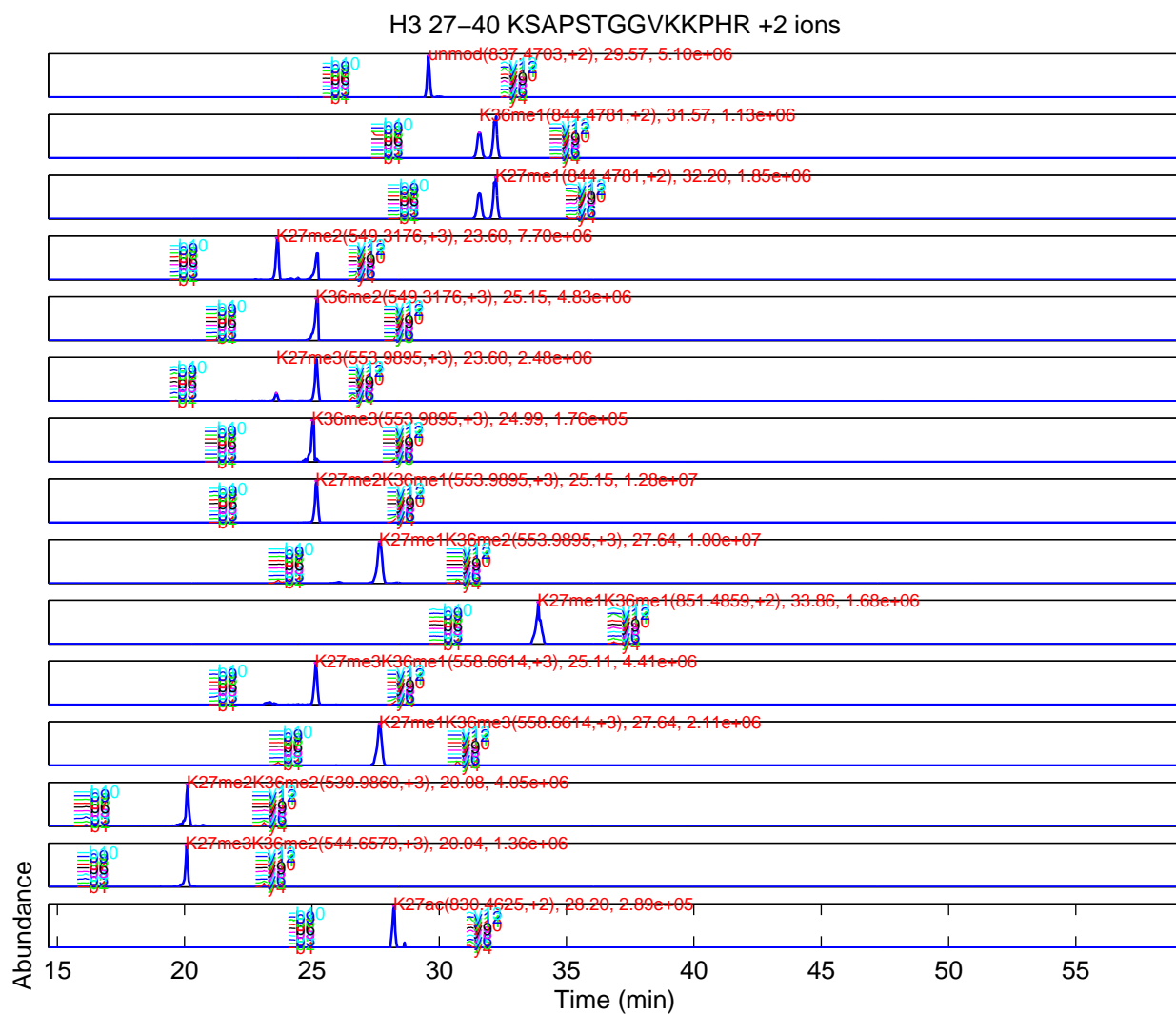
Figure S1E. Layout of H3.3 27−40 KSAPSTGGVKKPHR.

H4 4−17 GKGGKGLGKGGAKR +2 ions
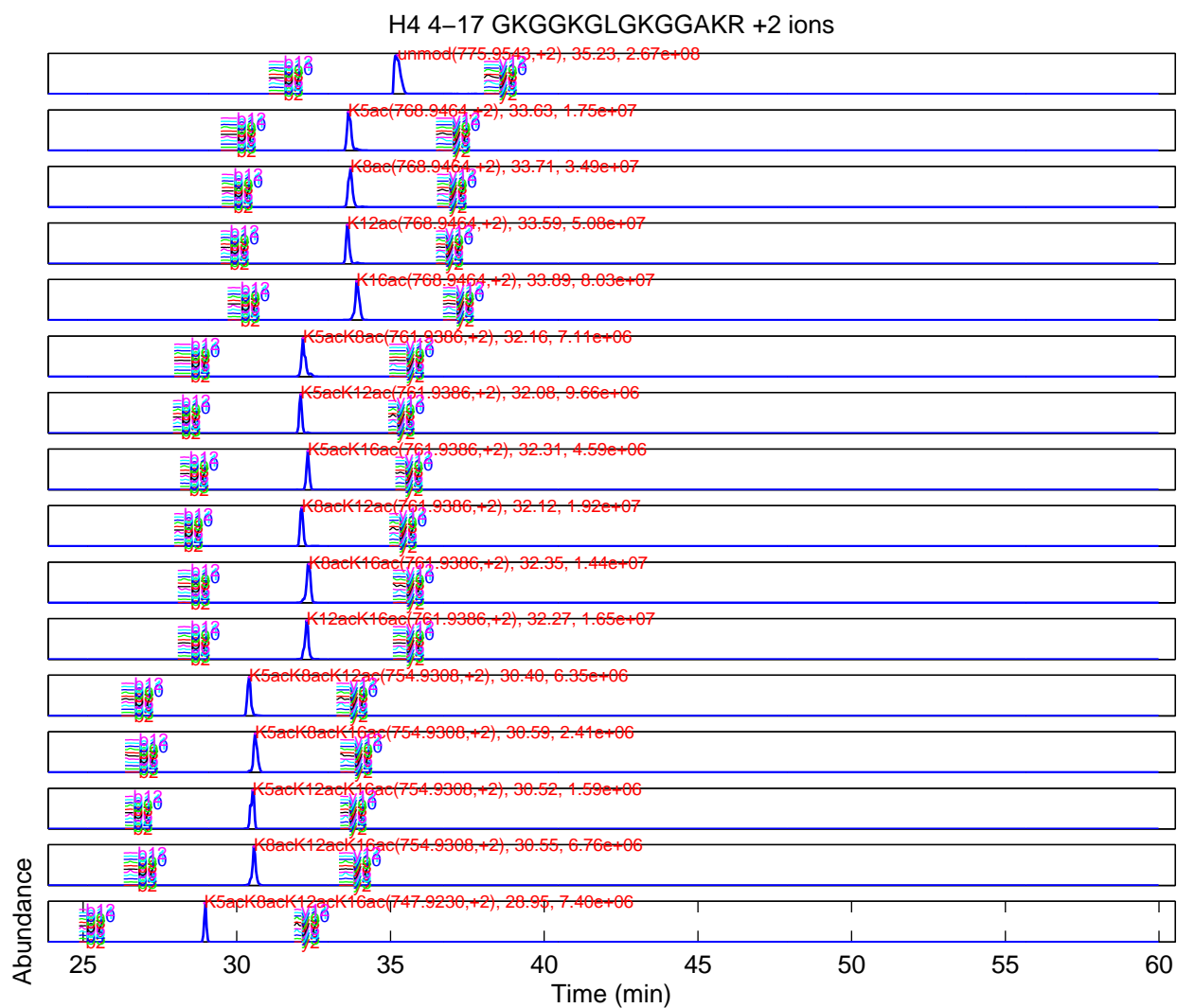
Figure S1F. Layout of H4 4−17 GKGGKGLGKGGAKR.
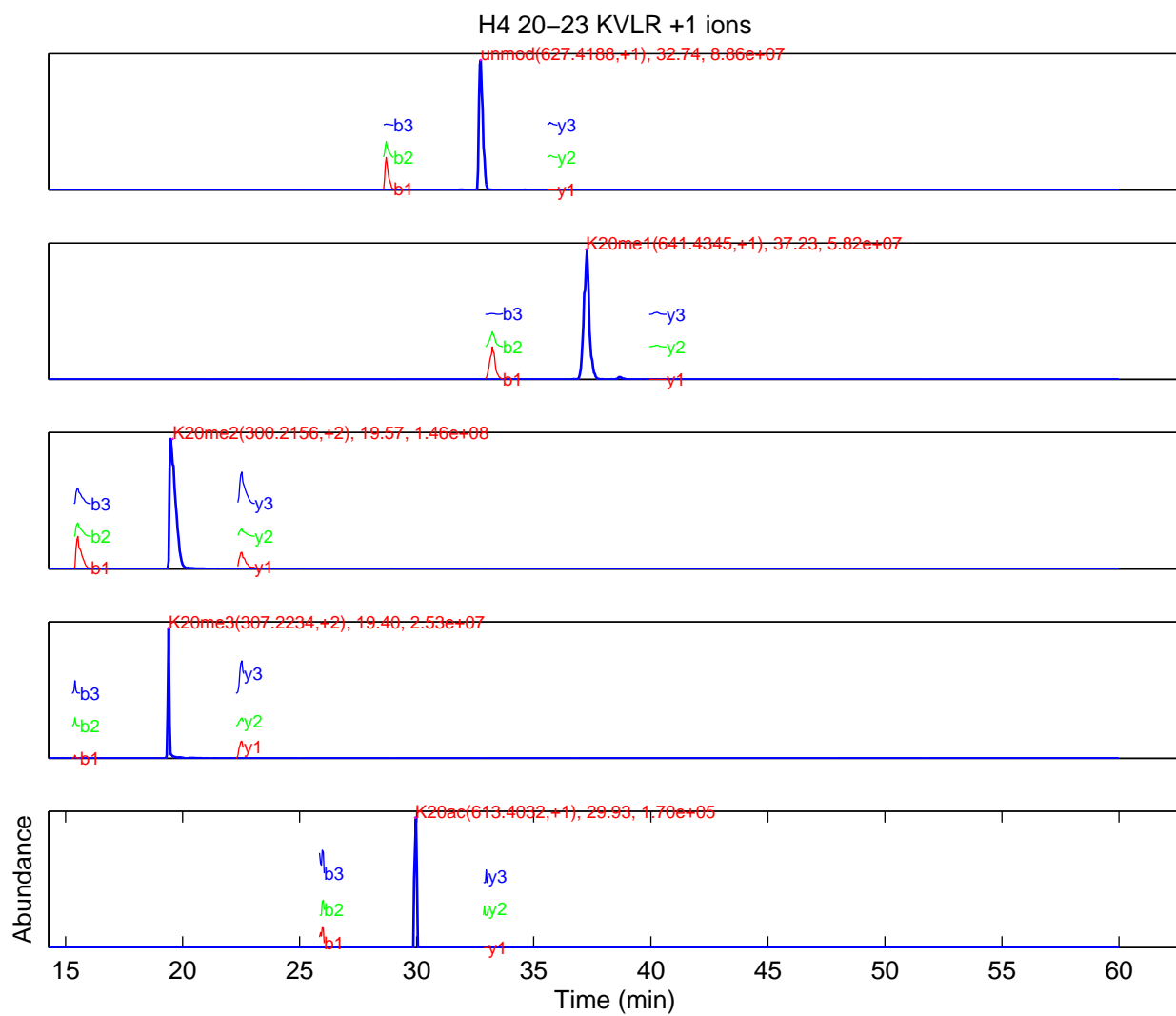
Figure S1G. Layout of H4 20−23 KVLR.
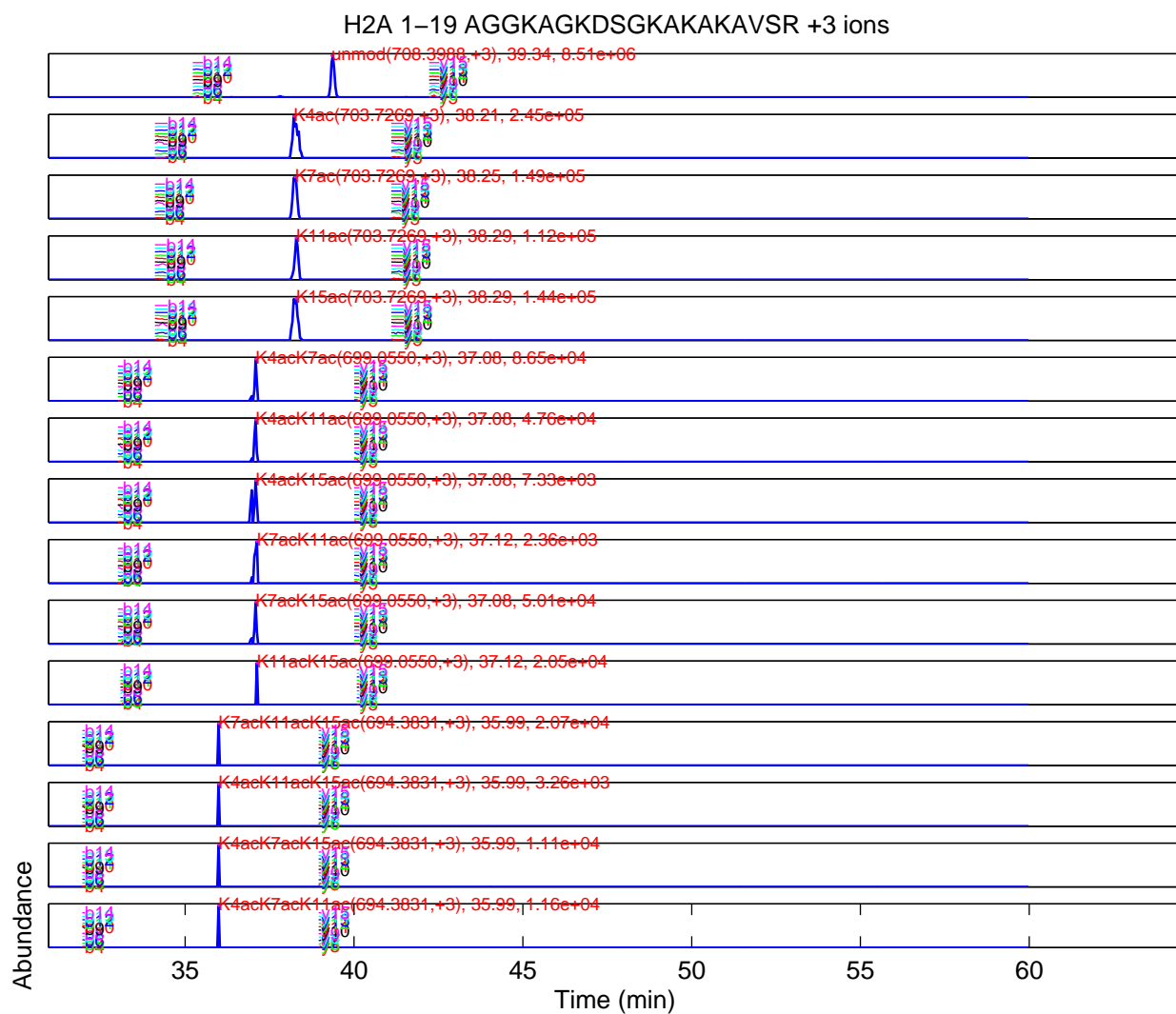
Figure S1H. Layout of H2A.V 1−19 AGGKAGKDSGKAKAKAVSR.

H2A 1−19 AGGKAGKDSGKAKTKAVSR +3 ions

unmod(718.4022,+3), 37.31, 6.71e+07
K4ac(713.7304,+3), 36.36, 1.17e+06
K7ac(713.7304,+3), 36.33, 2.37e+06
K11ac(713.7304,+3), 36.33, 2.99e+06
K15ac(713.7304,+3), 36.33, 7.35e+05
K4acK7ac(709.0586,+3), 35.08, 4.50e+05
K4acK11ac(709.0586,+3), 35.19, 1.12e+05
K4acK15ac(709.0586,+3), 34.58, 0.00e+00
K7acK11ac(709.0586,+3), 35.00, 3.91e+05
K7acK15ac(709.0586,+3), 35.19, 1.59e+05
K11acK15ac(709.0586,+3), 34.58, 0.00e+00
K7acK11acK15ac(704.3867,+3), 33.89, 2.03e+05
K4acK11acK15ac(704.3867,+3), 33.40, 0.00e+00
K4acK7acK15ac(704.3867,+3), 33.40, 0.00e+00
K4acK7acK11ac(704.3867,+3), 33.89, 2.33e+05

Figure S1I. Layout of H2A.Z 1−19 AGGKAGKDSGKAKTKAVSR.
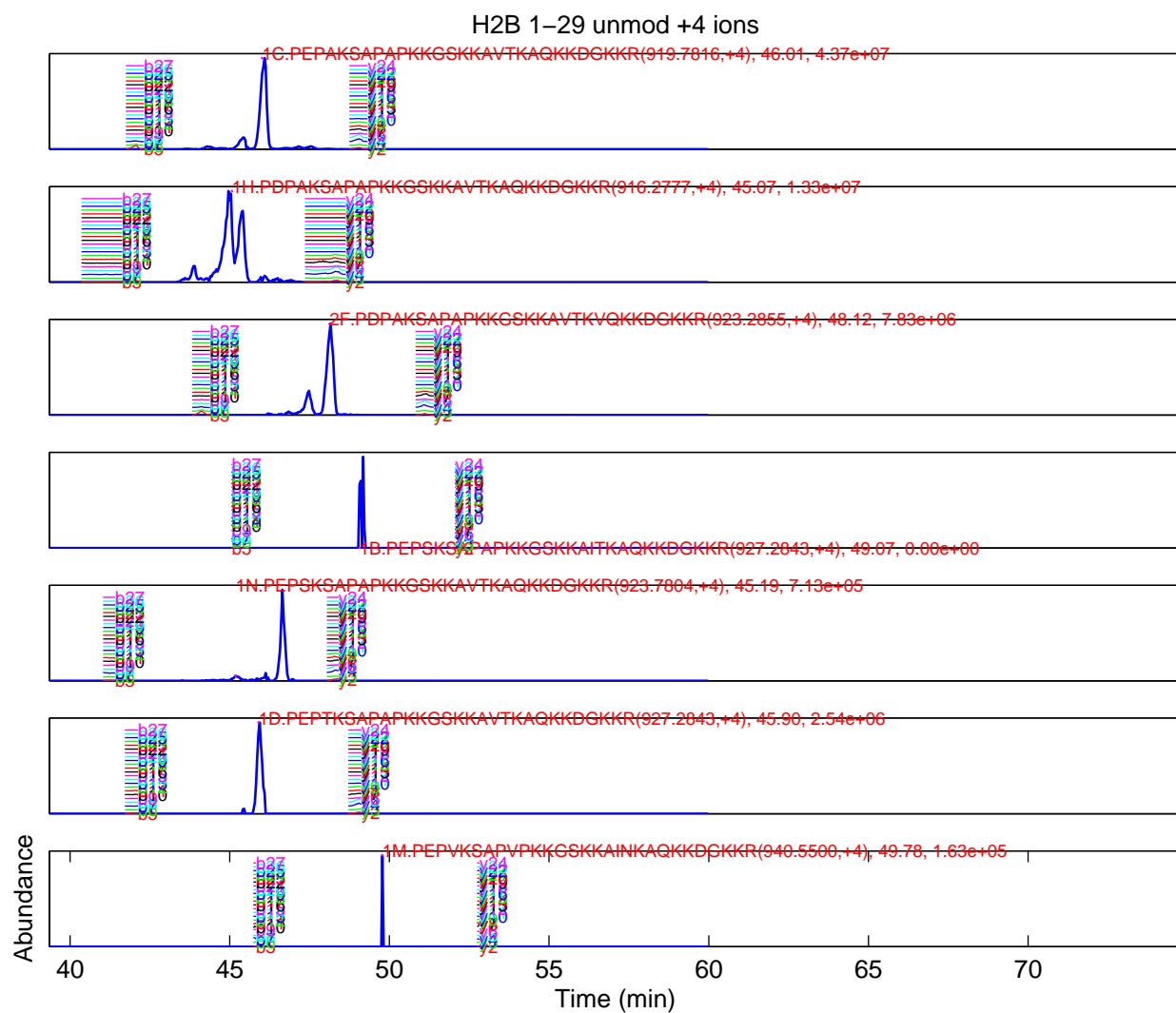
Figure S1J. Layout of H2B 1−29 variants.

Figure S2A. Isobaric peptides of H3 9−17 K9ac/K14ac.
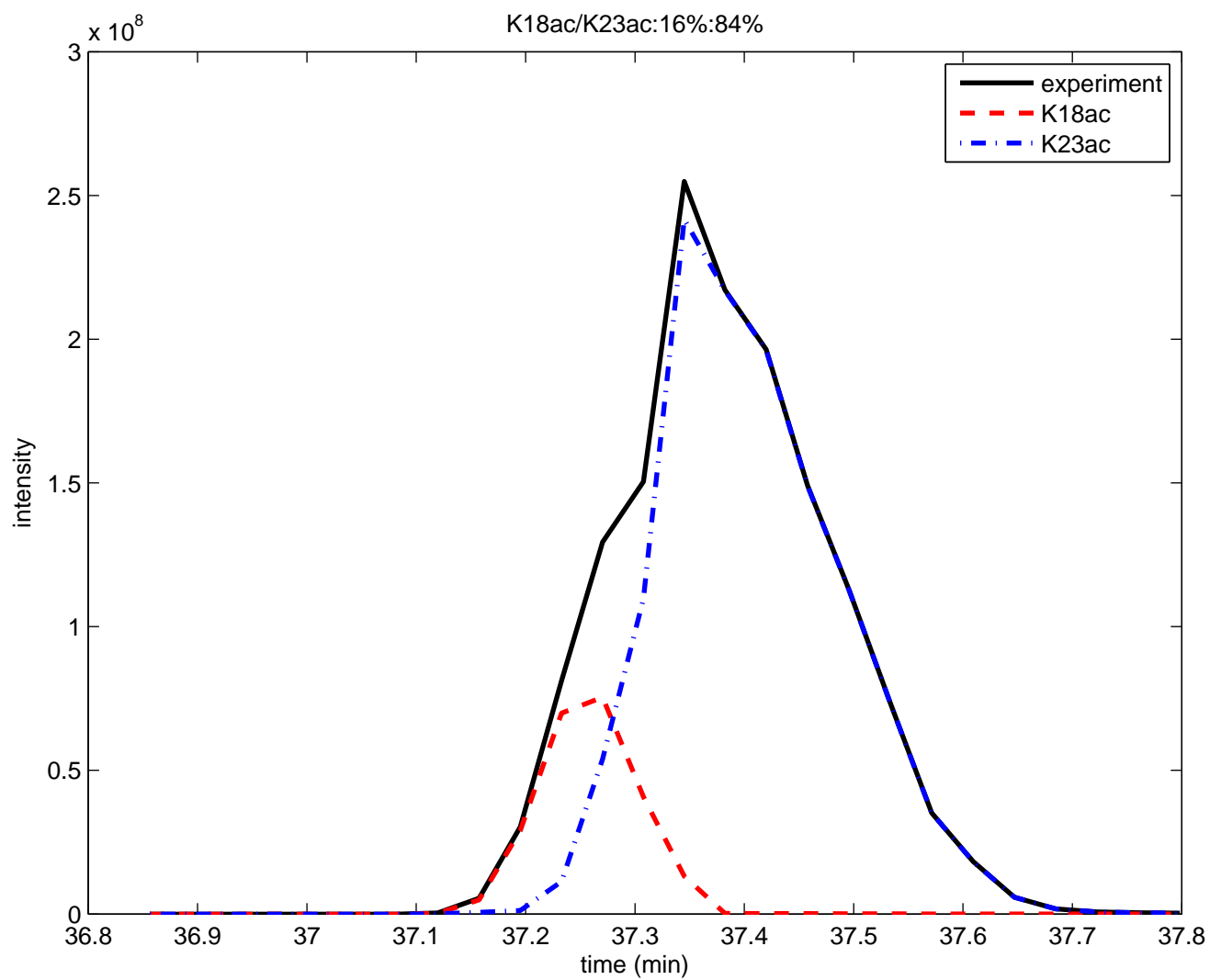
Figure S2B. Isobaric peptides of H3 18−26 K18ac/K23ac.
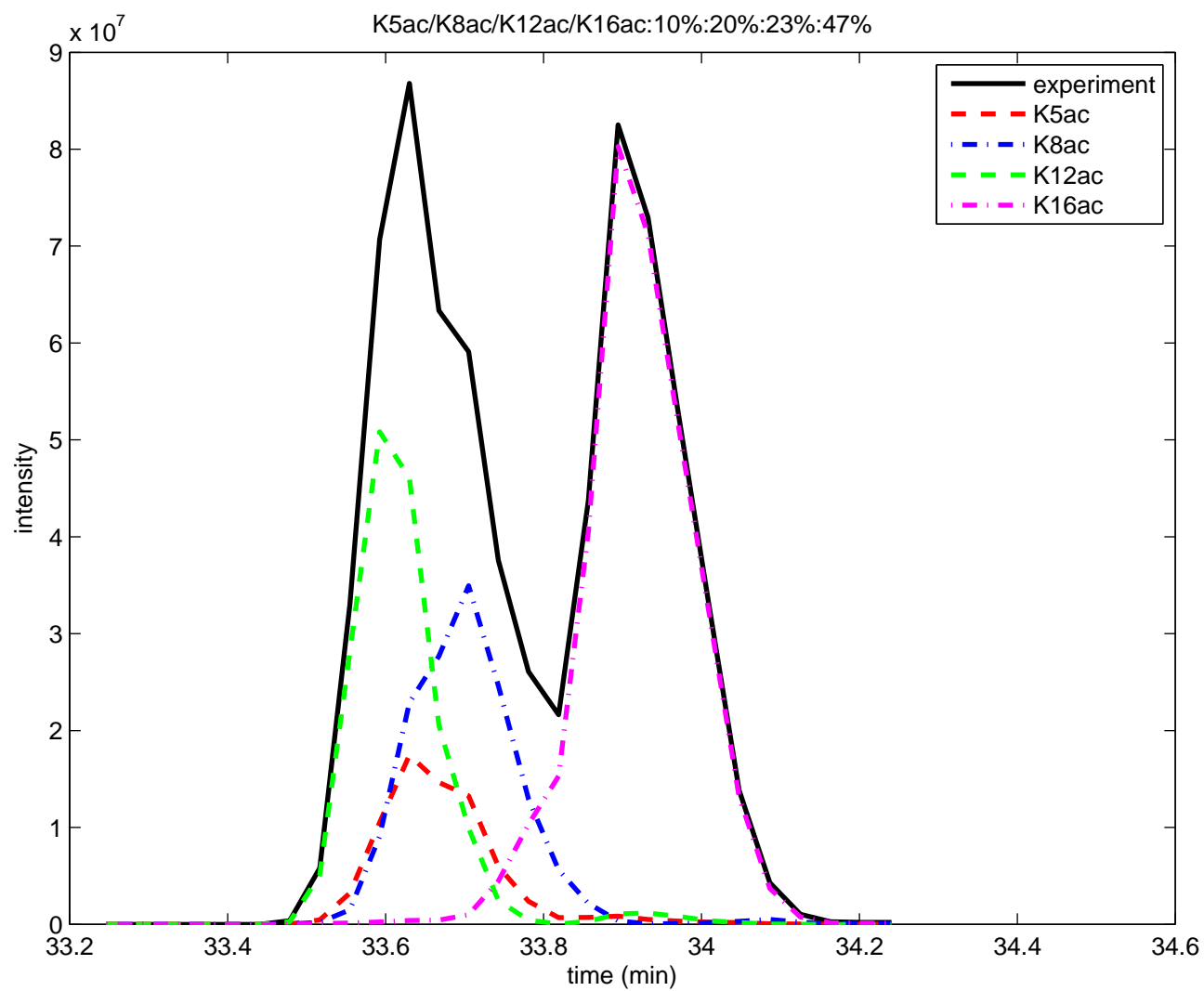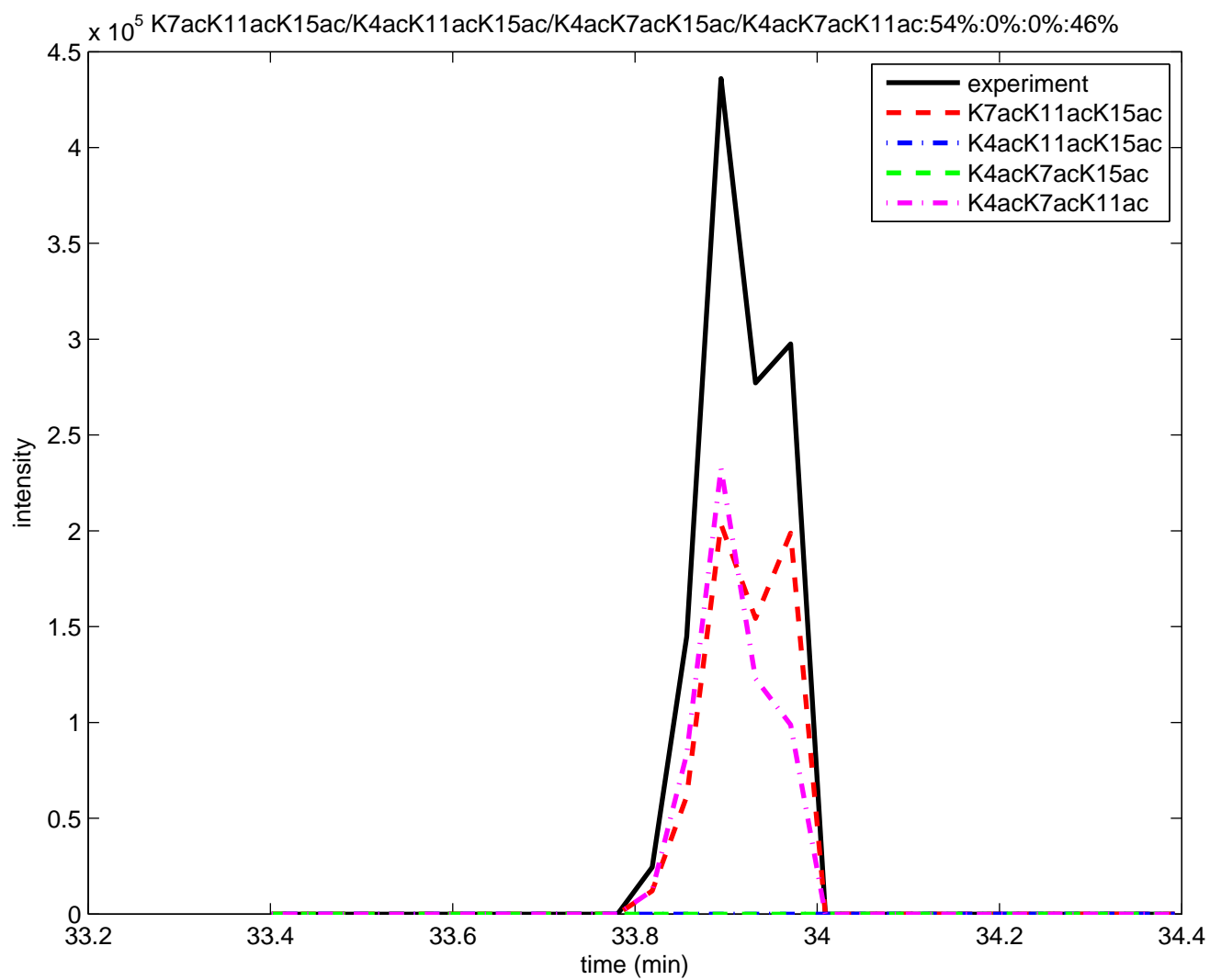
Figure S2C. Isobaric peptides of H4 4−17 1ac.
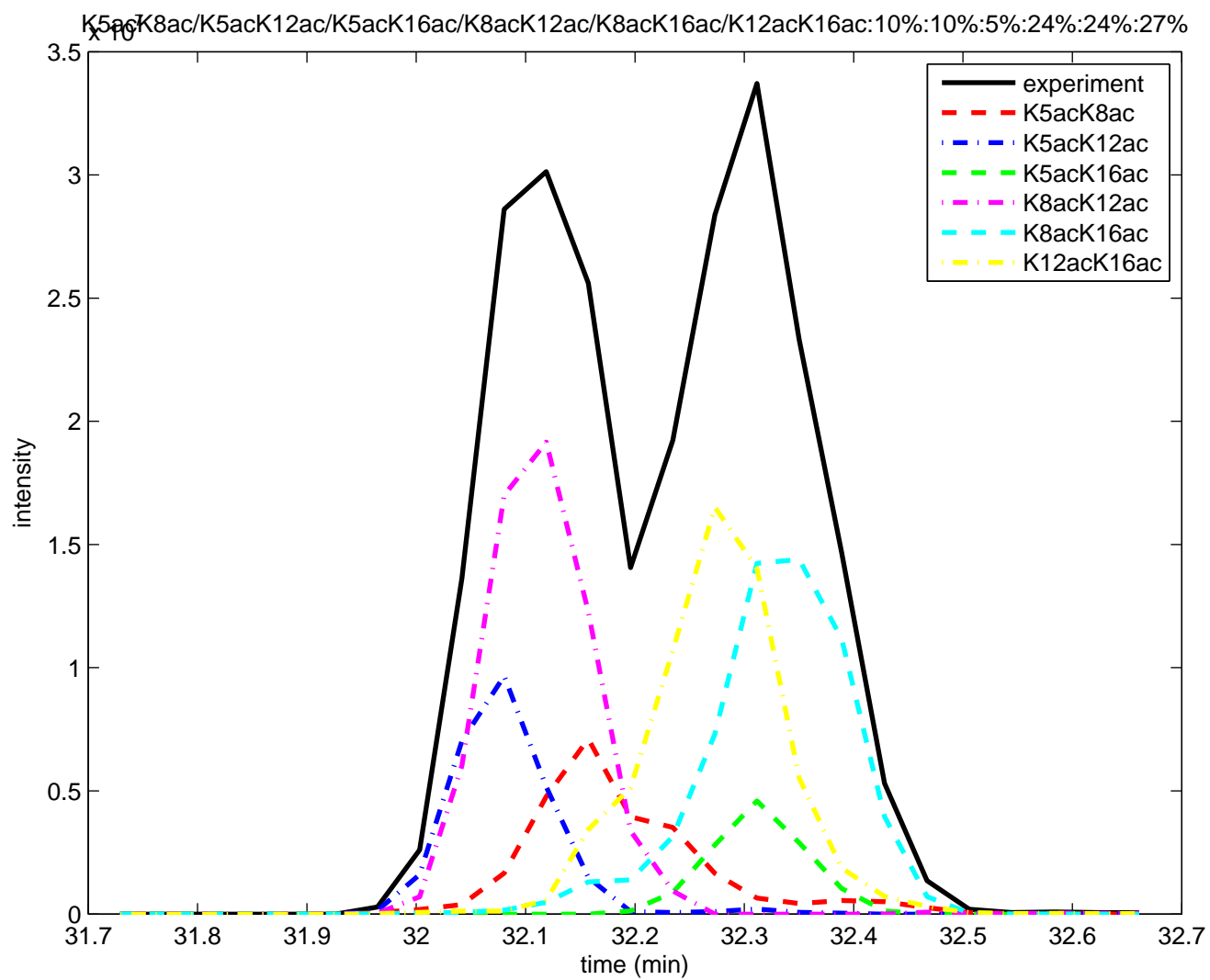
Figure S2D. Isobaric peptides of H4 4−17 2ac.
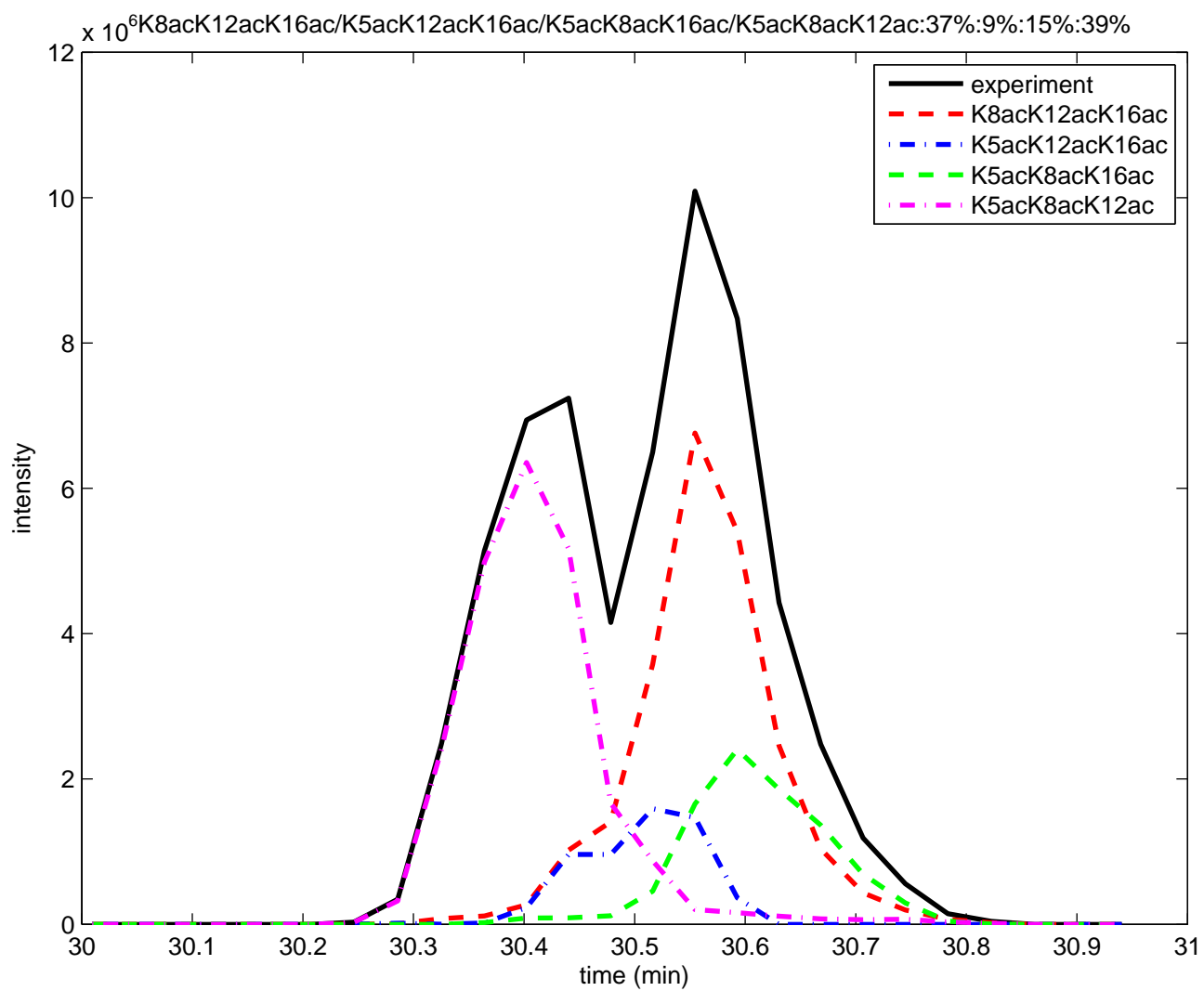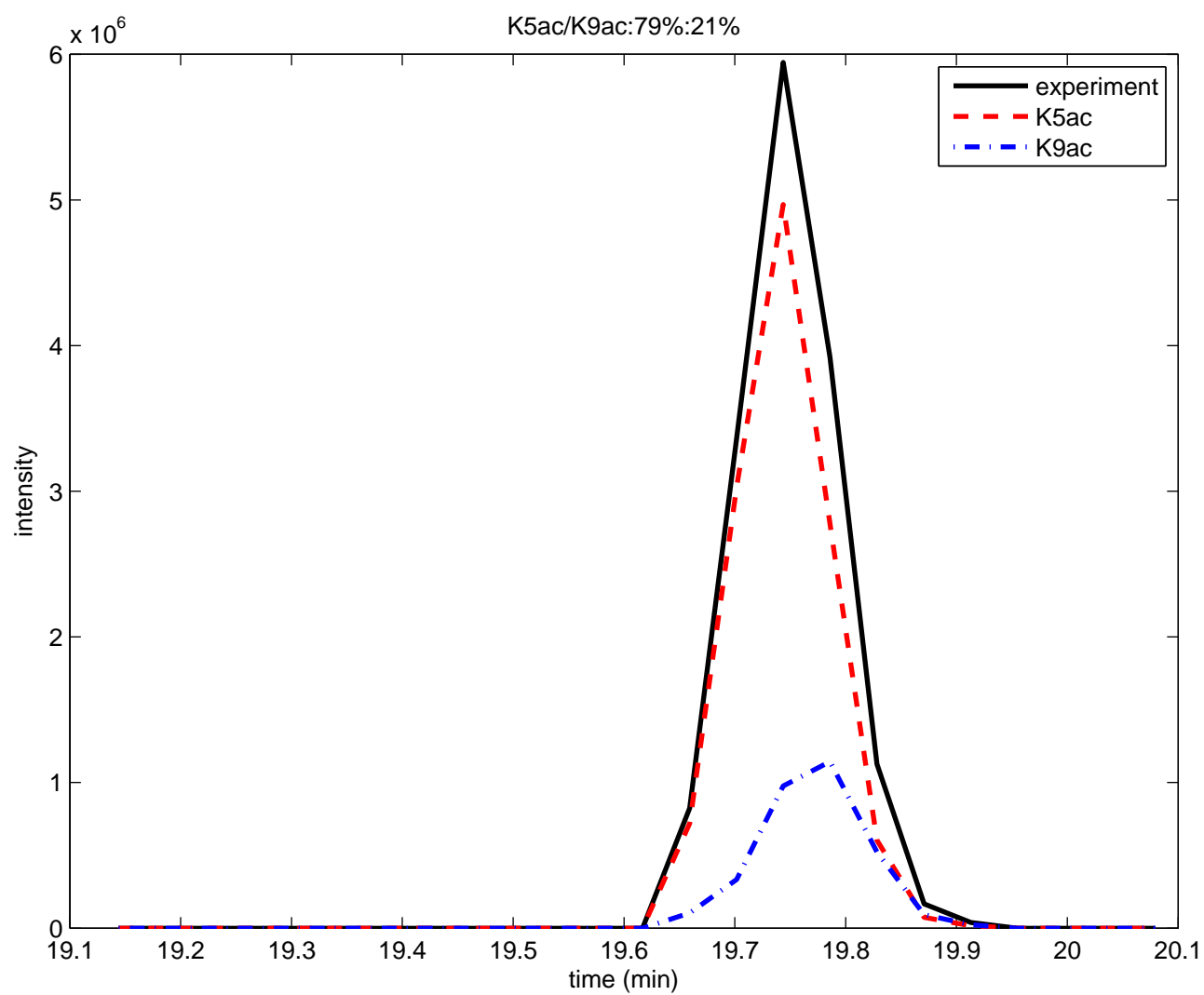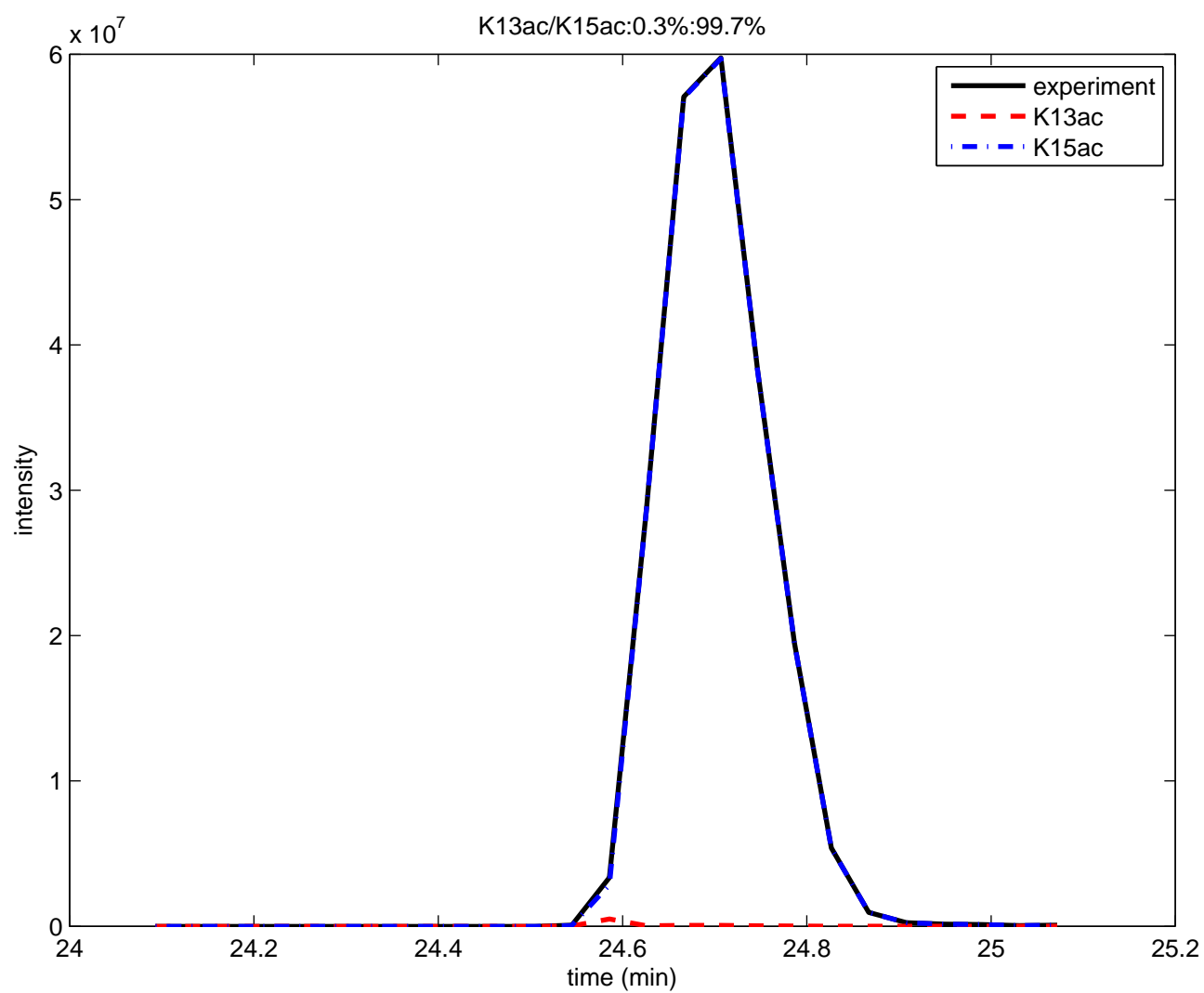
Figure S2E. Isobaric peptides of H4 4−17 3ac.
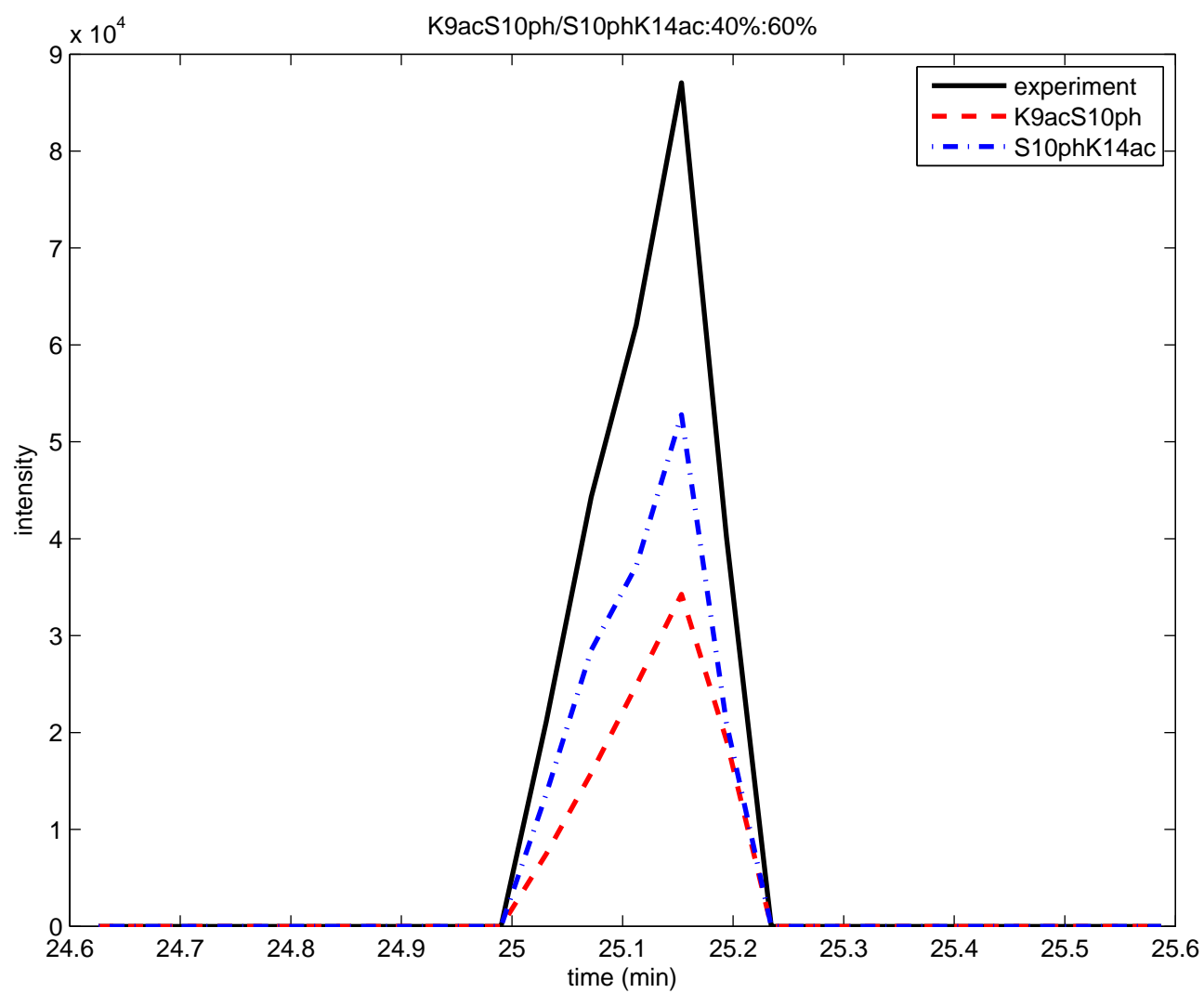
Figure S2F. Isobaric peptides of H3 9−17 K9acS10ph/S10phK14ac.
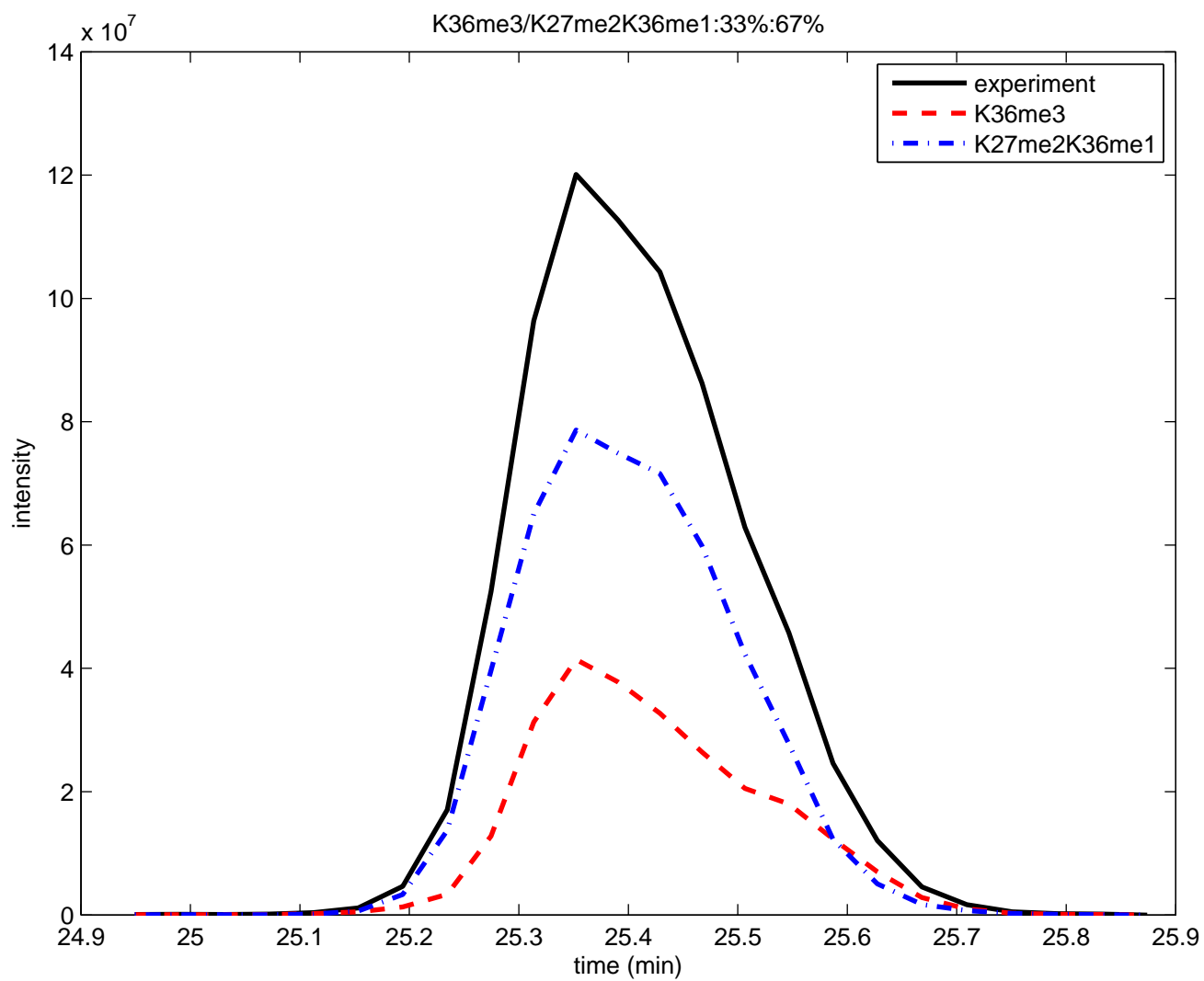
Figure S2G. Isobaric peptides of H3.1/2 27−40 K36me3/K27me2K36me1.
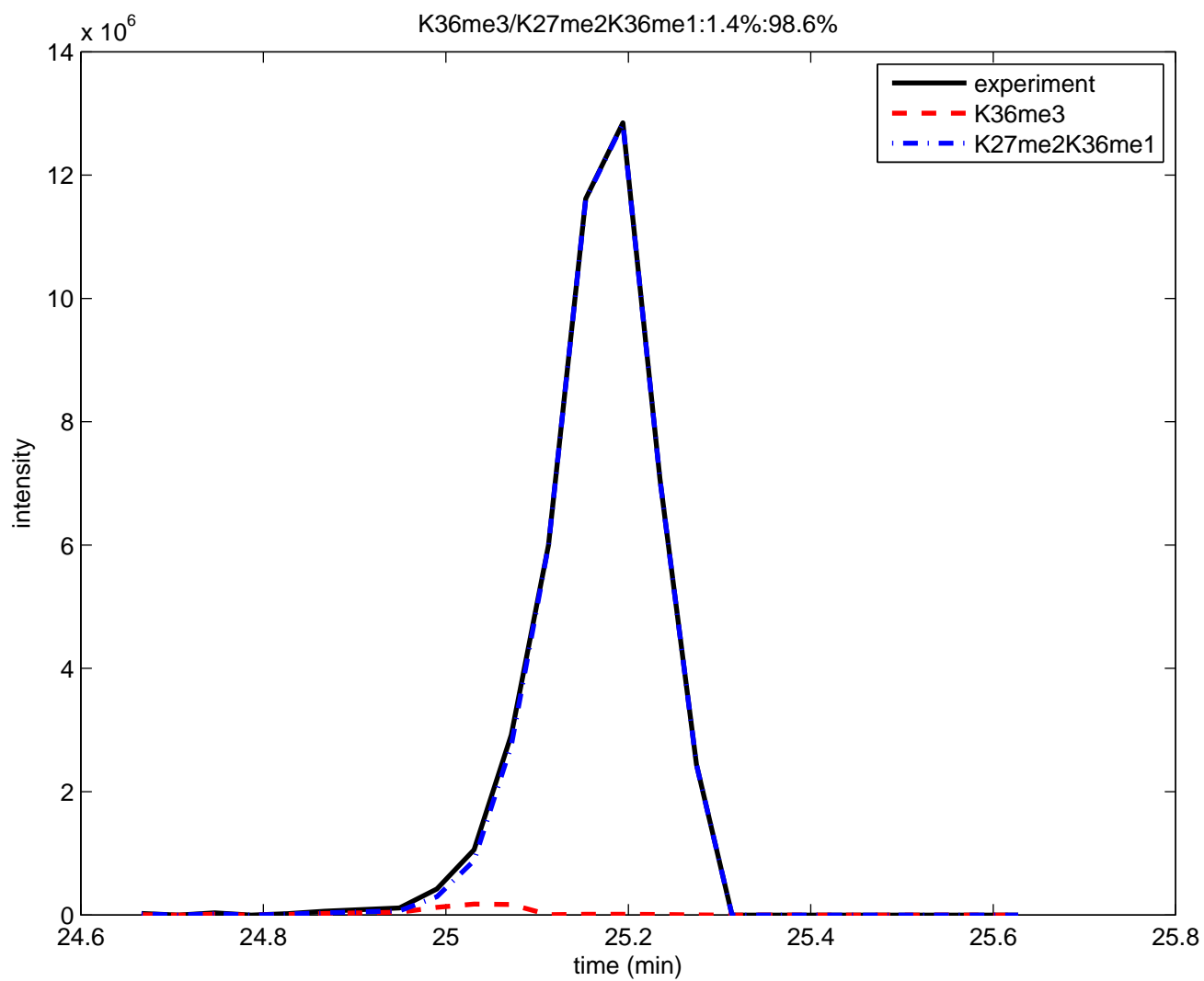
Figure S2H. Isobaric peptides of H3.3 27−-40 K36me3/K27me2K36me1.
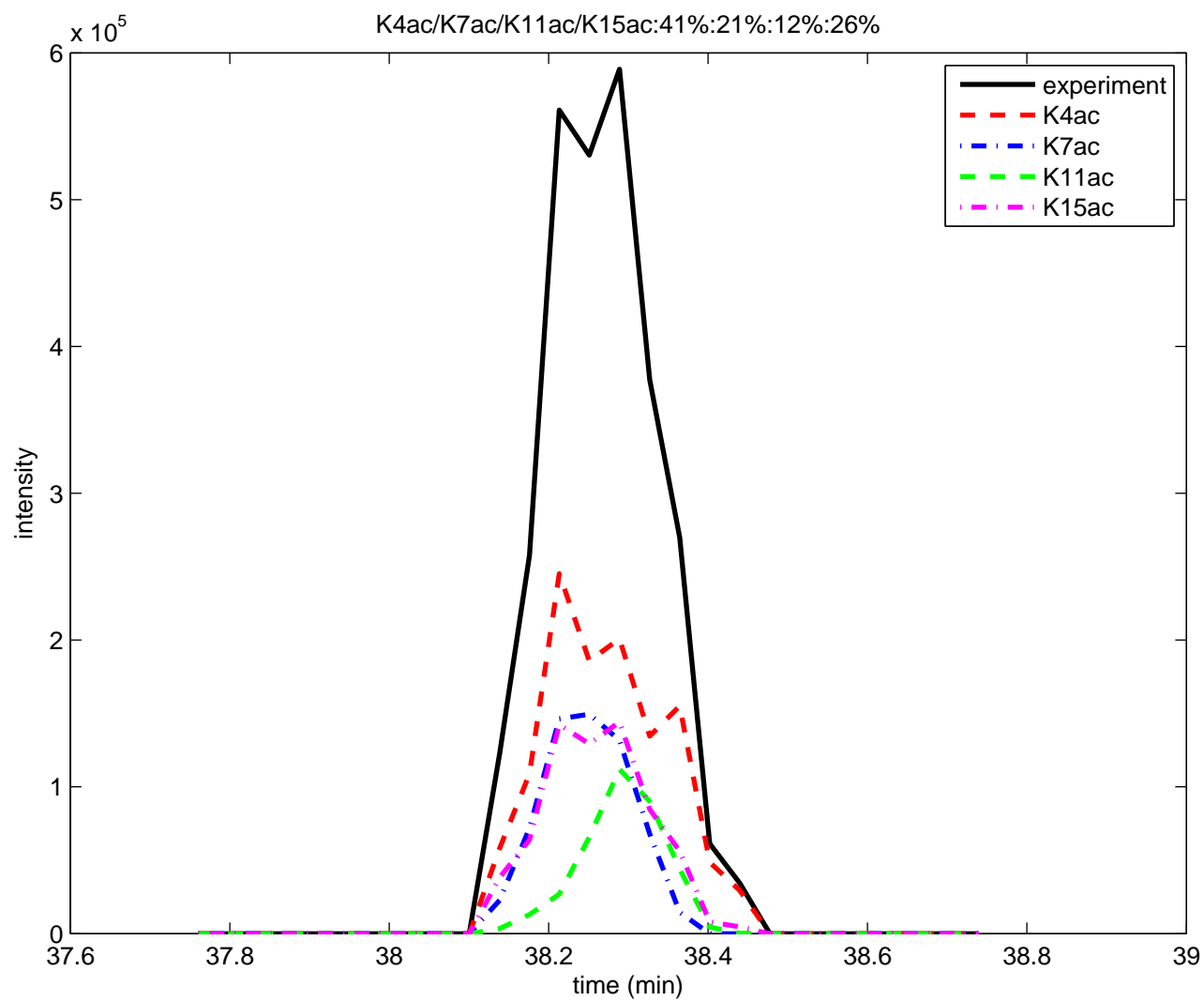
Figure S2I. Isobaric peptides of H2AV 1–-19 1ac.
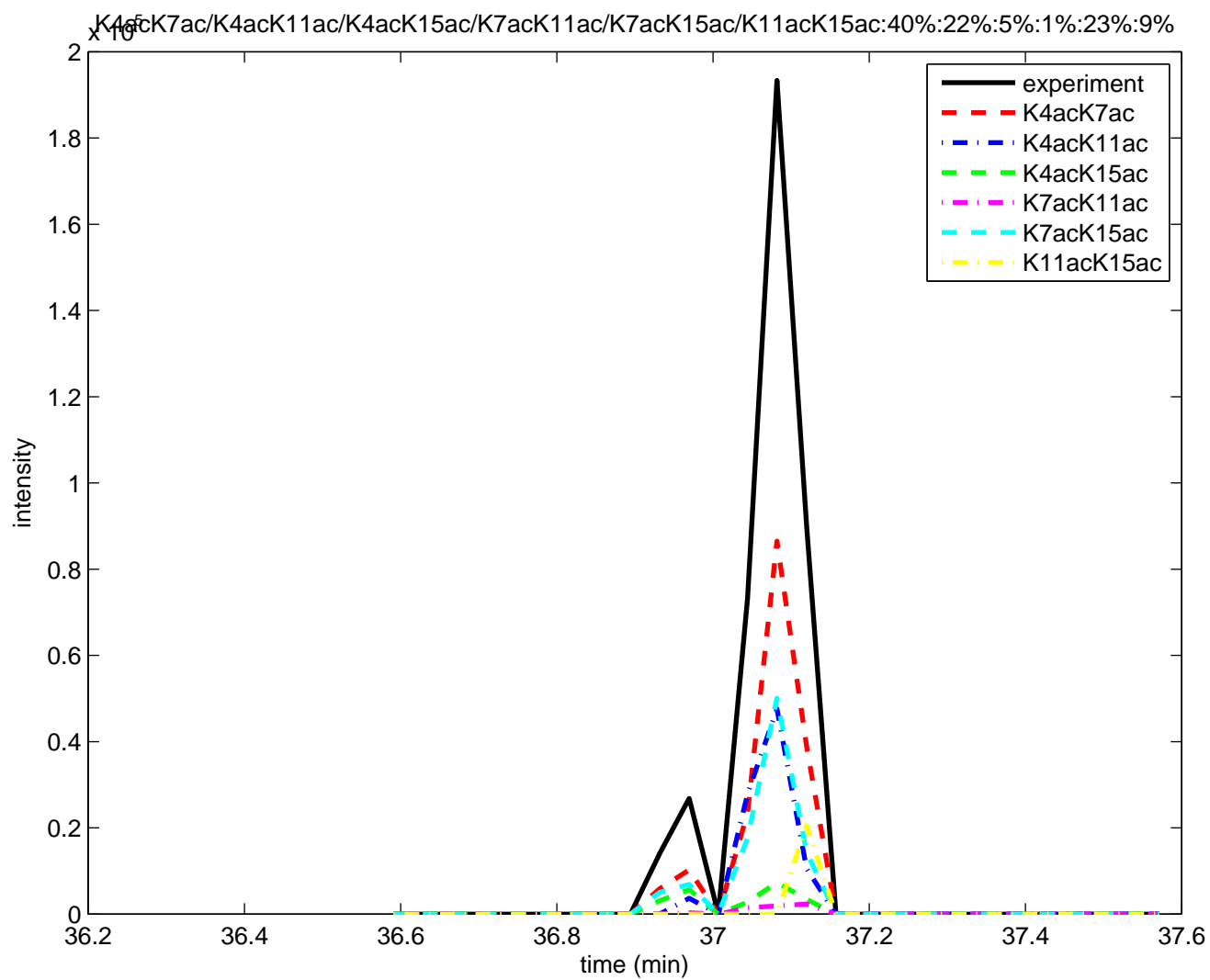
Figure S2J. Isobaric peptides of H2AV 1−-19 2ac.
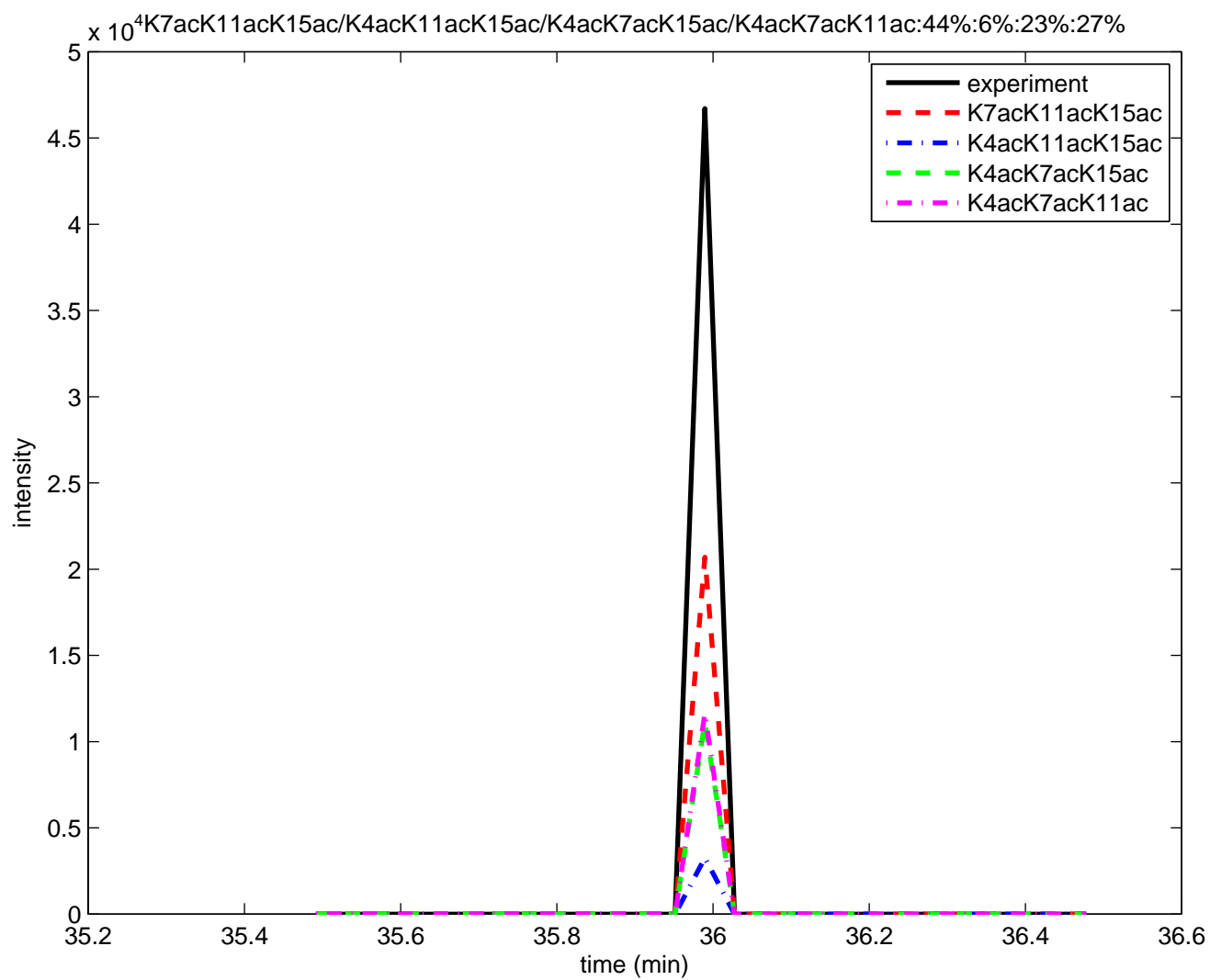
Figure S2K. Isobaric peptides of H2AV 1−-19 3ac.
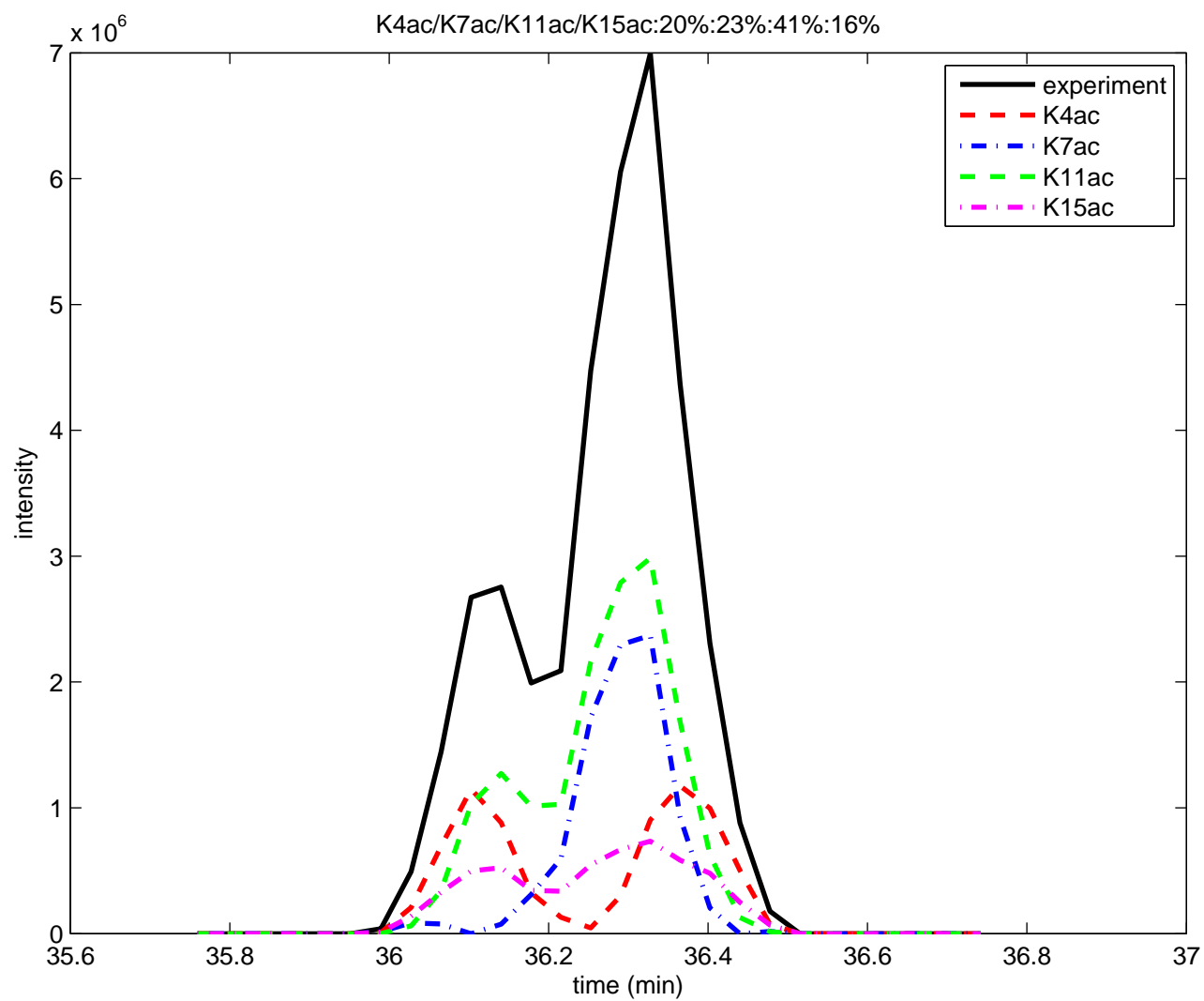
Figure S2L. Isobaric peptides of H2AZ 1--19 1ac.
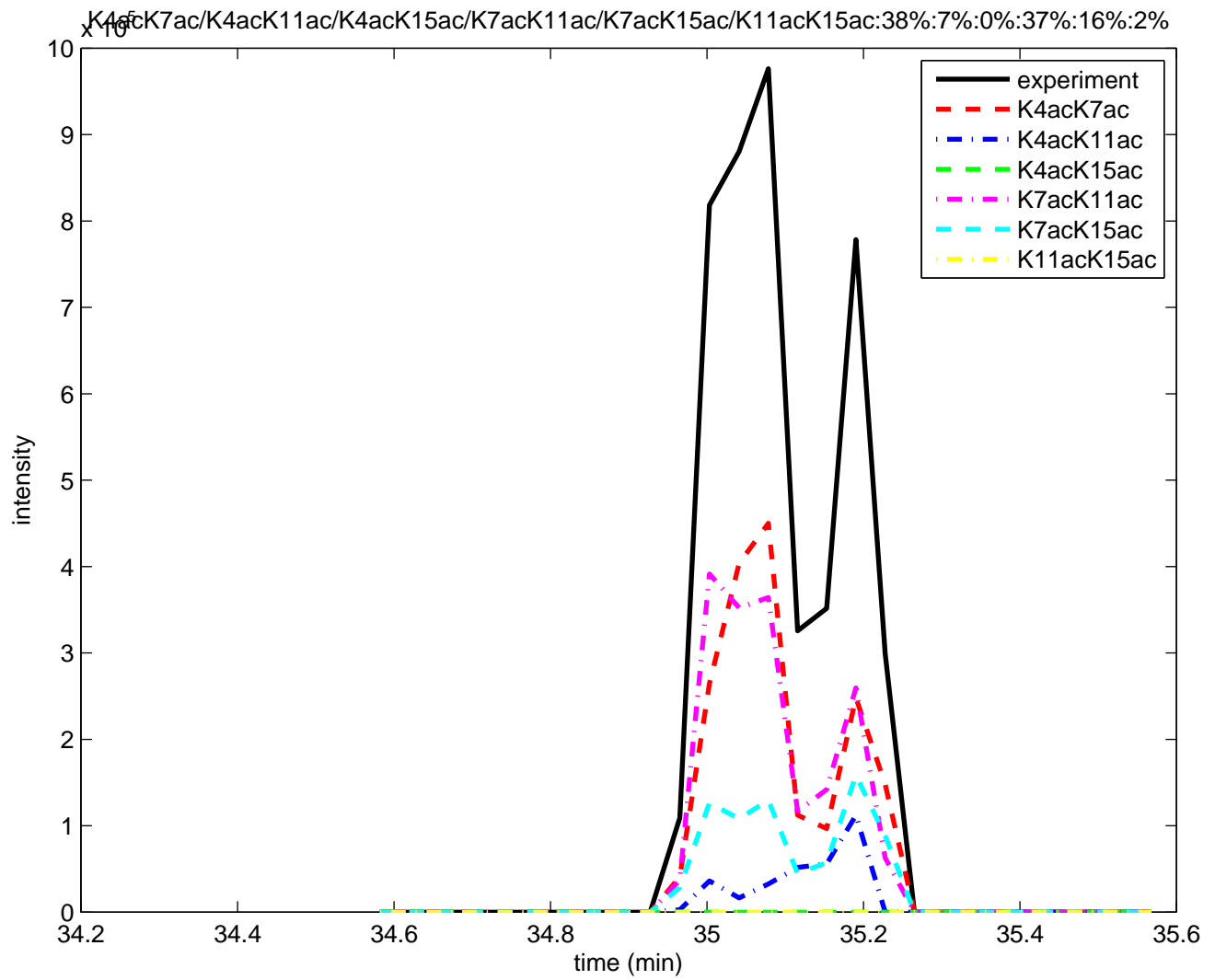
Figure S2M. Isobaric peptides of H2AZ 1−-19 2ac.

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

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

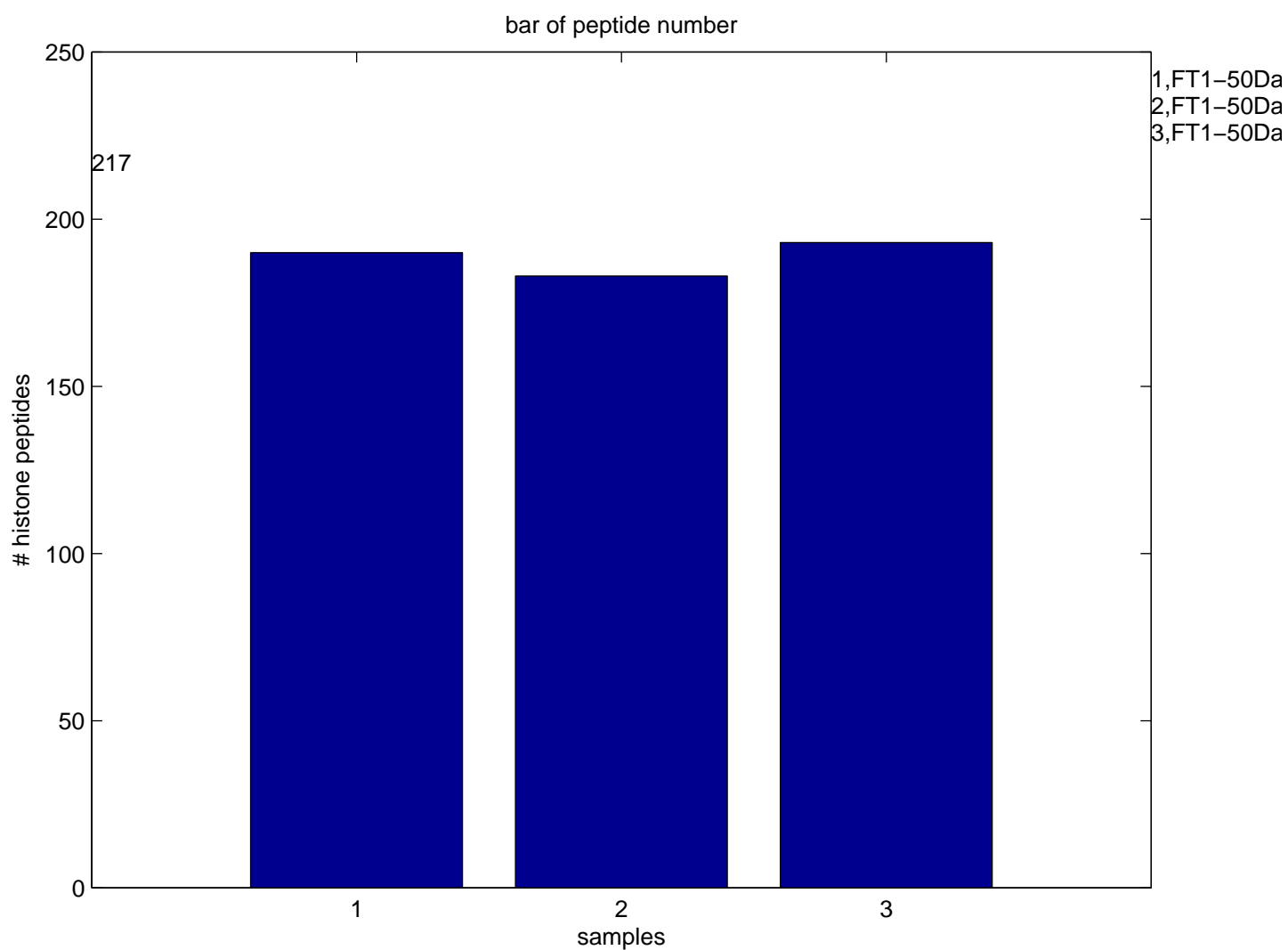**Figure S2P. Isobaric peptides of H2A 12−-17 K13ac/K15ac.**

Figure S3. MS/MS spectra for H3 K9ac and K14ac.

bar of peptide number

217

1,FT1−50Da
2,FT1−50Da
3,FT1−50Da

histone peptides

250

200

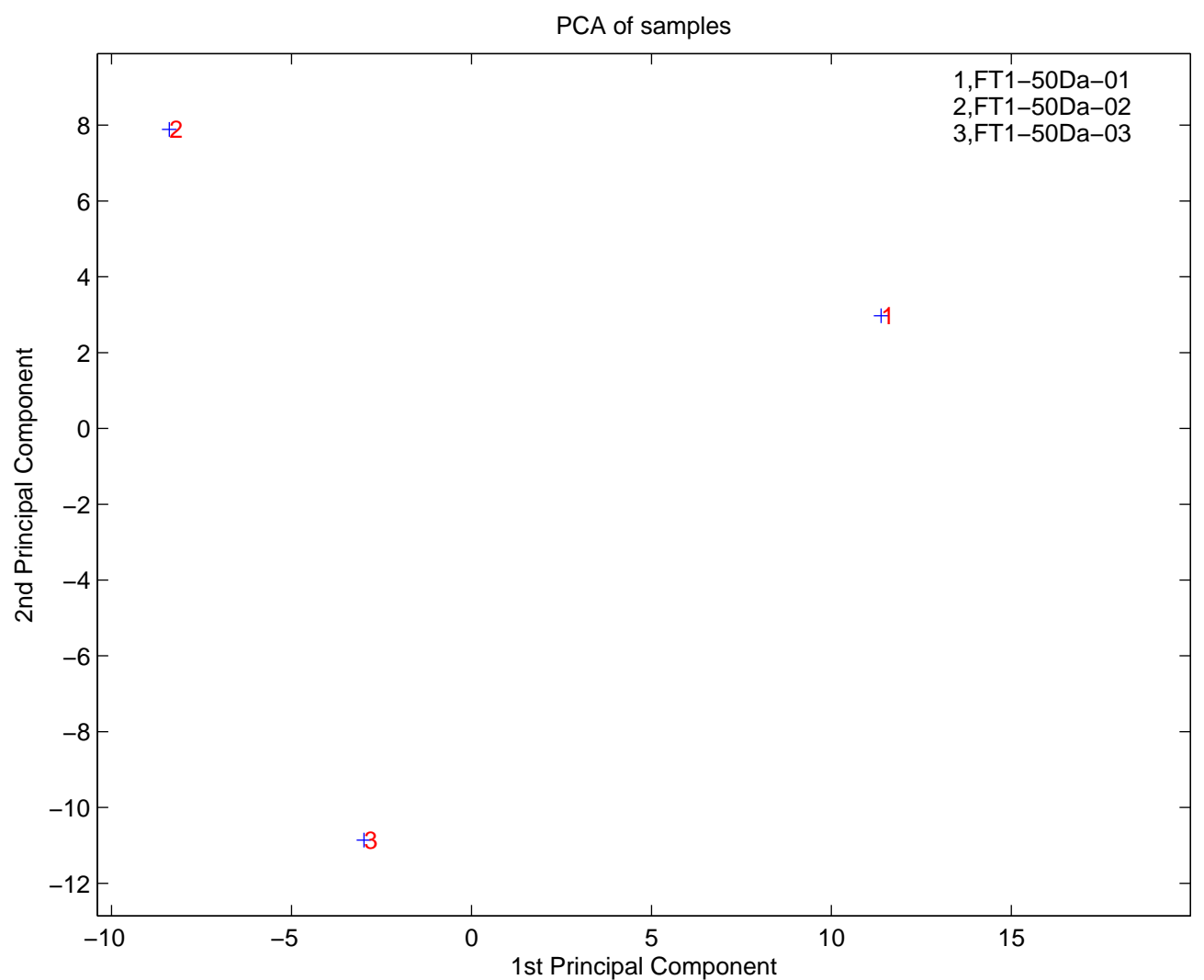150

100

50

0

1   2   3
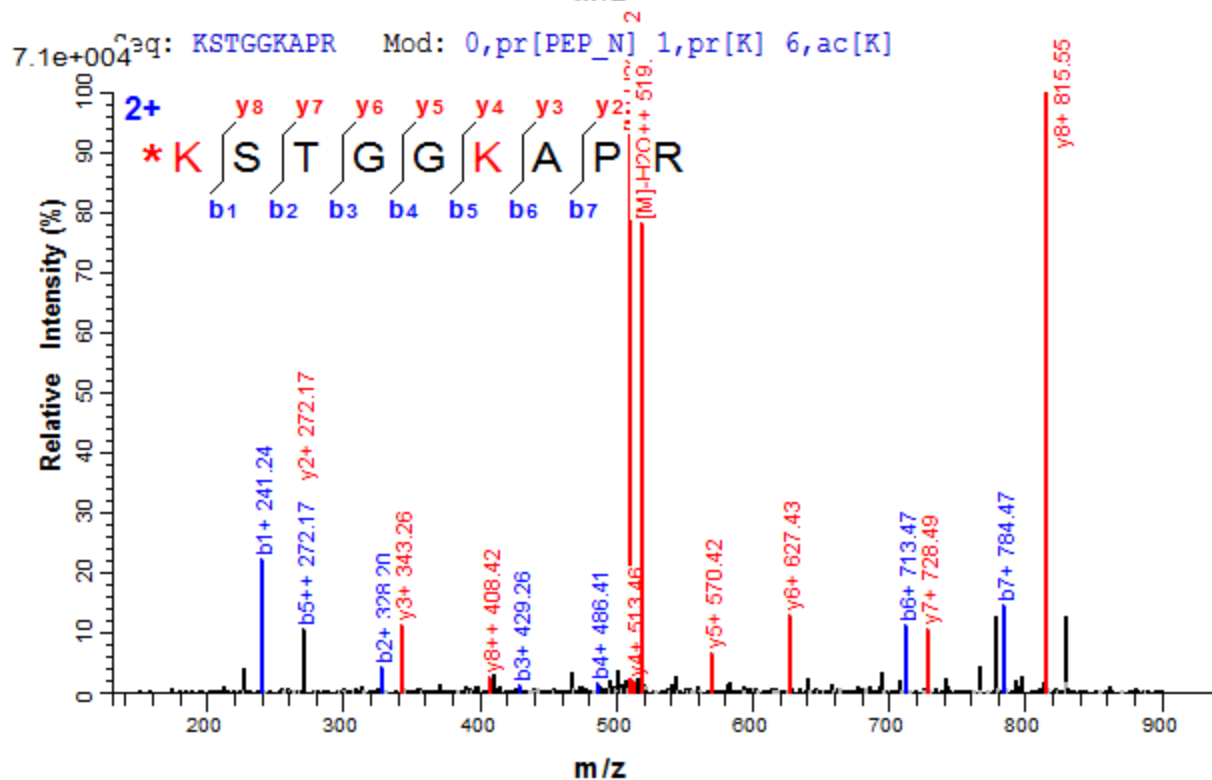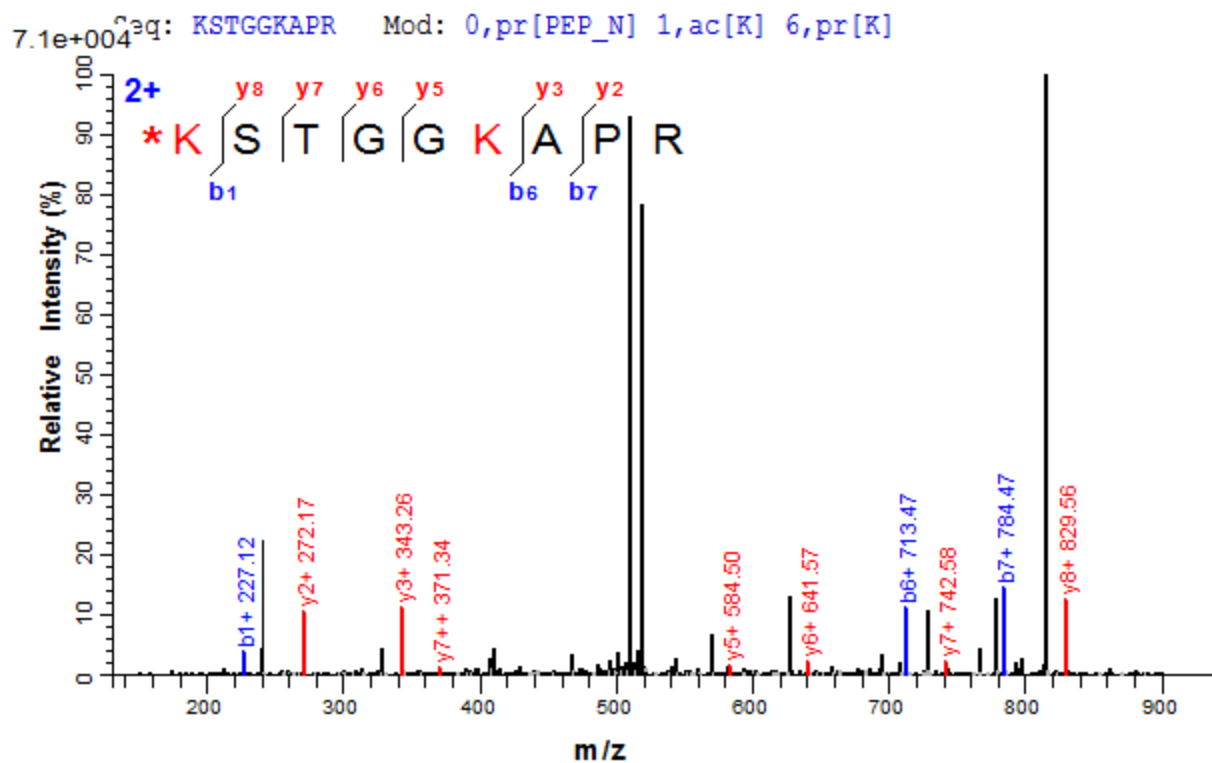
samples

There are 217 histone peptides theoretically. The number of identified peptides in each sample is shown.

Figure S4. Bar plot represents the number of histone peptides quantified by EpiProfile.
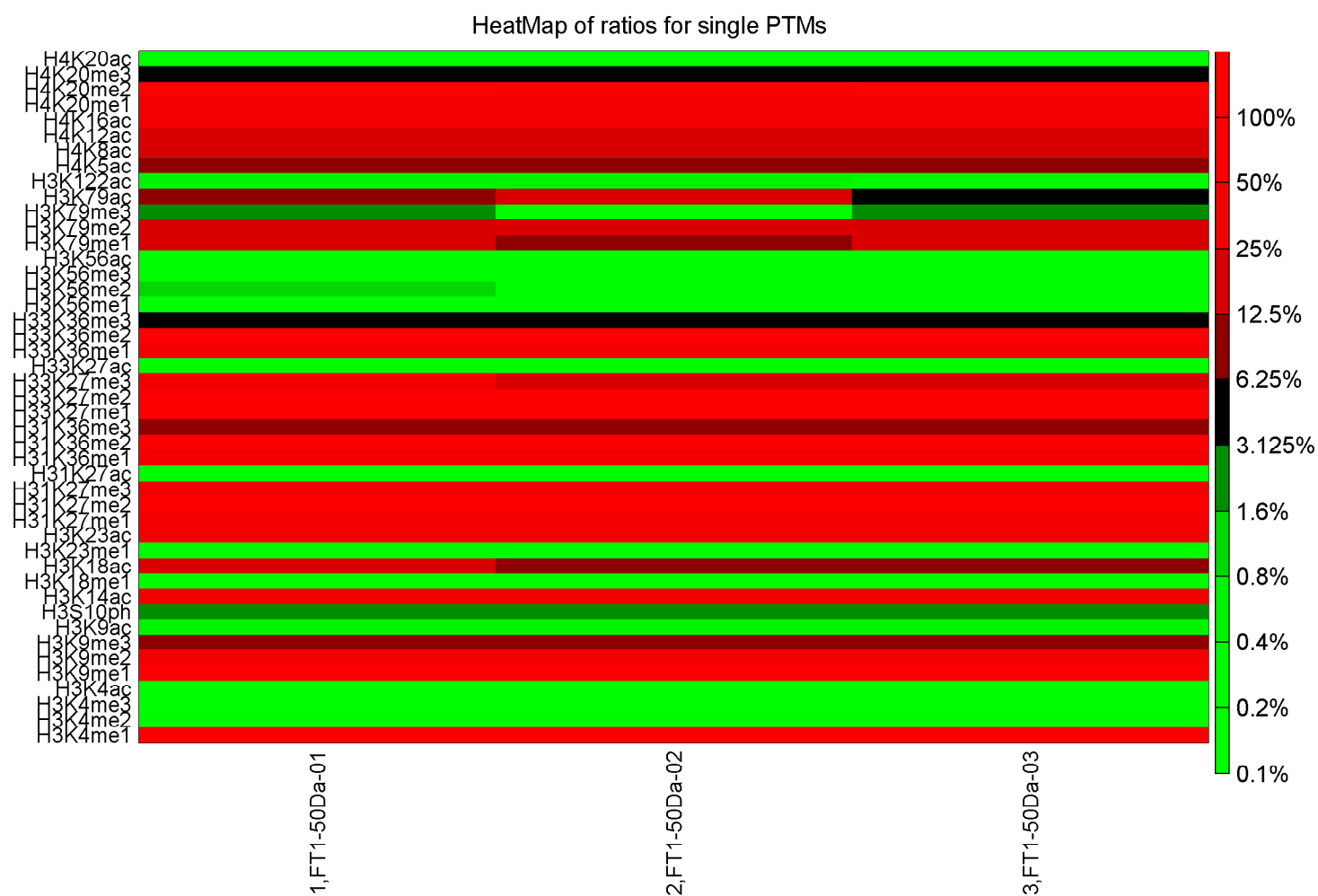
boxplot of peptide intensity
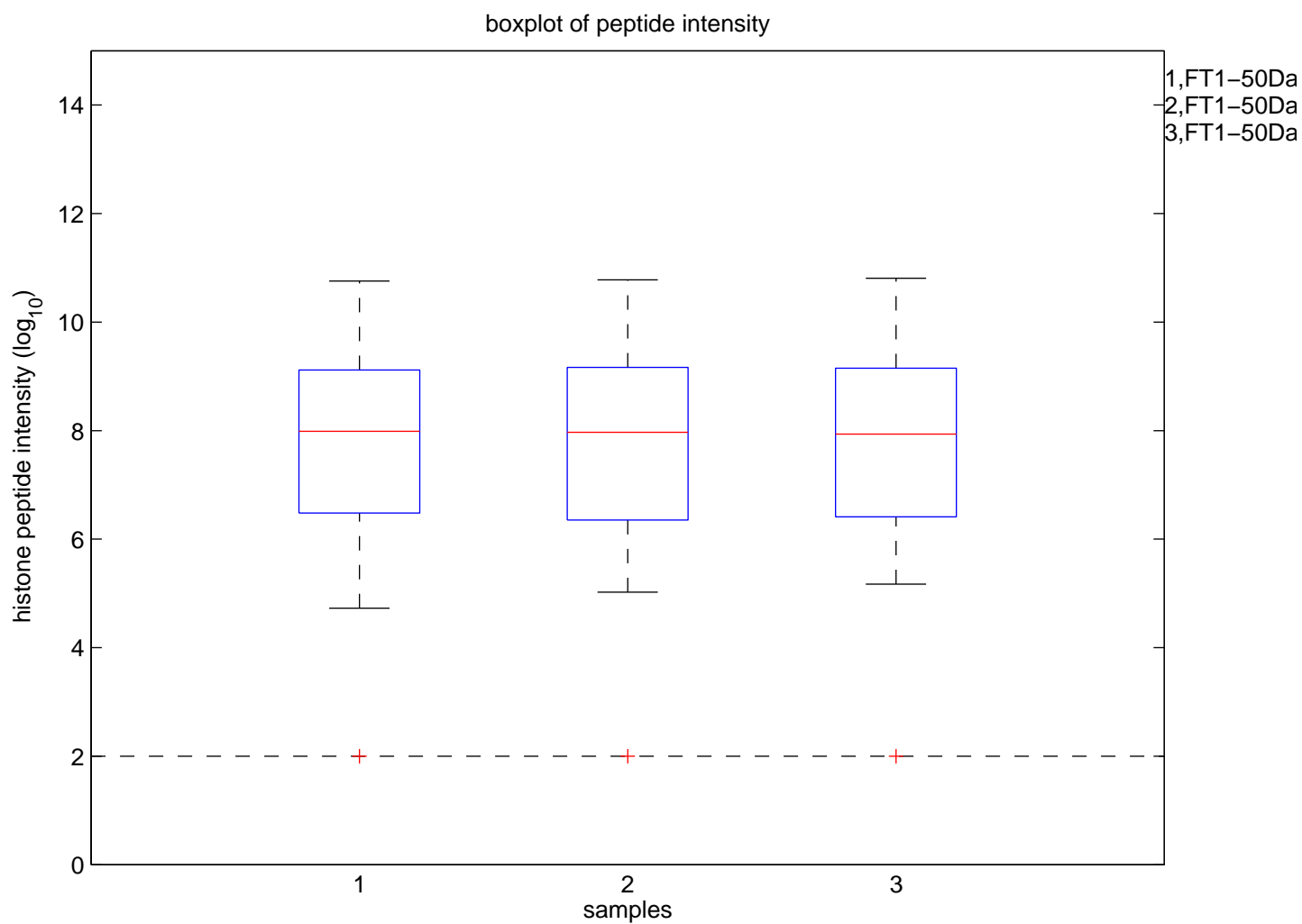
Boxplot produces a distribution of peptide intensity in each sample. There is one box per sample.
On each box, the central mark is the median, the edges of the box are the 25th and 75th percentiles,
and the whiskers extend to the most extreme data points not considered outliers.

Figure S5. Box plot displays the Log10 transformed total peptide intensity.

Figure S6. Principal component analysis (PCA) of all performed runs.

Figure S7. Heatmap represents the relative abundance of single histone PTMs.