Supporting Information

Characterization of Complete Histone Tail Proteoforms Using Differential Ion Mobility Spectrometry

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Author Contributions

#The two senior authors of this work.

EXPERIMENTAL SECTION

Intavis ResPep Settings

Solutions

- Fmoc-aa solution: 650 mM solution in DMF
- Activator: 600 mM HBTU in DMF
- Base: N-Methylmorpholine in DMF 0.81:1 v/v
- Capping solution: 5% acetic anhydrate in DMF
- Deprotection solution: 20% Piperidine in DMF

ResPep program. We subjected the amino acids 1 – 8 to double coupling for 10 min, amino acids 9 - 18 to double coupling for 30 min, and amino acids 19 - 26 to triple coupling for 50 min.

Cycle: 1 - 8

5 Deprotection 120 µL, Piperidine -> Peptides

6 Deprotection 80 µL, Piperidine -> Peptides

7 WashColumns 150 µL, Reservoir -> Peptides, 3x

8 WashColumns 200 µL, Reservoir -> Peptides, 3x

9 WashColumns 250 µL, Reservoir -> Peptides, 2x

10 Coupling 46.1 (activator) + 12.5 (base) + 5 (DMF) + 39.4 (derivative) ->
Peptides – 10 min reaction time

11 Coupling 46.1 (activator) + 12.5 (base) + 5 (DMF) + 39.4 (derivative) ->
Peptides – 10 min reaction time

12 Capping 120 µL, Capping - reaction time 5 min

13 WashColumns 150 µL, Reservoir - >Peptides, 3x

14 WashColumns 200 µL, Reservoir -> Peptides, 2x

15 WashColumns 250 µL, Reservoir -> Peptides, 2x

Cycle: 9 - 18

16 Deprotection 120 µL, Piperidine -> Peptides

17 Deprotection 80 µL, Piperidine -> Peptides

18 WashColumns 150 µL, Reservoir -> Peptides, 3x

19 WashColumns 200 µL, Reservoir -> Peptides, 3x

20 WashColumns 250 µL, Reservoir -> Peptides, 2x

21 Coupling 46.1 (activator) + 12.5 (base) + 5 (DMF) + 39.4 (derivative) ->

Peptides – 30min reaction time

22 Coupling 46.1 (activator) + 12.5 (base) +5 (DMF) + 39.4 (derivative)->

Peptides – 30min reaction time

23 Capping 120 µL, Capping -> Peptides - reaction time 5 min

24 WashColumns 150 µL, Reservoir - >Peptides, 3x

25 WashColumns 200 µL, Reservoir -> Peptides, 2x

26 WashColumns 250 µL, Reservoir -> Peptides, 2x

Cycle: 19 - max (-> 25)

27 Deprotection 120 µL, Piperidine -> Peptides

28 Deprotection 120 µL, Piperidine -> Peptides

29 WashColumns 150 µL, Reservoir -> Peptides, 3x

30 WashColumns 200 µL, Reservoir -> Peptides, 3x

31 WashColumns 250 µL, Reservoir -> Peptides, 2x

32 Coupling 51.5 (activator) + 14 (base) + 5 (DMF) + 43.5 (derivative) ->

Peptides – 50min reaction time

33 Coupling 51.5 (activator) + 14 (base) + 5 (DMF) + 43.5 (derivative)->

Peptides – 50min reaction time

34 Coupling 51.5 (activator) + 14 (base) + 5 (DMF) + 43.5 (derivative)->

Peptides – 50min reaction time

35 Capping 120 µL, Capping -> Peptides - reaction time 5 min

36 WashColumns 150 µL, Reservoir -> Peptides, 3x

37 WashColumns 200 µL, Reservoir -> Peptides, 2x

38 WashColumns 250 µL, Reservoir -> Peptides, 2x

Final

39 WashColumns 150 µL, Reservoir -> Peptides

40 WashColumns 200 µL, DCMwash -> Peptides

41 WashColumns 200 µL, Reservoir -> Peptides

42 Deprotection 120 µL, Piperidine -> Peptides

43 Deprotection 100 µL, Piperidine -> Peptides

44 Deprotection 100 µL, Piperidine -> Peptides

45 WashColumns 200 µL, Reservoir -> Peptides, 2x

46 WashColumns 200 µL, Reservoir -> Peptides, 2x

47 WashColumns 250 µL, Reservoir -> Peptides, 2x

48 WashColumns 250 µL, Ethanol -> Peptides, 3x

49 WashColumns 250 µL, DCMwash -> Peptides

50 RinseNeedle 2500 / 2500 uL

HPLC conditions

The Waters column was used at ambient temperature to purify the Part I and full histone tail employing gradient 1 and the Part II employing gradient 2. Solvents were: buffer A (aqueous) 0.1% TFA in H2O; buffer B (organic) 0.1% TFA in 9:1 Acetonitrile/H2O. The elution rate was 5 mL/min.

Gradient 1

1. Column equilibration 0% B - 6 min
2. Sample loading 1 - 3 min
3. 0 - 7% buffer B in 2 min
4. 7 - 16% buffer B in 15 min
5. 16 - 100% buffer B in 1 min
6. Column wash 0% B - 3 min

Gradient 2

7. Column equilibration 0% B - 6 min
8. Sample loading 1 - 3 min
9. 0 - 14% buffer B in 2 min
10. 14 - 23% buffer B in 15 min
11. 16 - 100% buffer B in 1 min
12. Column wash 0% B - 3min

Statistical Evaluation of the Orthogonality of Separations Across Charge States

To assess the orthogonality of separations across charge states, we found the EC values at maximum signal intensity for trimethylated, acetylated, and phosphorylated variants in each (Table S1) and statistically tested their pairwise linear correlation between every charge state and all others. The analysis has utilized the R stats package, with the cor.test() function at default settings using the statistics based on Pearson's product moment correlation coefficient (Table S2). Of the 30 charge state pairs, only two exhibited significant correlations ($p < 0.05$): me3 tails at $z = 9$ and 12 and p tails at $z = 8$ and 10. The adjustment for multiple testing (using the Benjamini-Hochbeck method implemented in p.adjust() function) has produced the final answer of no significant correlation.

Results and Discussion

Figures

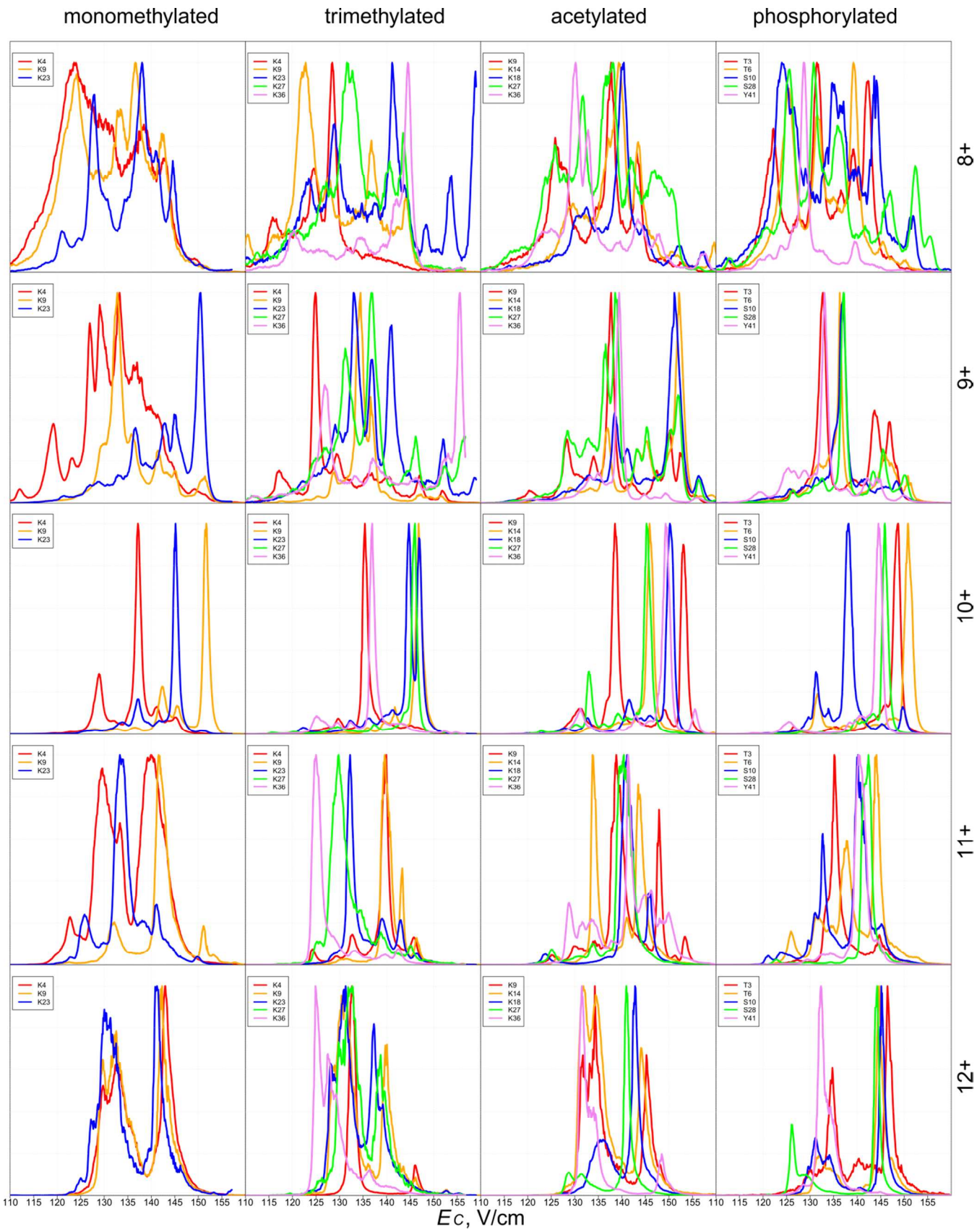


Figure S1. FAIMS spectra for all 18 PTM localization variants of monomethylated (me), trimethylated (me3), acetylated (ac), and phosphorylated (p) histone tail ions in this work (charge states z = 8 - 12).

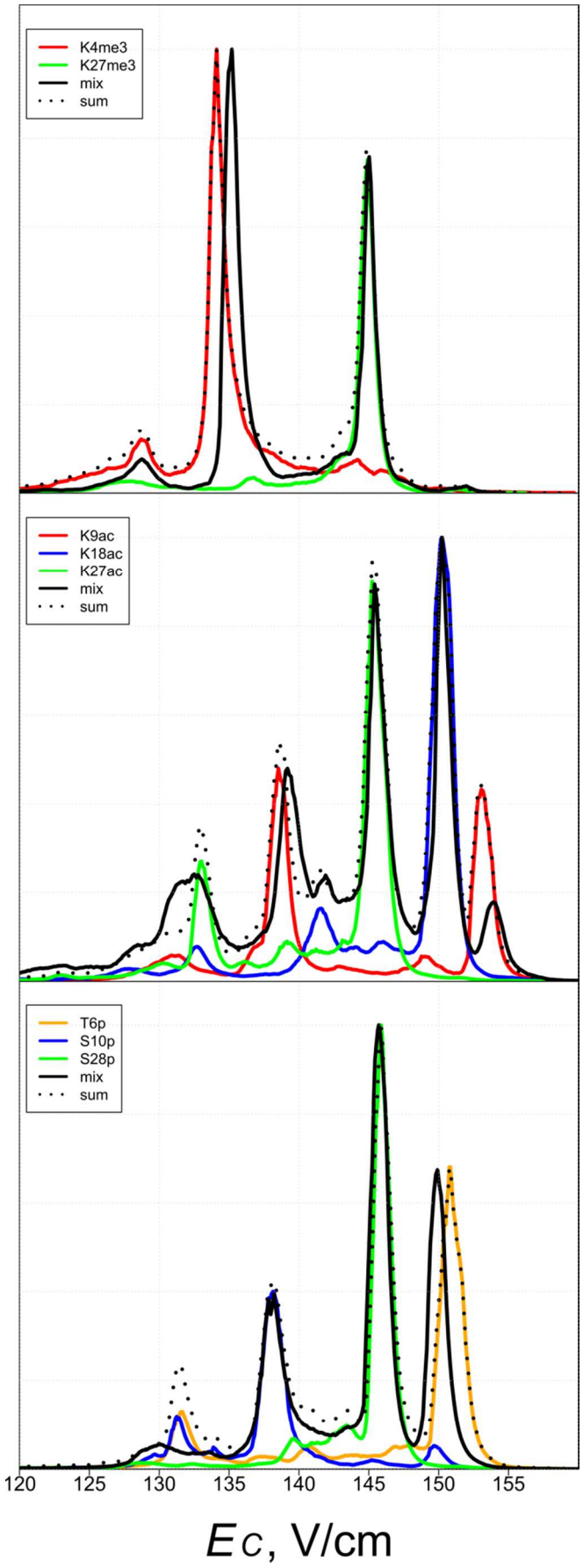


Figure S2. Validation of FAIMS separation by comparison of the spectra measured for individual variants and their computed additions with the spectra for variant mixtures.

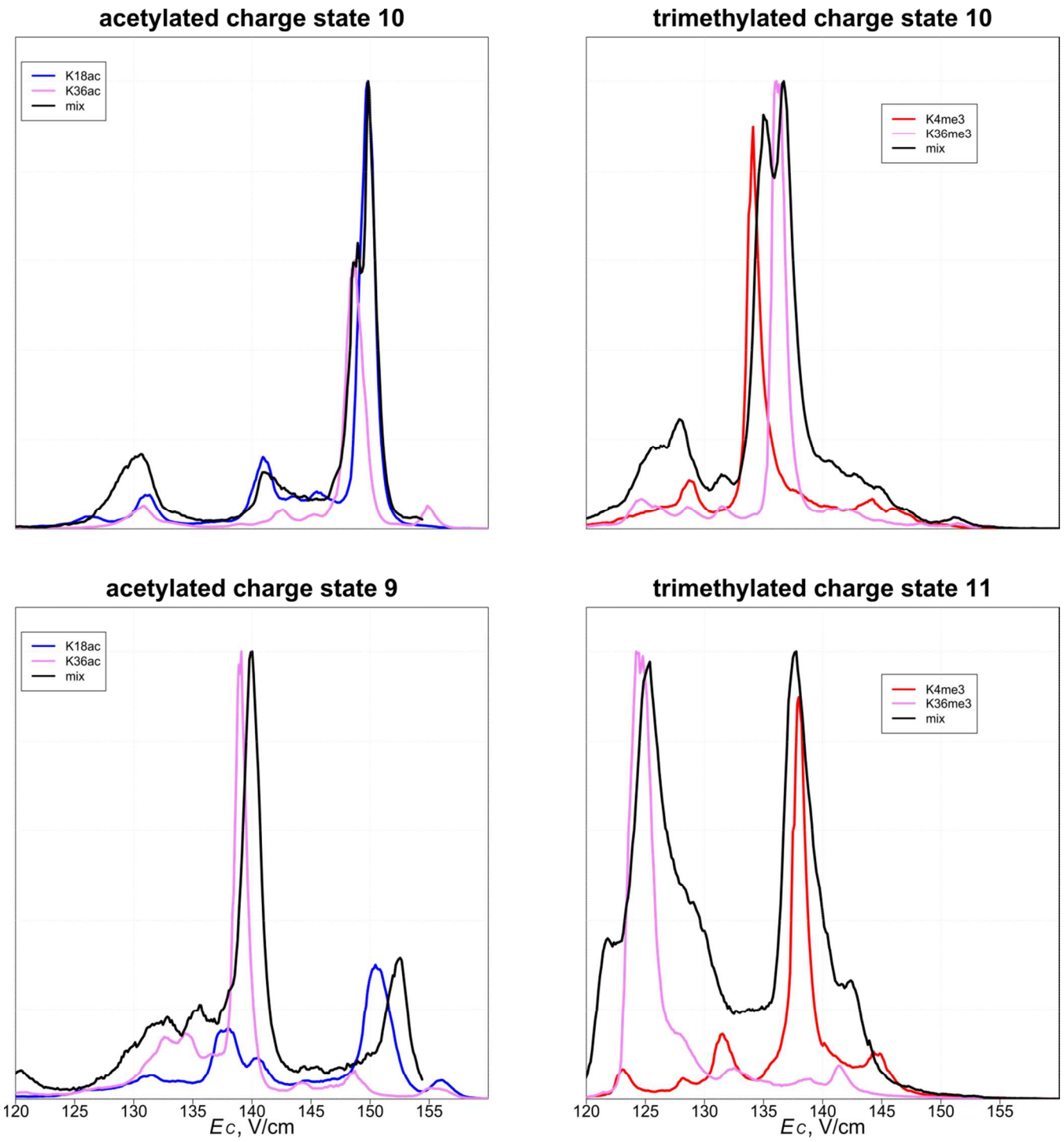


Figure S3. Examples of orthogonal separations at different charge states. The variants not resolved at z = 10 are separated baseline at other charge states.

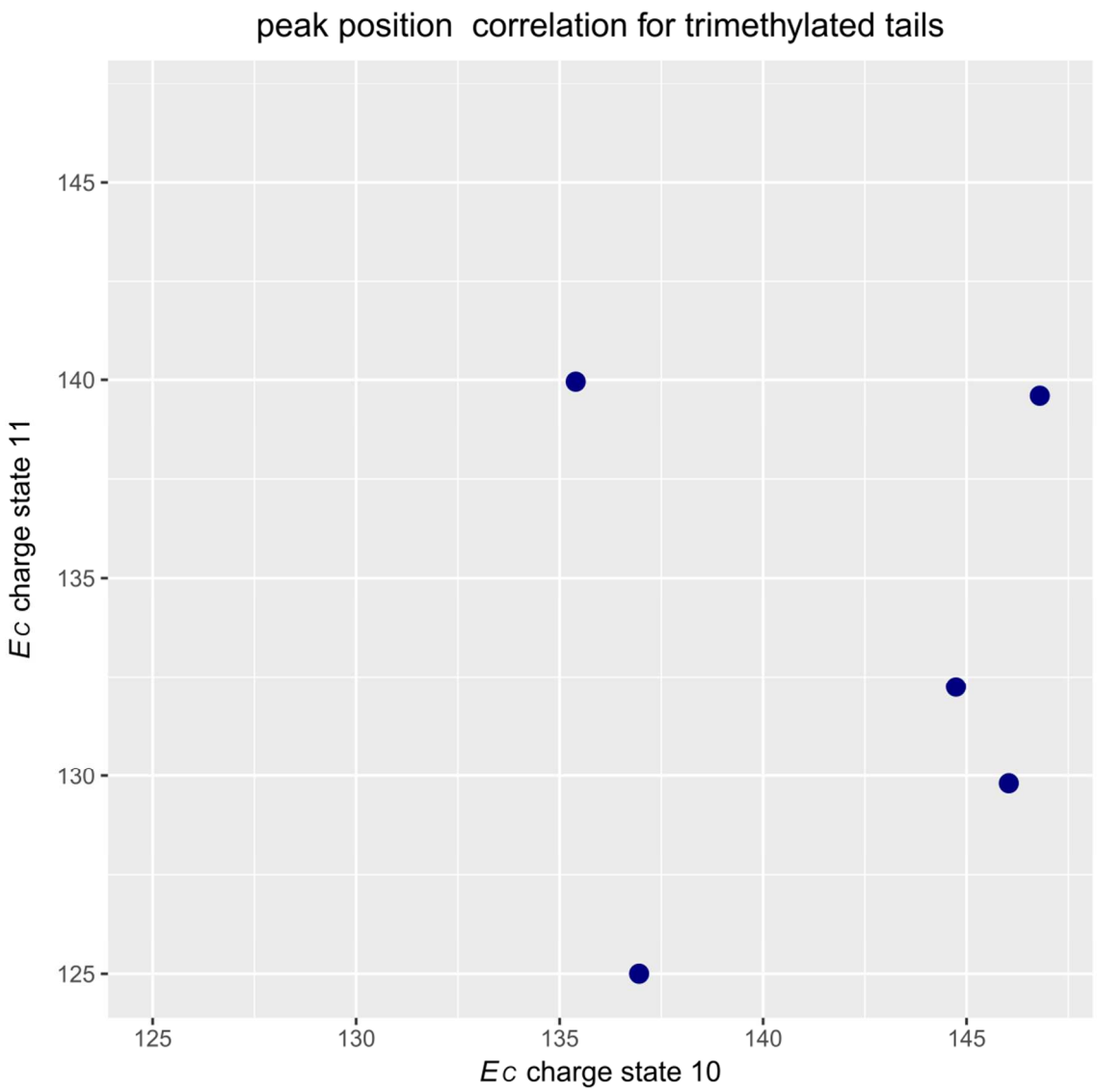


Figure S4A. The FAIMS separations of me3 variants at z = 10 and 11 are uncorrelated.

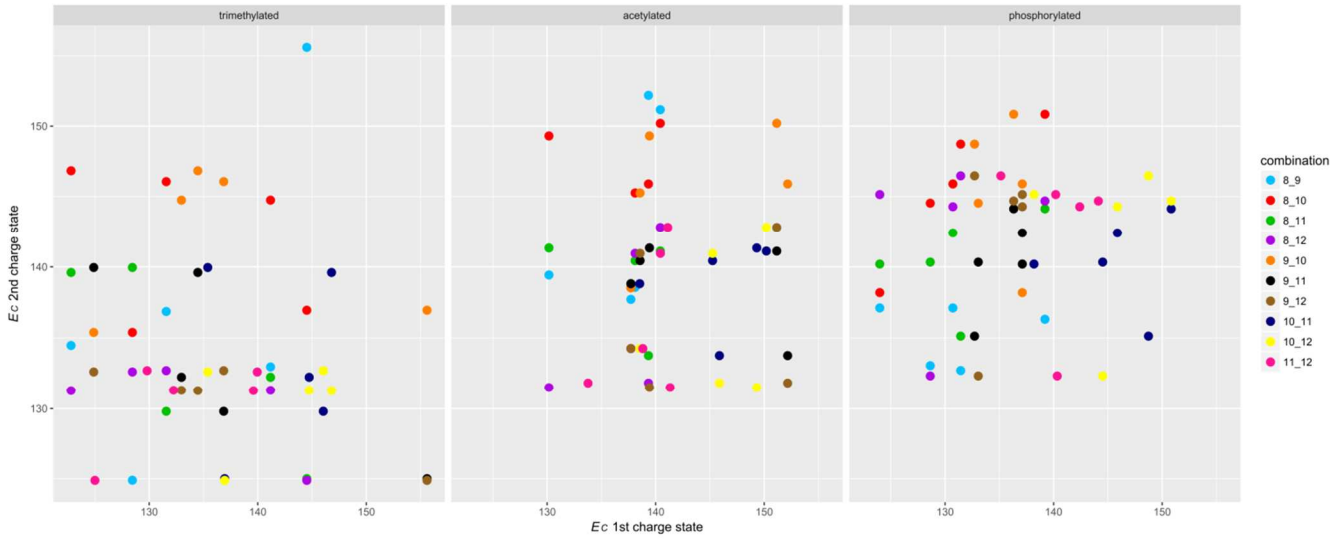


Figure S4B. Overall lack of correlation between FAIMS separations of m3, ac, and p variants across charge states.

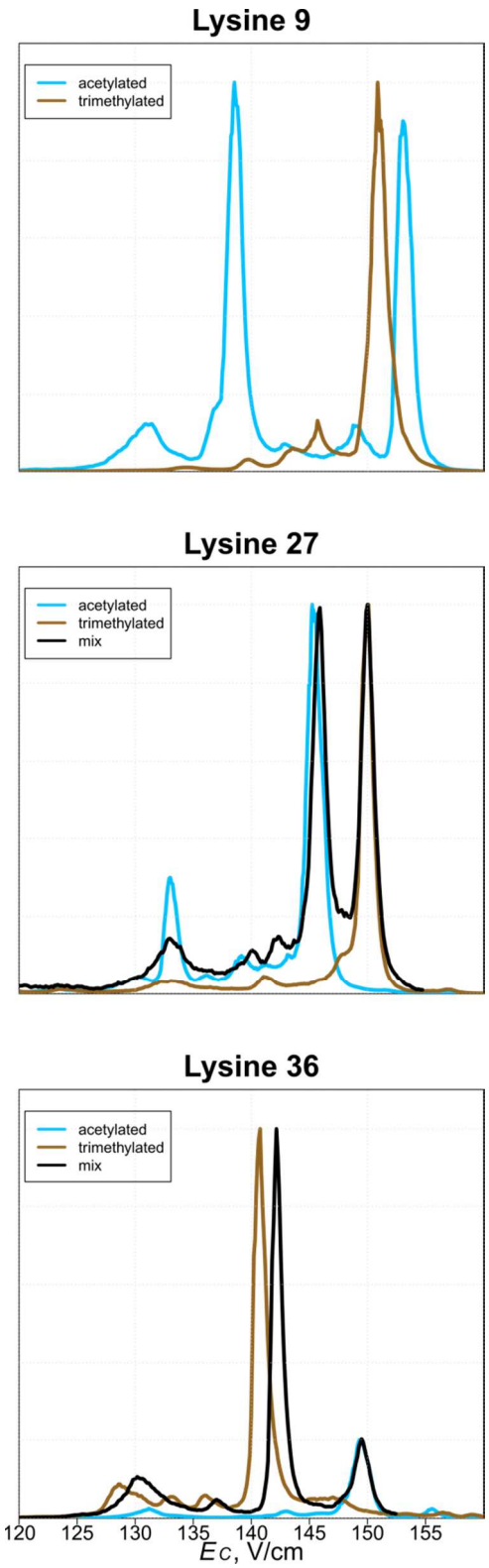
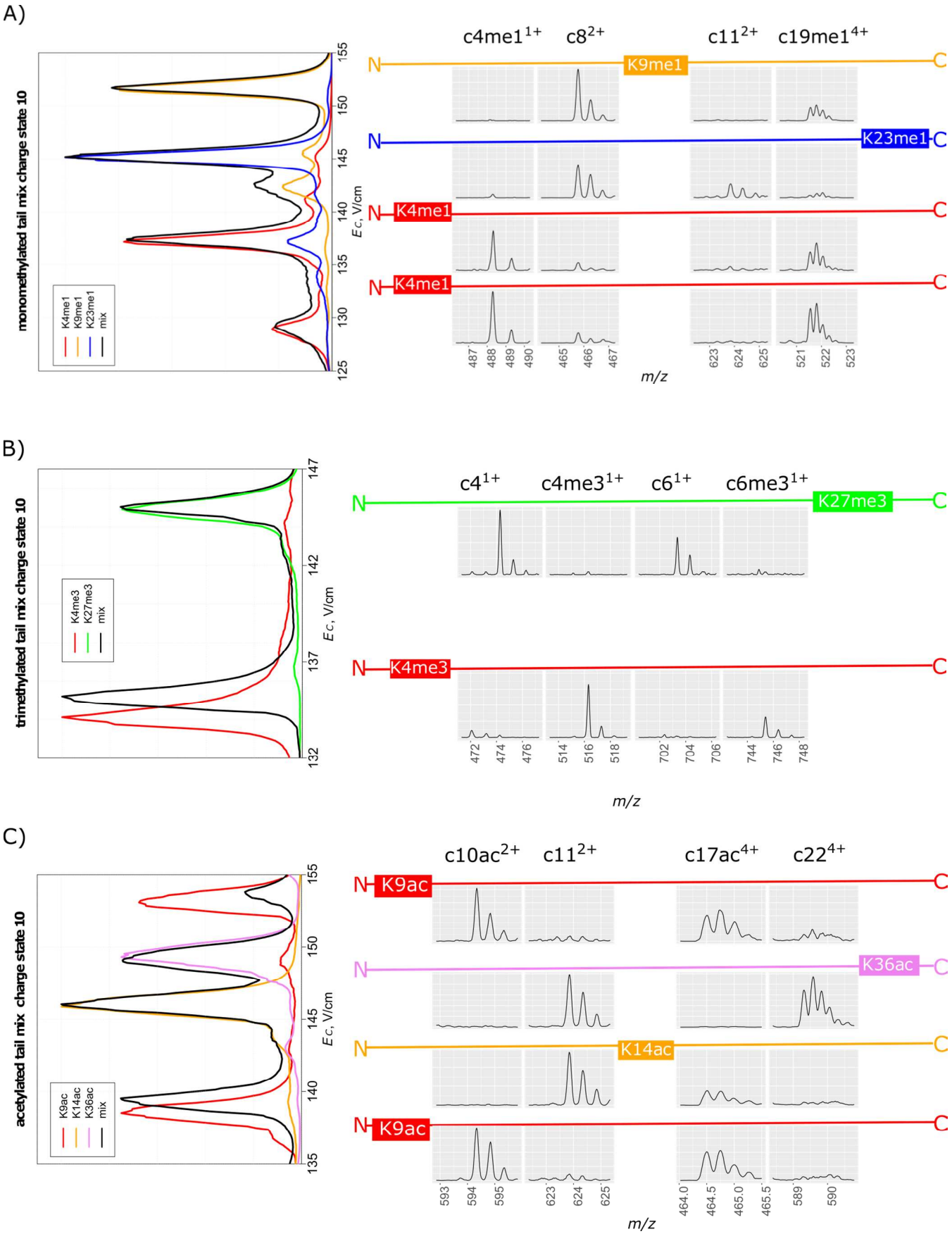


Figure S5. Normalized FAIMS spectra for the tails trimethylated or acetylated at the same K position and their mixtures.


A)
monomethylated tail mix charge state 10
K4me1
K9me1
K23me1
mix
Ec, V/cm
c4me1 1+
c8 2+
c11 2+
c19me1 4+
K9me1
K23me1
K4me1
K4me1
m/z
B)
trimethylated tail mix charge state 10
K4me3
K27me3
mix
Ec, V/cm
c4 1+
c4me3 1+
c6 1+
c6me3 1+
K27me3
K4me3
m/z
C)
acetylated tail mix charge state 10
K9ac
K14ac
K36ac
mix
Ec, V/cm
c10ac 2+
c11 2+
c17ac 4+
c22 4+
K9ac
K36ac
K14ac
K9ac
m/z

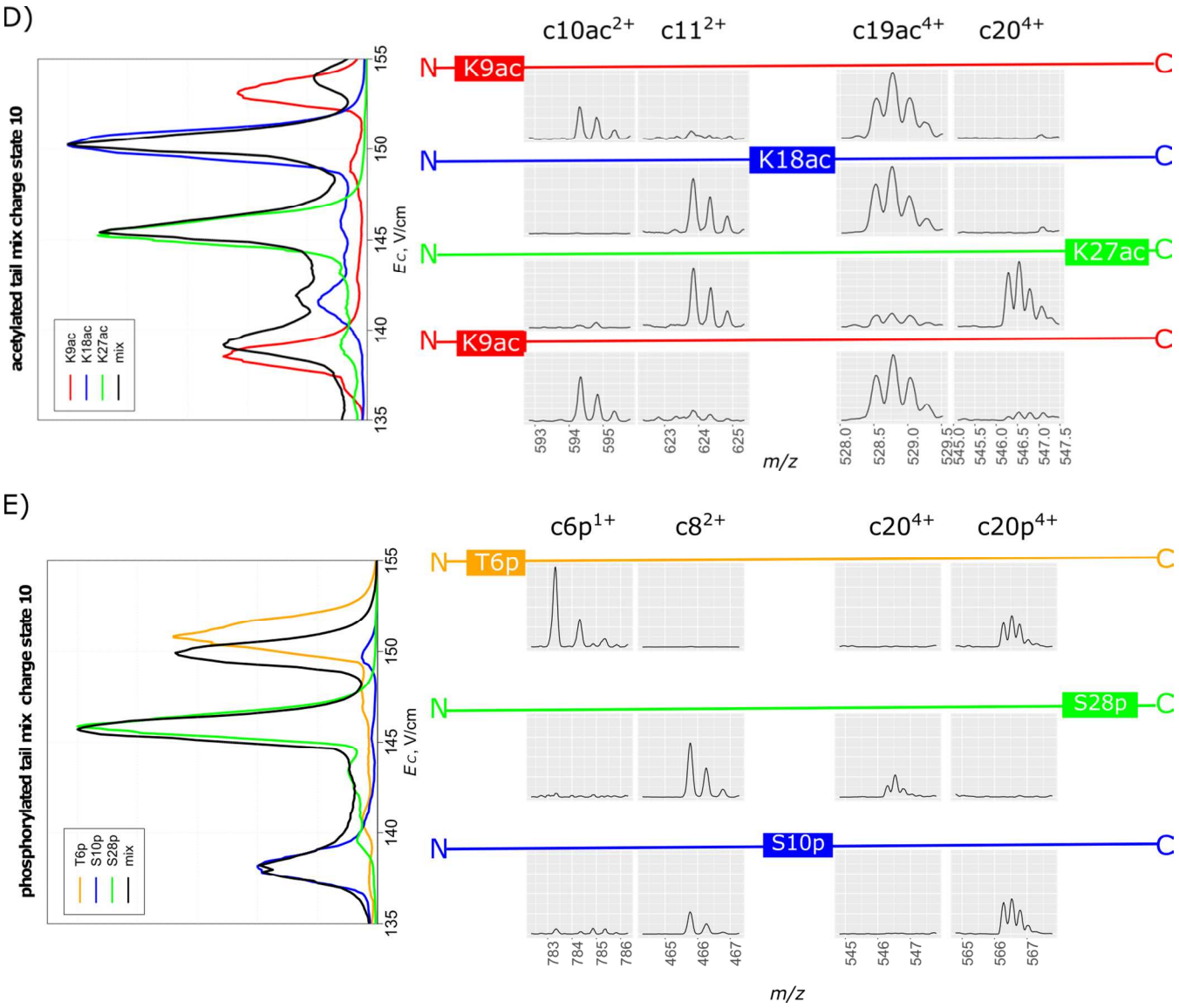


Fig. S6. Validation of FAIMS separation of representative binary and ternary variant mixtures by ETD MS/MS (zoom mode):

A. K4me1/K9me1/K23me1

B. K4me3/K27me3

C. K9ac/K14ac/K36ac

D. K9ac/K18ac/K27ac

E. T6p/S10p/S28P

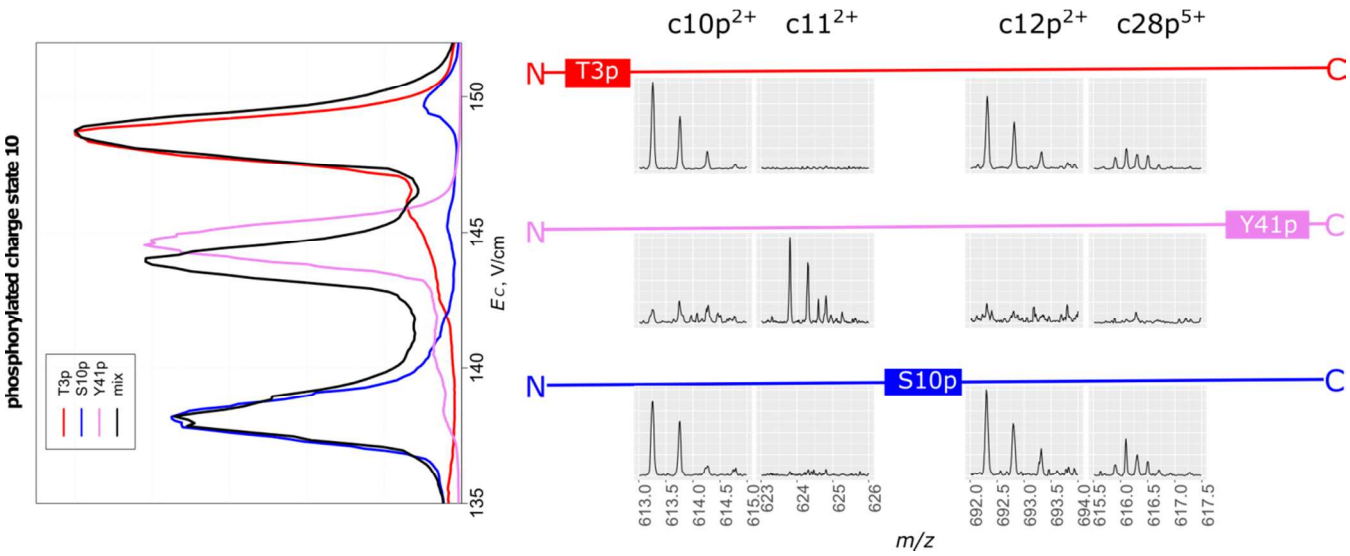


Fig. S7. Validation of FAIMS separation for the T3p/S10p/Y41p variant mixture by ETD MS/MS in the ultrazoom mode.

Table S1. Values of EC (V/cm) for tallest FAIMS spectral peaks: all variants and charge states.

	z = 8	z = 9	z =10	z = 11	z = 12
K4me3	128.5	124.9	135.4	140	132.6
K9me3	122.8	134.5	146.8	139.6	131.3
K23me3	141.2	133	144.7	132.2	131.3
K27me3	131.6	136.9	146	129.8	132.7
K36me3	144.5	155.6	137	125	124.9
K9ac	137.7	137.7	138.5	138.8	134.3
K14ac	139.3	152.2	145.9	133.8	131.8
K18ac	140.4	151.2	150.2	141.1	142.8
K27ac	138.1	138.6	145.2	140.4	141
K36ac	130.2	139.4	149.3	141.3	131.5
T3p	131.4	132.7	148.7	135.1	146.4
T6p	139.2	136.3	150.8	144.1	144.7
S10p	124	137.1	138.2	140.2	145.1
S28p	130.7	137.1	145.9	142.4	144.3
Y41p	128.6	133.1	144.5	140.3	132.3
	z = 8	z = 9	z =10	z = 11	z = 12
K4me3	128.5	124.9	135.4	140	132.6

Table S2. Statistics of correlations between separations in all charge state pairs for trimethylated, acetylated, and phosphorylated variants.

Charge state pair	PTM	Correlation coefficient (R)	R2	Correlation p-value	Adjusted p-value
8, 9	me3	0.64	0.41	0.24	0.91
8, 10	me3	-0.31	0.10	0.61	0.93
8, 11	me3	-0.84	0.70	0.08	0.59
8, 12	me3	-0.68	0.46	0.21	0.90
9, 10	me3	-0.15	0.02	0.81	0.93
9, 11	me3	-0.84	0.71	0.07	0.59
9, 12	me3	-0.92	0.85	0.03	0.40
10, 11	me3	0.08	0.01	0.90	0.93
10, 12	me3	0.42	0.18	0.48	0.92
11, 12	me3	0.68	0.47	0.21	0.90
8, 9	ac	0.55	0.31	0.33	0.92
8, 10	ac	-0.20	0.04	0.75	0.93
8, 11	ac	-0.39	0.15	0.52	0.92
8, 12	ac	0.57	0.32	0.32	0.92
9, 10	ac	0.49	0.24	0.40	0.92
9, 11	ac	-0.50	0.25	0.40	0.92
9, 12	ac	0.13	0.02	0.84	0.93
10, 11	ac	0.31	0.09	0.62	0.93
10, 12	ac	0.24	0.06	0.69	0.93
11, 12	ac	0.50	0.25	0.39	0.92
8, 9	p	-0.02	0.00	0.98	0.98
8, 10	p	0.93	0.86	0.02	0.40

8, 11	p	0.39	0.15	0.51	0.92
8, 12	p	0.19	0.04	0.76	0.93
9, 10	p	-0.31	0.10	0.61	0.93
9, 11	p	0.70	0.49	0.19	0.90
9, 12	p	0.45	0.20	0.45	0.92
10, 11	p	0.08	0.01	0.90	0.93
10, 12	p	0.14	0.02	0.82	0.93