

## Supporting Information for

### Dynamic investigation of hypoxia-induced L-lactylation

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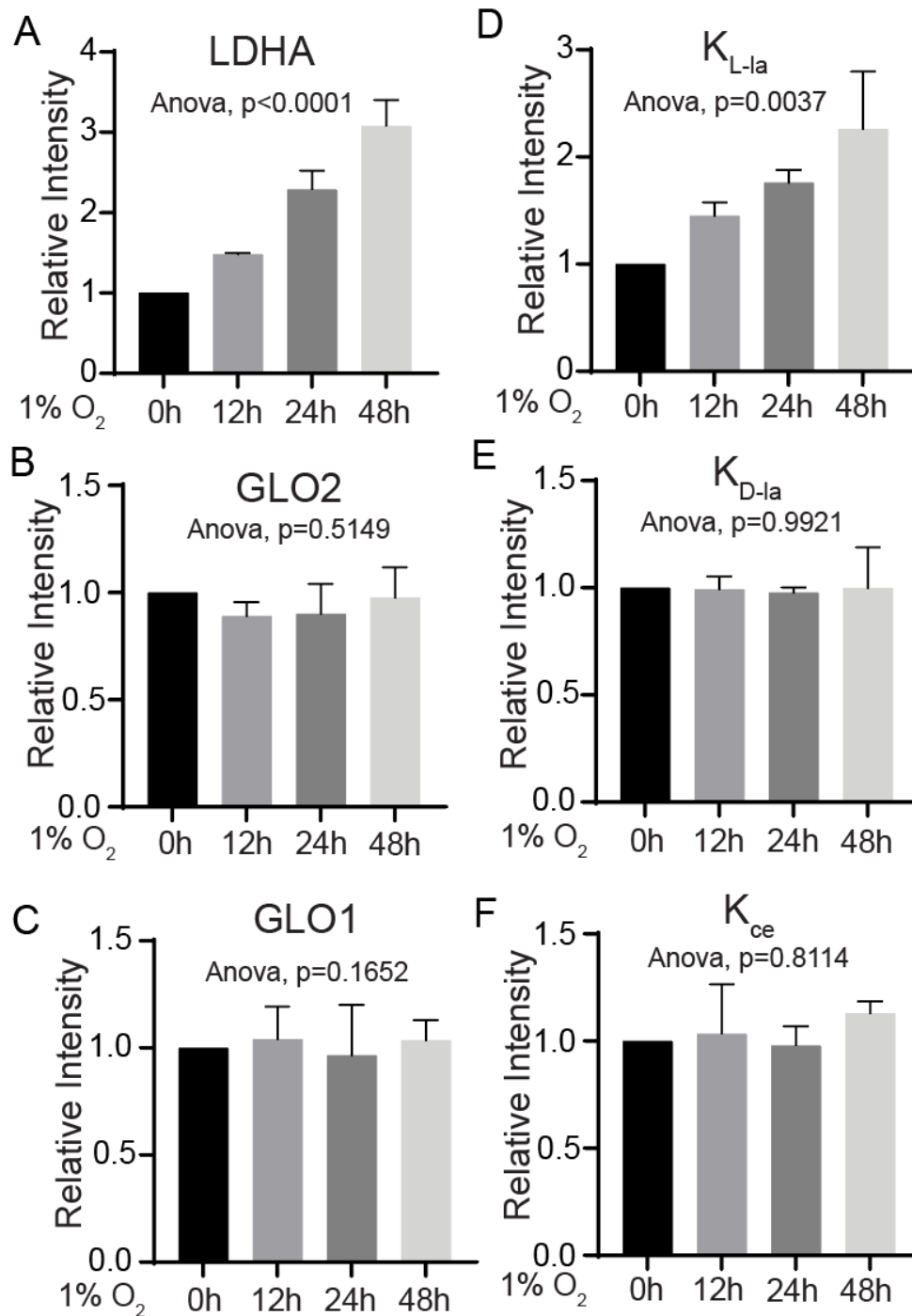
#### **This PDF file includes:**

Figures S1 to S12

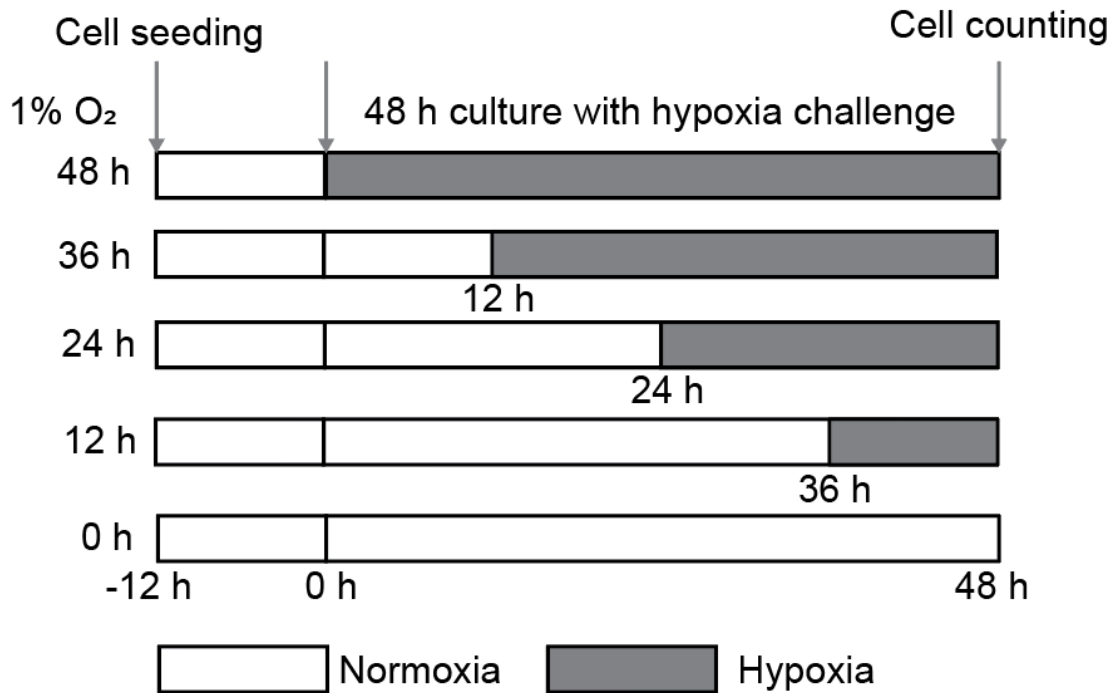
Table S1

Data S1

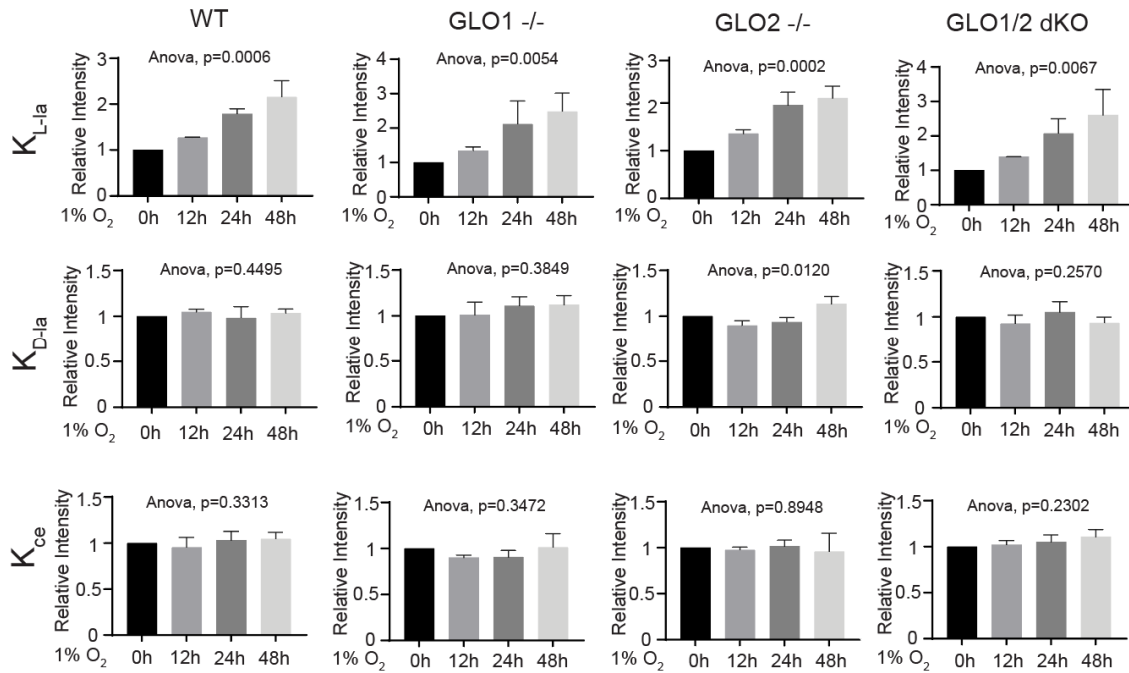
## I. Supplemental figures



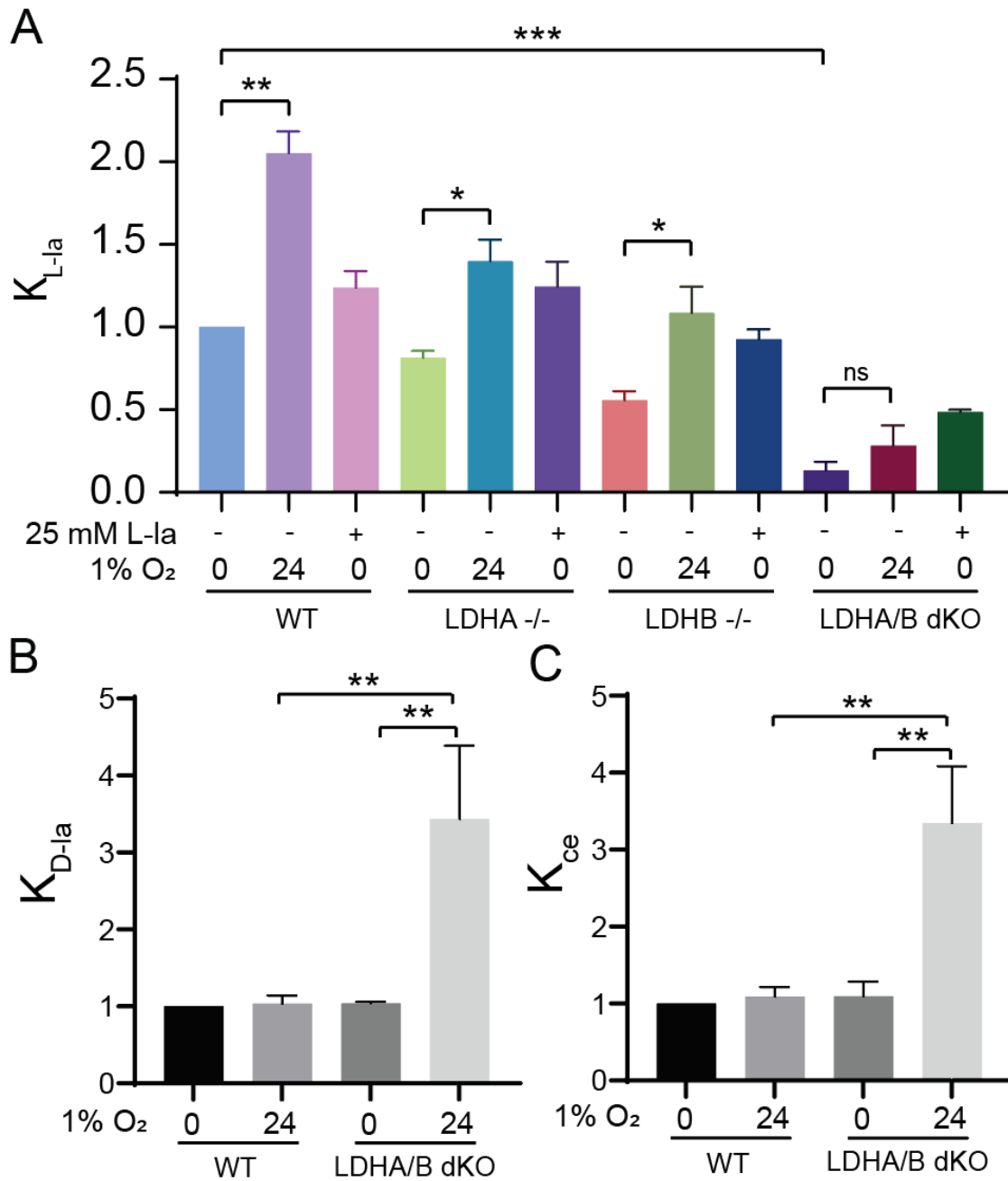
**Figure S1, related to Figure 2. Hypoxia induces K<sub>L-la</sub>, but not K<sub>D-la</sub> or K<sub>ce</sub>, in WT cancer cells.** Densitometry quantification of immunoblots for LDHA (A), GLO2 (B), and GLO1 (C), K<sub>L-la</sub> (D), K<sub>D-la</sub> (E), K<sub>ce</sub> (F), normalized by the loading control. In each experiment, values were then normalized to the normoxia group. Values are the mean of three experiments. Statistical analysis was performed by one-way ANOVA.



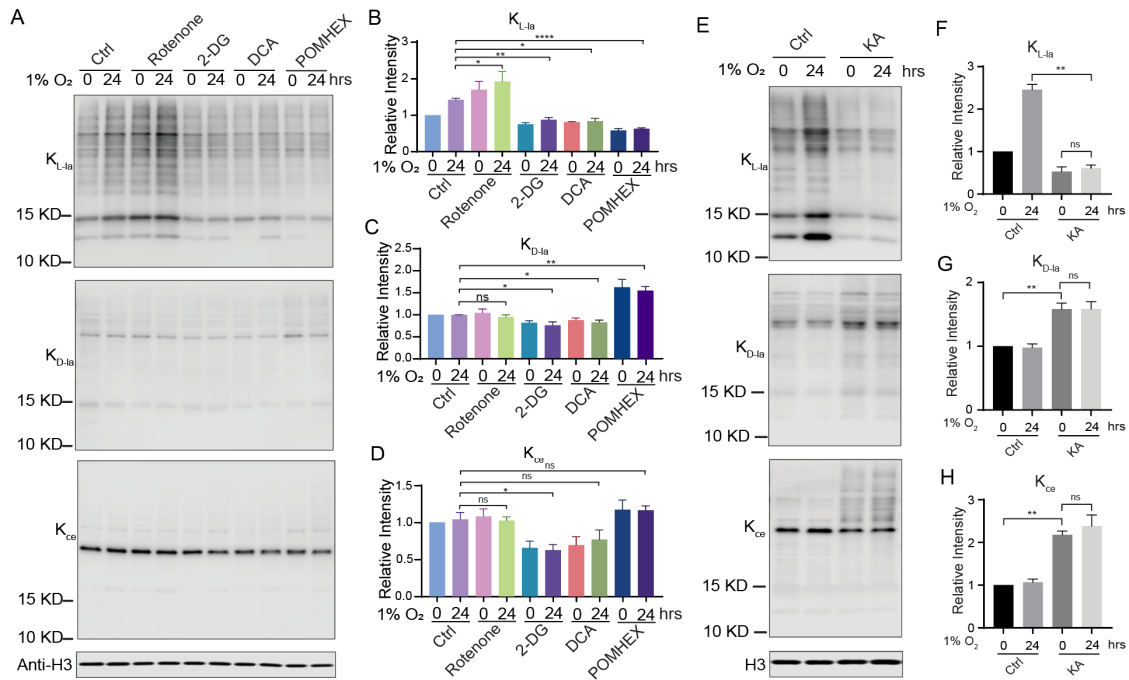
**Figure S2, related to Figure 3. Schematic workflow for evaluating cell adaptability to hypoxia.** For each cell line, cells were cultured for 12 h prior to hypoxia treatment. During the 48-h culture time, cells were treated with 48 h, 36 h, 24 h, 12 h, or 0 h hypoxia before measuring viability. Cell numbers were normalized to the normoxia group (0 h hypoxia) to assess hypoxia adaptation between different cell types.

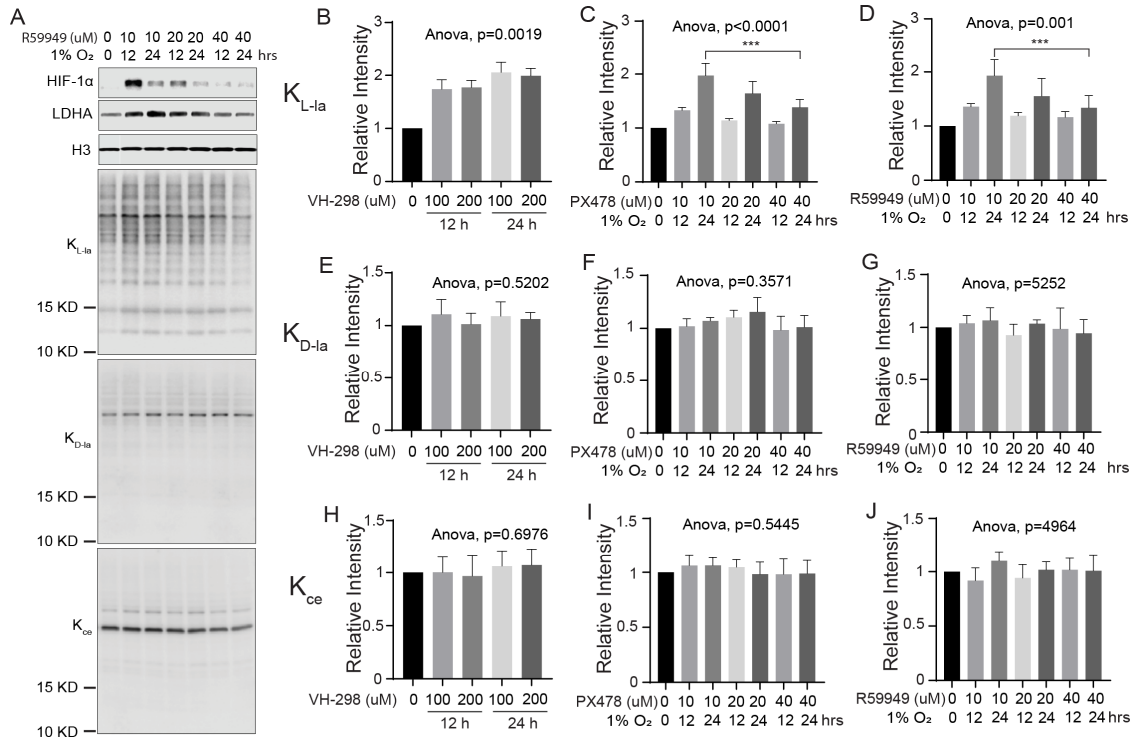


**Figure S3, related to Figure 3. Hypoxia induces K<sub>L-la</sub>, but not K<sub>D-la</sub> or K<sub>ce</sub>, in glyoxalase-deficient cells.** Densitometry quantification of immunoblots for K<sub>L-la</sub>, K<sub>D-la</sub>, and K<sub>ce</sub>, normalized by the loading control. In each experiment, values were then normalized to the normoxia group. Values are the mean of three experiments. Statistical analysis was performed by one-way ANOVA.

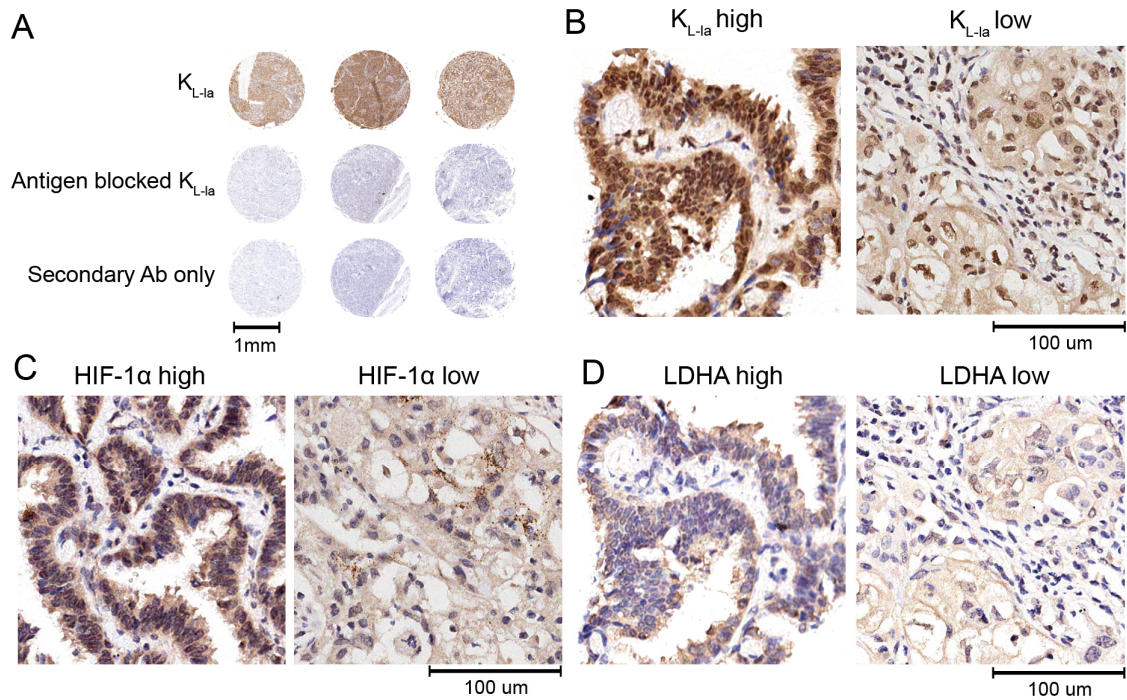


**Figure S4, related to Figure 4. Hypoxia-induced  $K_{L-la}$  levels in LDH-deficient cells.** Densitometry quantification of immunoblots for (A)  $K_{L-la}$ , (B)  $K_{D-la}$  and  $K_{ce}$  (C) was conducted and normalized by the loading control. In each experiment, values were then normalized to the control group (WT cells with no treatment). Values are the mean of two experiments for  $K_{L-la}$  and three experiments for  $K_{D-la}$  and  $K_{ce}$ . Statistical analysis was performed by student's t-test. \*:  $p < 0.05$ ; \*\*:  $p < 0.01$ ; \*\*\*:  $p < 0.001$ ; ns: not significant.

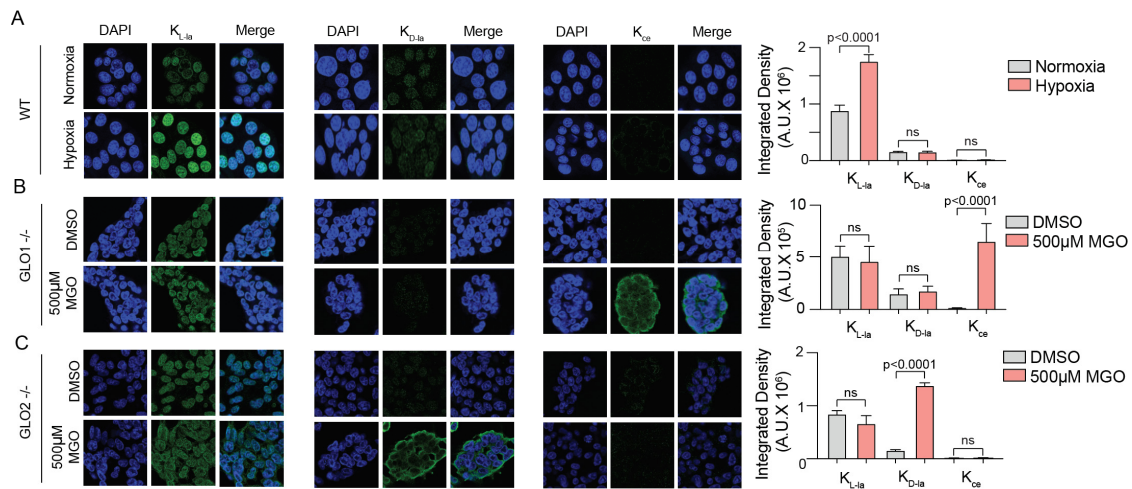




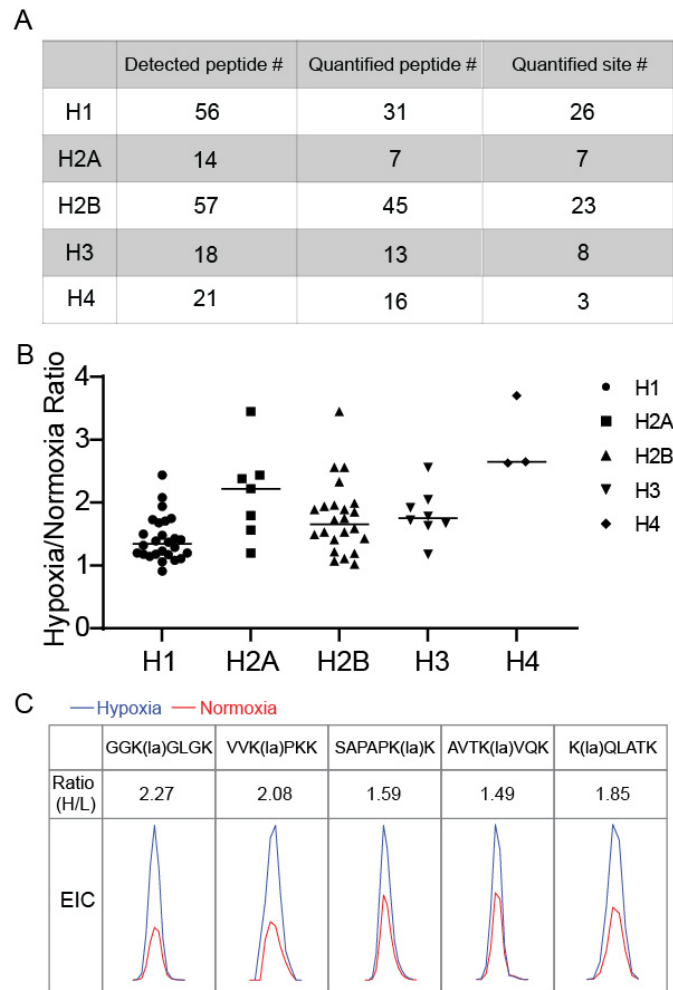
**Figure S6, related to Figure 5. K<sub>L-la</sub> correlates with HIF-1α.** (A) Immunoblots of whole-cell lysates of MCF-7 cells in response to hypoxia coupled with R59945, which inhibits HIF-1α-mediated transcription in response to hypoxia. (B-J) Densitometry quantification of immunoblots depicting whole-cell lysates of MCF-7 cells treated with hypoxia and different HIF-1α modulators for K<sub>L-la</sub> (B-D), K<sub>D-la</sub> (E-G), and K<sub>ce</sub> (H-J). In each experiment, quantification values were first normalized by the loading control, then normalized to the control group (WT cells with no treatment). Values are the mean of three experiments. Statistical analysis was performed by one-way ANOVA.



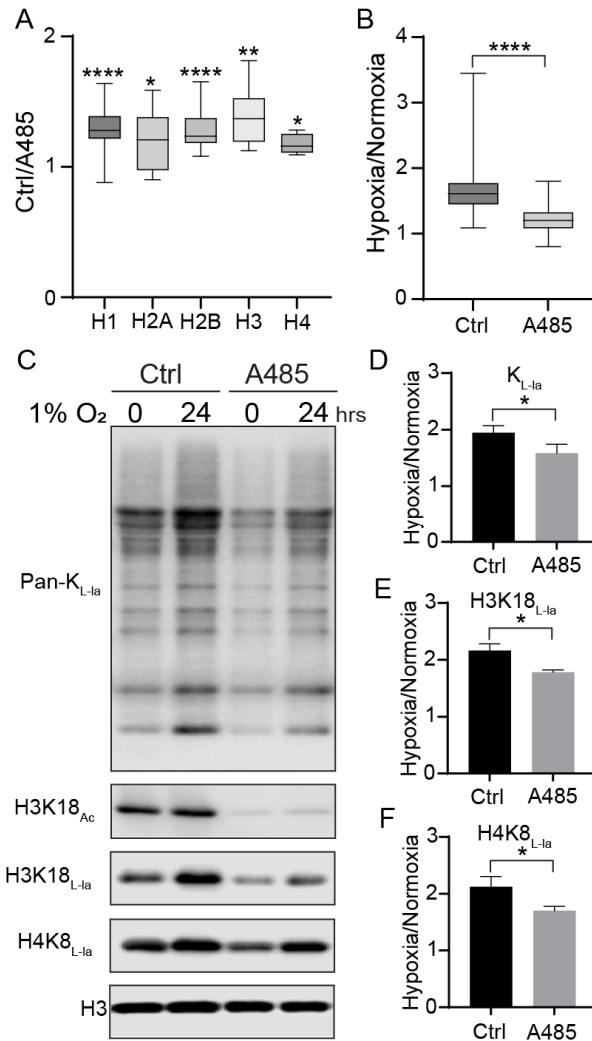
**Figure S7, related to Figure 5. Quality control and zoom-in view of the IHC staining results.** (A) Representative images from human lung adenocarcinoma tissue microarrays stained for K<sub>L-la</sub> (top), and two negative controls: blocking the K<sub>L-la</sub> antibody with a K<sub>L-la</sub> peptide library before conducting the staining (middle), or the primary antibody was omitted, while all other procedures remained the same (bottom). (B-D) Zoom-in view of K<sub>L-la</sub> (B), HIF-1α (C), and LDHA (D) staining as in Fig. 5D.



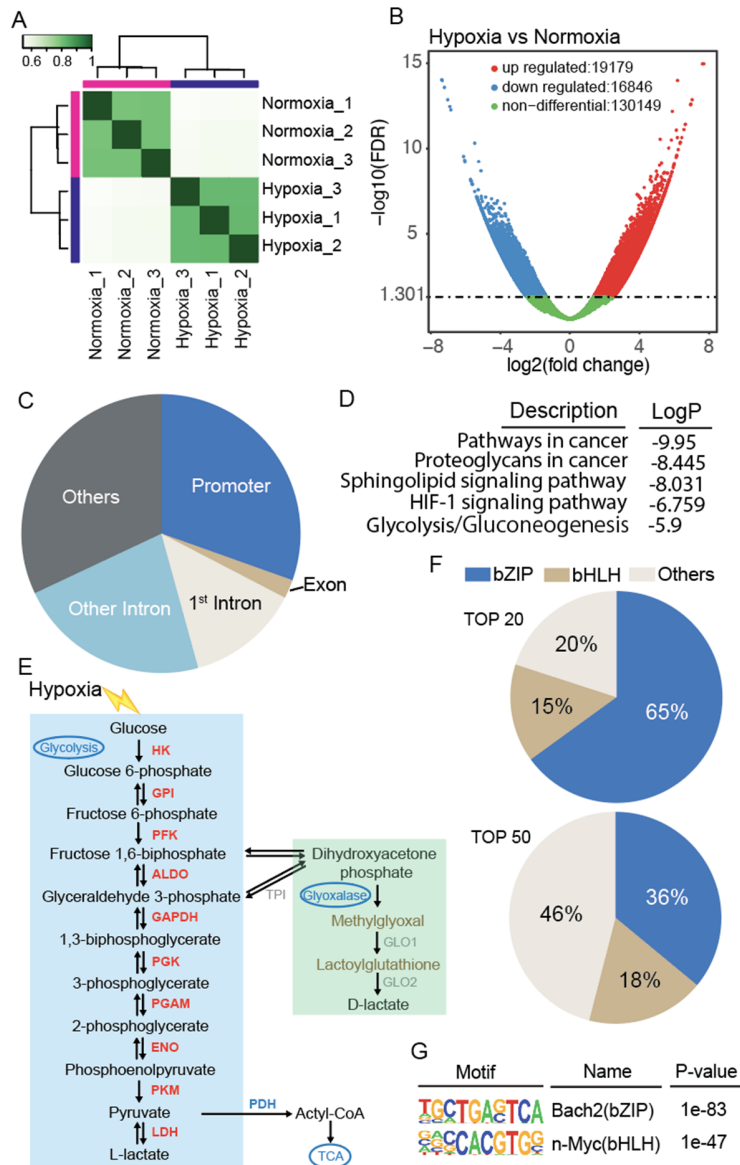
**Figure S8. Subcellular localization distinguishes hypoxia-induced K<sub>L-1a</sub> from K<sub>D-1a</sub> and K<sub>ce</sub>.** (A) Immunofluorescence staining was conducted on WT MCF-7 cells under hypoxic and normoxic conditions with K<sub>L-1a</sub>, K<sub>D-1a</sub>, and K<sub>ce</sub> antibodies. (B-C) Immunostaining images of HEK293T GLO1<sup>-/-</sup> (B) and GLO2<sup>-/-</sup> (C) cells treated with DMSO or 500 μM MGO for 6 hours. DAPI: 4',6-diamidino-2-phenylindole (nuclear stain). Statistical analysis was performed by student's t-test. ns: not significant.



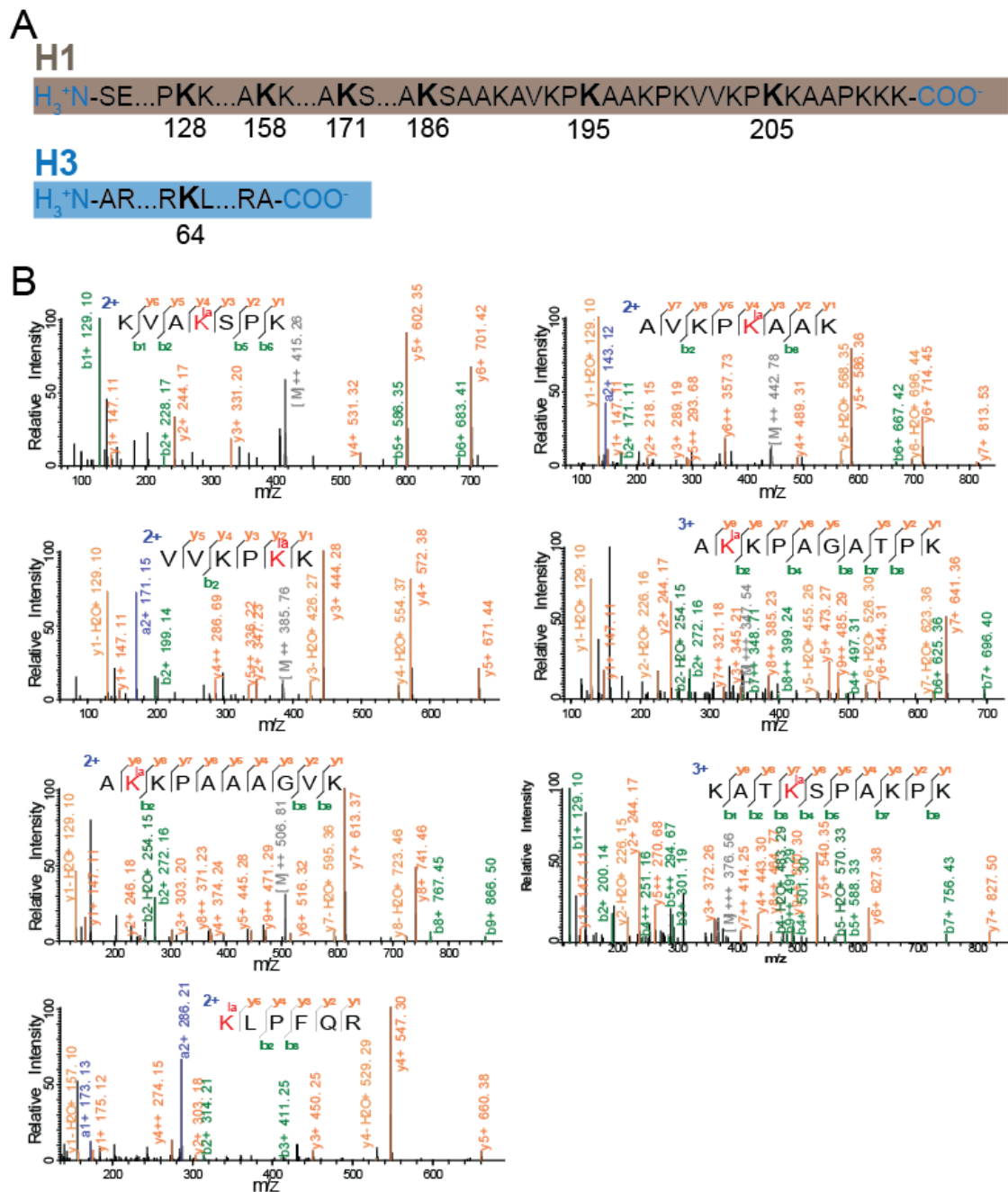
**Figure S9, related to Figure 6. Hypoxia-regulated K<sub>L-la</sub> sites.** (A) Statistics of proteomics results of K<sub>L-la</sub> peptides on histone proteins. (B) Distribution of hypoxia/normoxia ratios for quantified histone K<sub>L-la</sub> sites. The ratios are grouped by histone protein. (C) Ratios and chromatographs of representative K<sub>L-la</sub> peptides.



**Figure S10. p300/CBP drives hypoxia-induced K<sub>L-1a</sub>.** (A) Distribution of control/A485 ratios for quantified histone K<sub>L-1a</sub> sites. The ratios are grouped by histone proteins. (B) Distribution of hypoxia/normoxia ratios for quantified histone K<sub>L-1a</sub> sites, either treated with DMSO or 30  $\mu$ M A485. (C) Immunoblots of whole-cell lysates of MCF-7 cells treated with hypoxia and 30  $\mu$ M A485. (D-F) Densitometry quantification of immunoblots for K<sub>L-1a</sub> (D), H3K18<sub>L-1a</sub> (E), and H4K8<sub>L-1a</sub> (F), normalized by the loading control. Values are the mean of three experiments. Statistical analysis was performed by student's t-test. \*: p < 0.05; \*\*: p < 0.01; \*\*\*\*: p < 0.0001.



**Figure S11. Hypoxia-induced  $K_{L-1a}$  is associated with glycolysis gene activation.** (A) Heat-map of Pearson correlation coefficient among samples. The shade of color represents correlation coefficient. (B) Volcano plot illustrating differential peaks between normoxia and hypoxia groups. Each point represents a peak, with significantly upregulated genes in red and downregulated genes in blue, and non-significant genes in green. The horizontal dashed line indicates significance threshold of false-discovery rate (FDR). (C) Pie chat depicting the functional regions of the differential peaks. (D) Selected KEGG pathways associated with genes with promoters marked by hypoxia-induced H3K18<sub>L-1a</sub>. (E) Glycolysis and glyoxalase genes with promoters marked by H3K18<sub>L-1a</sub>. Red: hypoxia upregulated; blue: hypoxia downregulated; gray: unidentified. (F) HOMER motif analysis of all hypoxia-upregulated H3K18<sub>L-1a</sub> peaks. Pie charts demonstrate the distribution of the top 20 (upper) and 50 (lower) significant motifs. (G) Representative motifs from bZIP and bHLH transcription factors in (F).



**Figure S12. Newly identified histone K<sub>L-la</sub> marks.** (A) Seven new histone marks were identified from the proteomics data for studying hypoxia-regulated histone K<sub>L-la</sub>, of which six were on histone H1 and one was on histone H3. (B) The peptide-spectrum matches (PSMs) of these newly identified histone marks were confirmed by manual verification.

## II. Supplemental tables

**Table S1. Quantified hypoxia/normoxia ratios of histone K<sub>L-1a</sub> sites.**

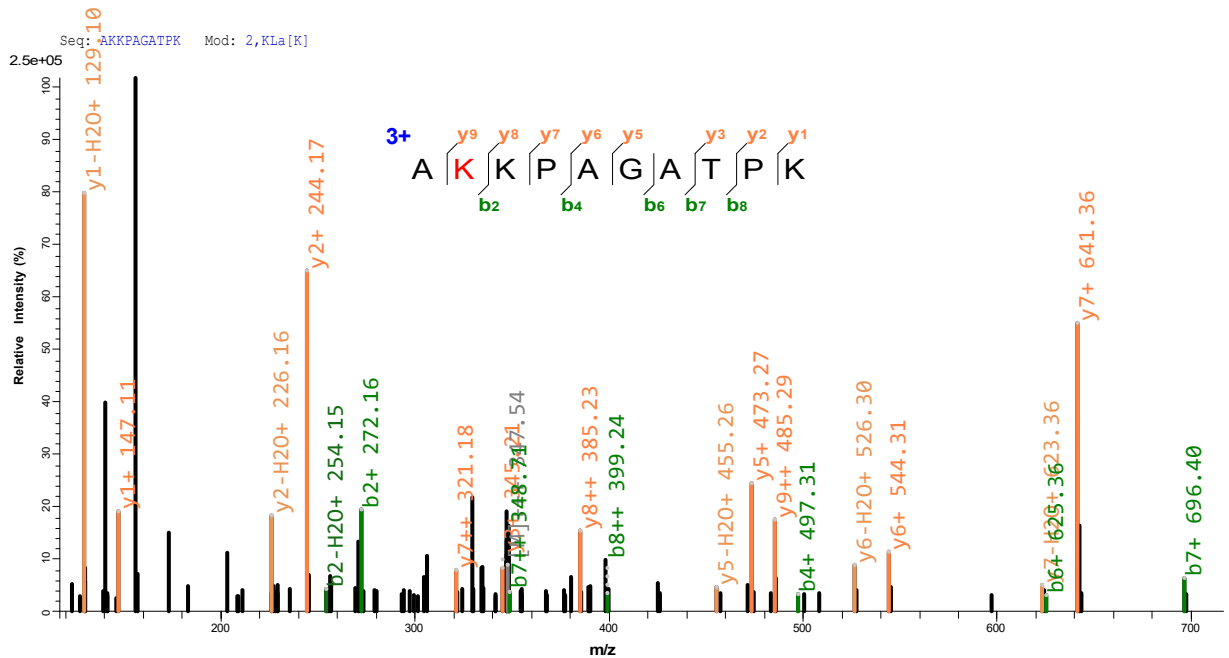
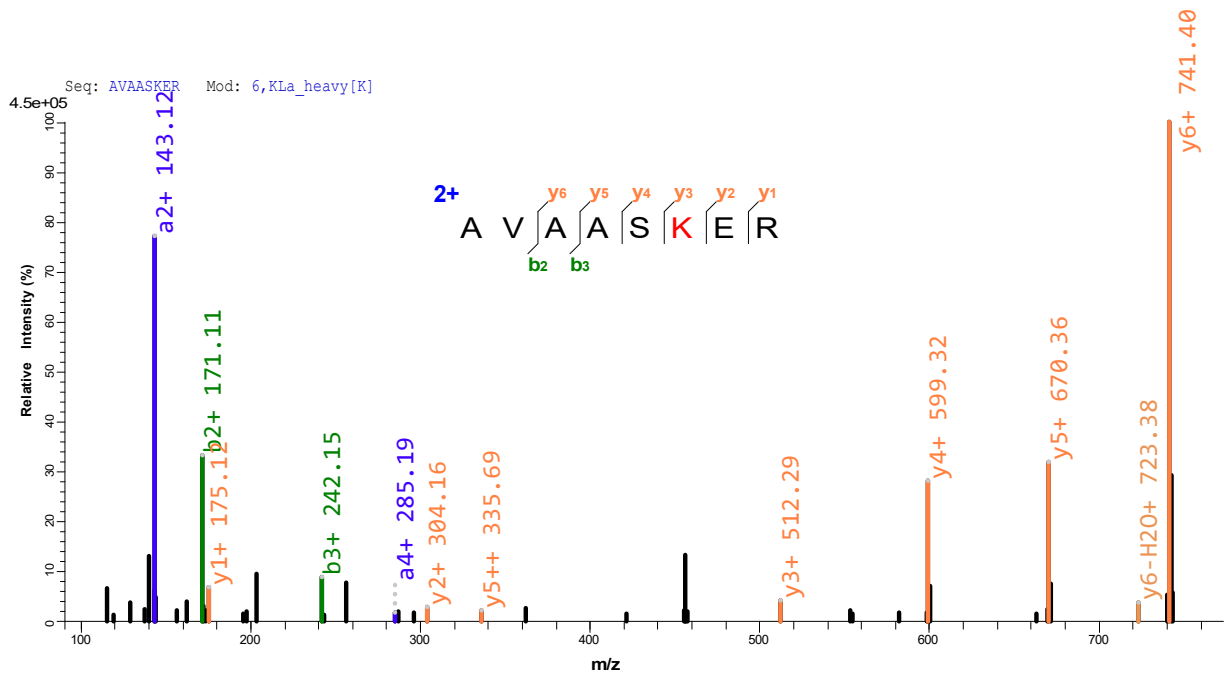
<b>Kla Site</b>	<b>Hypoxia/Normoxia</b>	<b>Kla Site</b>	<b>Hypoxia/Normoxia</b>
H1.2K33	1.75	H1.2K51	2.44
H1.2K62	1.39	H1.2K89	1.23
H1.2K96	1.2	H1.2K105	0.91
H1.2K158	1.48	H1.2K167	1.5
H1.2K171	1.15	H1.2K190	1.73
H1.2K195	1.92	H1.2K200	1.18
H1.2K205	2.08	H1.5K36	1.32
H1.5K131	1.68	H1.5K159	1.29
H1.5K167	1.18	H1.5K187	1.06
H1.5K193	1.31	H1.1K187	1.41
H1.3K140	1.2	H1.3K148	1.71
H1.3K168	1.11	H1.3K178	1.43
H1.4K158	1.17	H1.4K167	1.08
H2AK9	2.38	H2AK36	1.79
H2AK95	2.22	H2AJK9	2.44
H2AZK11	3.45	H2AZ15	1.56
H2AX127	1.2	H2B1CK5	2.56
H2B1CK11	1.89	H2B1CK12	1.89
H2B1CK15	1.94	H2B1CK16	1.96
H2B1CK20	1.53	H2B1CK23	1.19
H2B1CK85	1.07	H2B1CK108	3.45
H2B1CK116	1.49	H2B1CK120	2.33
H2B1DK5	1.59	H2B1DK11	2.56
H2B1MK5	1.43	H2B1MK11	1.75
H2B1MK15	1.41	H2B1MK16	1.72
H2B1MK20	1.53	H2B2FK5	1.85
H2B2FK11	1.11	H2B2FK15	1.22
H2B2FK16	1.99	H2B2FK20	1.02
H3K9	2.56	H3K14	1.92
H3K18	2.05	H3K23	1.72
H3K27	1.64	H3K56	1.18

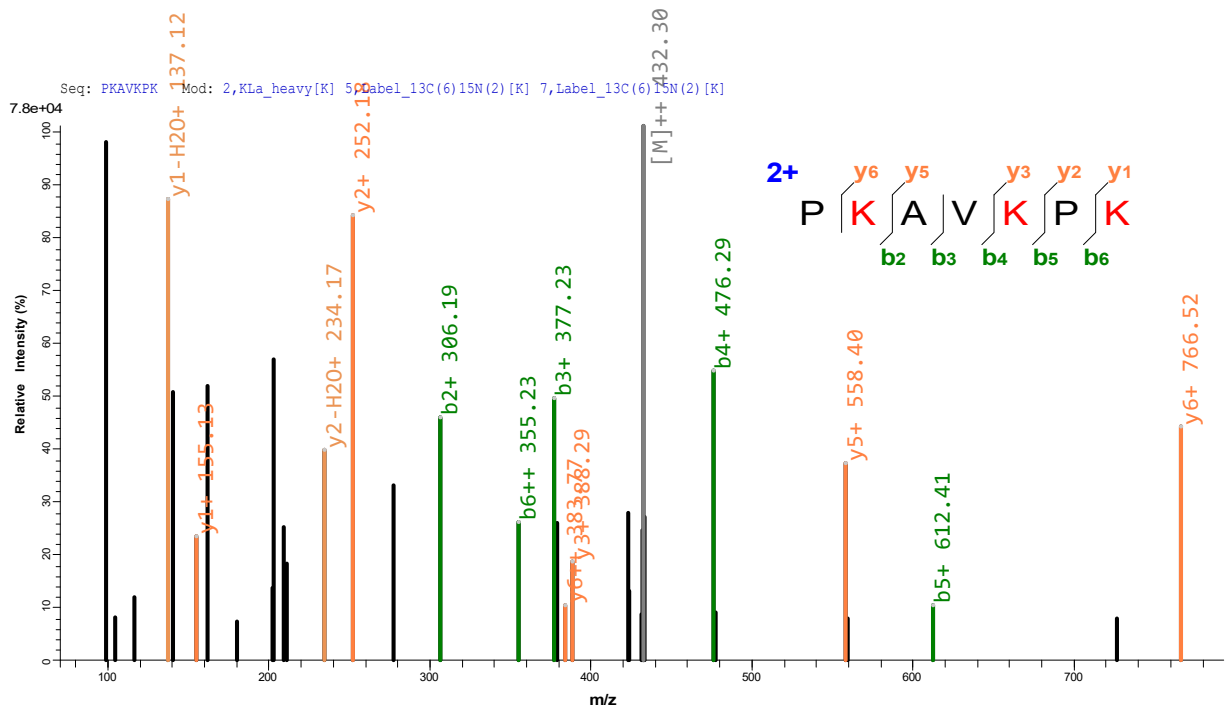
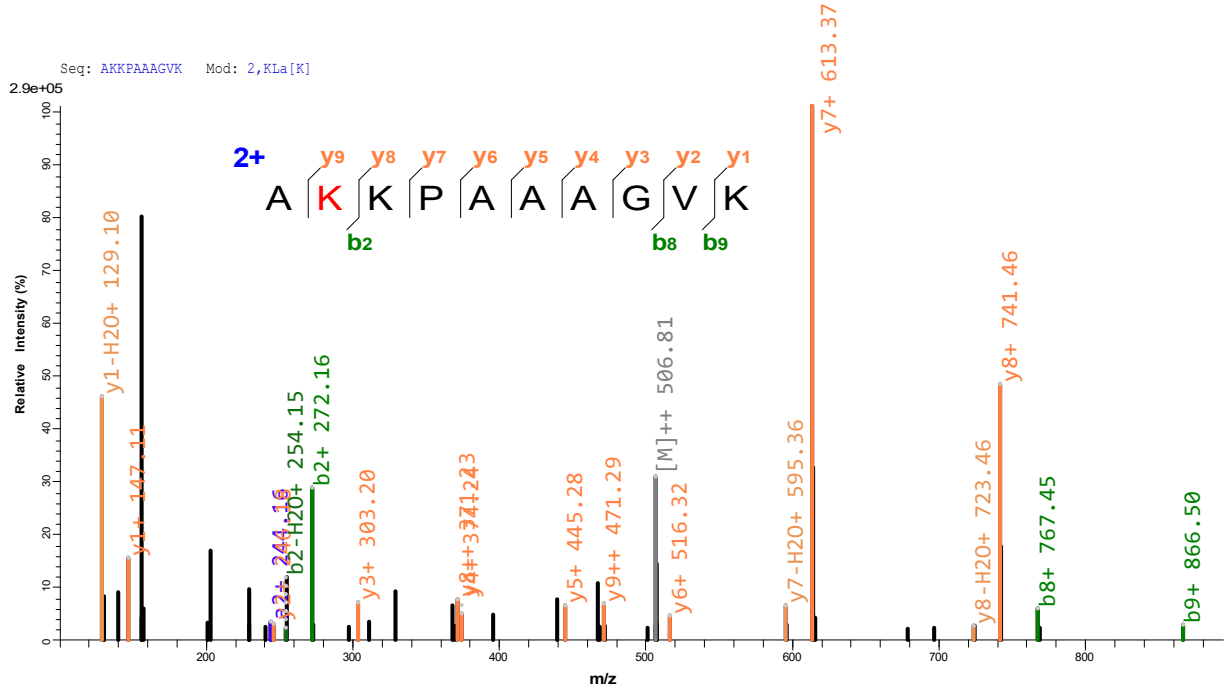
H3K79	1.67	H3K122	1.785
H4K5	3.7	H4K8	2.65
H4K12	2.63		

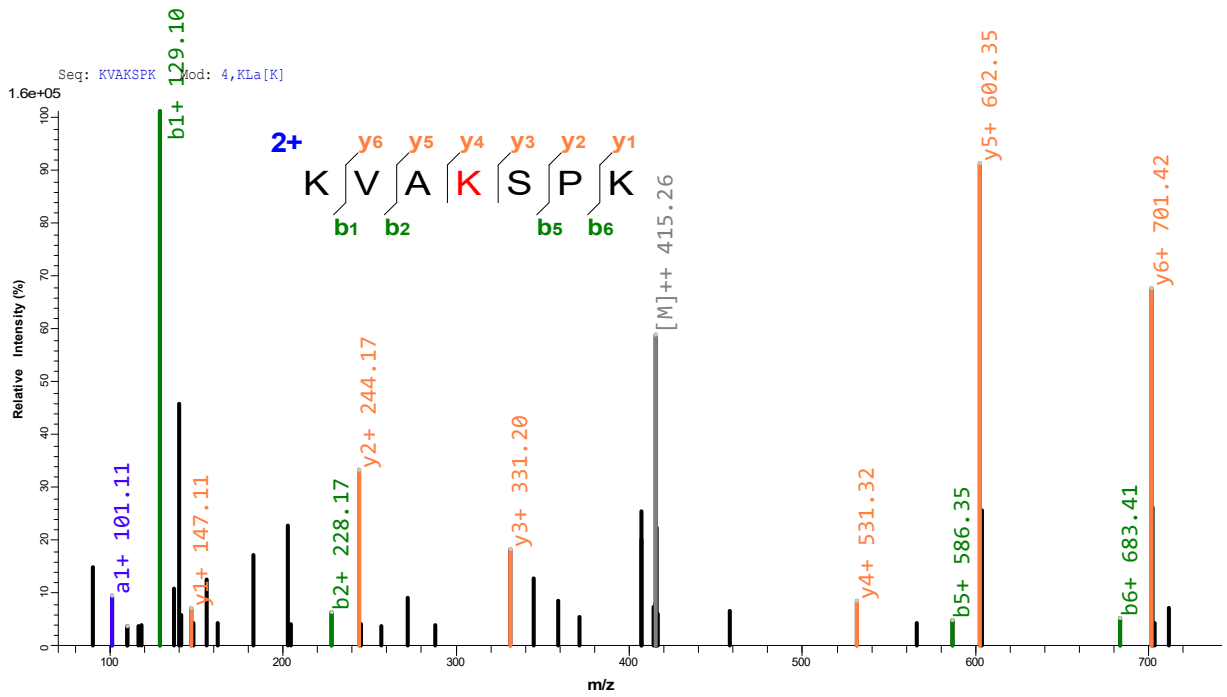
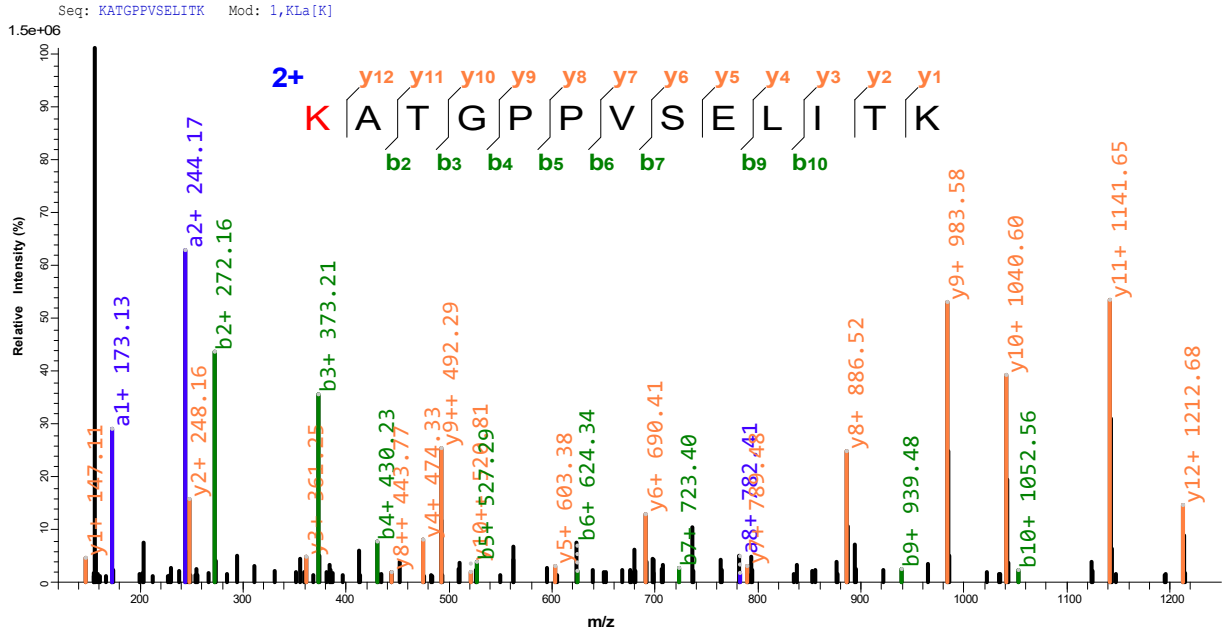
### III. Supplemental data

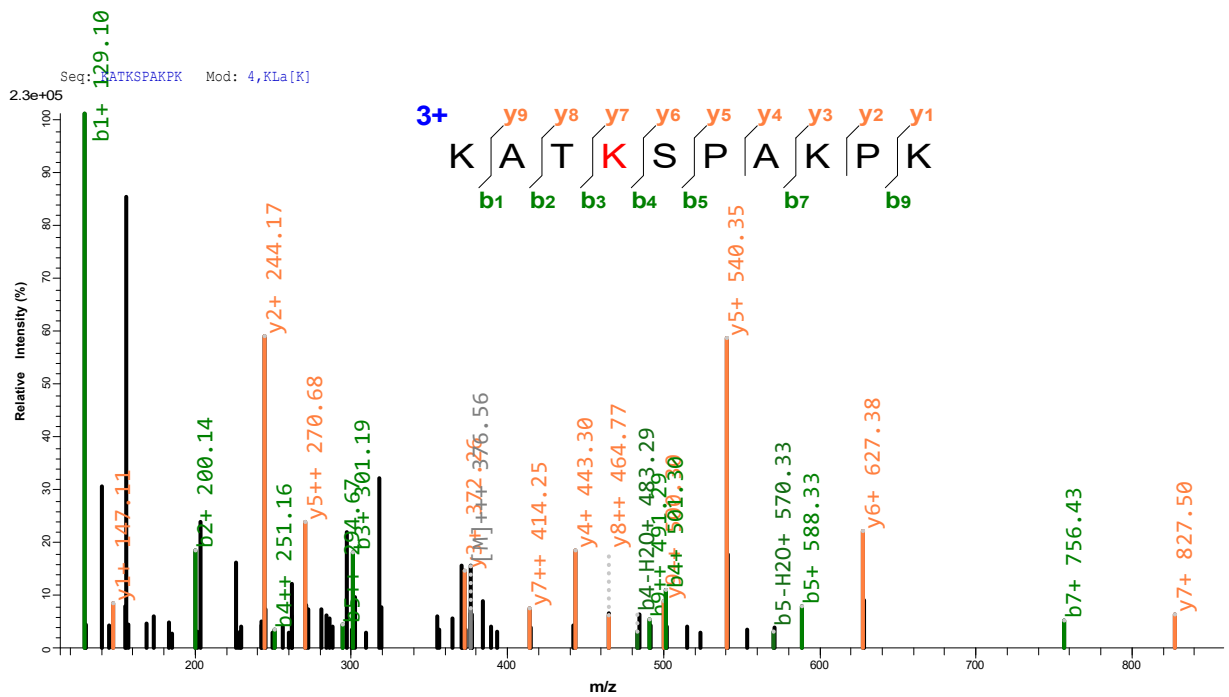
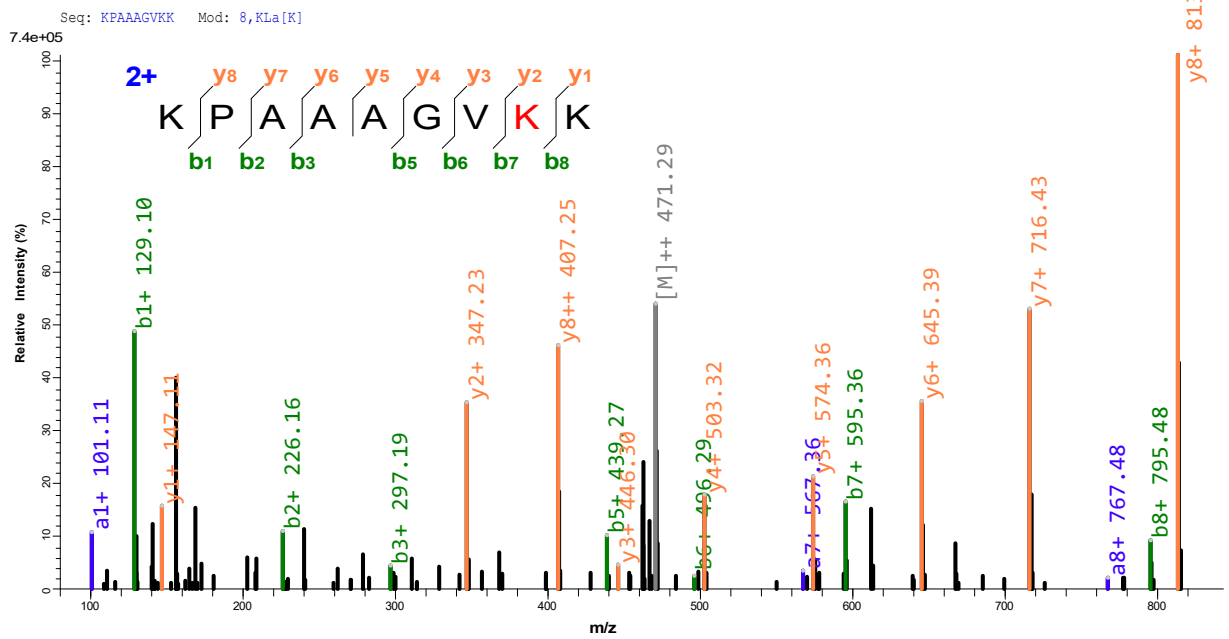
#### Data S1. PSMs for quantified K<sub>L</sub>-la peptides

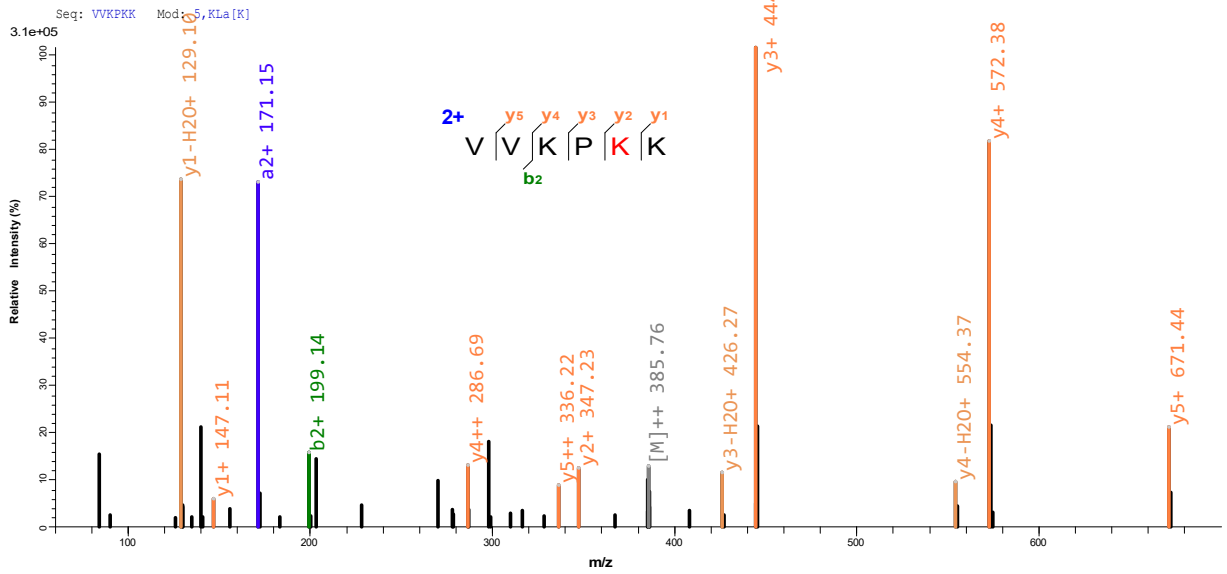
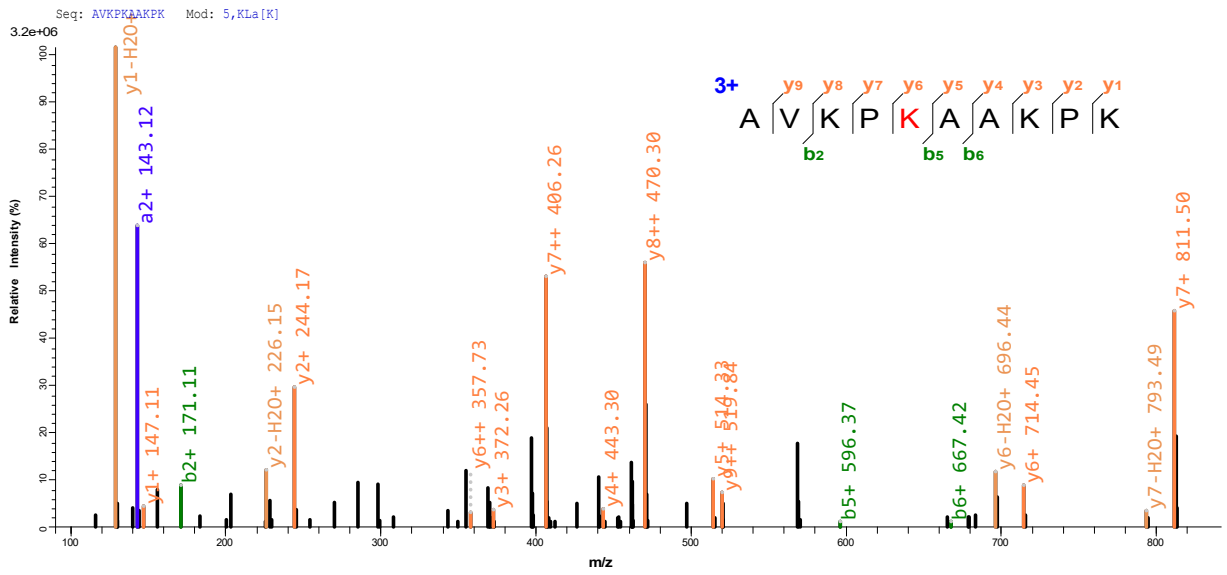
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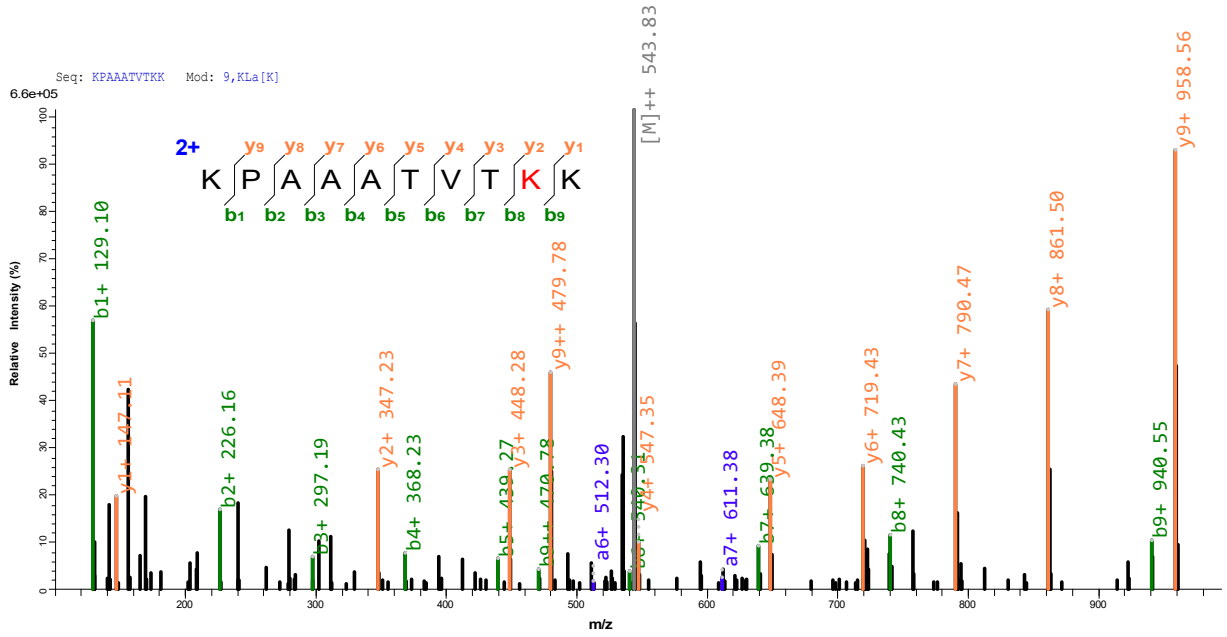
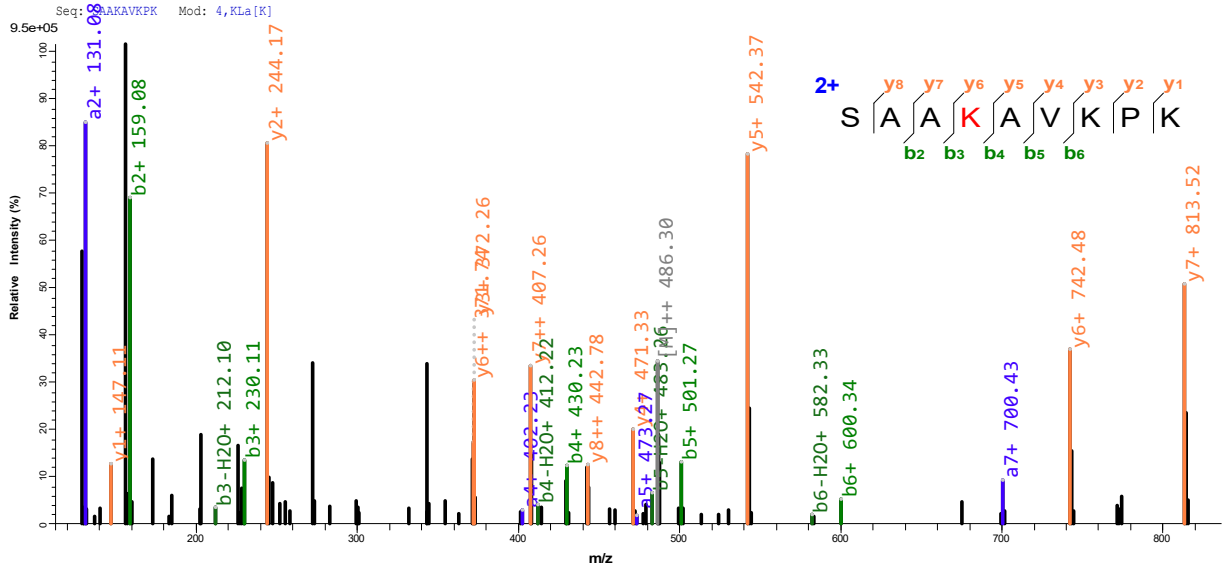


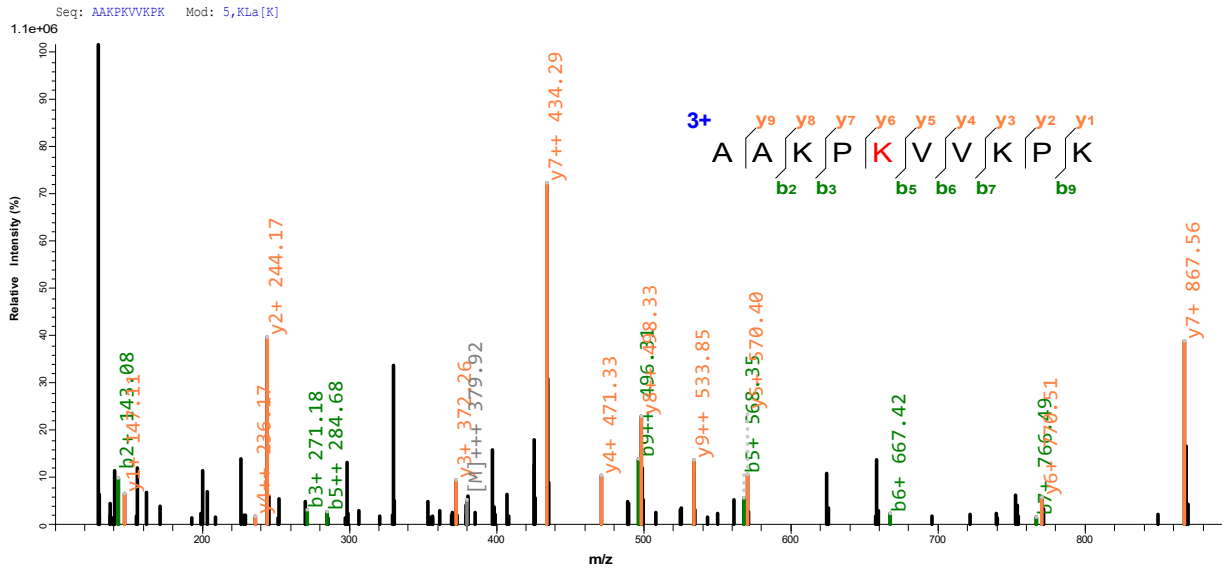
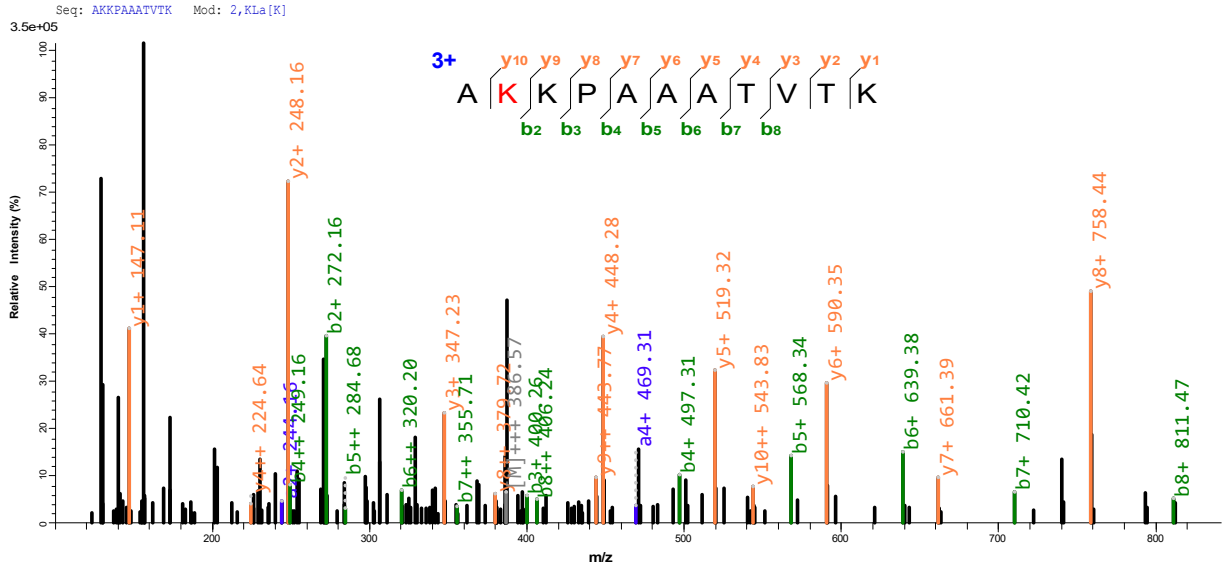


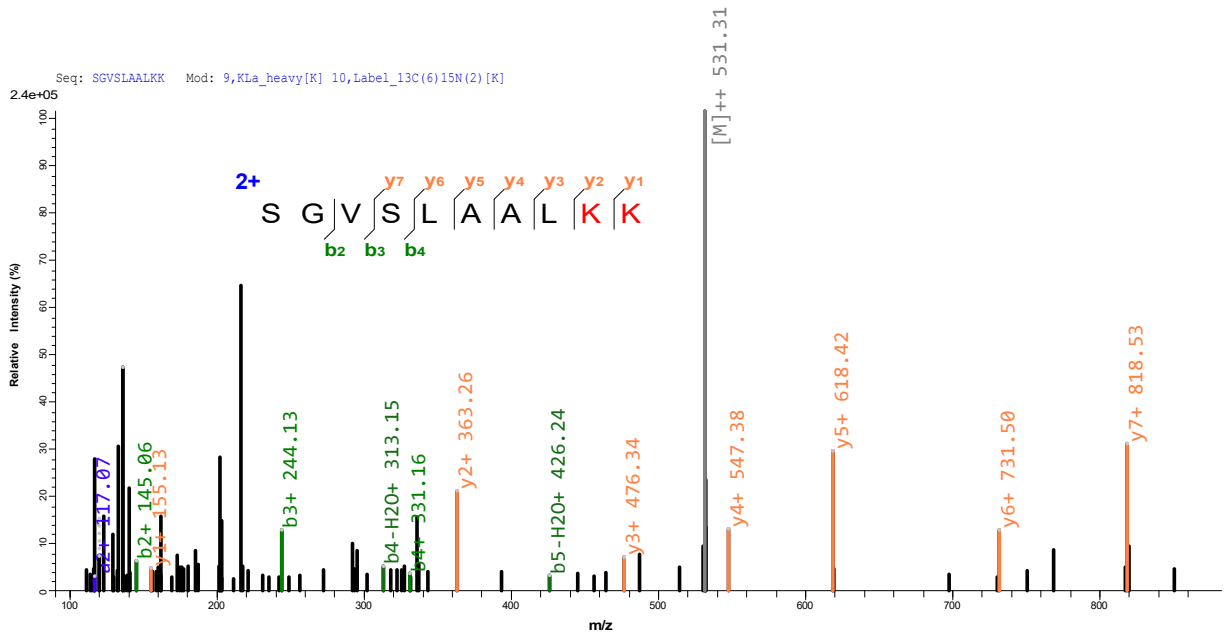
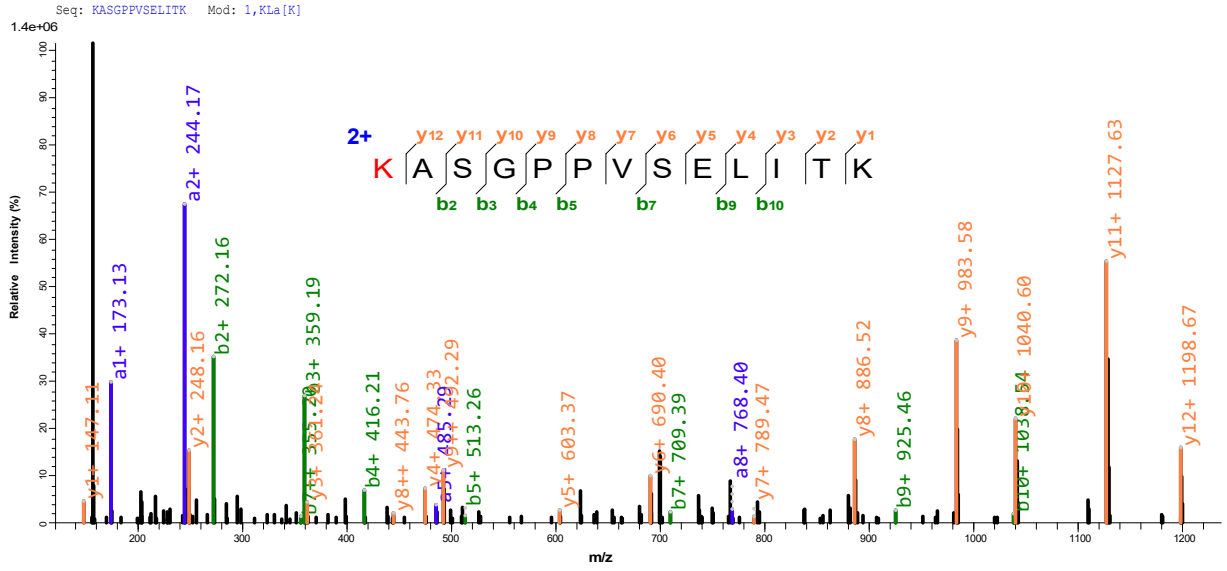


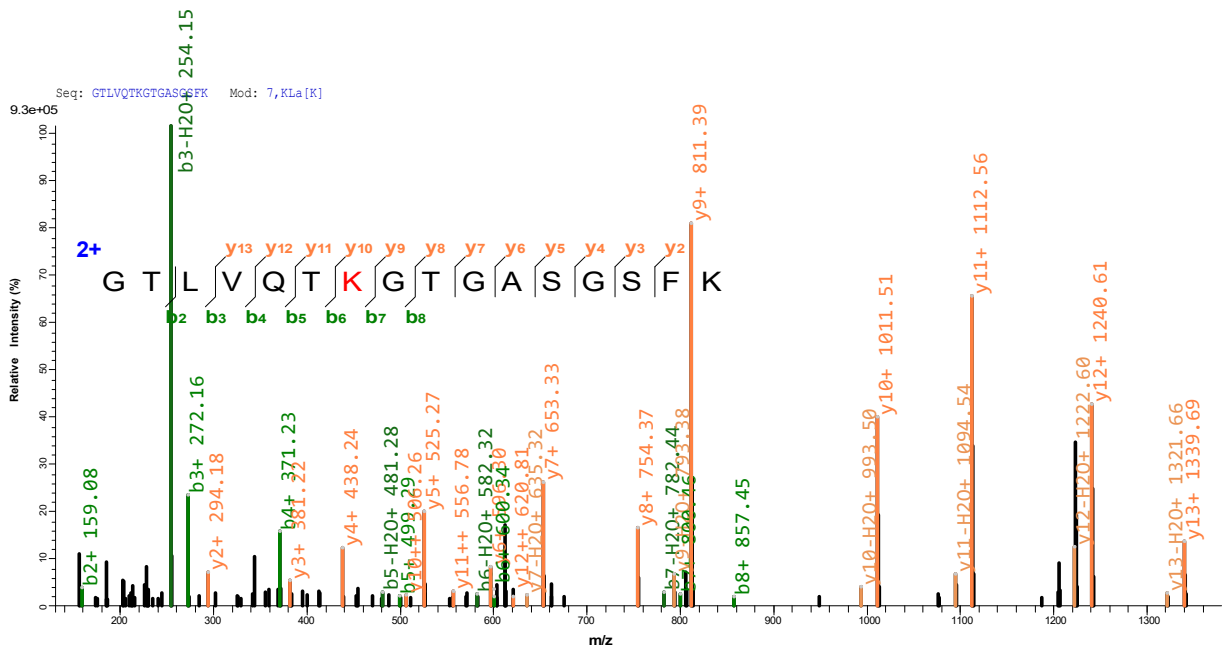
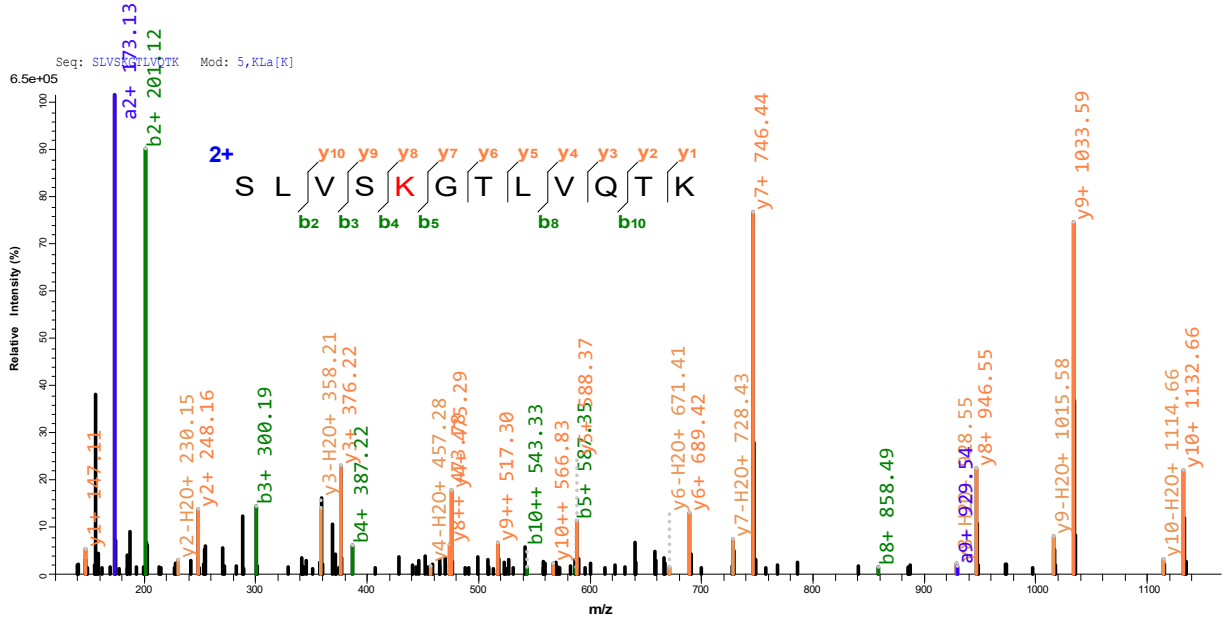


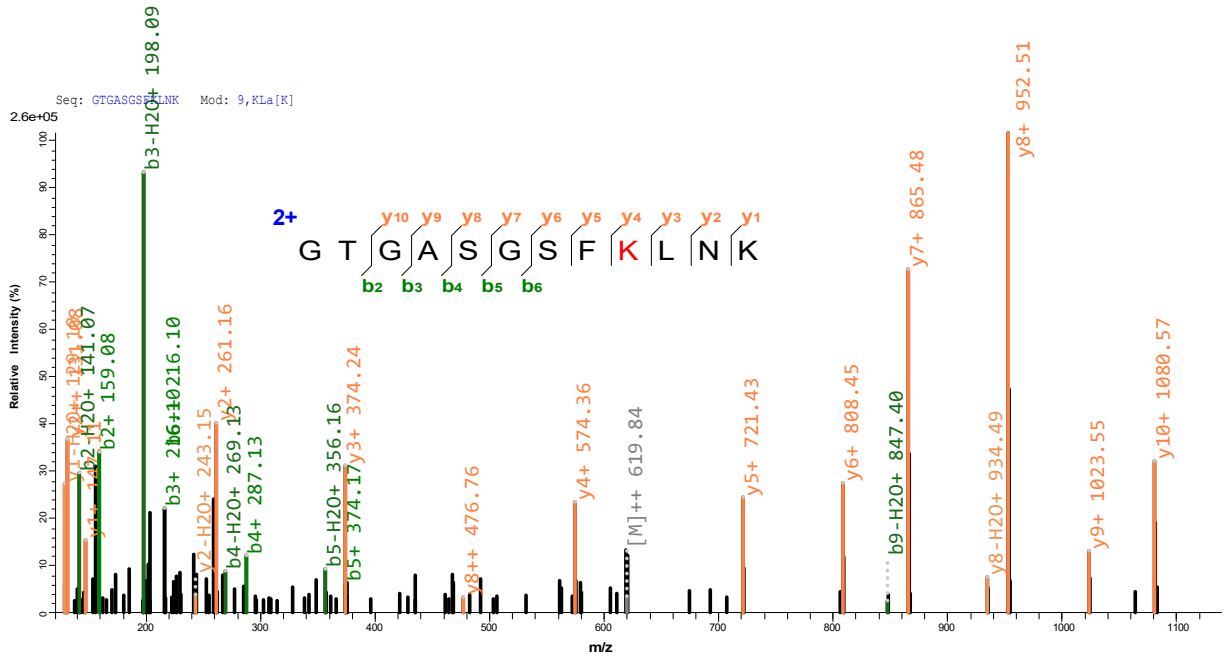
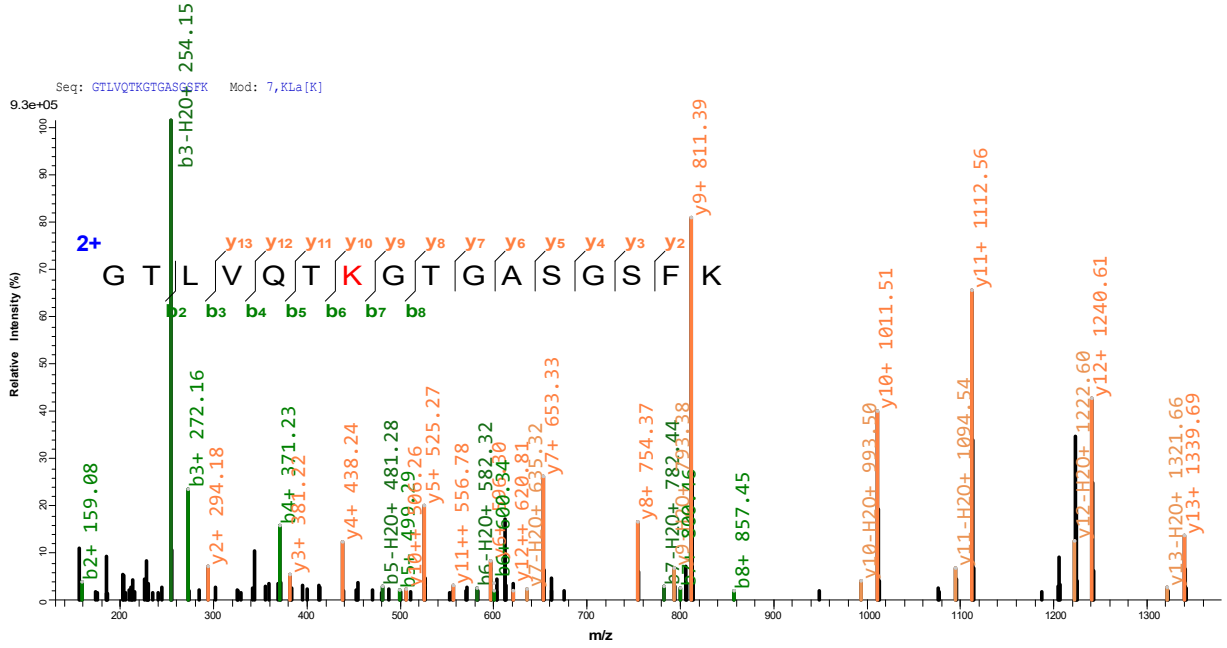


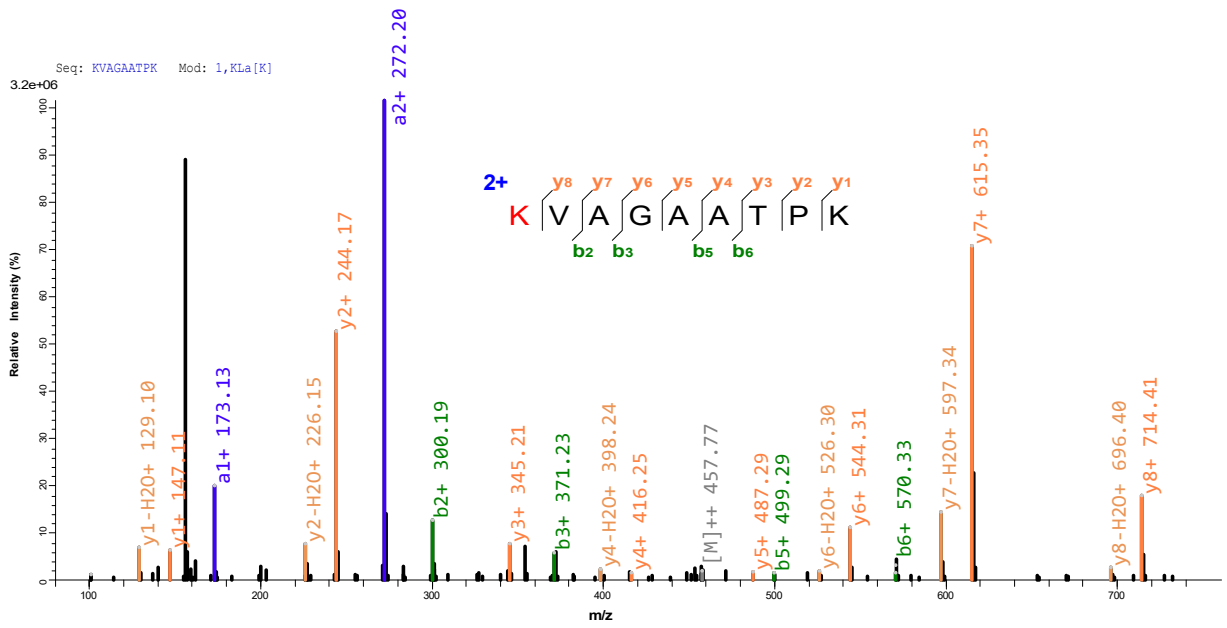
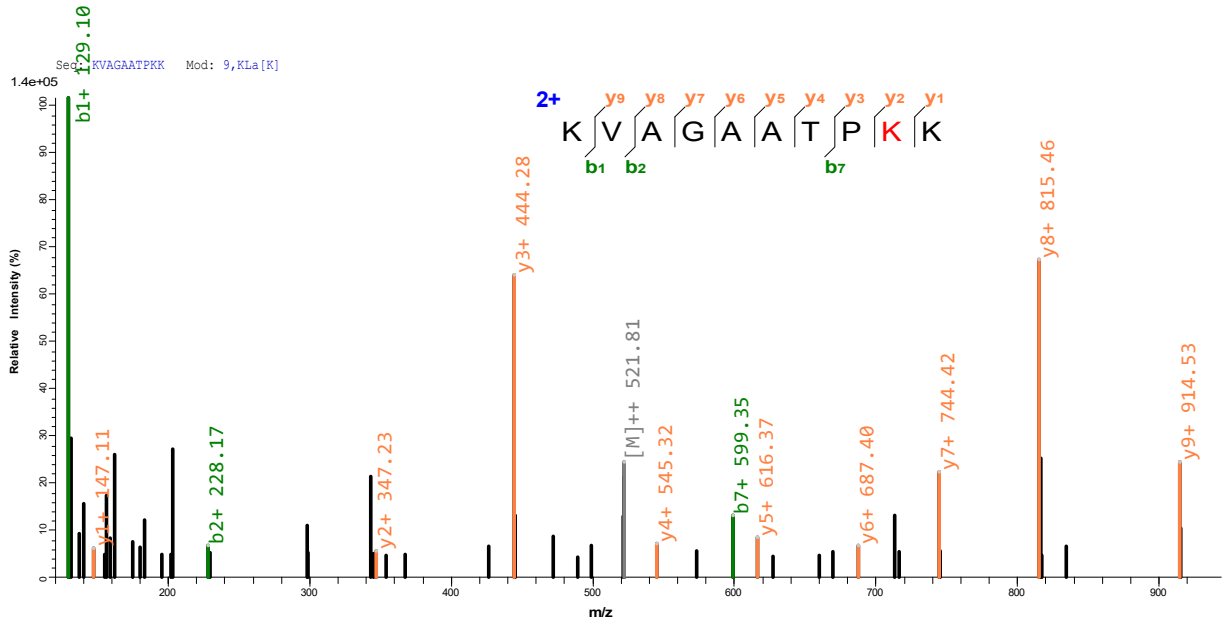


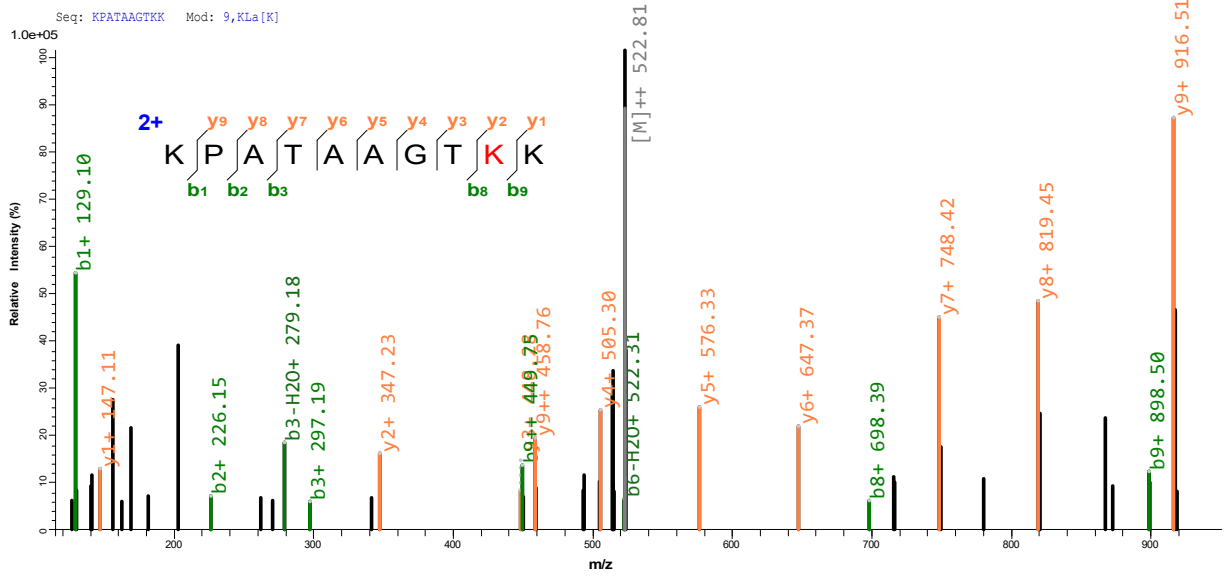
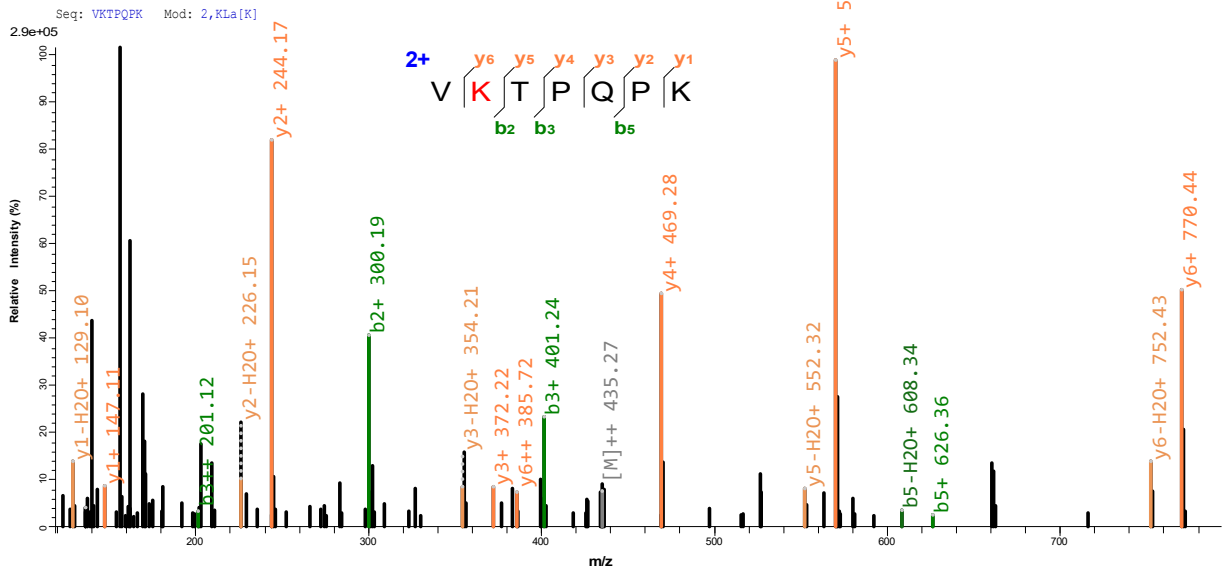


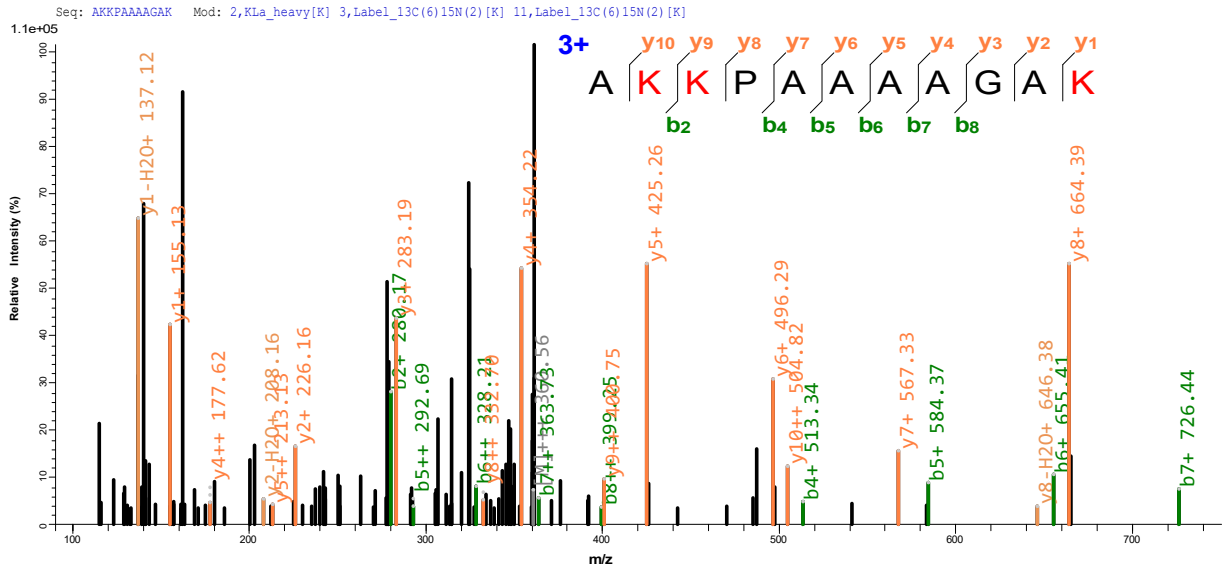
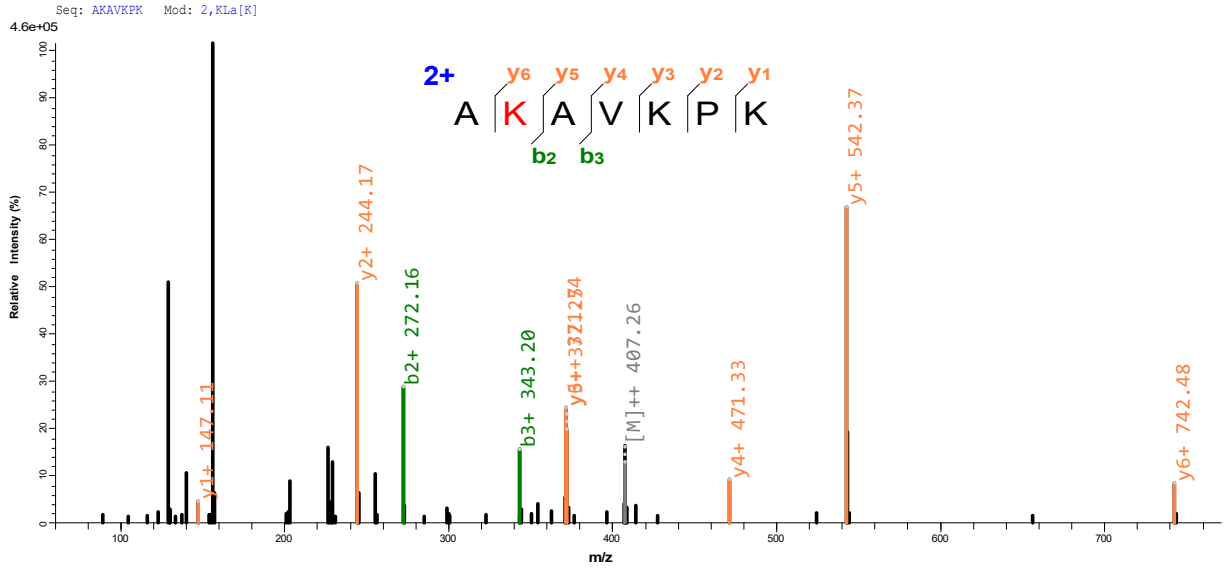


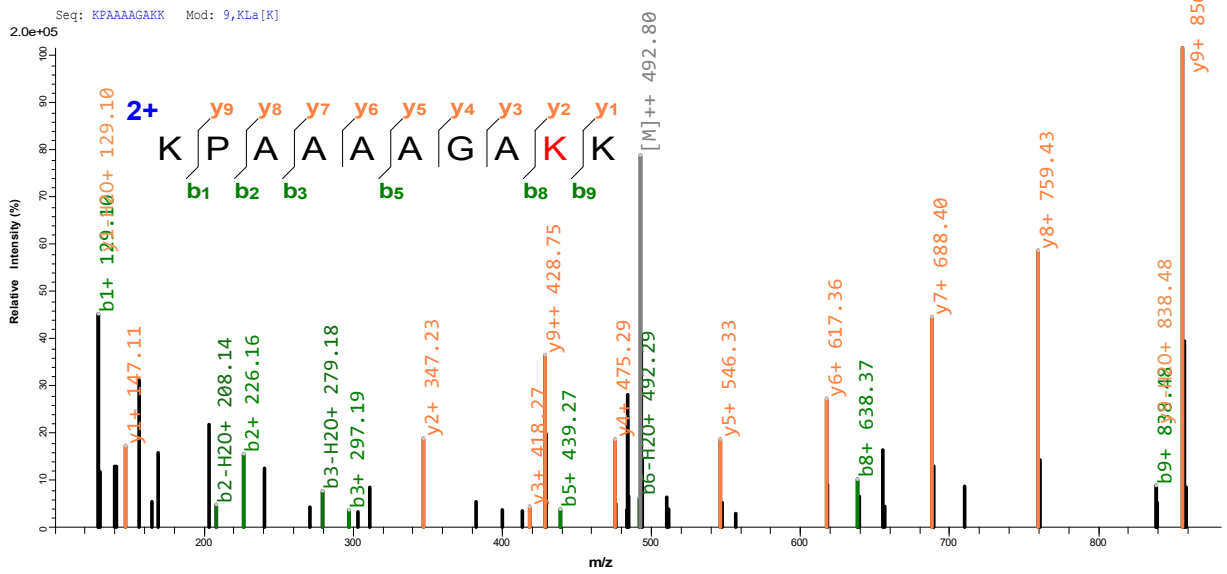




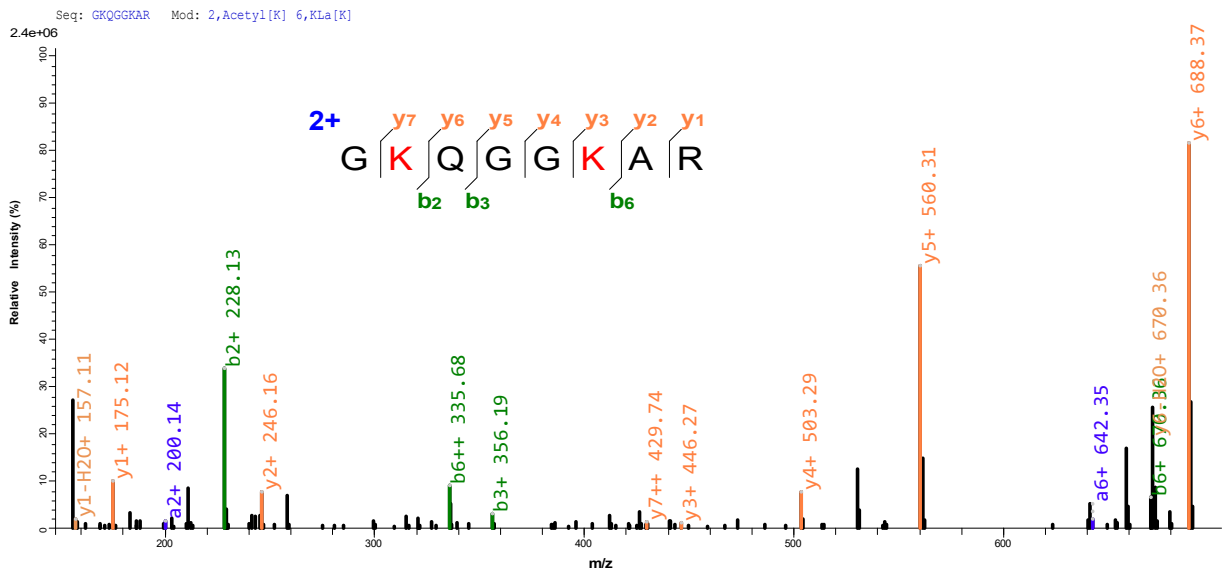


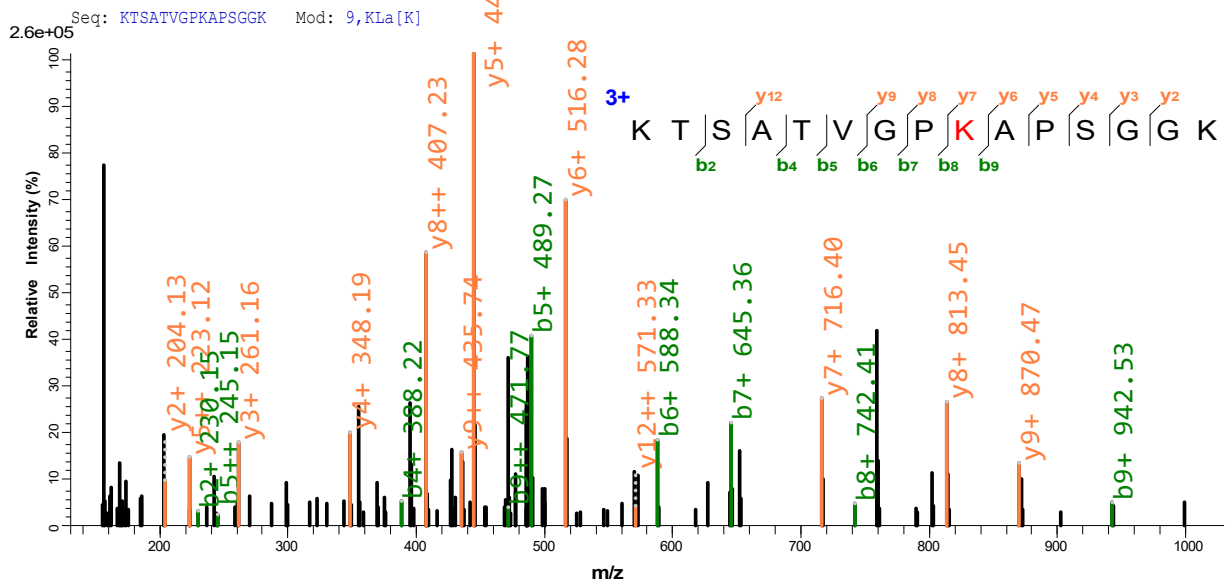
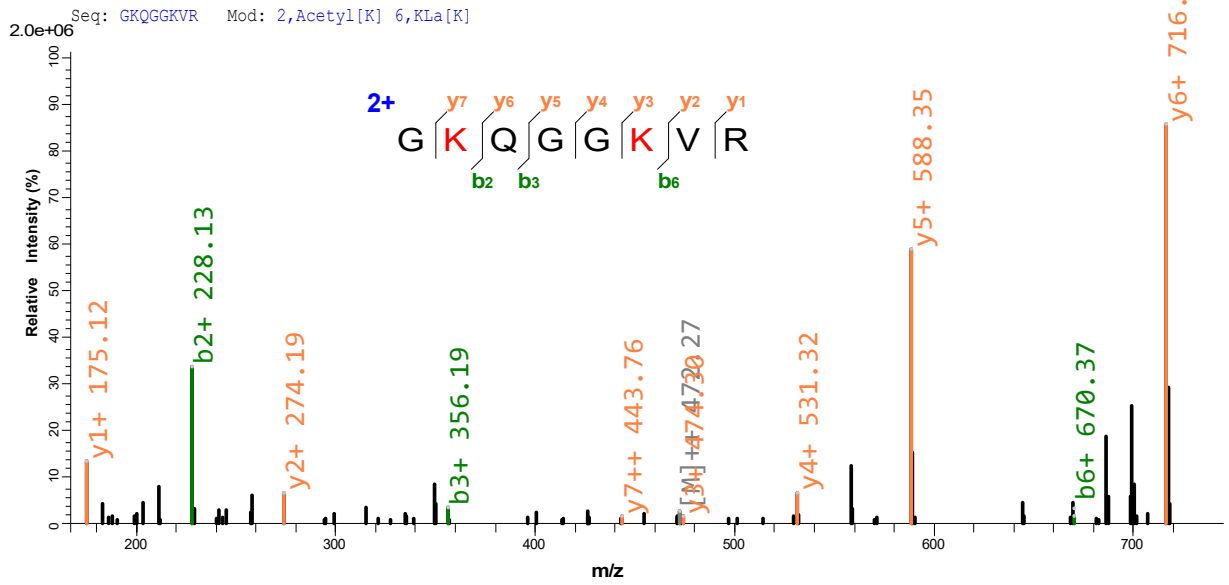


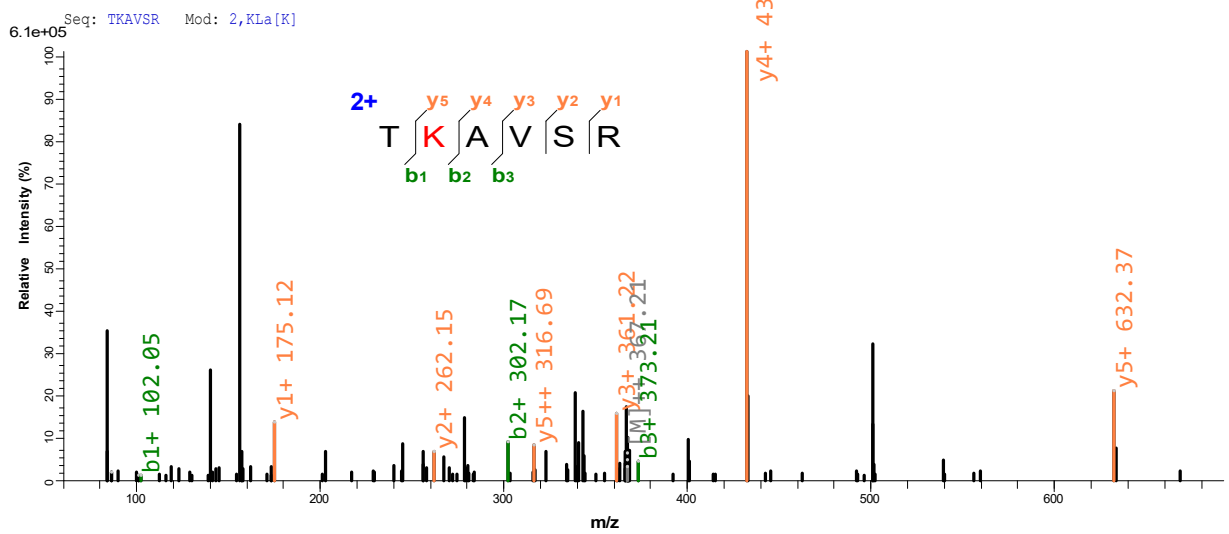
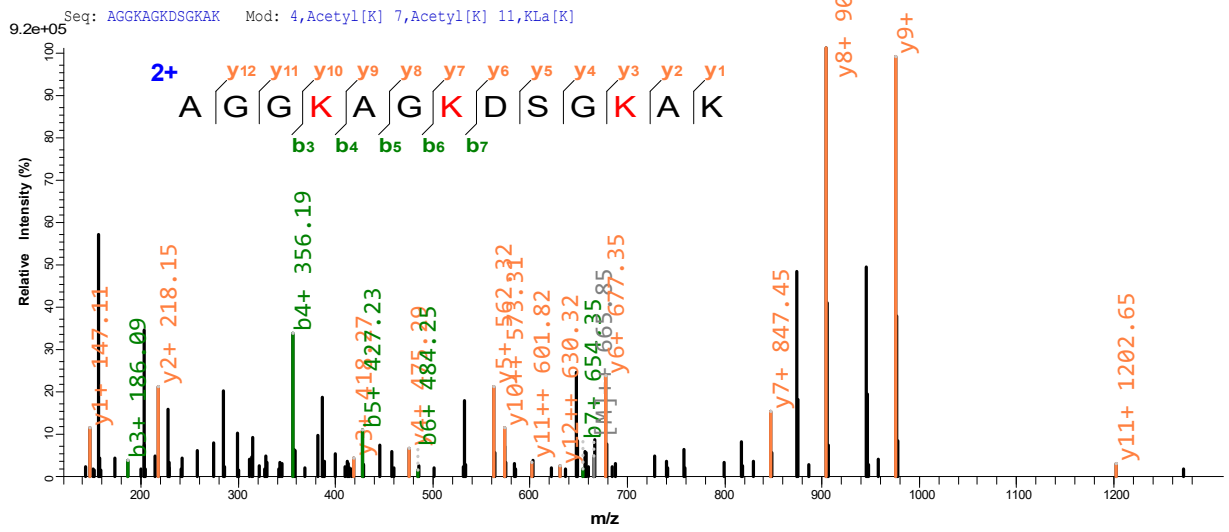


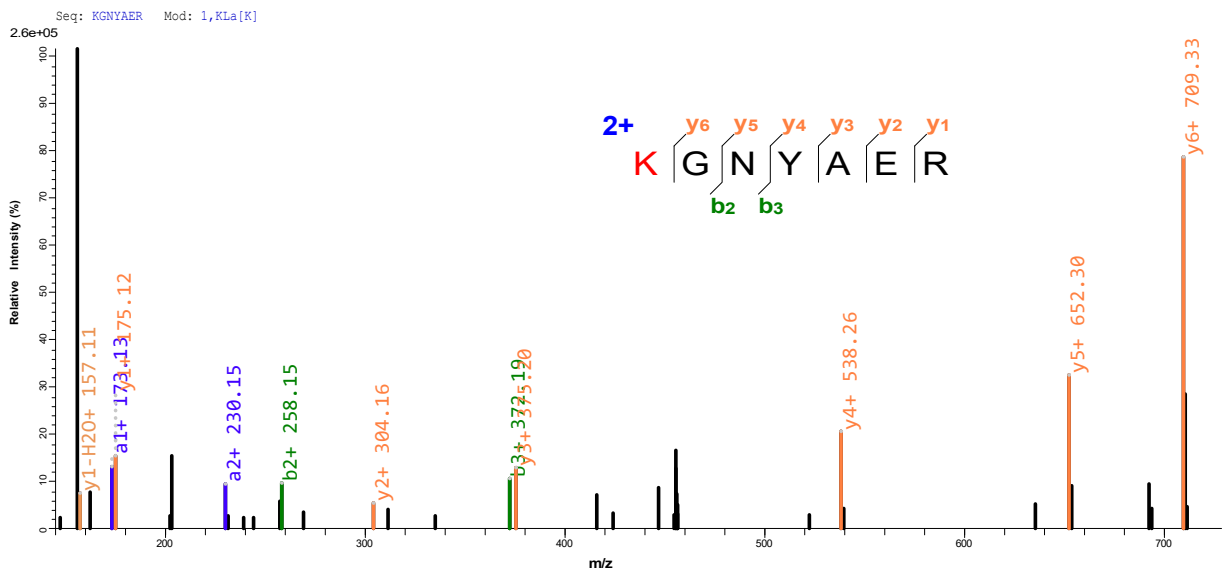
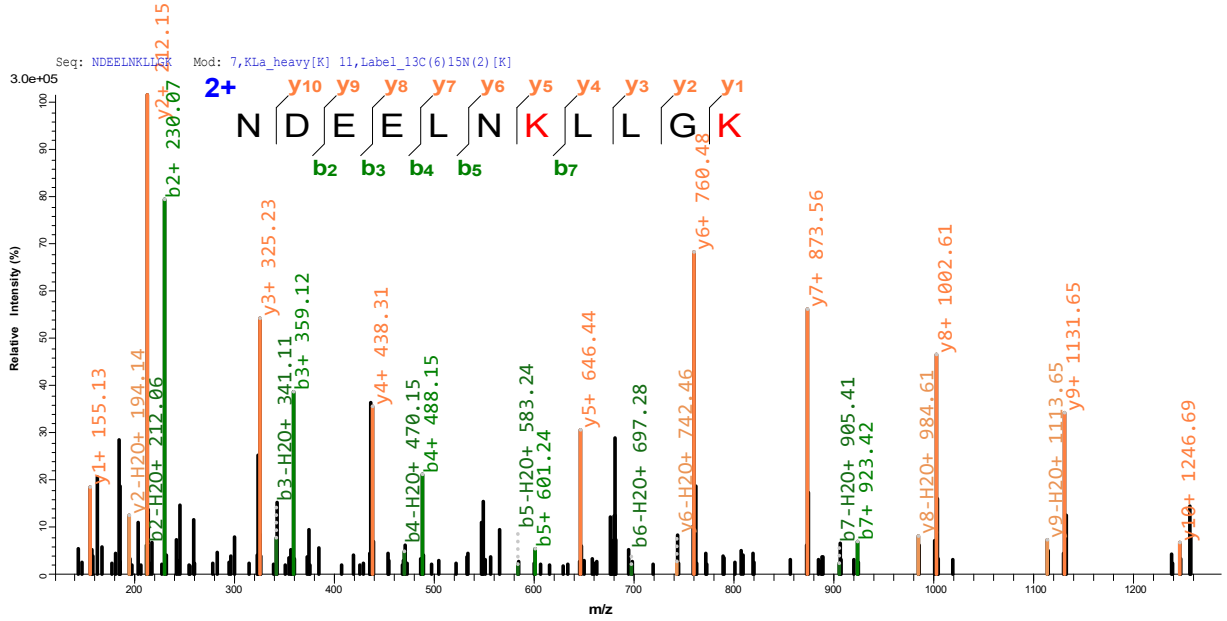


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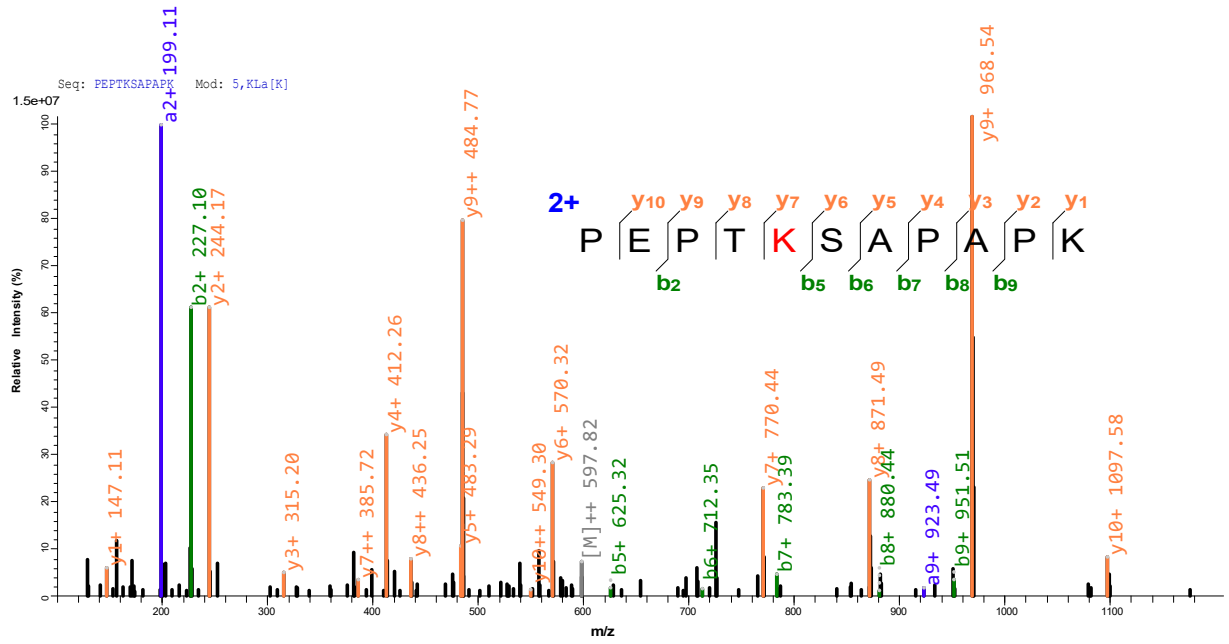
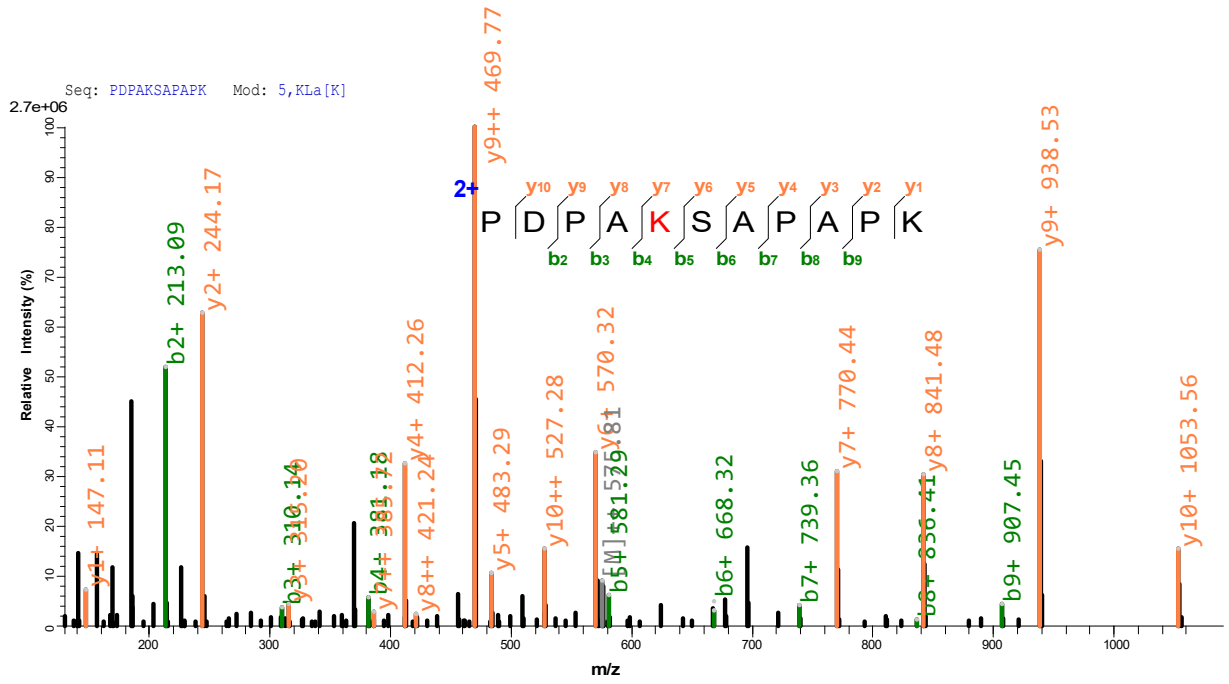


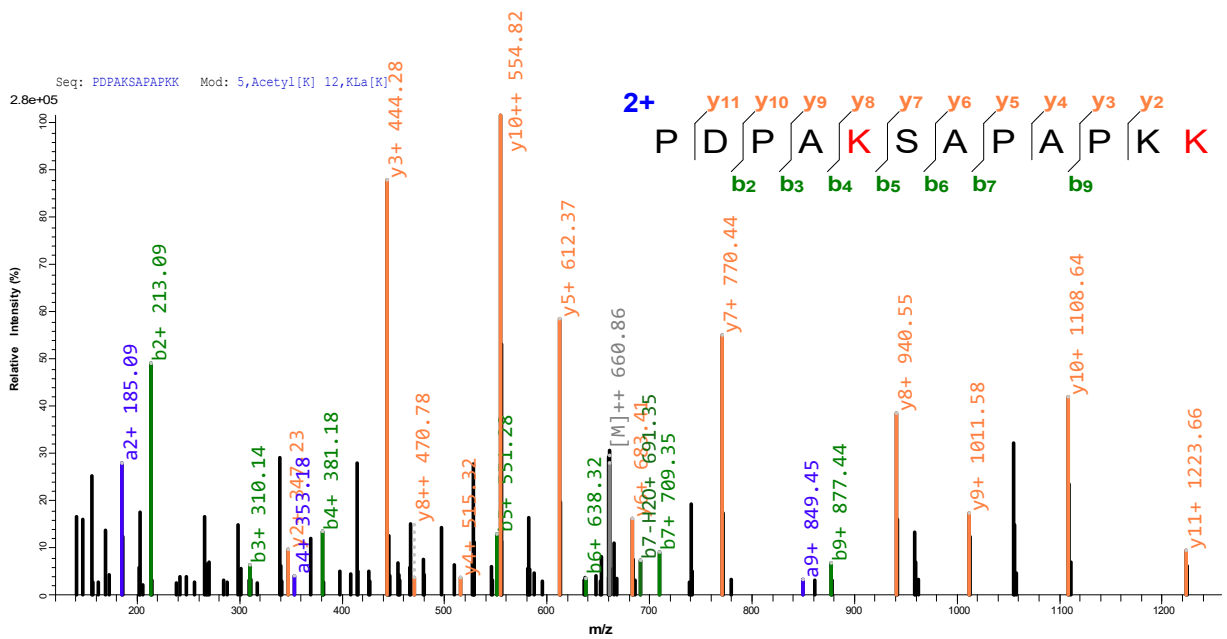
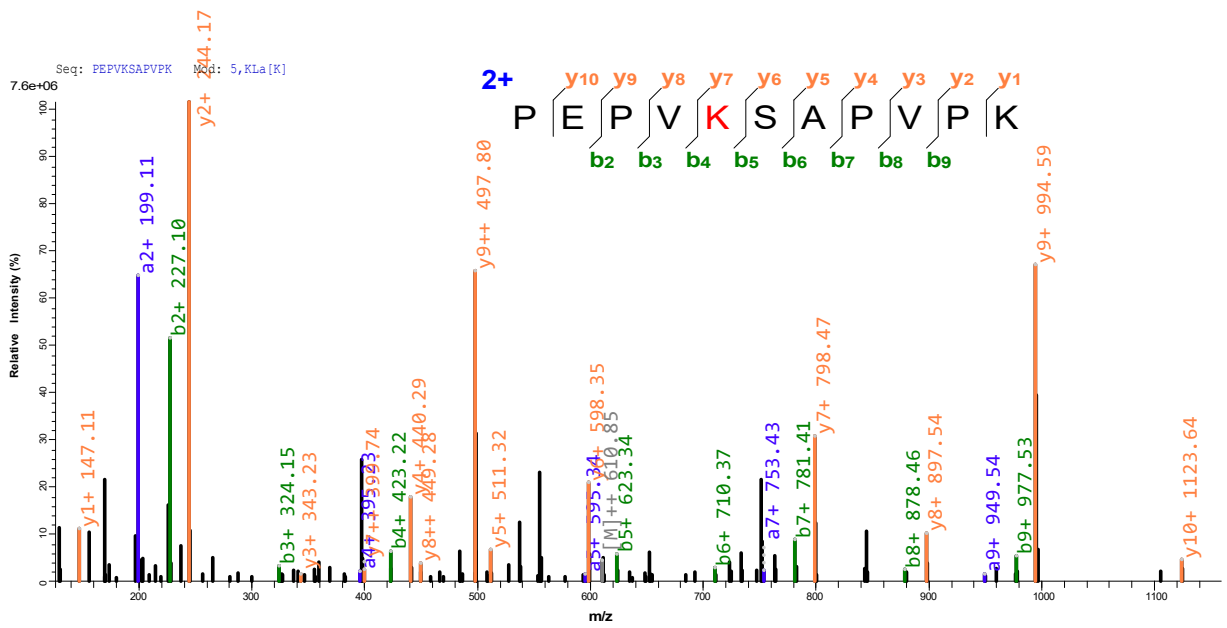


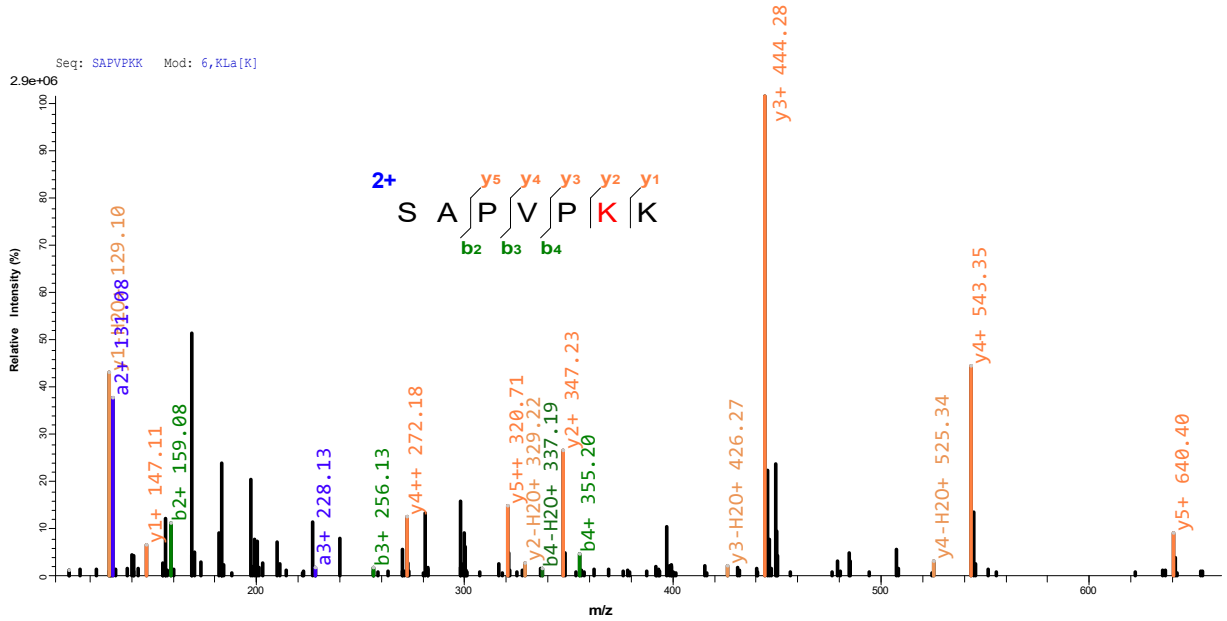
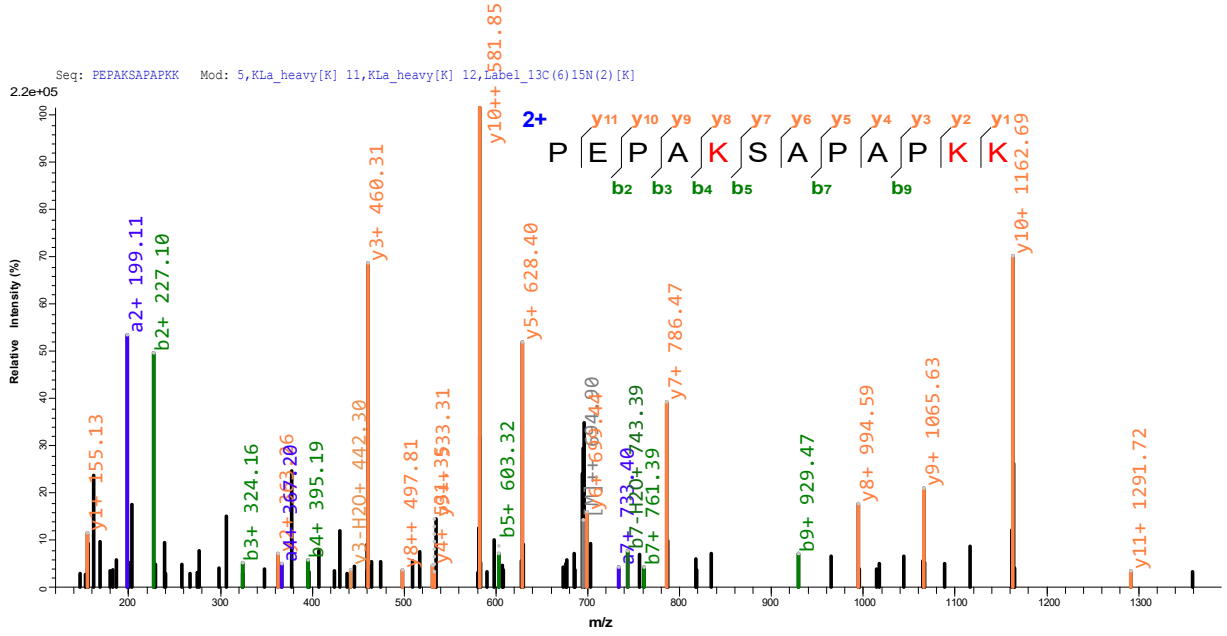


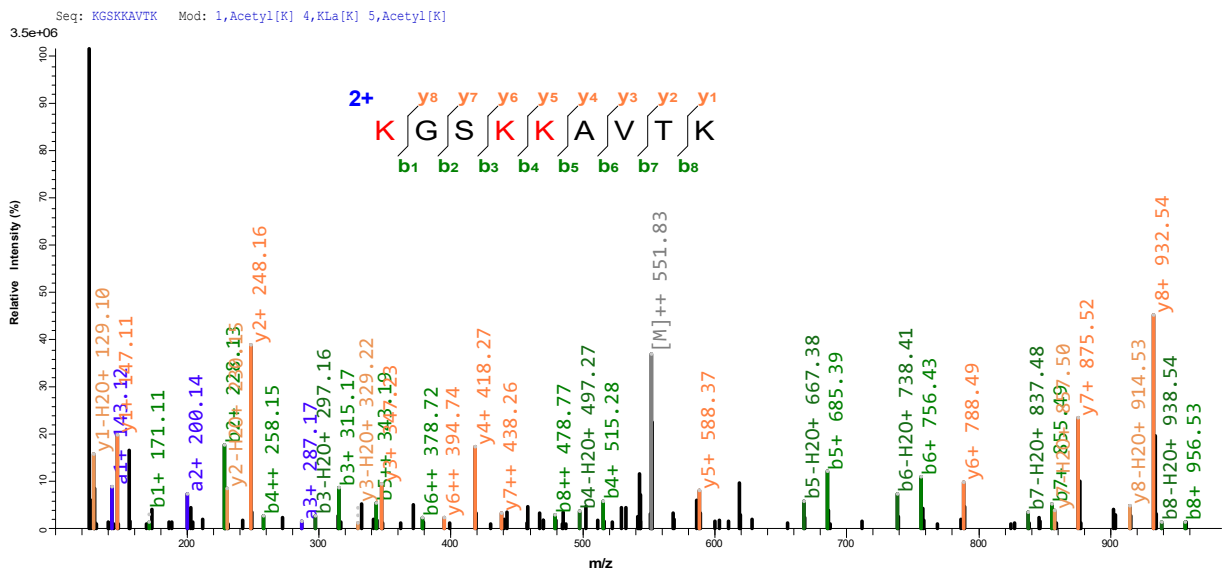
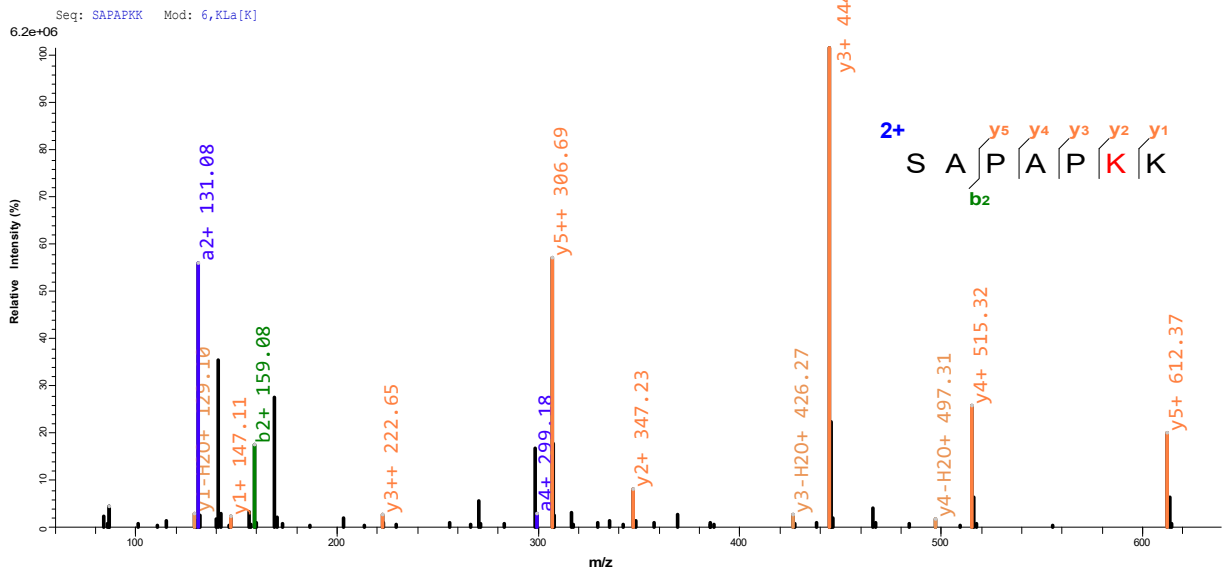


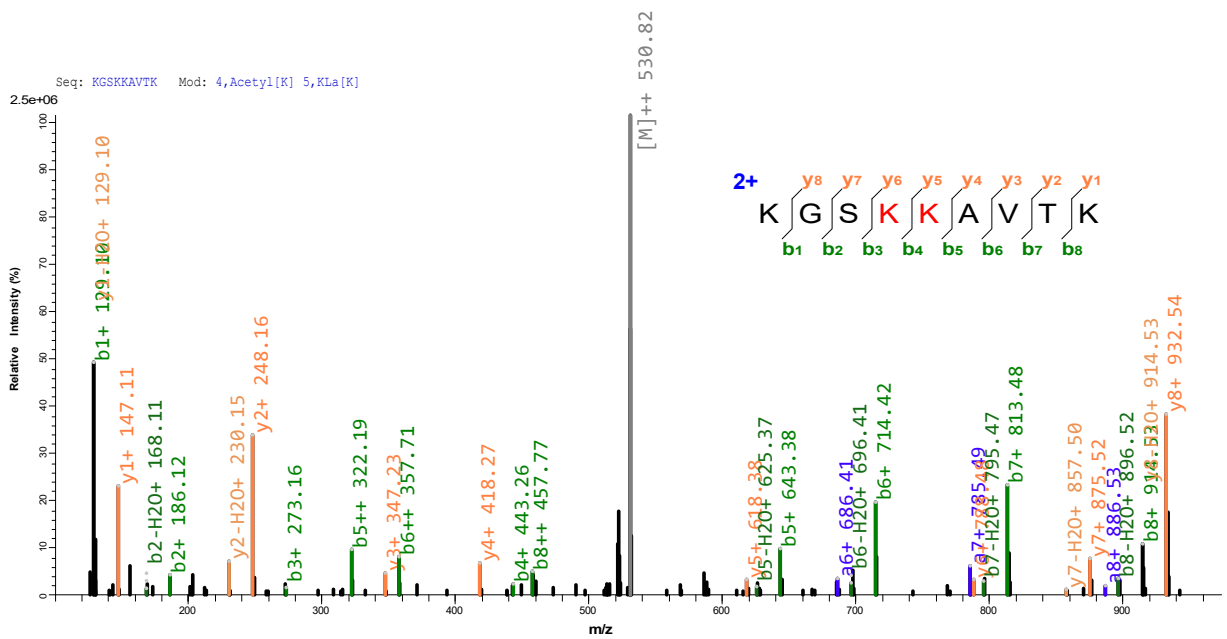
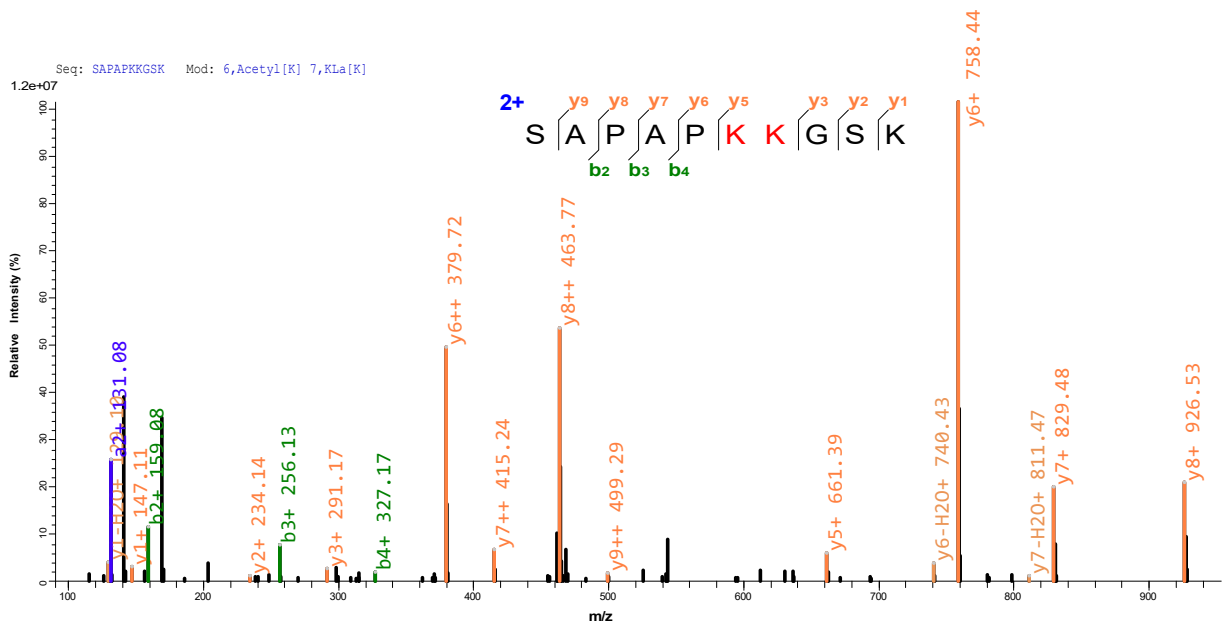
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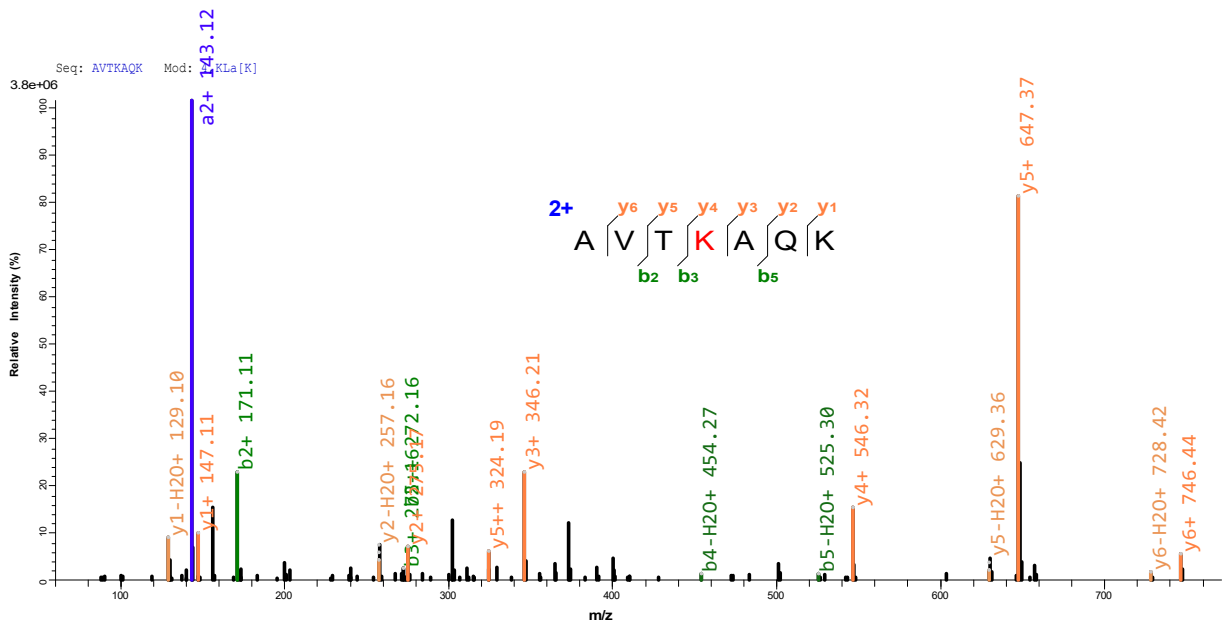
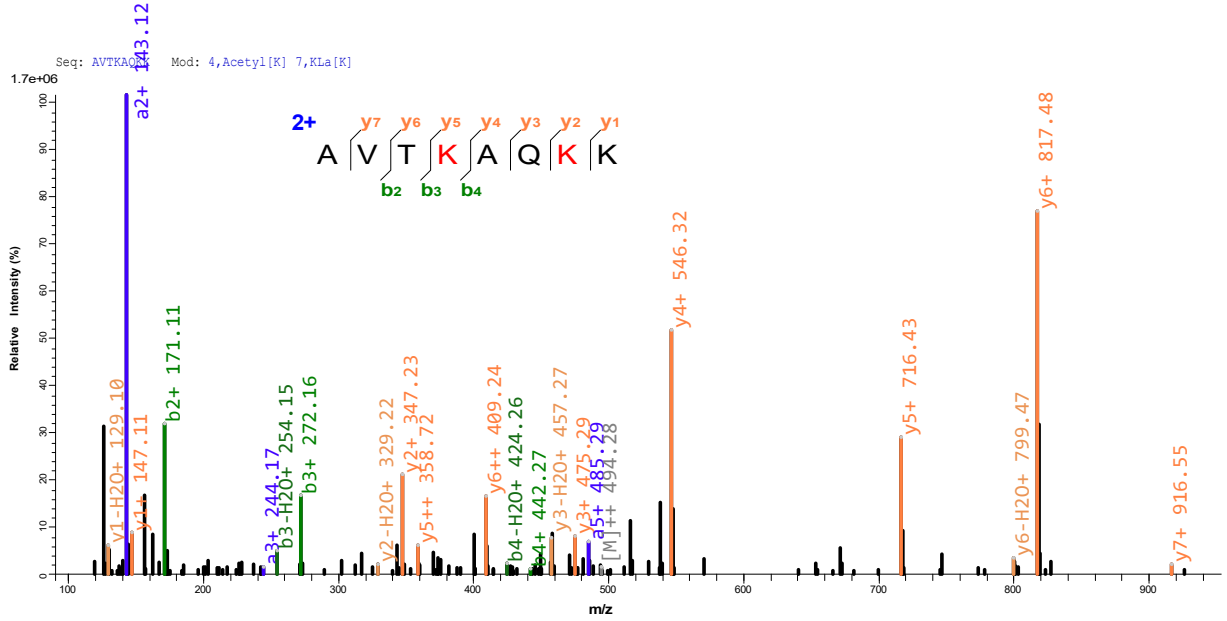


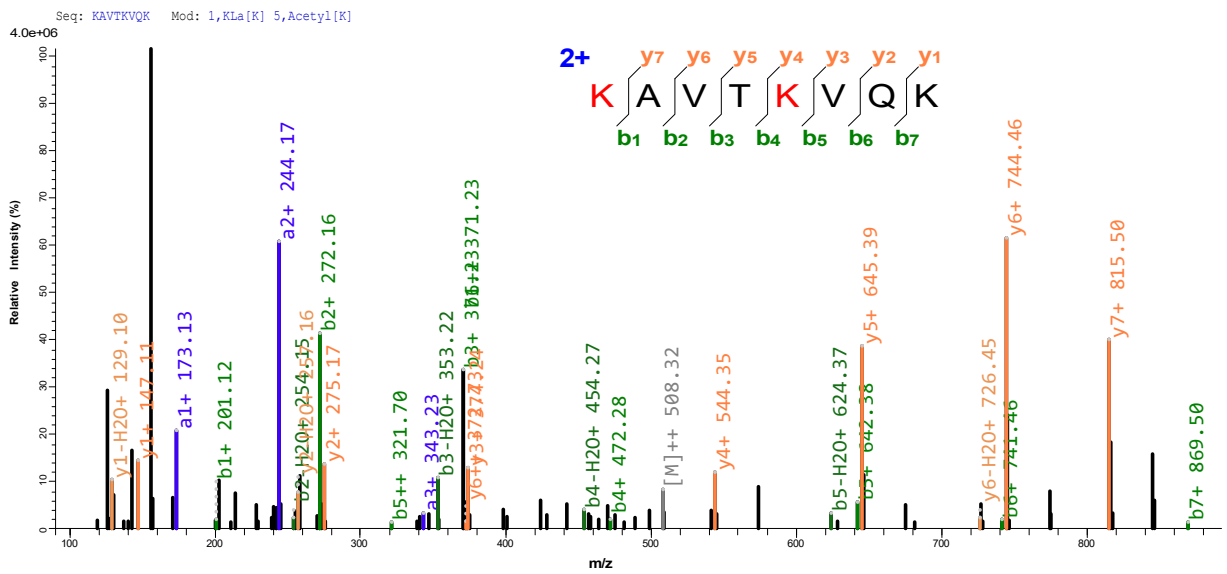
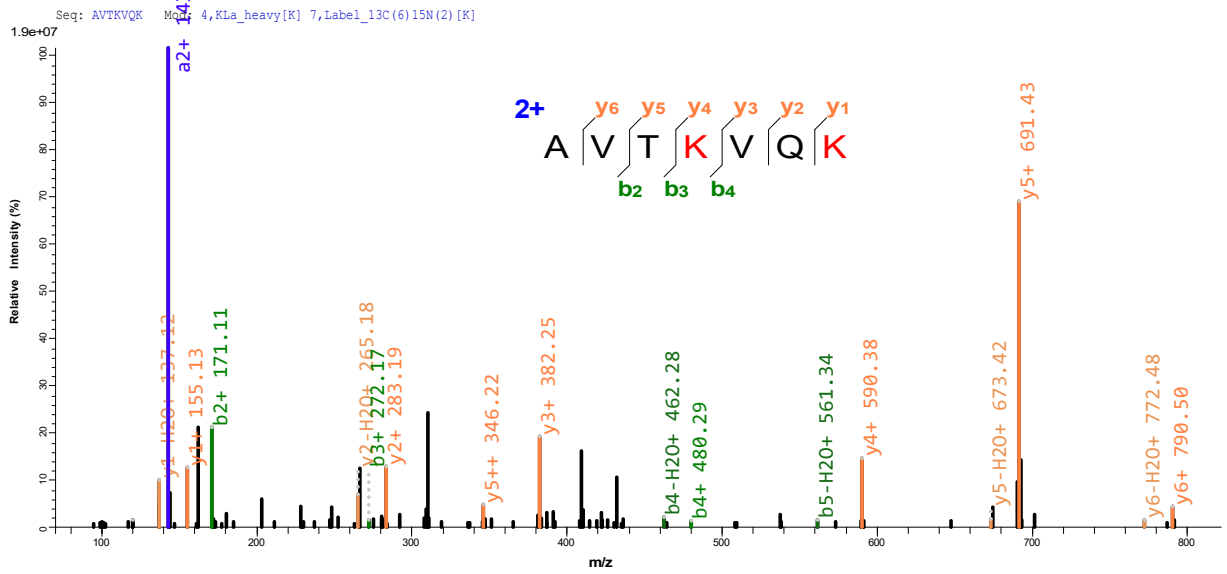


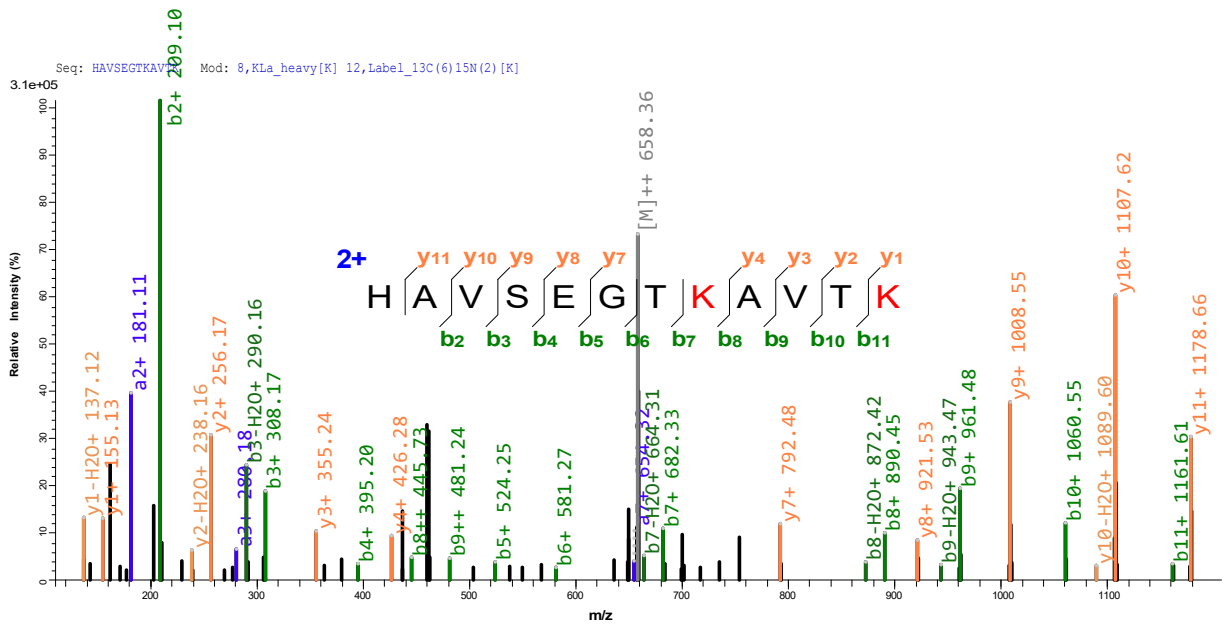
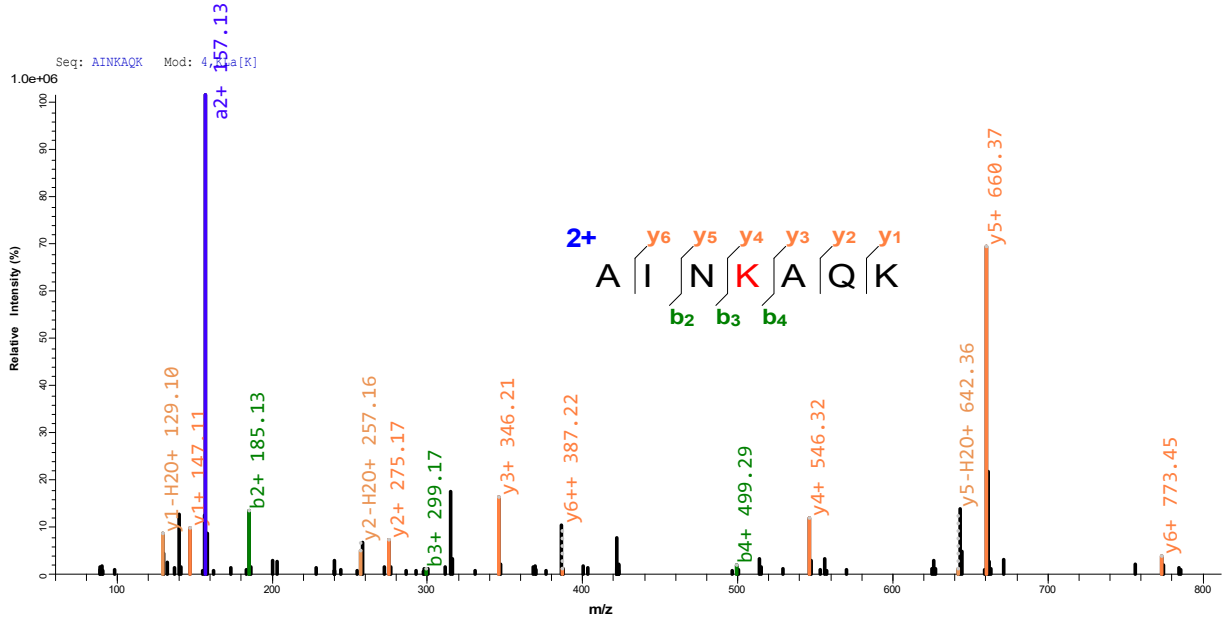


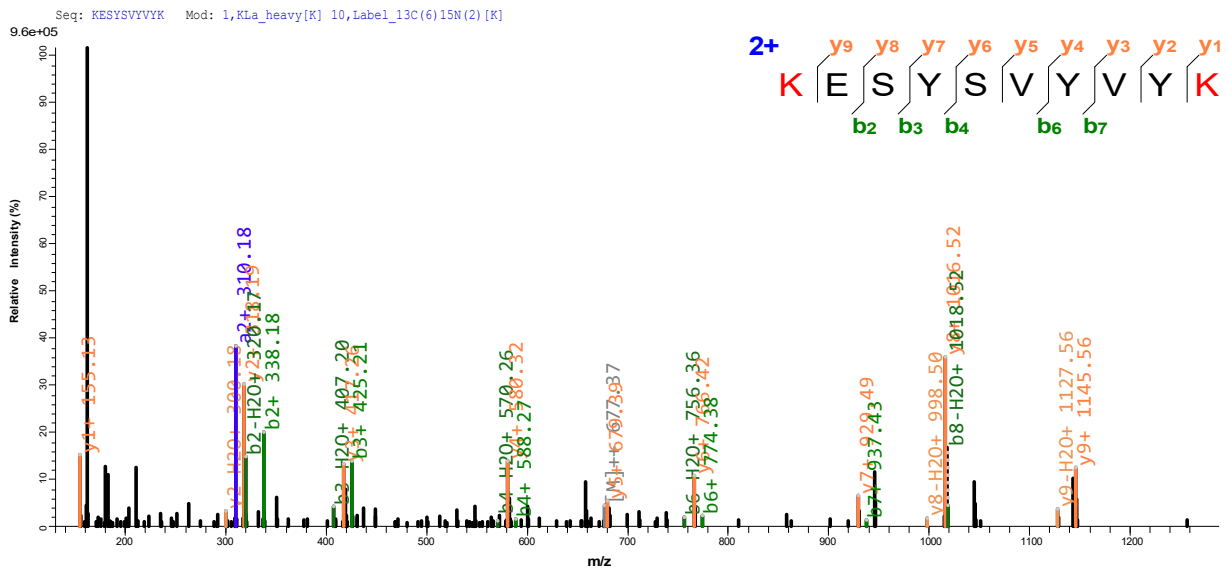
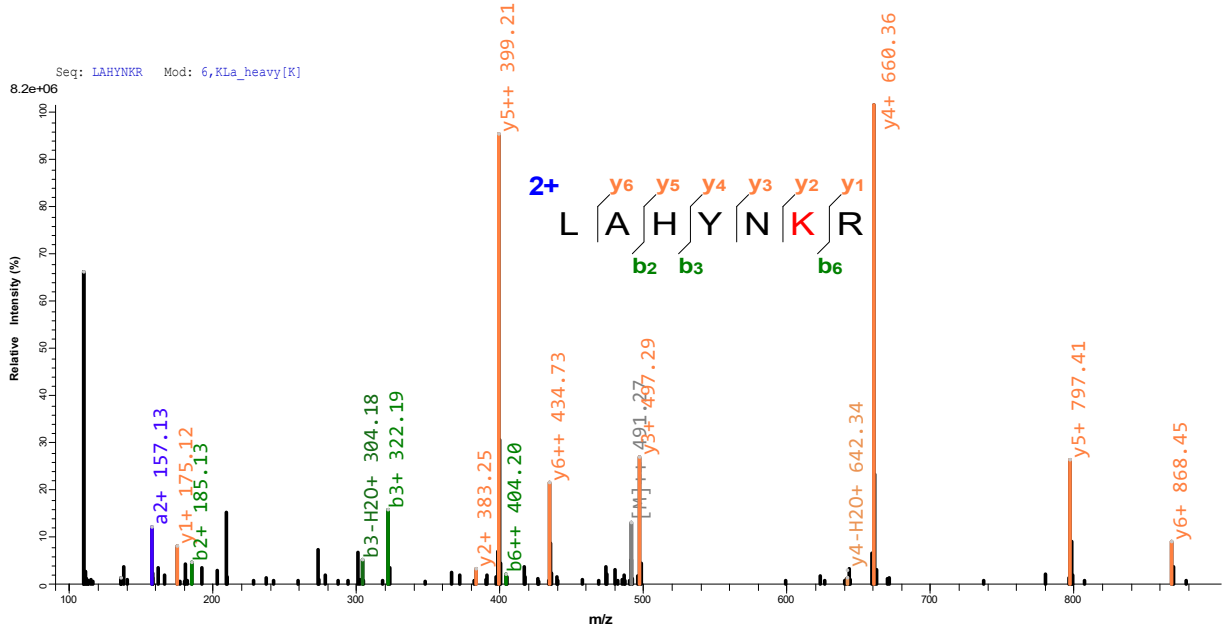


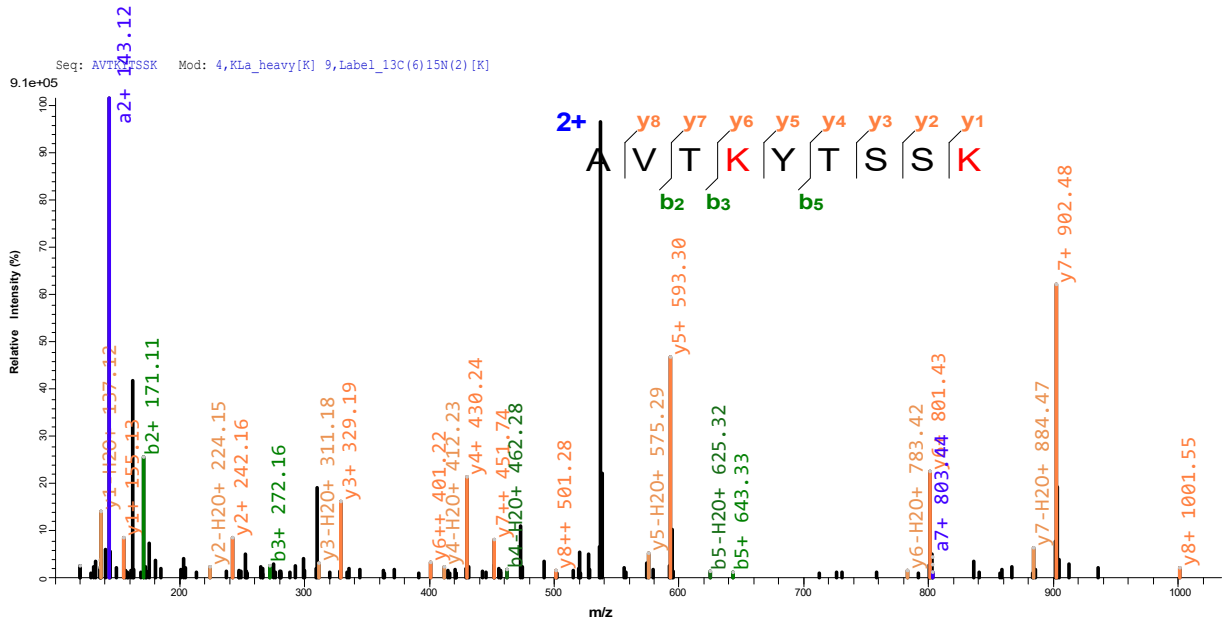
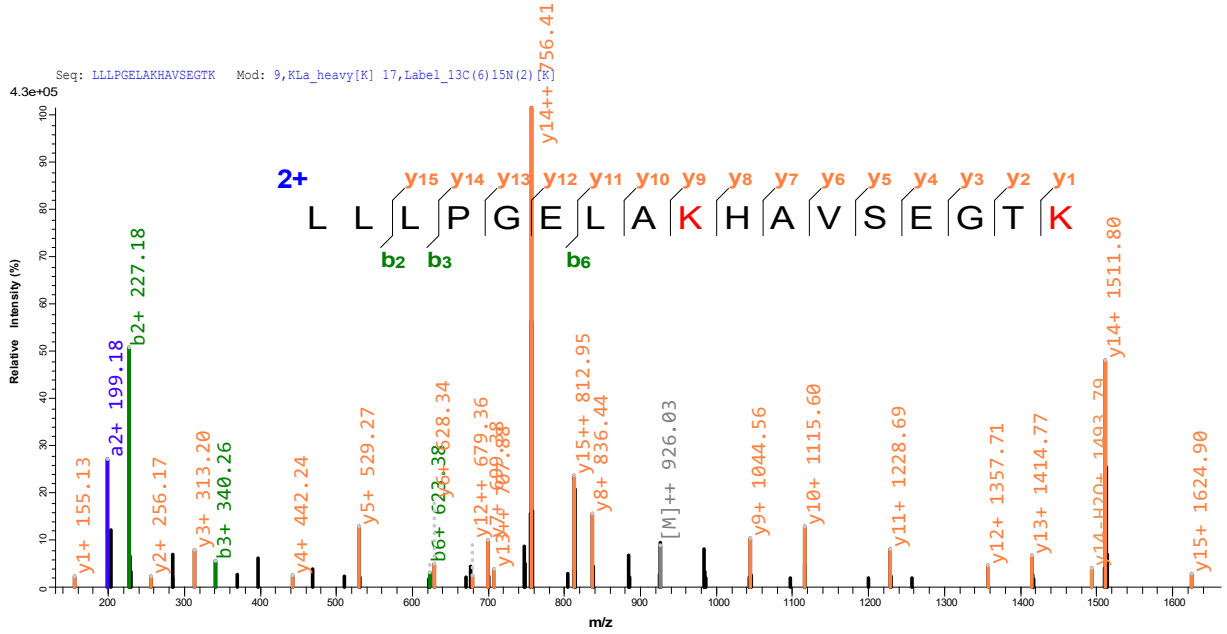




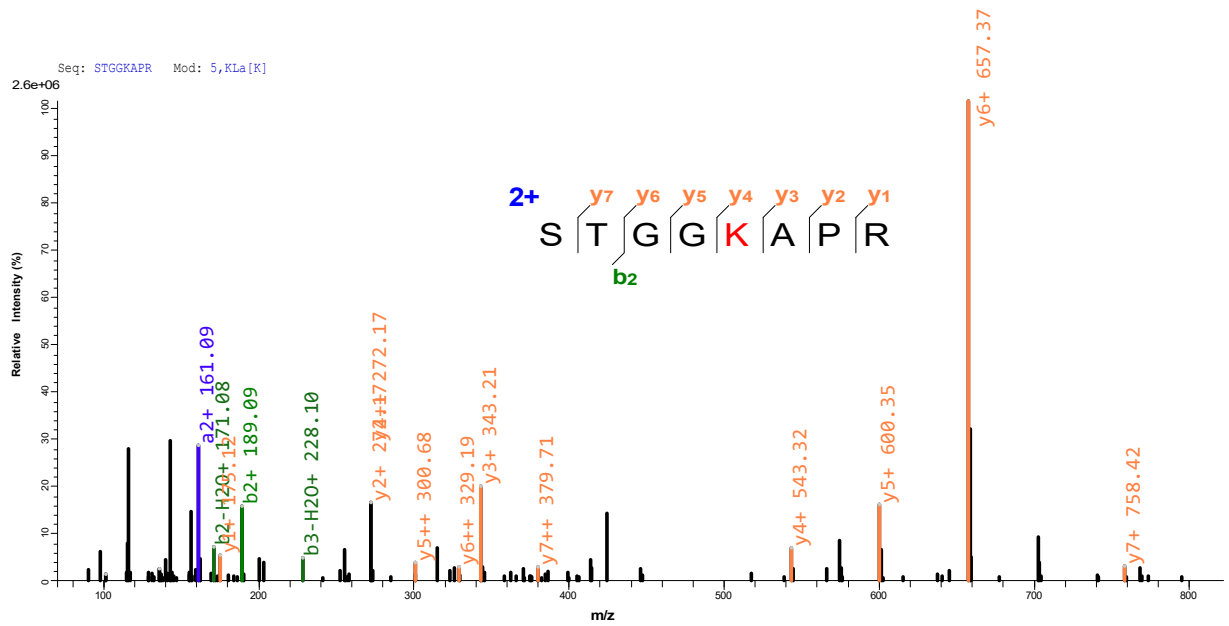
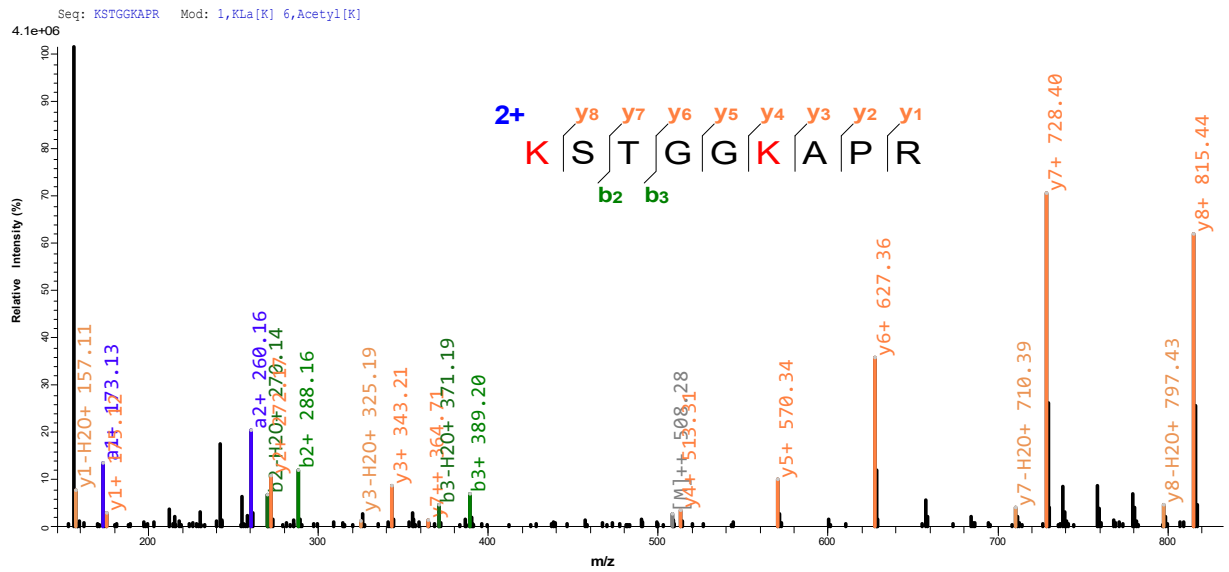


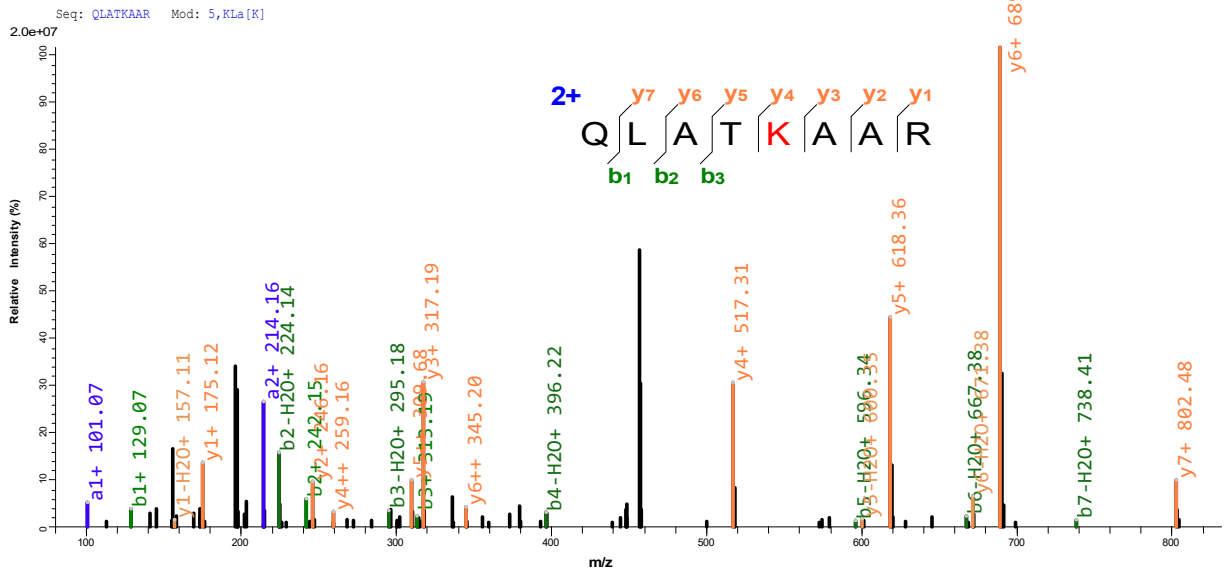
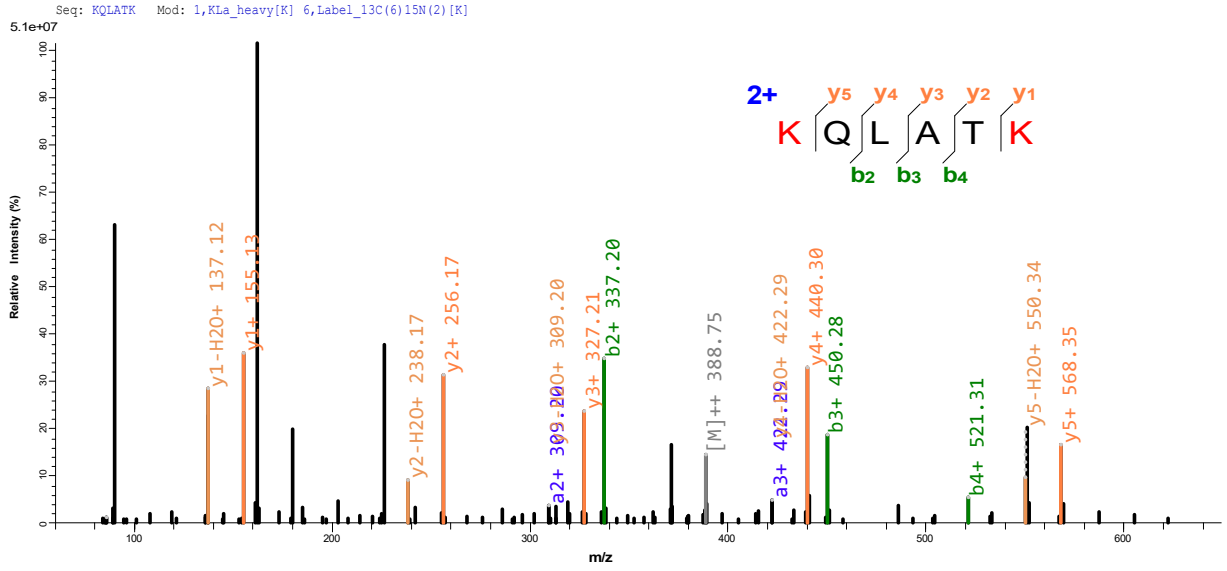


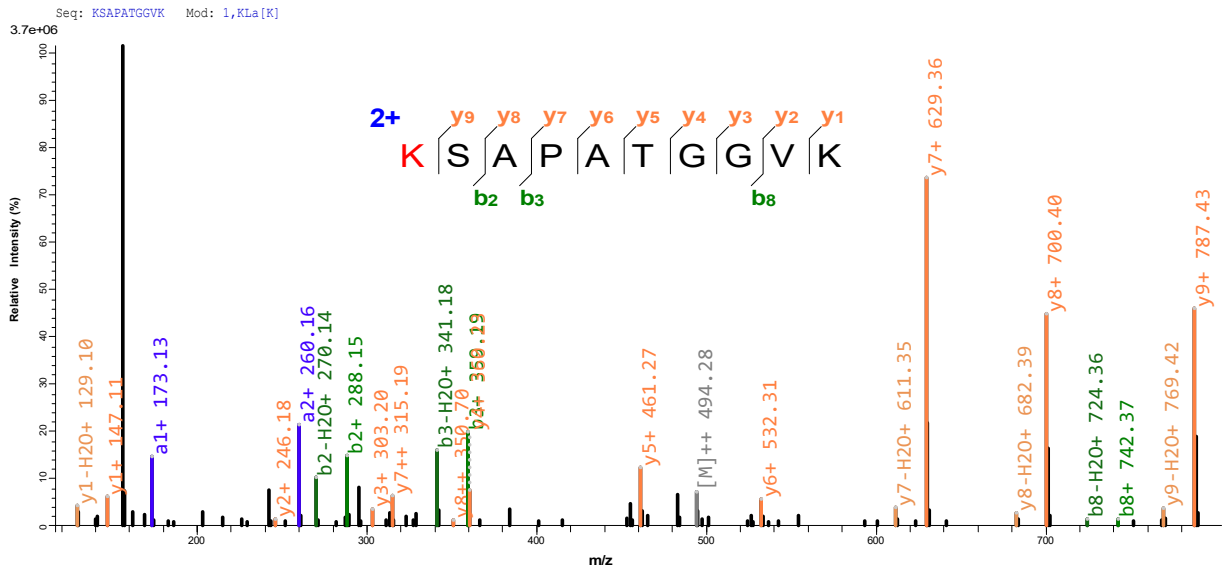
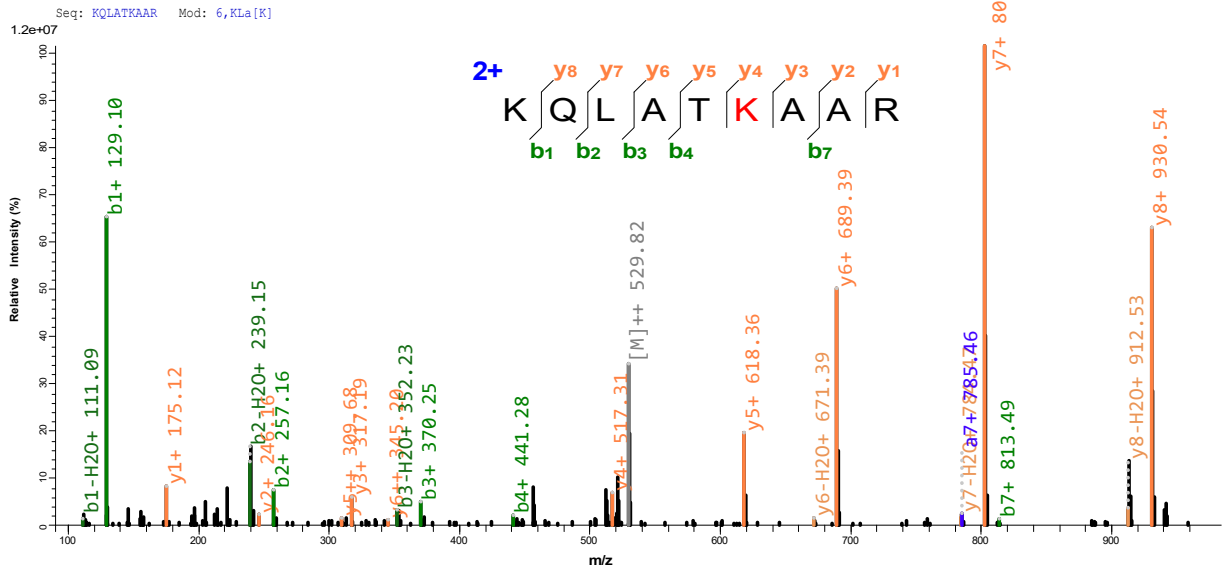


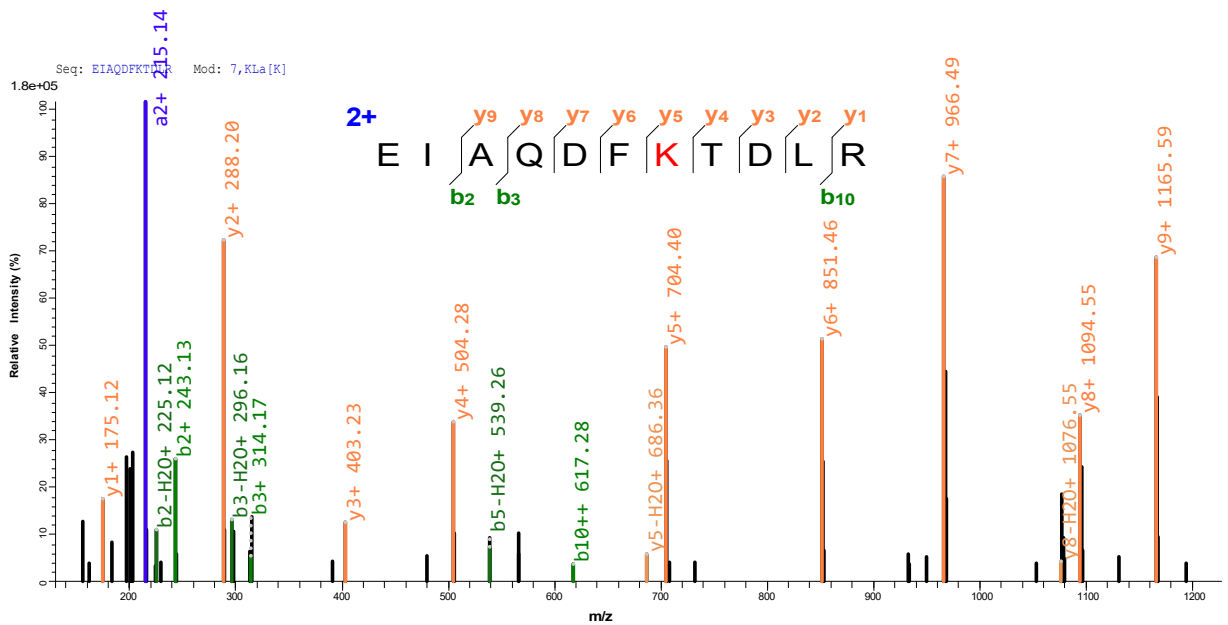
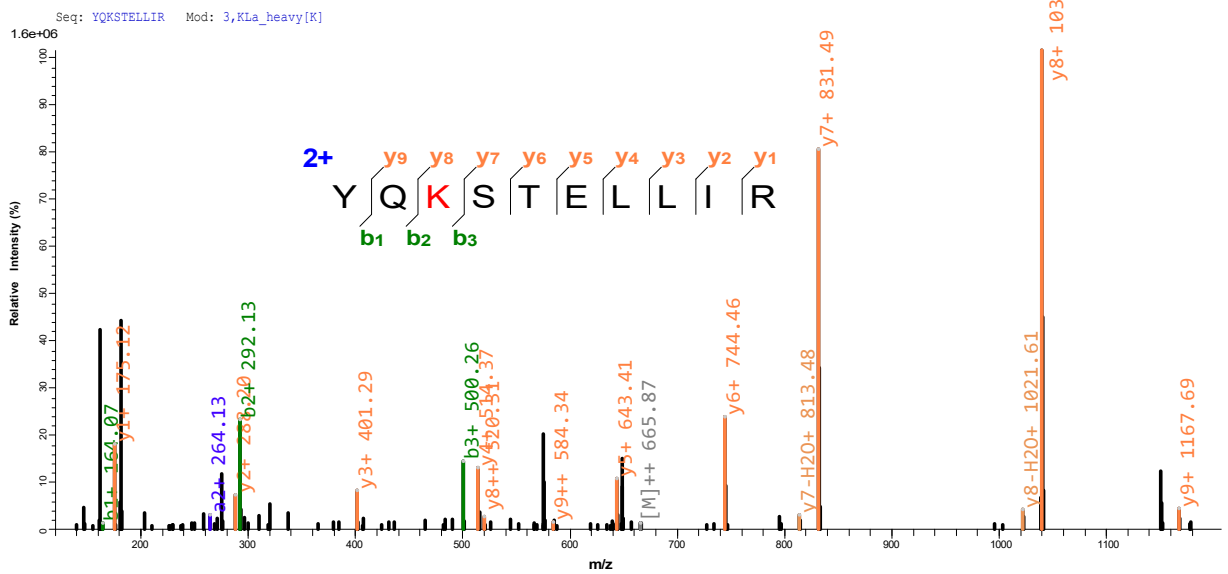


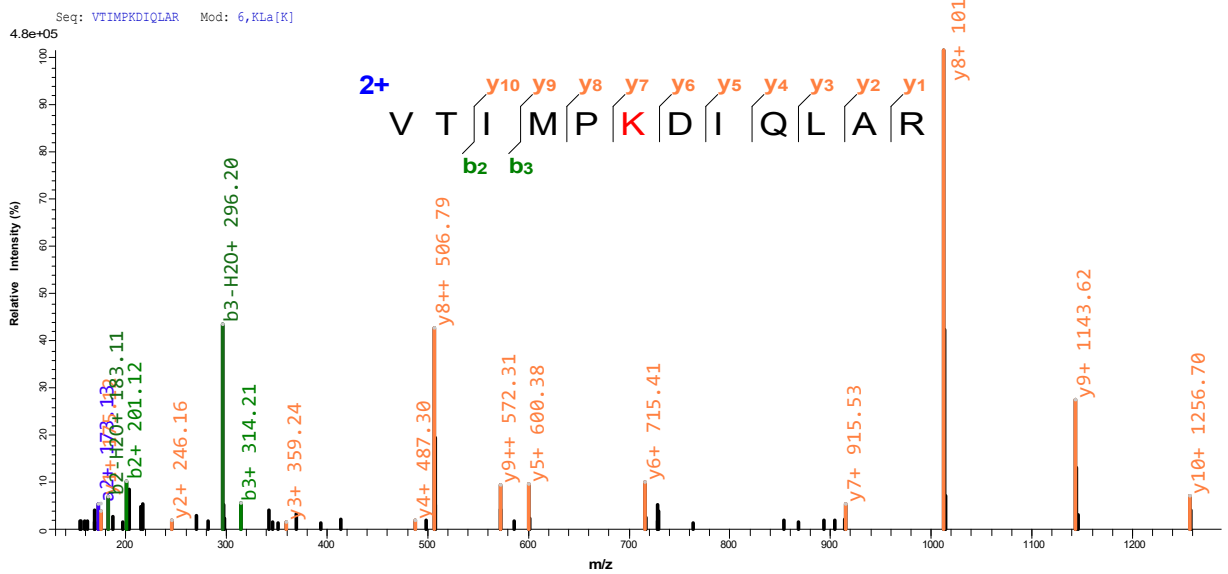
# H3











## H4

