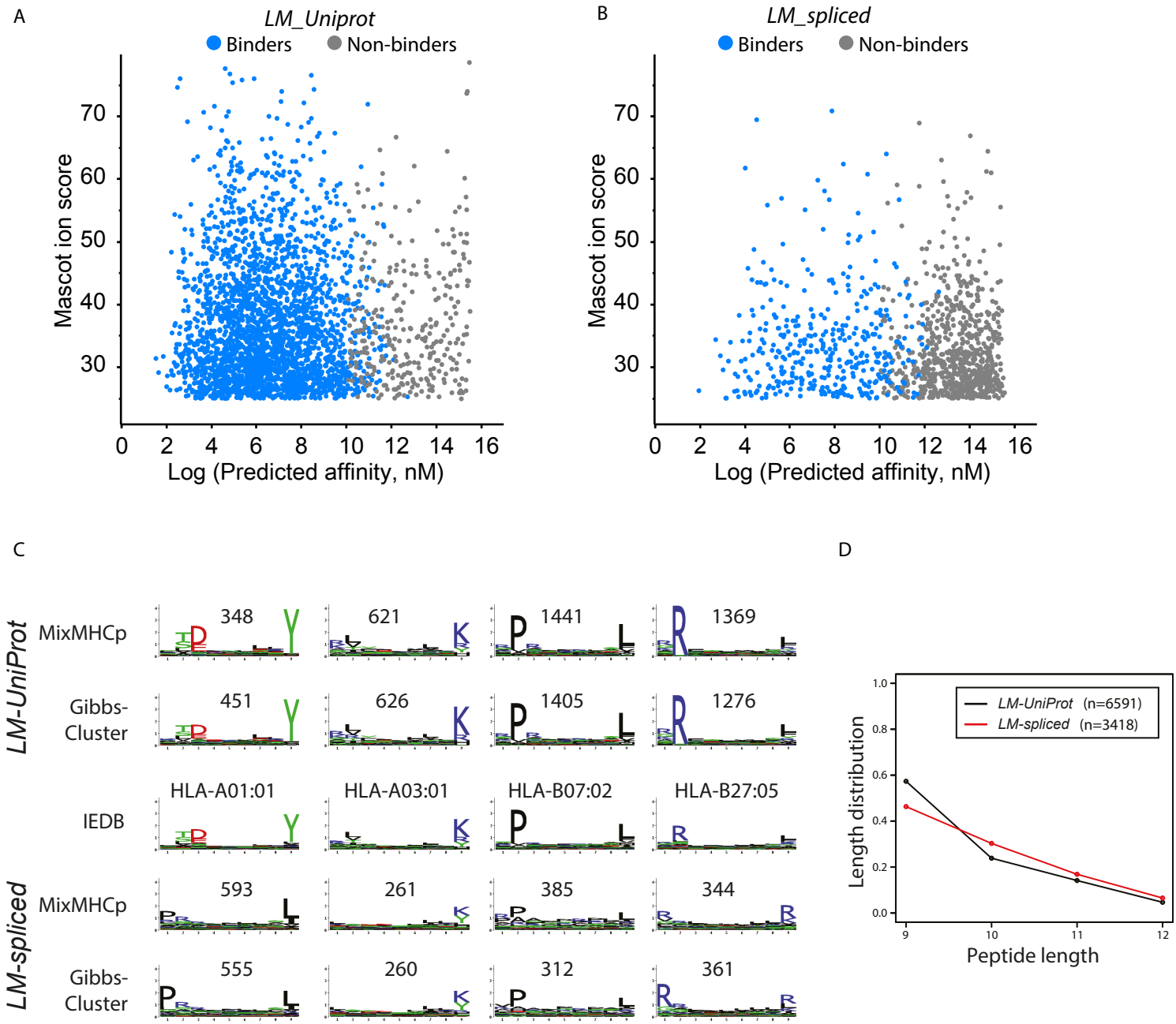
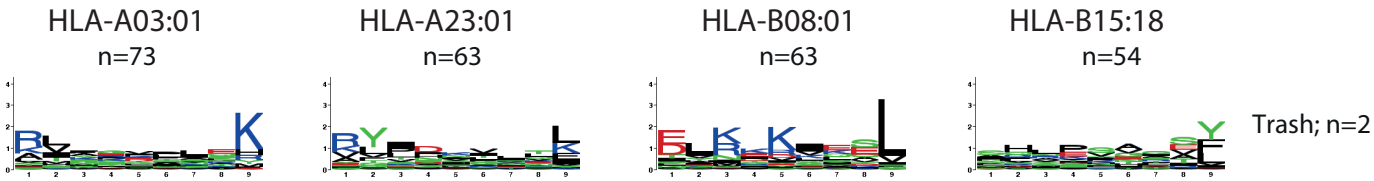
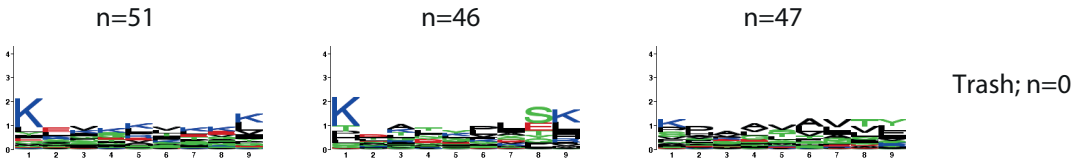


Supplemental Figure 1: Re-analysis of the Liepe et al. data available as Supplemental Data 2. A) Correlation of the predicted binding affinity by NetMHCpan and MS/MS identification scores of peptides identified by Liepe et al. as *LM_UniProt* (A) and *LM_spliced* (B) in the Fib dataset. Binders (rank top 2%) are marked in blue and non-binders in gray C) Motif deconvolution analysis with MixMHCp and GibbsCluster of the 9-mer *LM-UniProt* and *LM-spliced* peptides identified by Liepe et al. in the GR-LCL 2D dataset and comparison to known logos from IEDB for HLA-A01:01, HLA-A03:01 and HLA-B07:02 and HLA-B27:05. Motifs found in *LM-UniProt* peptides are highly reproducible and comparable to the known motifs from IEDB, while this is not the case for motifs found in *LM-spliced* peptides. D) Length distribution of the *LM-UniProt* and *LM-spliced* peptide identified by Liepe et al. in the GR-LCL 2D dataset.

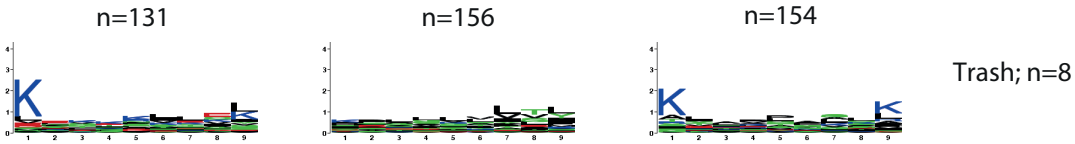
A 9 mer peptides assigend by MaxQuant as UniProt while originally identified as *LM_spliced* by Mascot



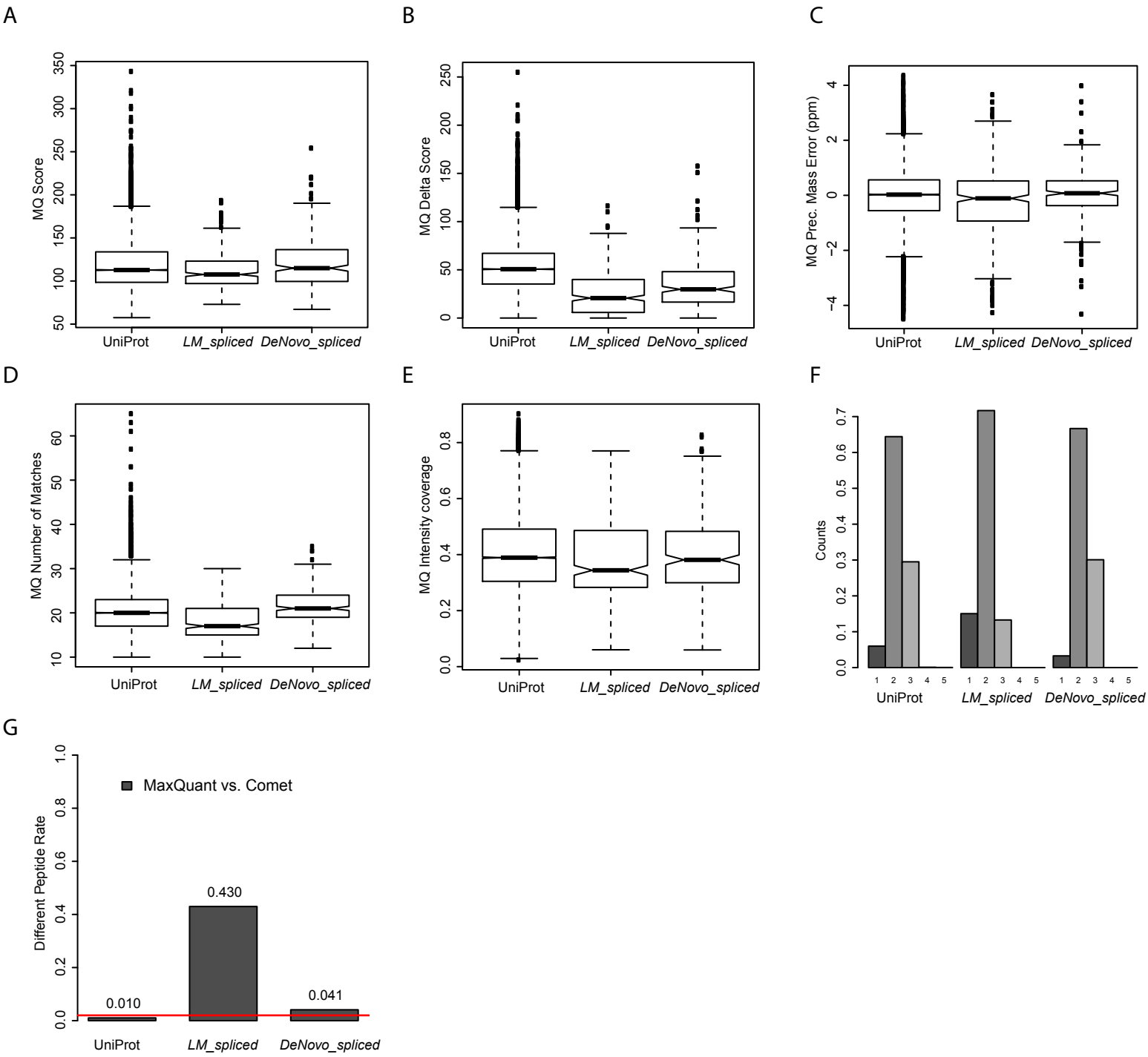
B 9 mer peptides Mascot assigned as *LM_spliced* while MaxQuant as Uniprot



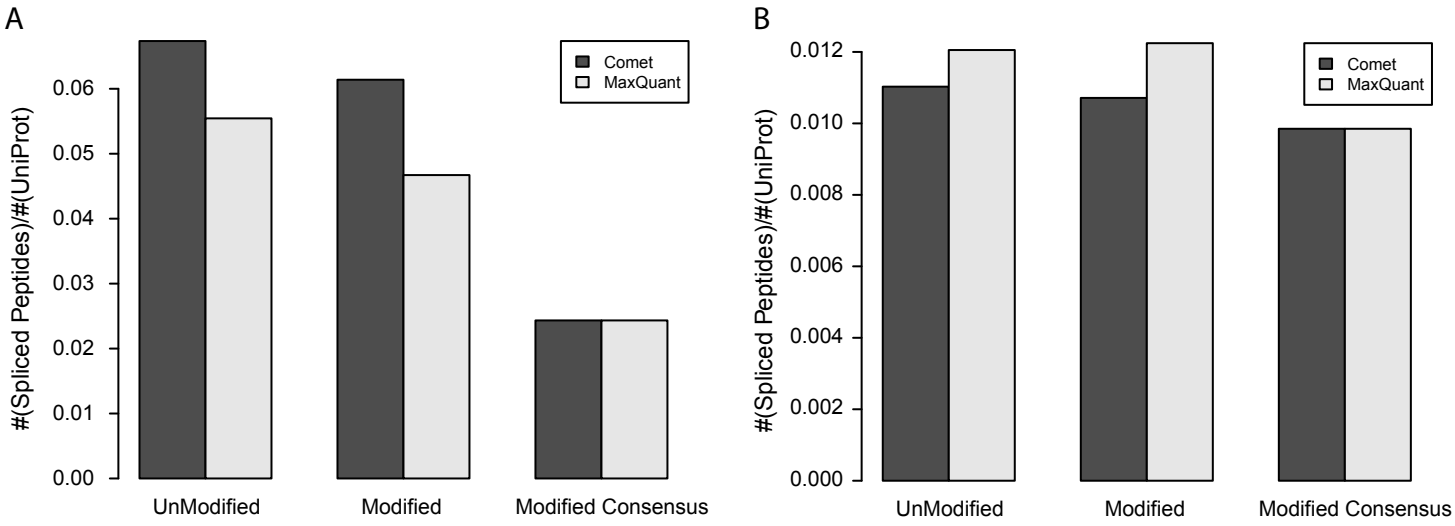
C All remianing 9 mer *LM_spliced* after removal of PSMs assigend by MaxQuant as Uniprot



Supplemental Figure 2: Motif deconvolution analysis with MixMHCp of the 9-mer peptide fraction of A) sequences matching UniProt proteins by MaxQuant that the same MS/MS scans have been originally identified as *LM_spliced* by Liepe et al., B) Mascot assigened *LM_spliced* peptides that MaxQuant assigned the same MS/MS scans as UniProt matches, and C) remaining *LM_spliced* peptides after removal of conflicting PSMs that have been assigned as UniProt in MaxQuant search. The list of peptides has been extracted from Supplemental Data 6.

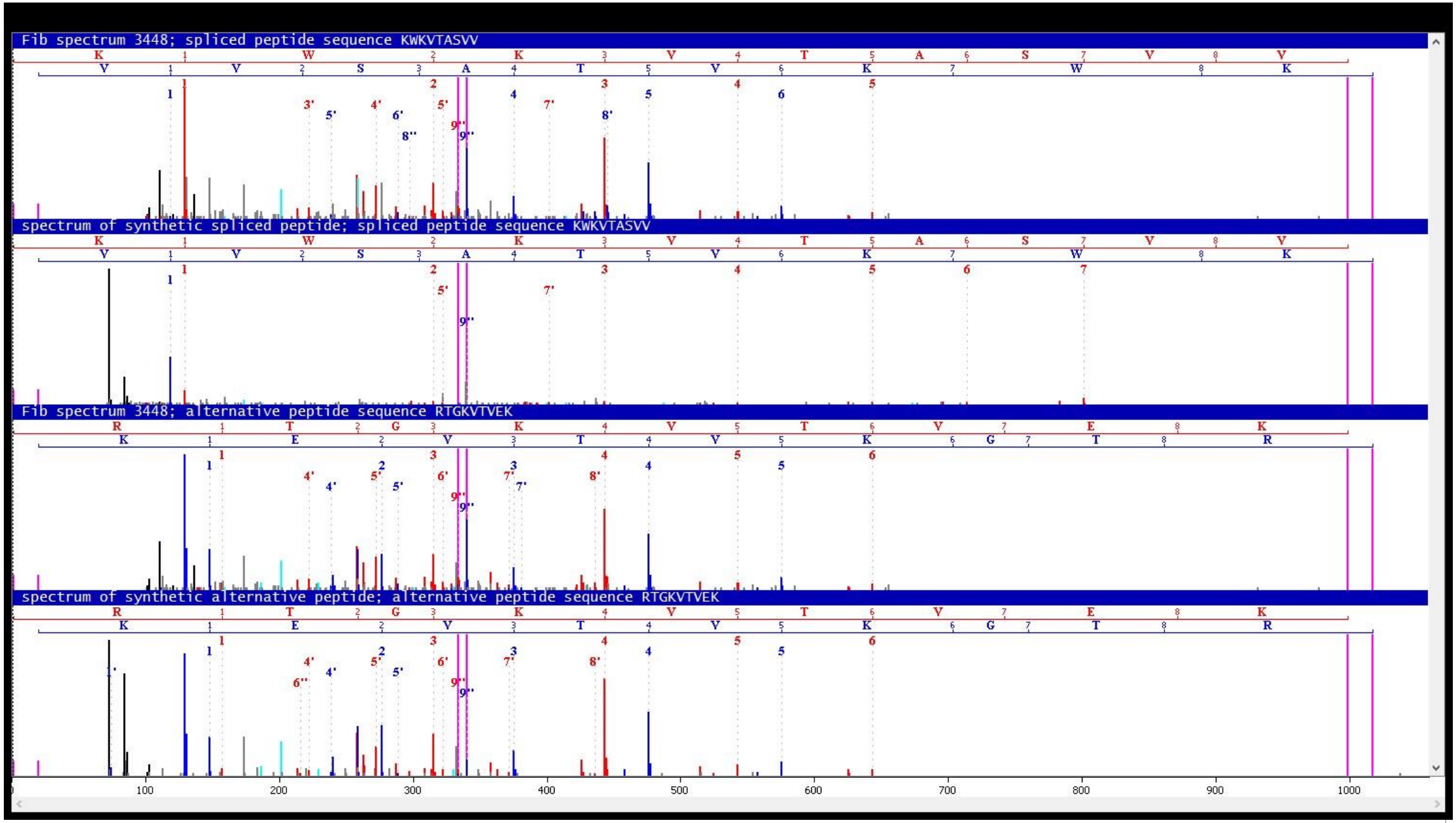


Supplemental Figure 3: Compared to UniProt and *DeNovo_spliced* peptides, *LM_spliced* peptides were characterized with A) lower Andromeda score for the best MS/MS spectrum, B) lower score difference to the second best identified peptide, C) higher absolute precursor mass deviation, D) fewer peaks matching to the predicted fragmentation spectrum, E) lower fraction of total MS/MS peak intensity matched, and F) a larger fraction of singly charged MS/MS spectra matched. G) Rate of disagreement in peptide identification between MaxQuant and Comet for MS/MS scans matched to Uniprot, *LM_spliced* and *DeNovo_spliced* peptides.



Supplemental Figure 4: Ratio of spliced peptide count and UniProt peptide count for the *LM_spliced* (A) and *DeNovo_spliced* (B) peptides for all Fib samples. Variable modifications are as specified in the text. For 'Modified consensus' the peptide counts are calculated on the subset of PSMs where Comet and MaxQuant agreed, therefore, the values for Comet and MaxQuant are the same.

Supplemental Figure 5: Comparison of MS/MS annotations of endogenous and synthetic counterparts of 21 pairs of *LM_spliced* and their UniProt alternatives, and of the three *LM_spliced* peptides. For the 21 pairs we provide: MS/MS annotation of endogenous HLA-Ip as *LM_spliced* peptides, MS/MS annotation of the synthetic counterpart of the *LM_spliced*, MS/MS annotation of the same endogenous HLA-Ip as an alternative UniPro*t* peptide, and MS/MS annotation of the synthetic counterpart of the alternative UniProt peptide. For the three *LM_spliced* peptides we provide MS/MS annotations of endogenous HLA-Ip and of the synthetic counterparts.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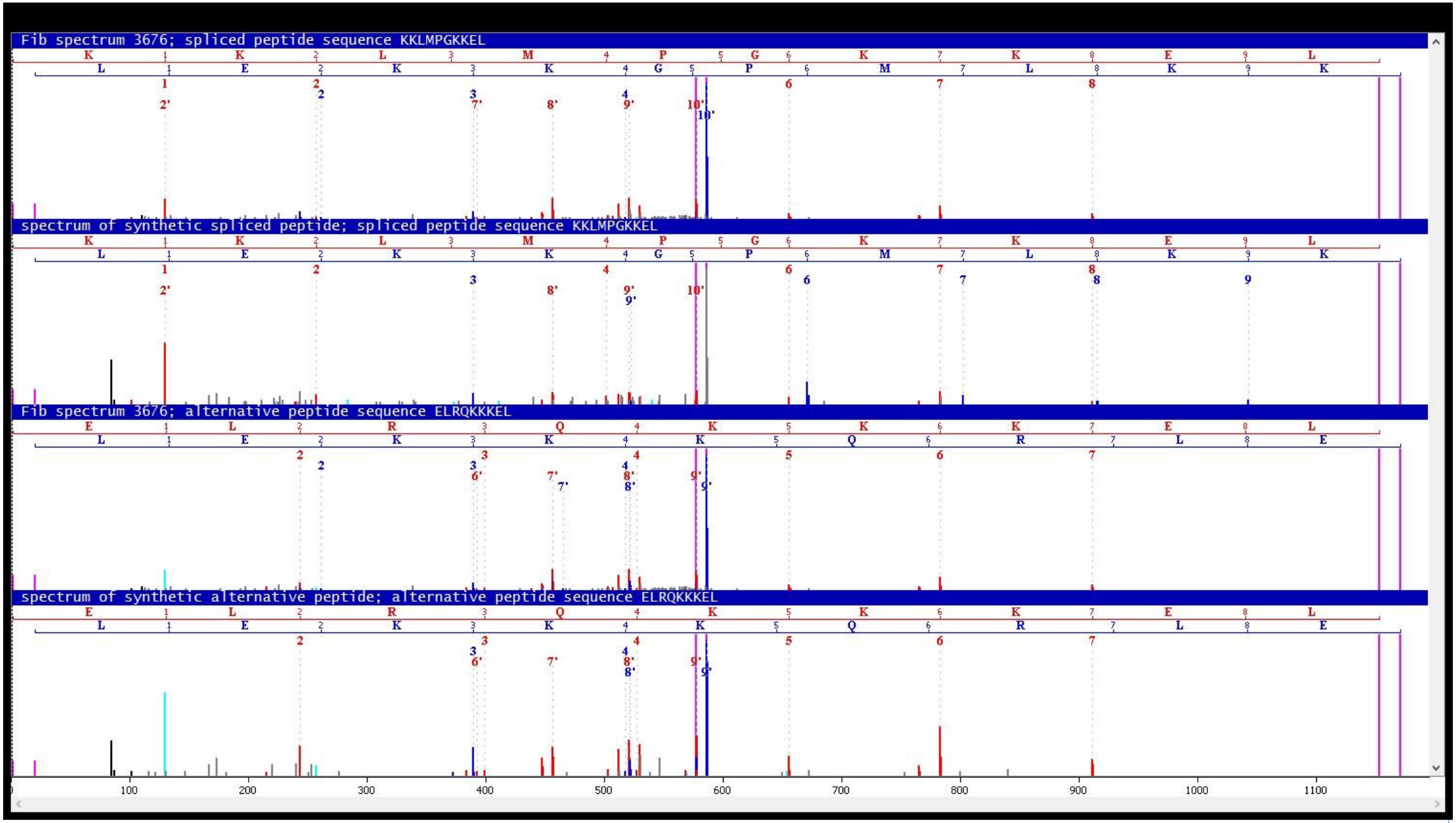
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spectrum of synthetic spliced peptide; spliced peptide sequence KKLMPGKKEL
Fib spectrum 3676; alternative peptide sequence ELRQKKKEL
spectrum of synthetic alternative peptide; alternative peptide sequence ELRQKKKEL
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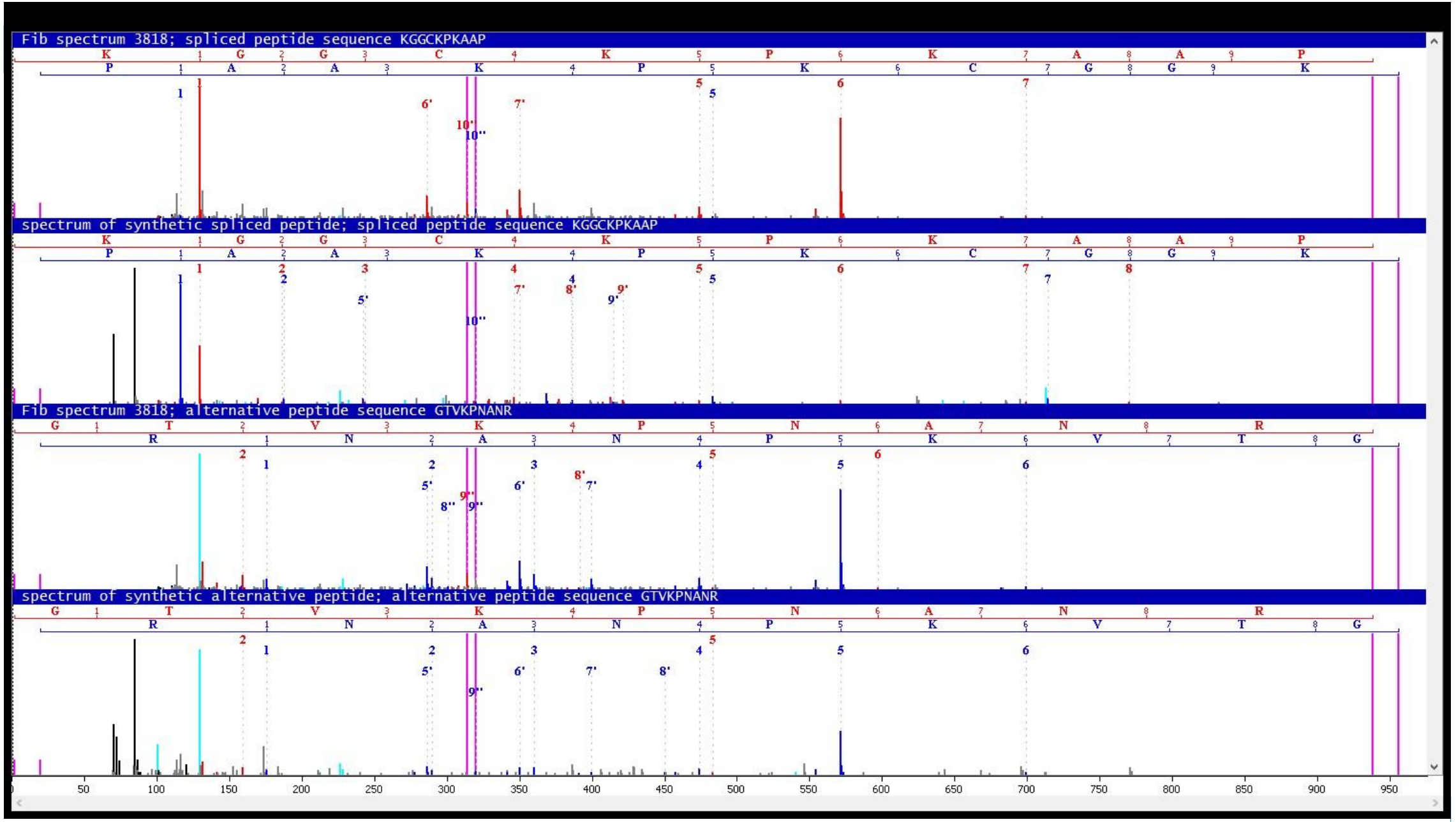
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Fib spectrum 3818; alternative peptide sequence GTVKPNANR
spectrum of synthetic alternative peptide; alternative peptide sequence GTVKPNANR
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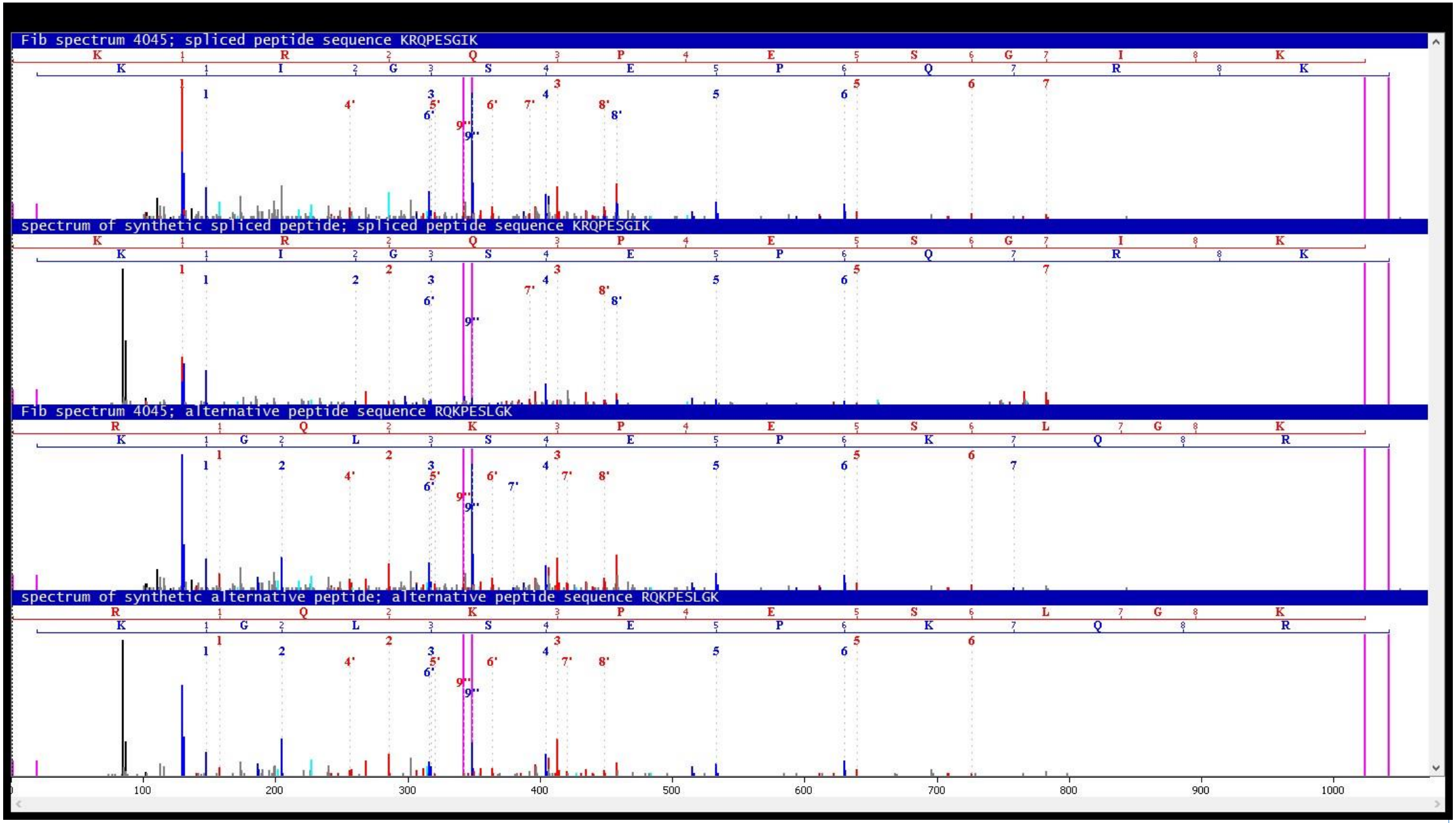
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Fib spectrum 4045; alternative peptide sequence RQKPESLGK
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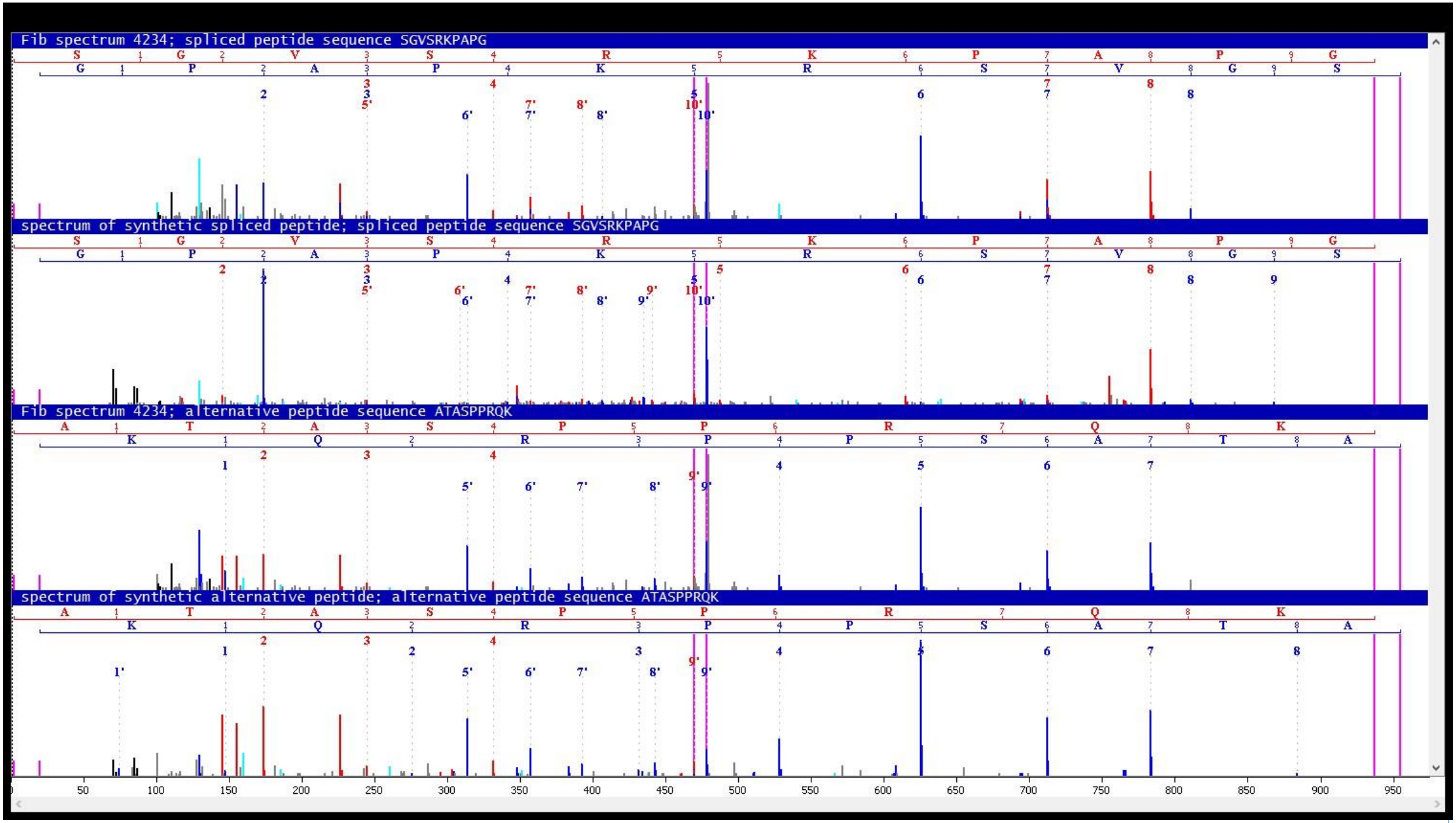
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spectrum of synthetic alternative peptide; alternative peptide sequence ATASPPRQK
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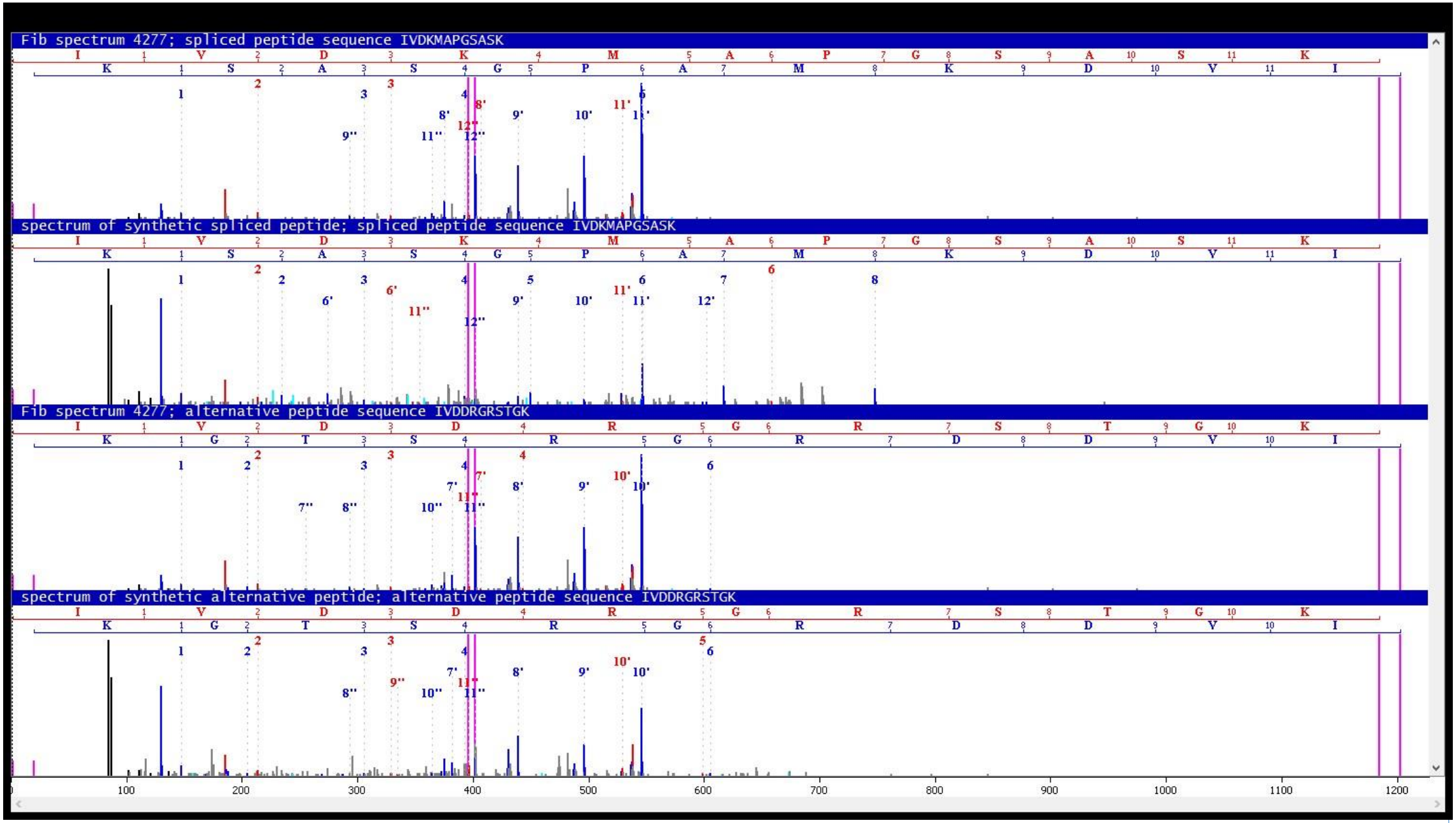
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Fib spectrum 4277; alternative peptide sequence IVDDRGRSTGK
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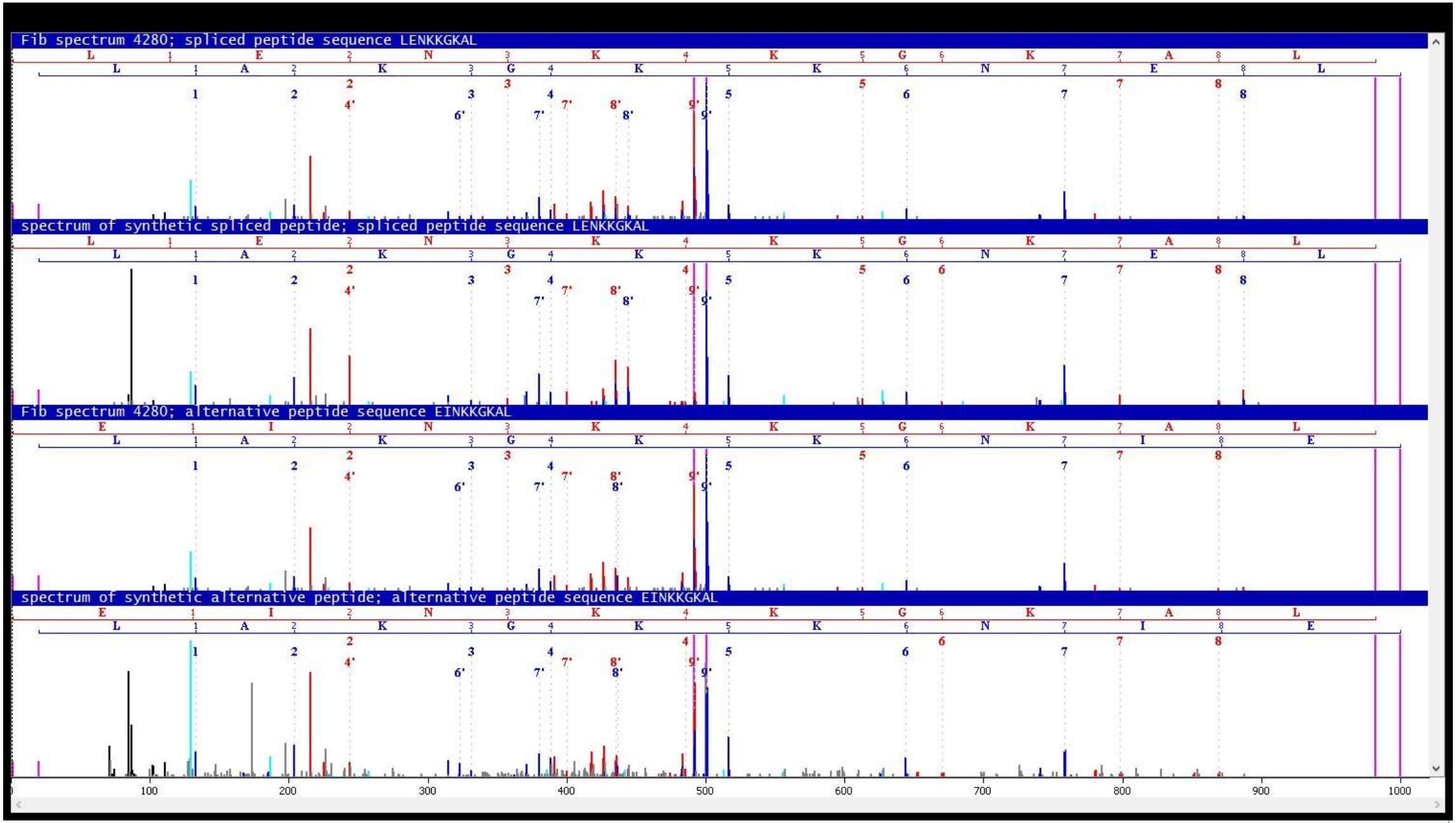
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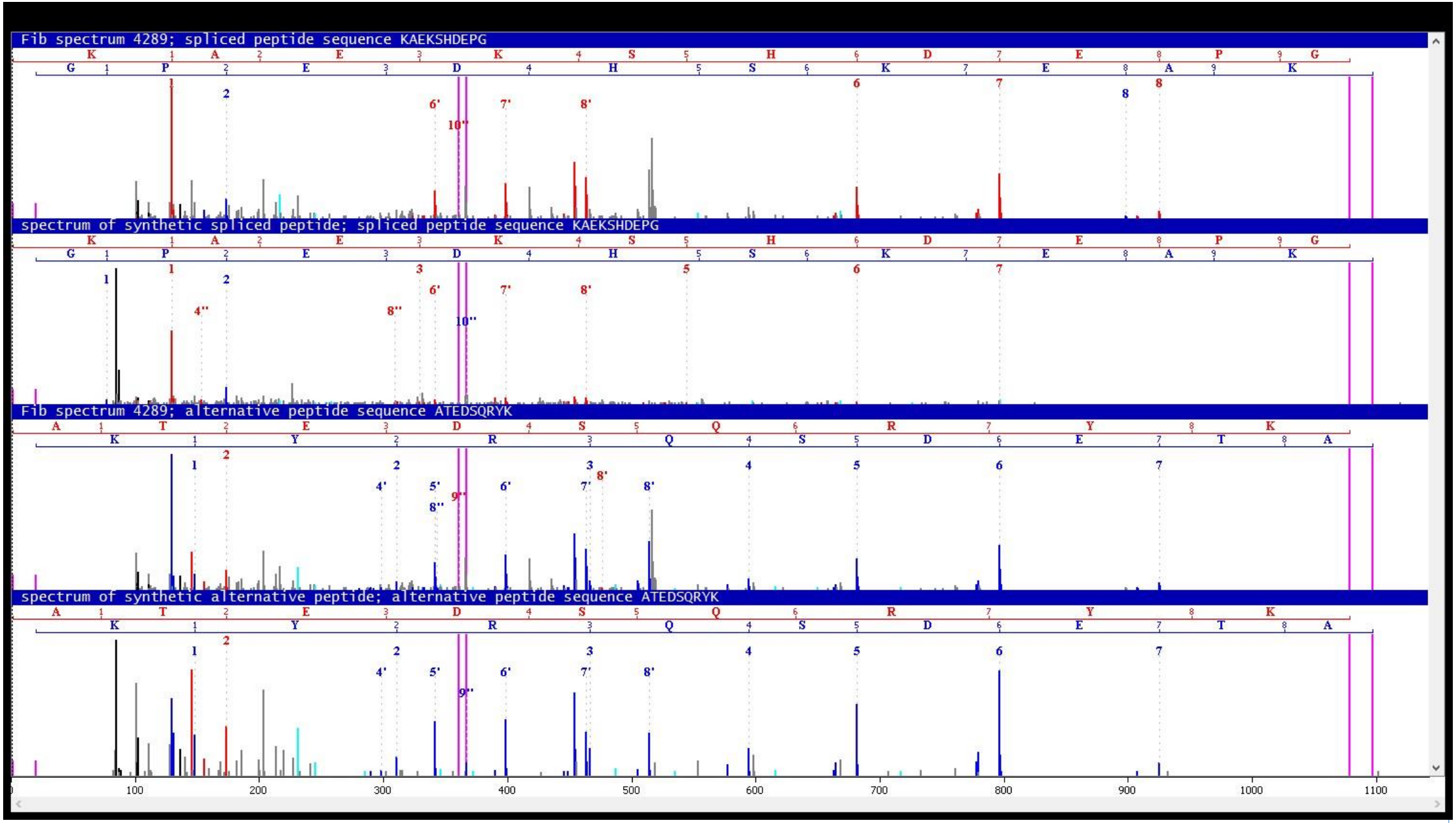
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Fib spectrum 4289; alternative peptide sequence ATEDSQRYK
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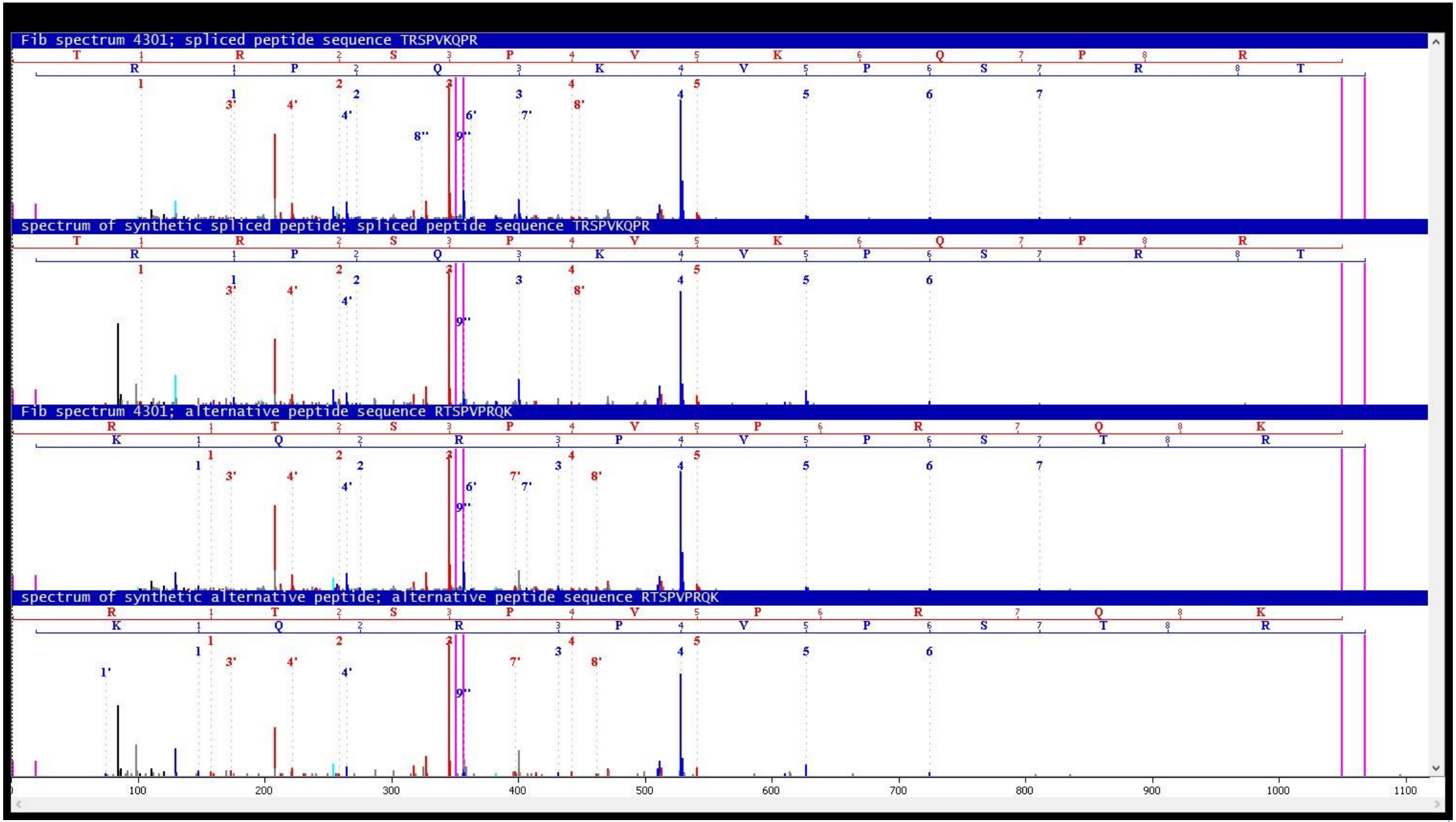
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Fib spectrum 4301; alternative peptide sequence RTSPVPRQK
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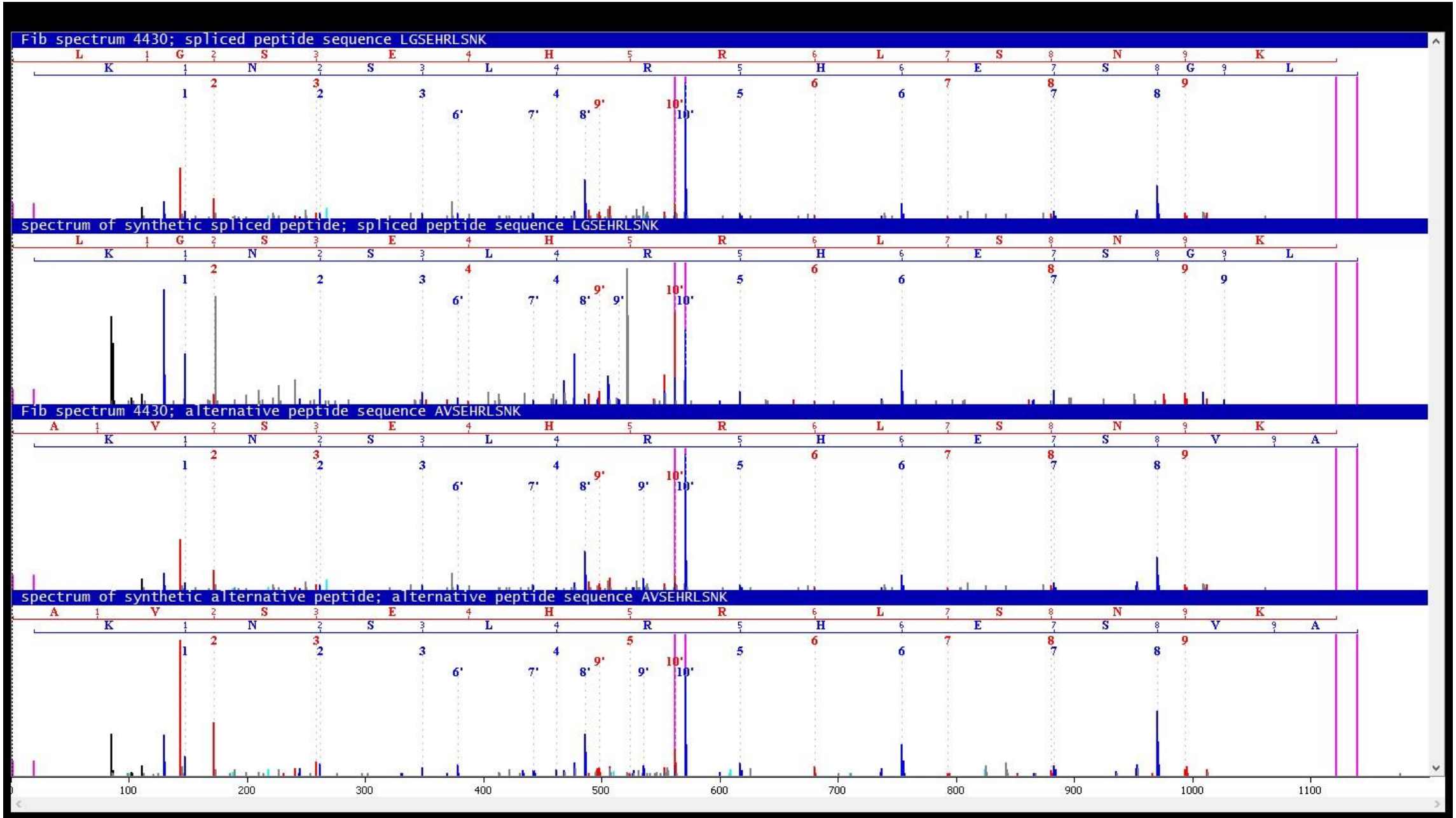
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spectrum of synthetic spliced peptide; spliced peptide sequence LGSEHRLSNK
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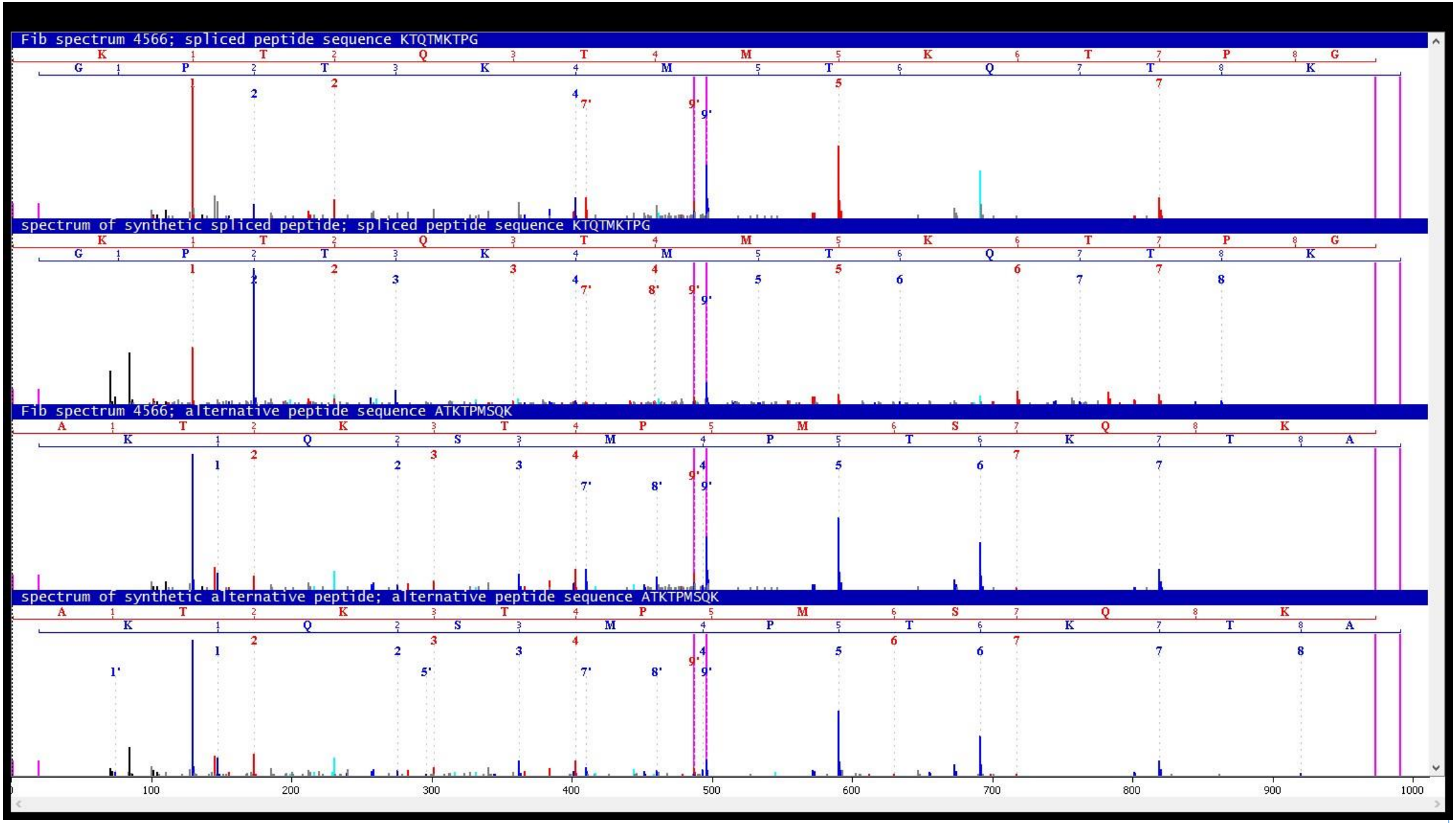
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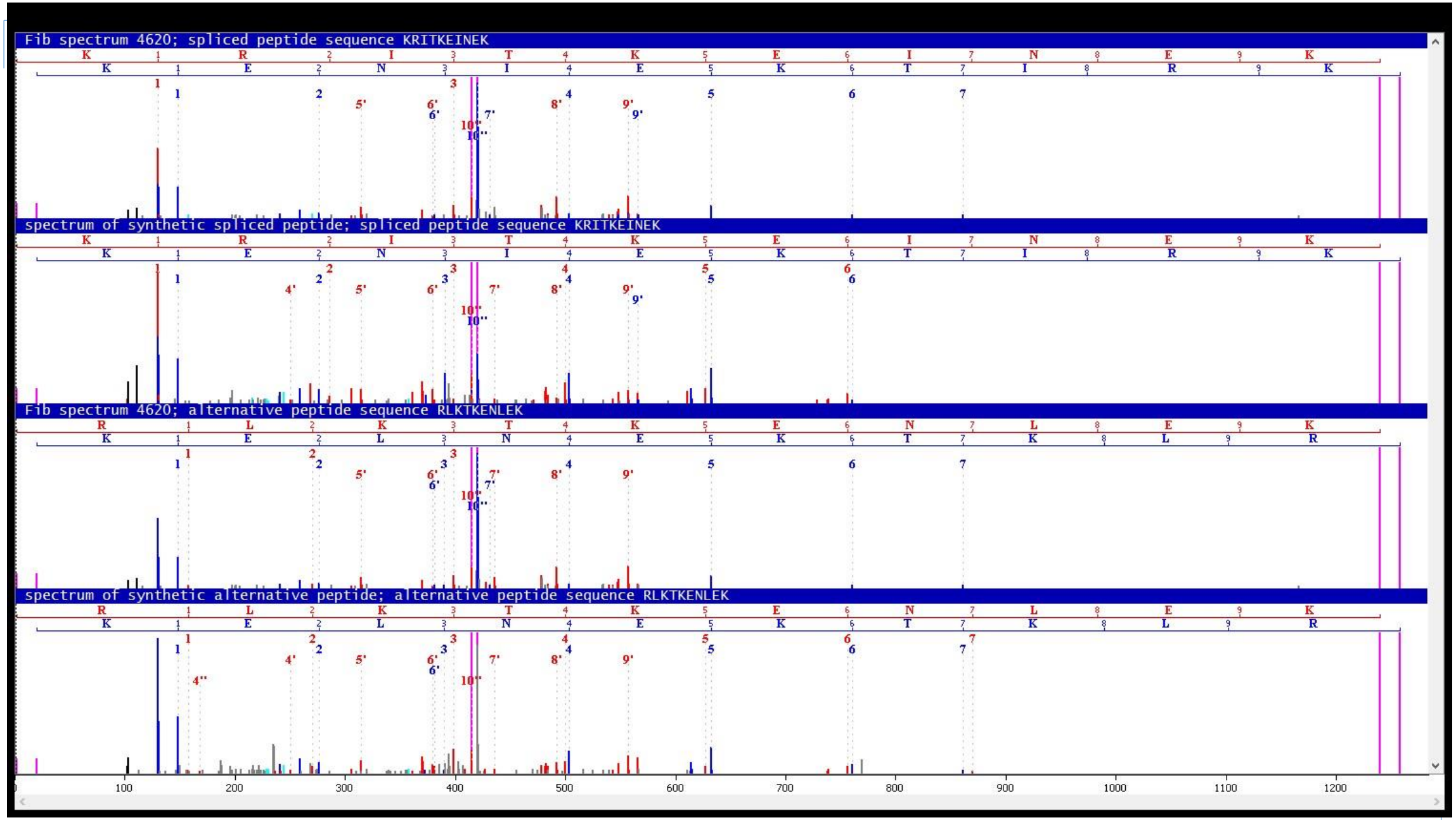
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Fib spectrum 4620; alternative peptide sequence RLKTKENLEK
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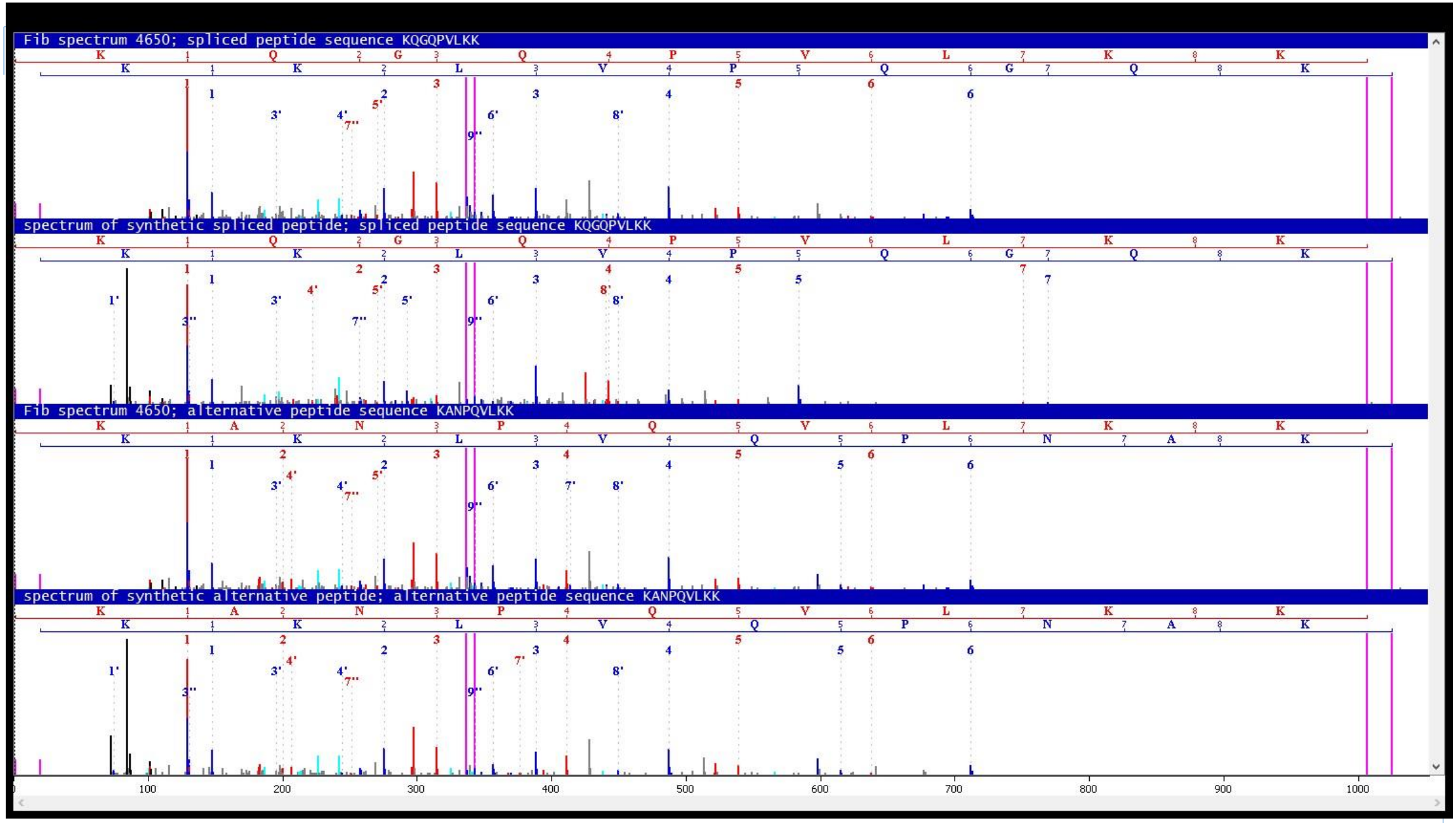
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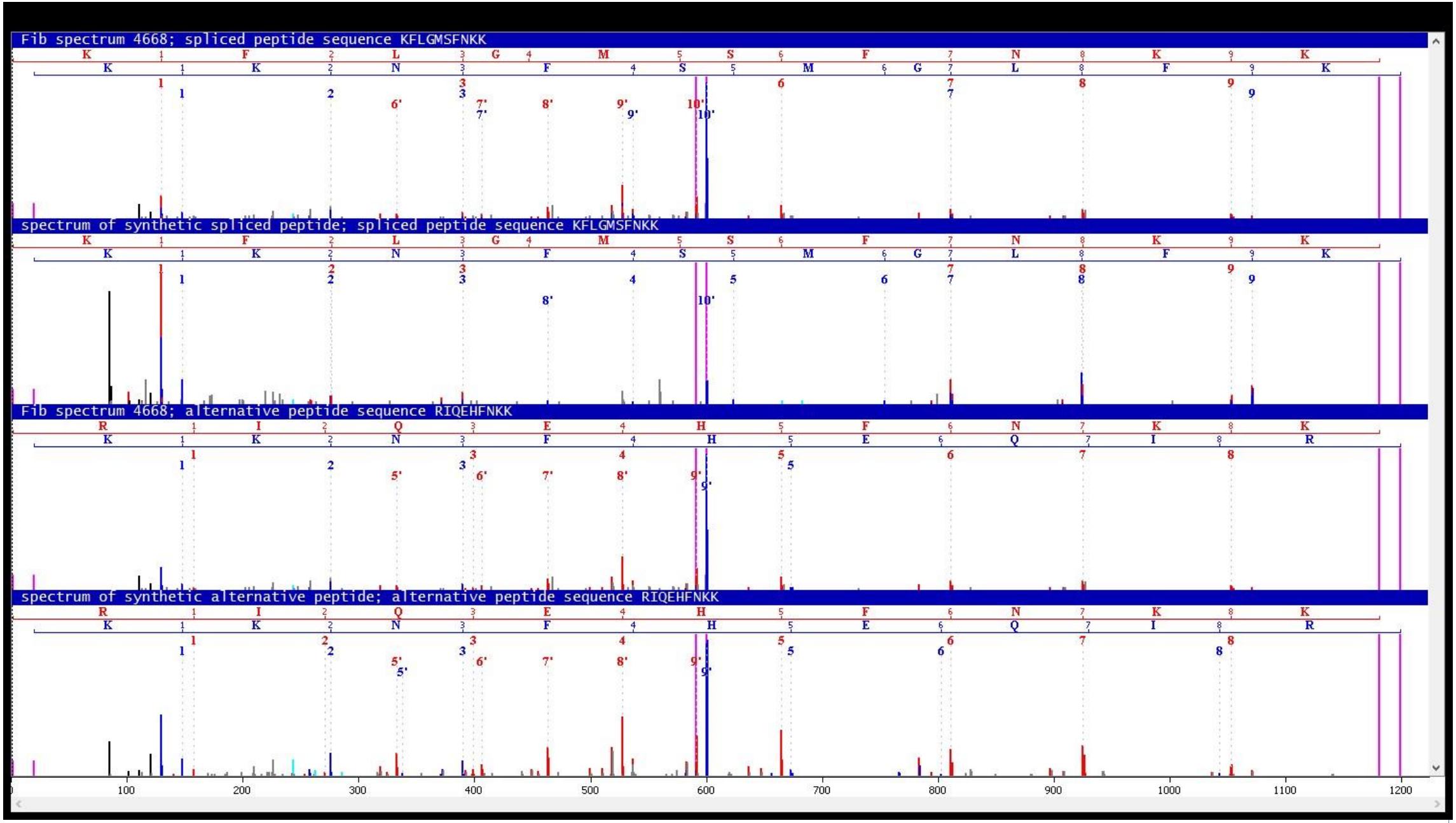
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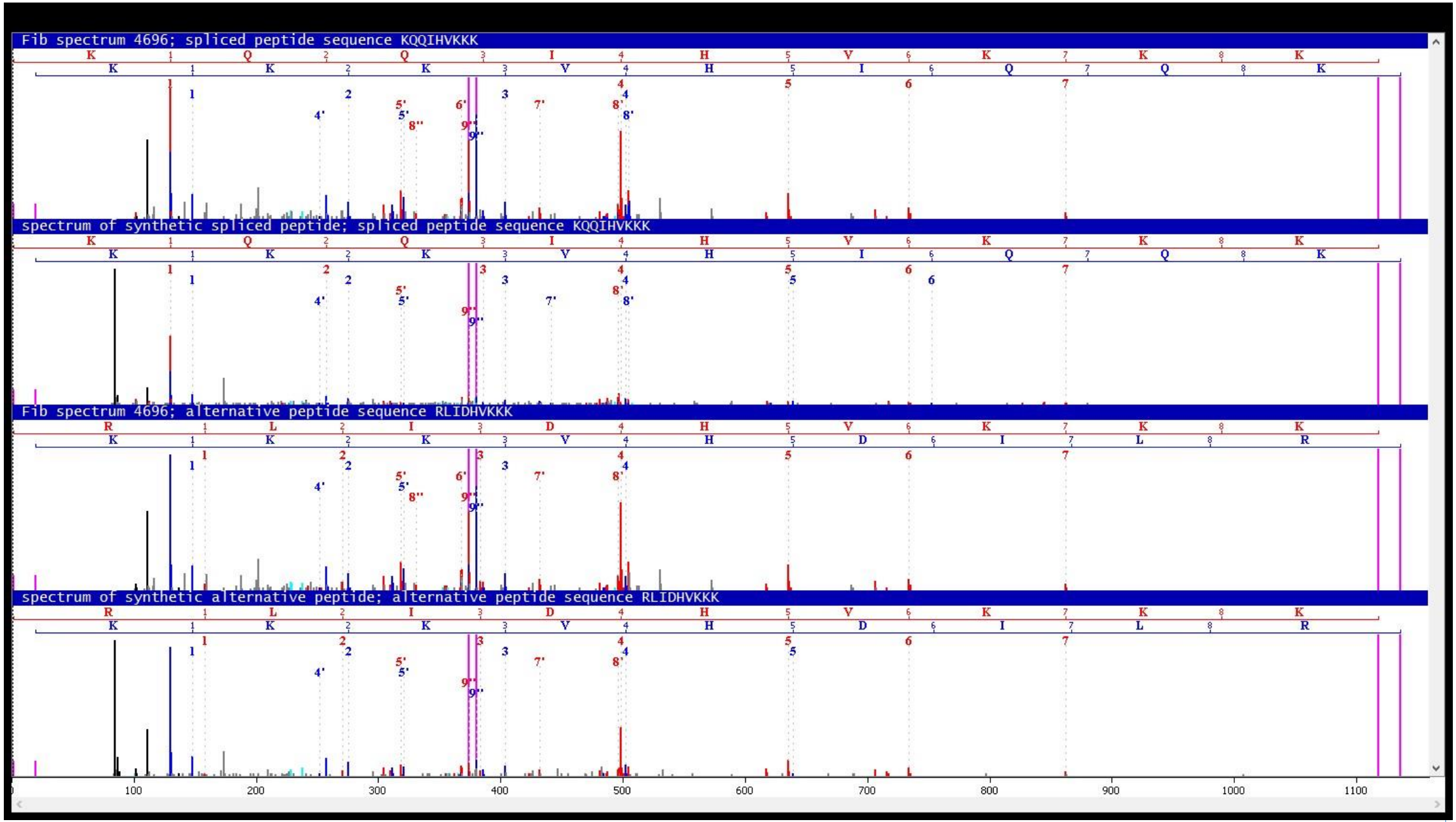
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Fib spectrum 4696; alternative peptide sequence RLIDHVKKK
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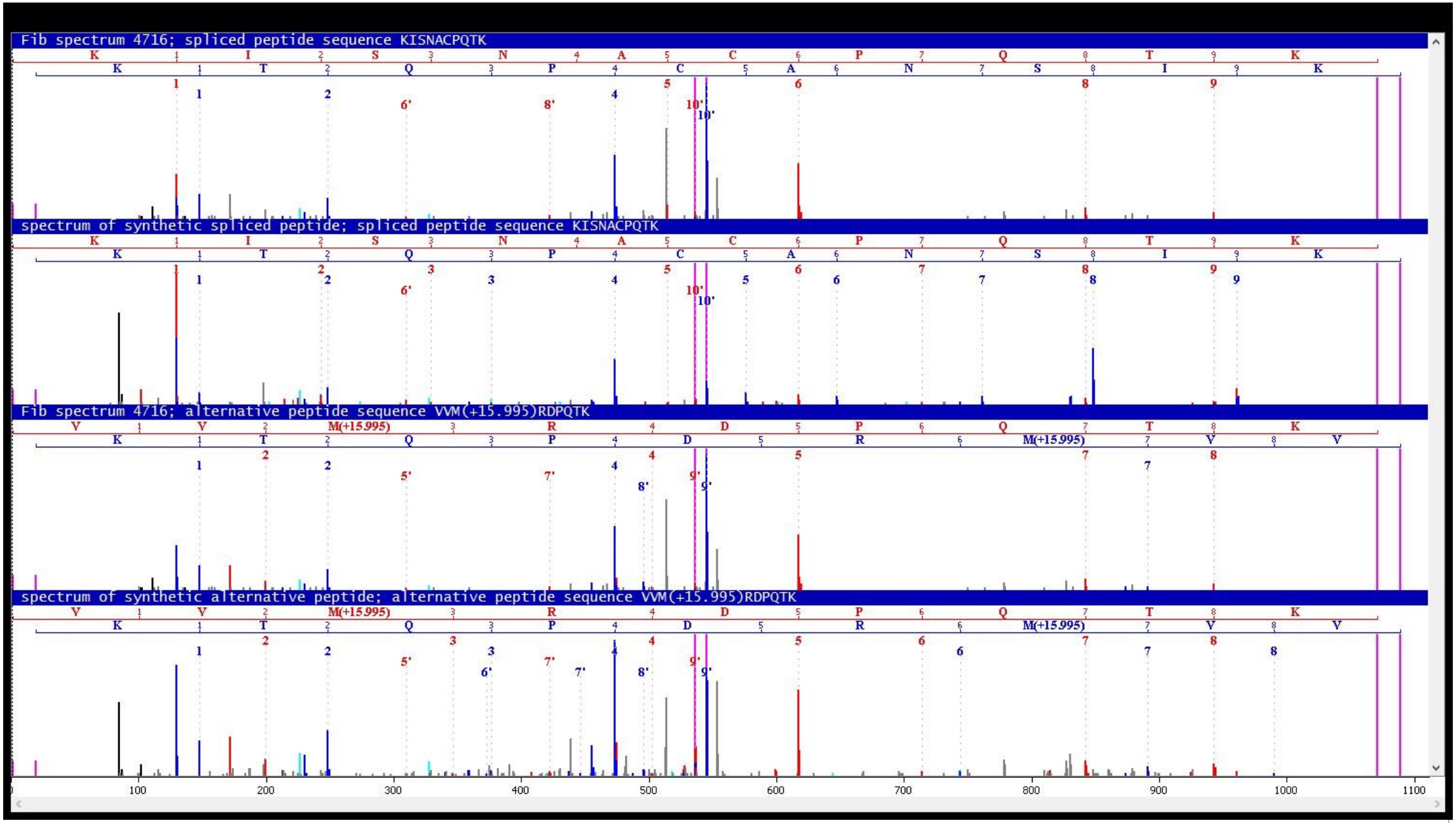
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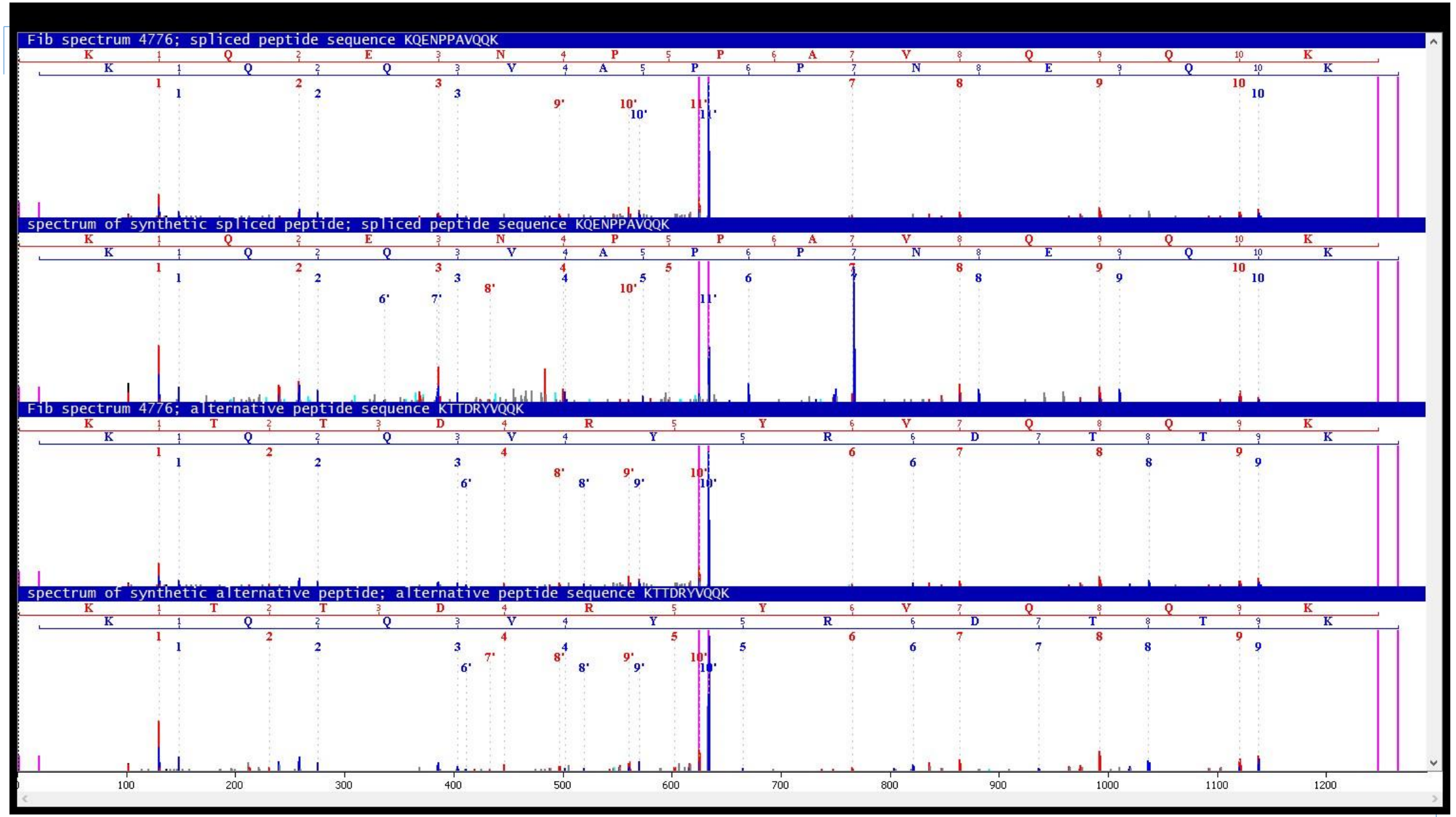
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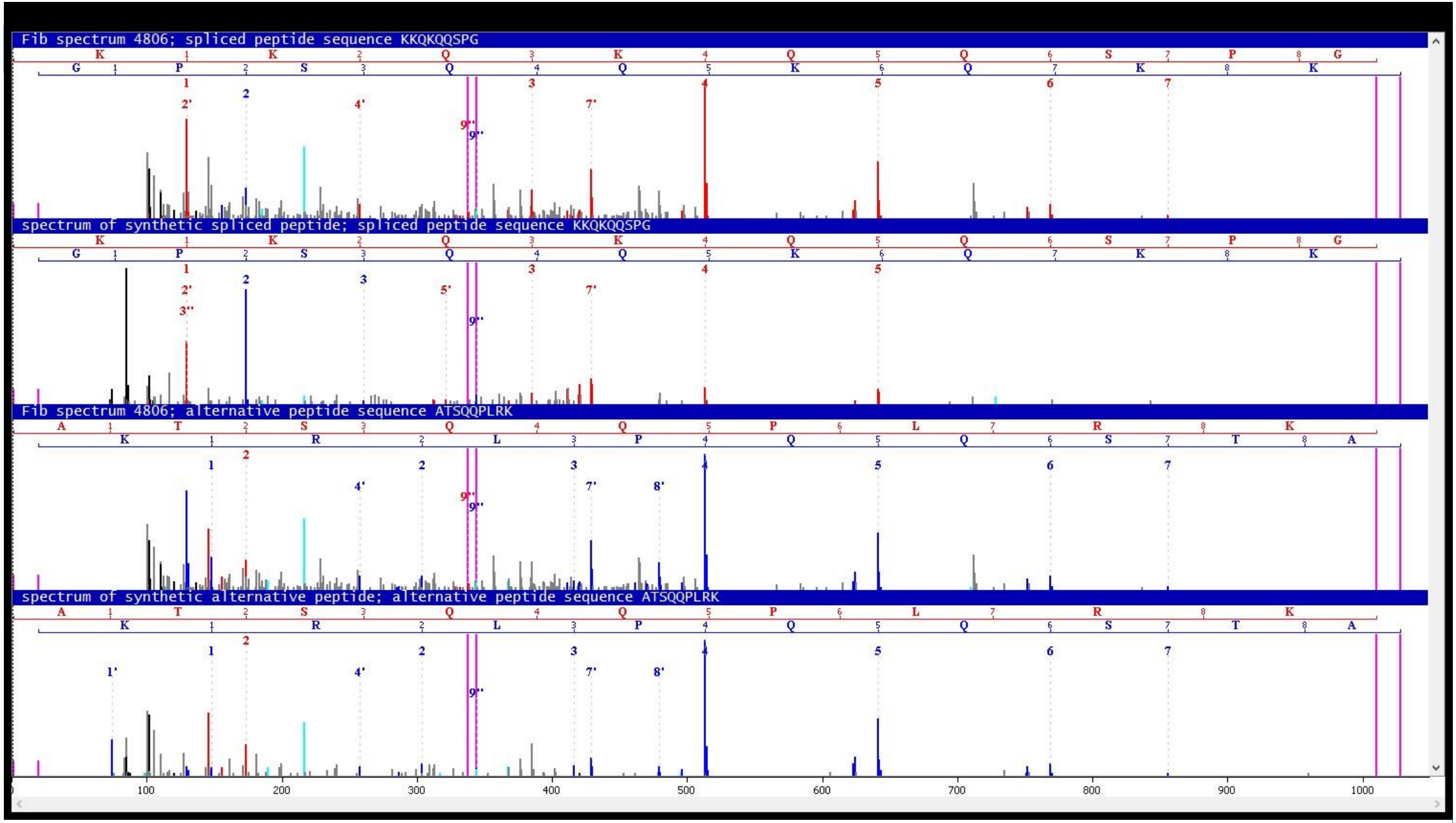
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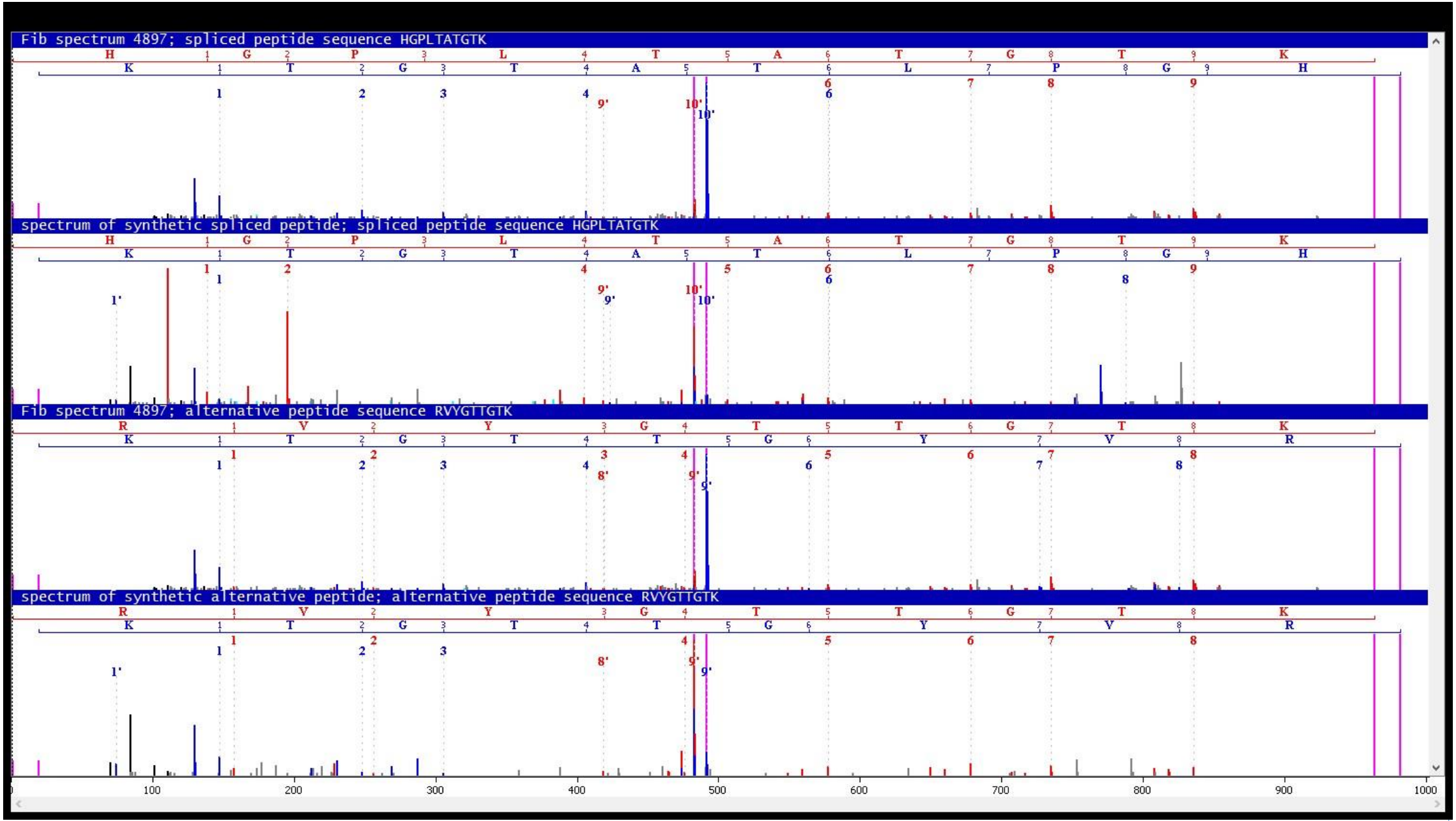
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Fib spectrum 4897; alternative peptide sequence RVYGTTGTK
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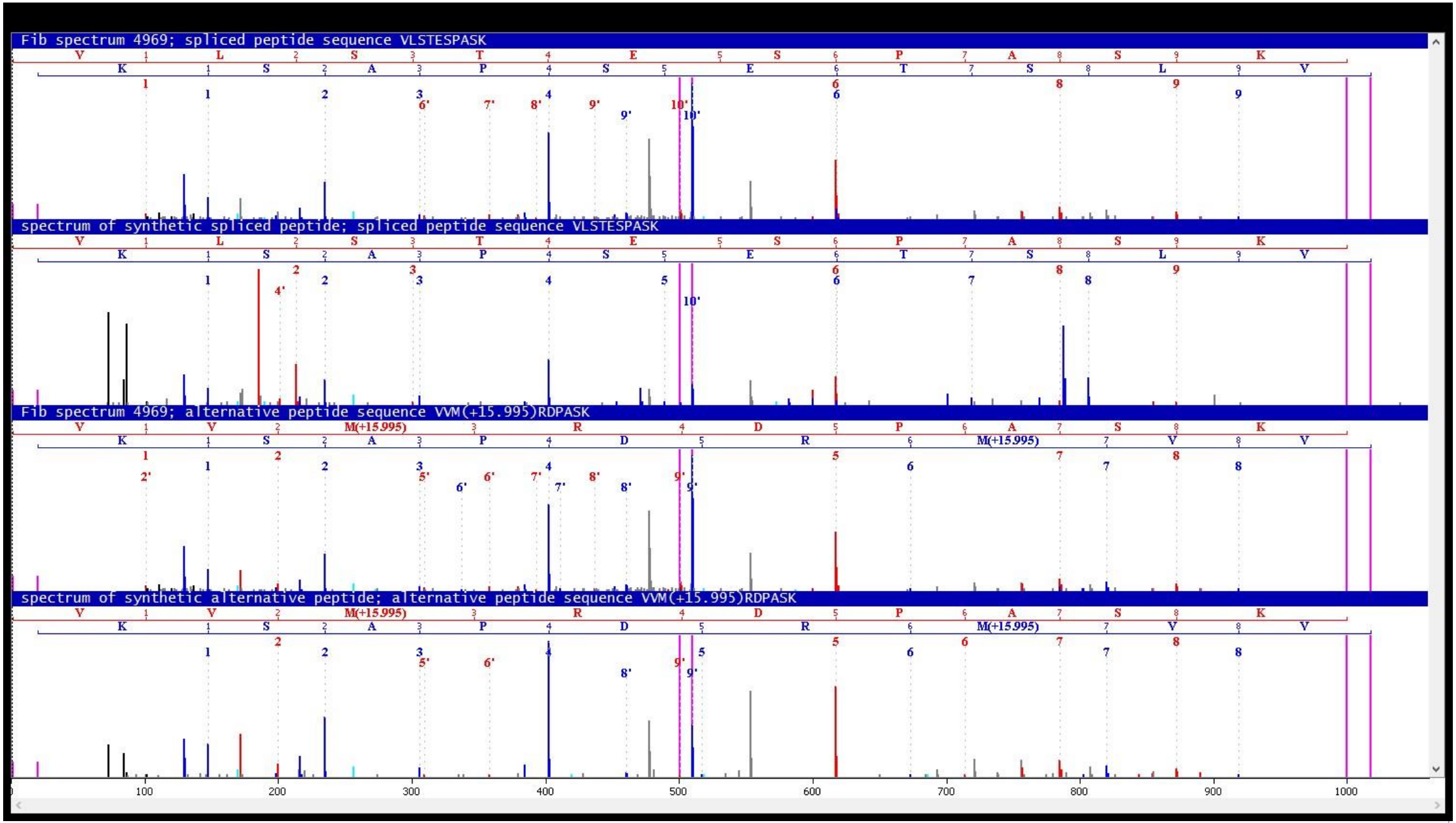
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spectrum of synthetic spliced peptide; spliced peptide sequence VLSTESPASK
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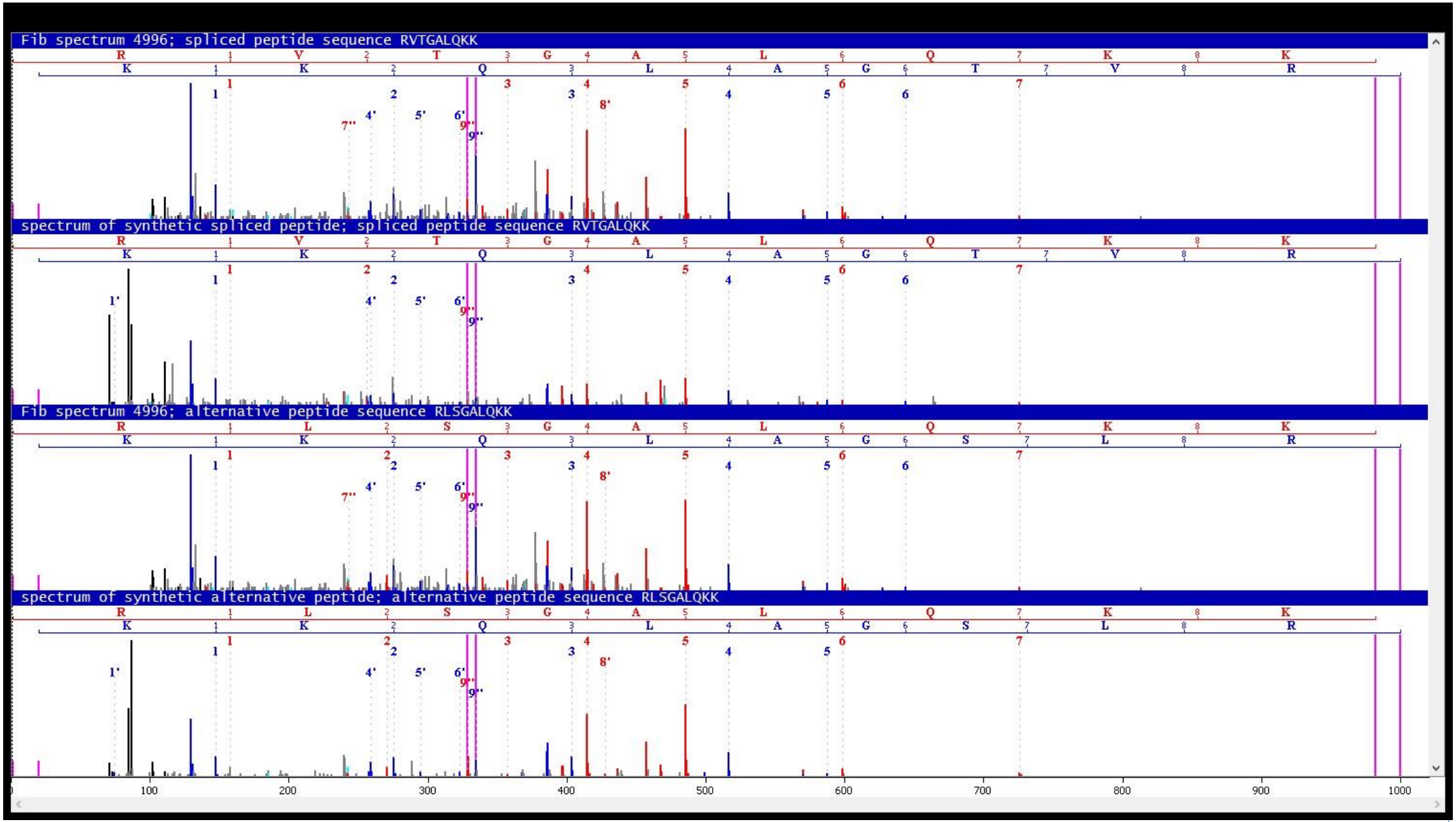
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Fib spectrum 4996; alternative peptide sequence RLSGALQKK
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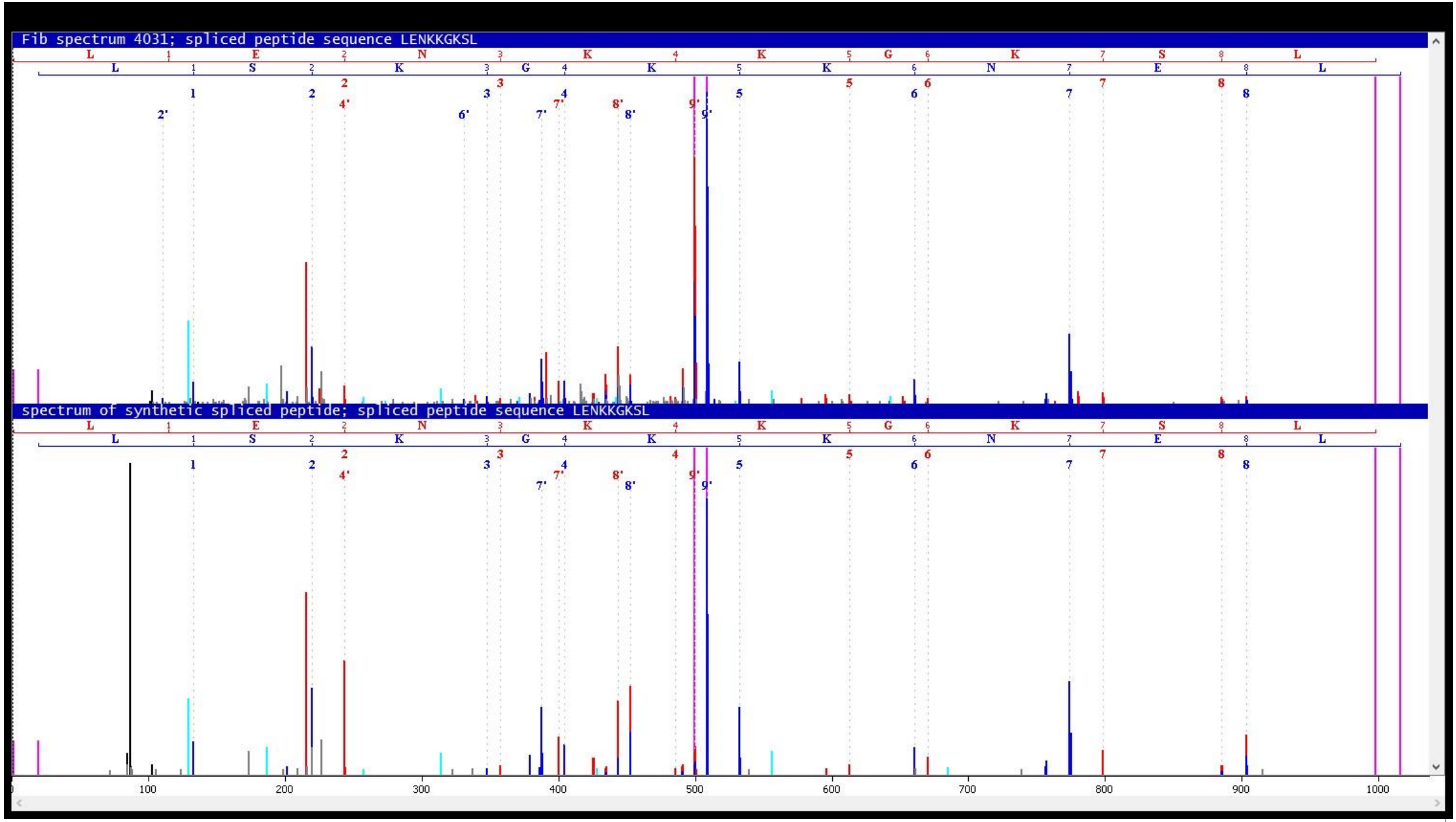
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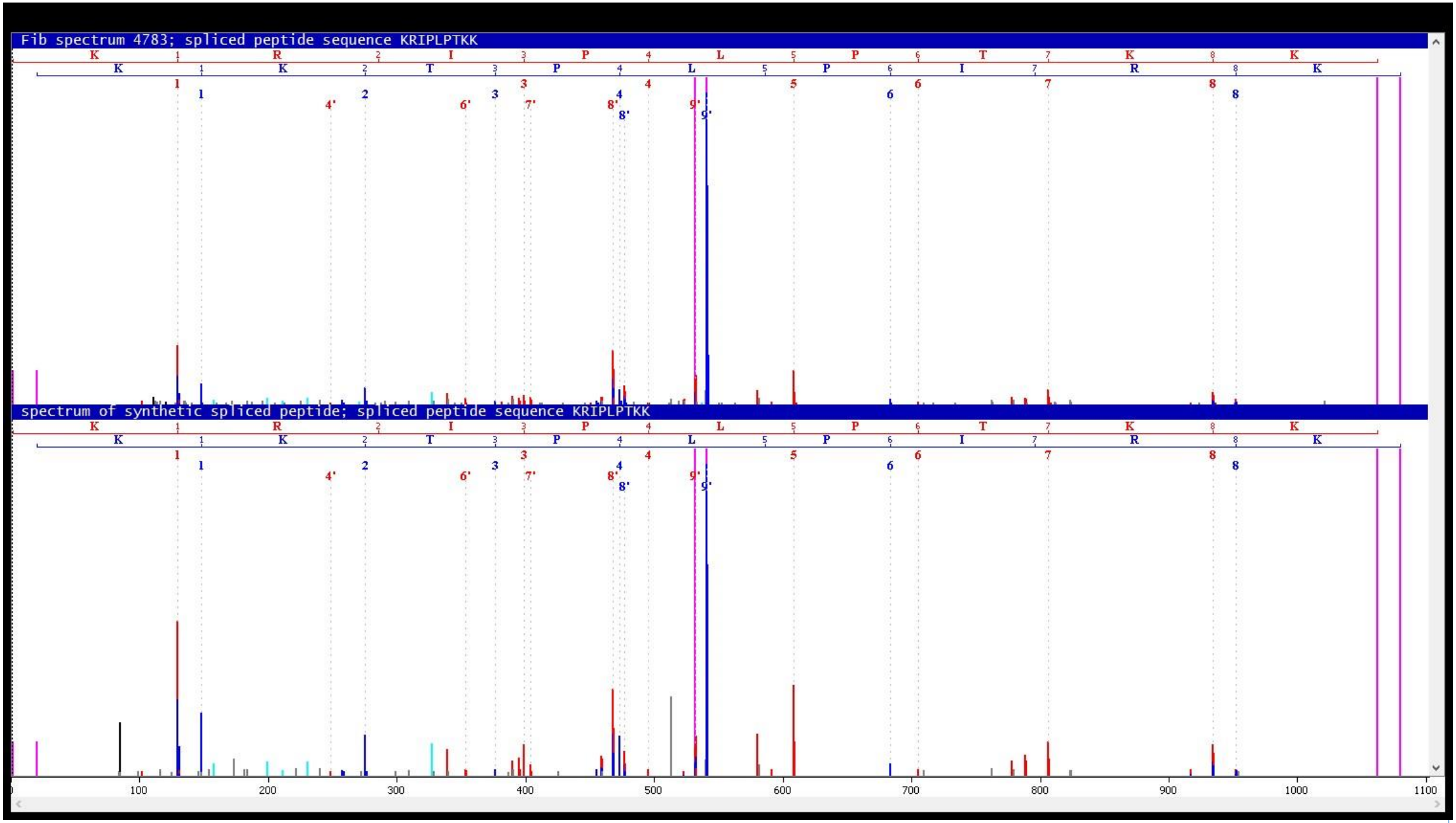
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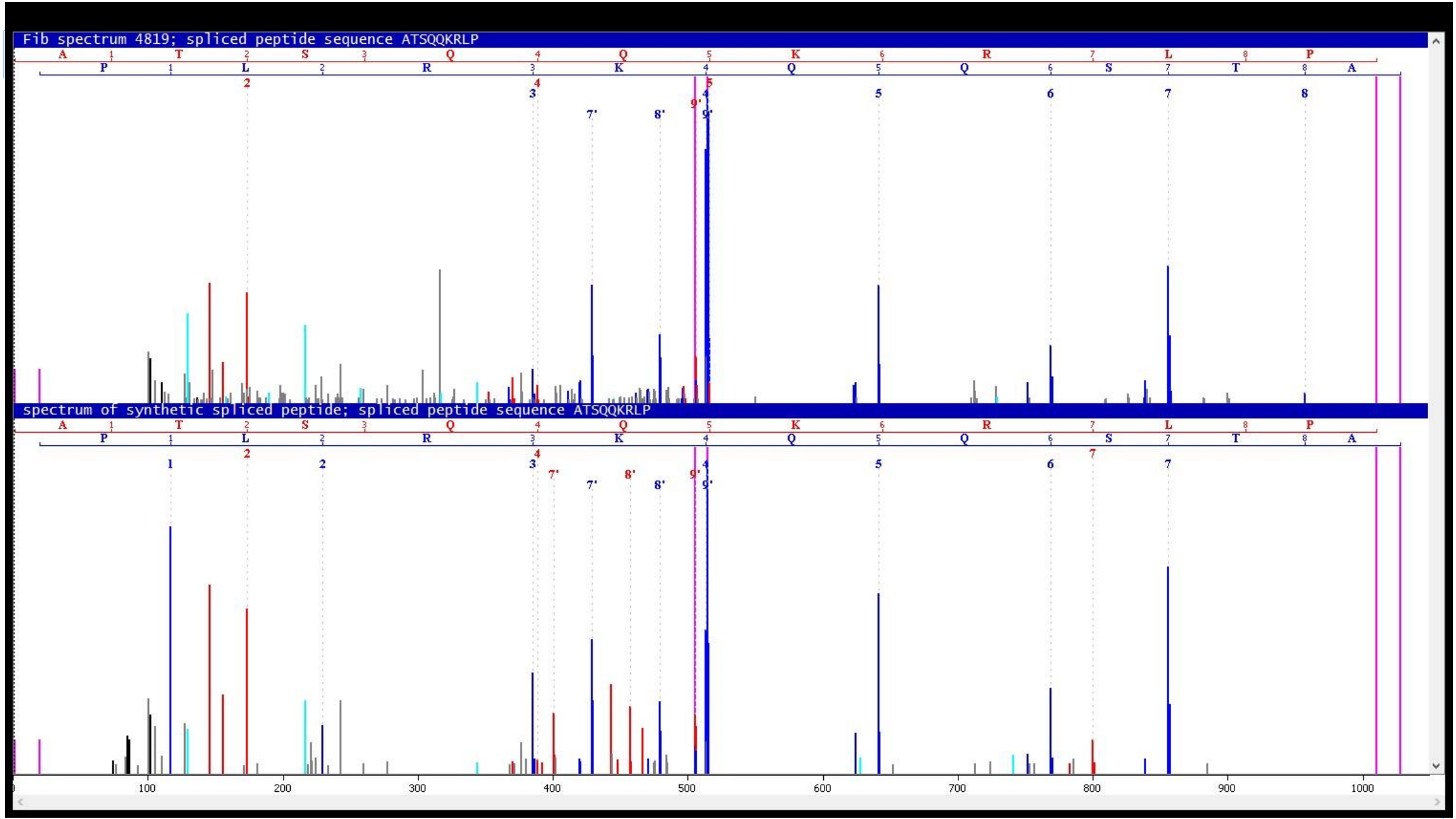
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P 1 L 2 R 3 K 4 Q 5 Q 6 S 7 T 8 A
spectrum of synthetic spliced peptide; spliced peptide sequence ATSQQKRLP
A 1 T 2 S 3 Q 4 Q 5 K 6 R 7 L 8 P
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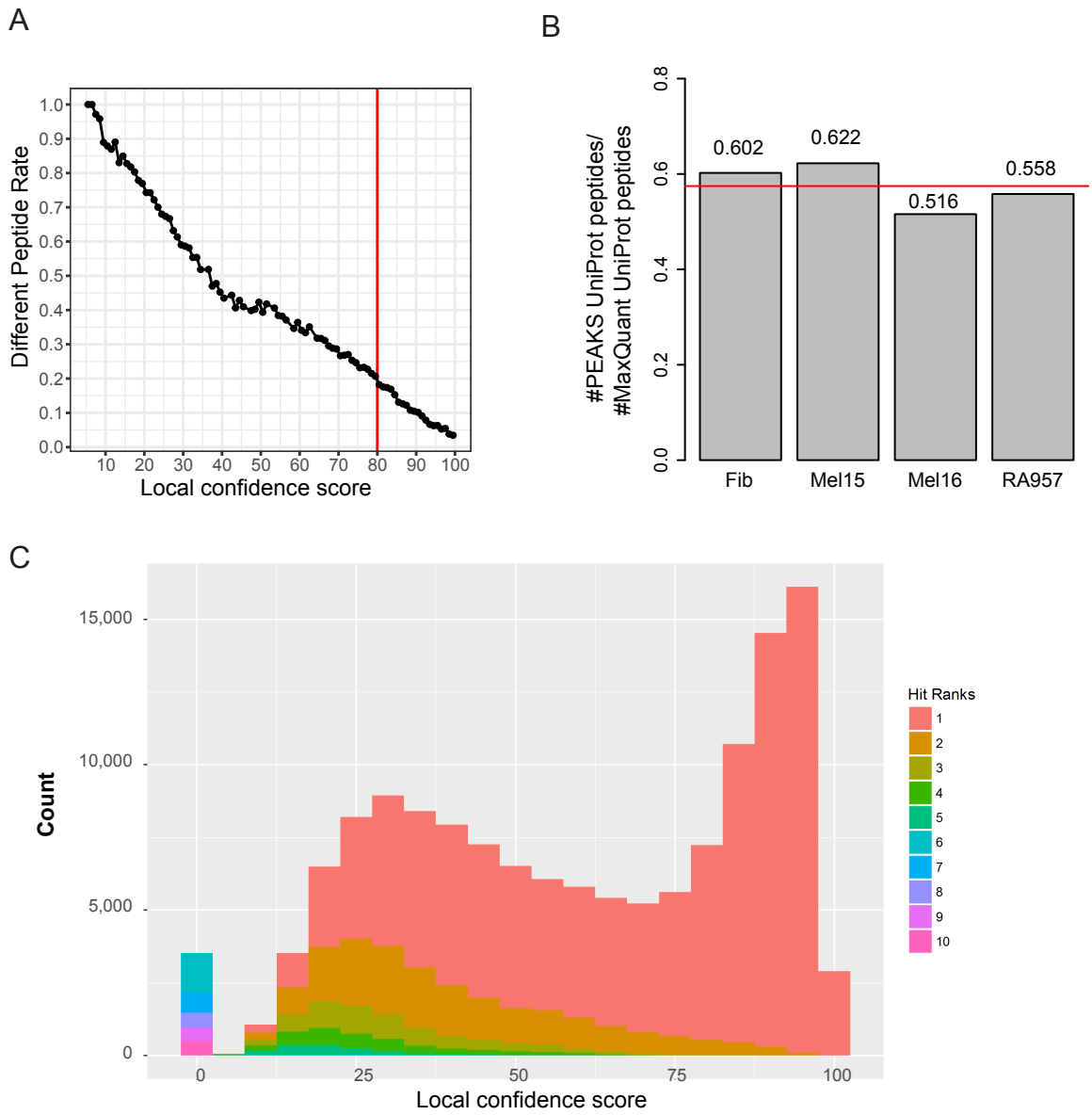
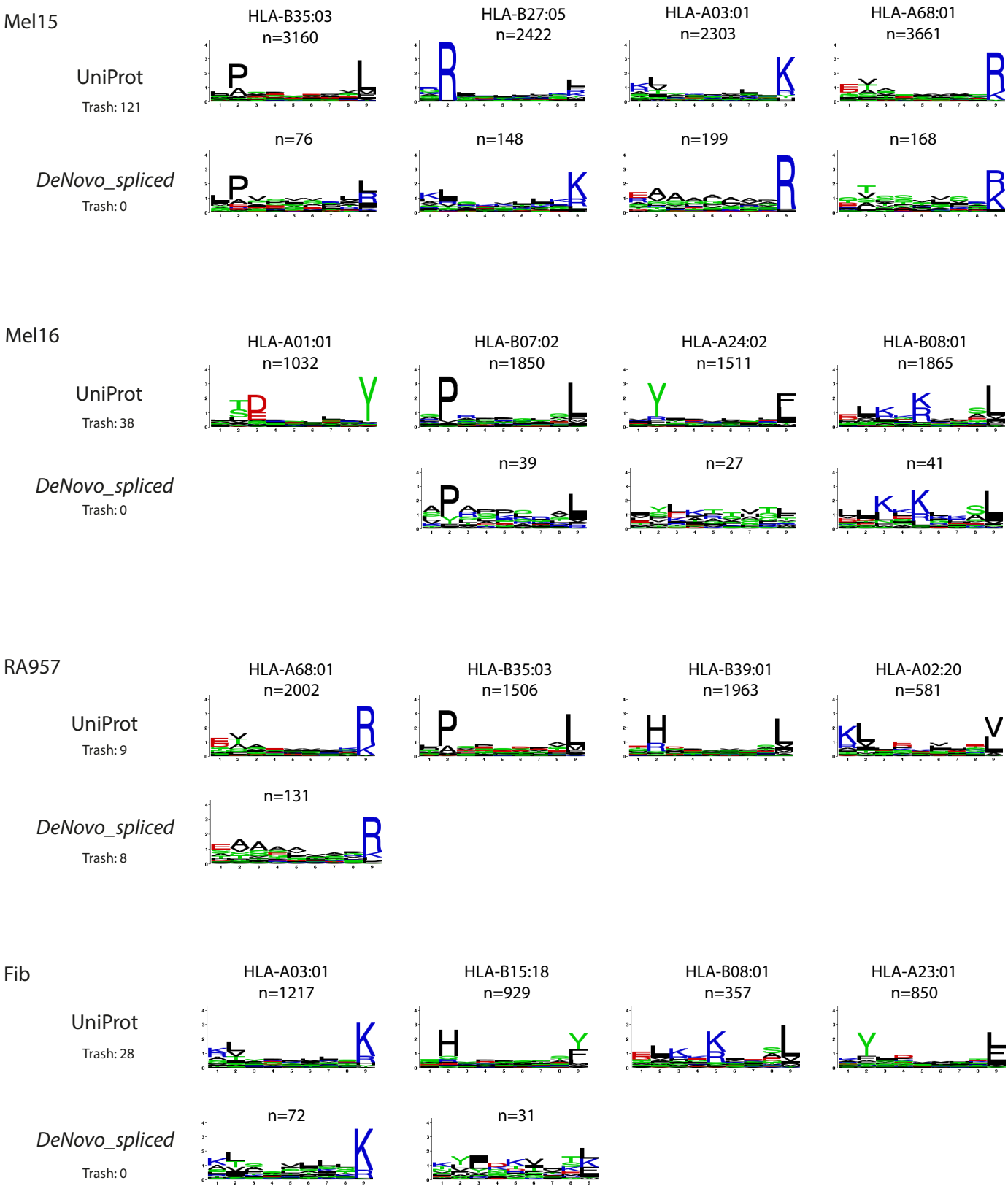
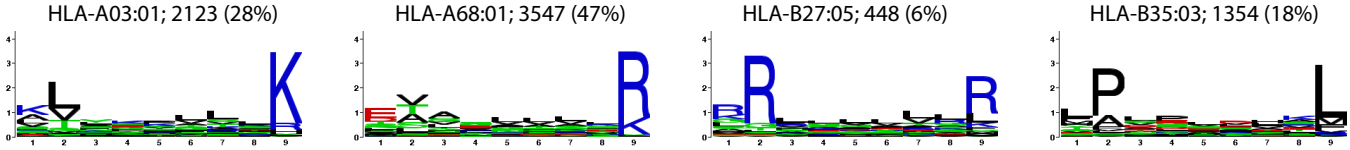


Supplemental Figure 6: A) Estimation of the error rate in PEAKS results by the fraction of Mel15 UniProt PSMs (y-axis) with a local confidence score higher than a threshold (x-axis), for which PEAKS assigned a different peptide compared to MaxQuant. Since the MaxQuant FDR is in agreement with the Comet FDR and Mascot FDR for the UniProt peptides (Figure 1 C), we assume that the error by MaxQuant is indeed about 1% and most of the sequence differences in this case are caused by erroneous PEAKS matches. PEAKS shows a FDR of about 20% at a local confidence score of 80 (red line). B) Fraction of UniProt HLA peptides identified by PEAKS at a local confidence score of 80 compared to MaxQuant UniProt peptides at spectrum level of FDR 1% for the different samples. C) A histogram presenting for each PSM the local confidence scores of the ten best matches identified by PEAKS in Mel15 data as an example.



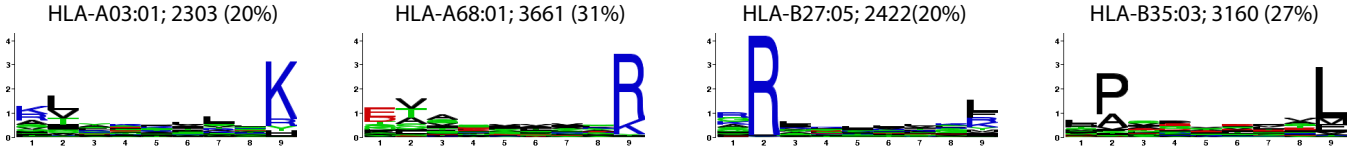
Supplamental Figure 7: Sequence motifs for the 9-mer *DeNovo_spliced* peptides and UniProt peptides identified by MaxQuant in Mel15, Mel16, RA957 and Fib samples.

A. PEAKS



Trash cluster: 4 (0.1%)

B. MaxQuant



Trash cluster: 121 (1%)

Supplemental Figure 8: A) Clustering analysis with MixMHCp of HLA-Ip identified as UniProt hits by PEAKS in Mel15 sample revealed three dominant clusters referring to HLA-B35:03, HLA-A68:01 and HLA-A03:01 and only 6% of the peptides clustered into the motif of the HLA-B27:05 allele. B) Ssimilar clustering analysis of HLA-Ip identified as UniProt hits by MaxQuant from the same MS data revealed four dominant clusters and 20% of the peptides clustered into the HLA-B27:05 motif.