

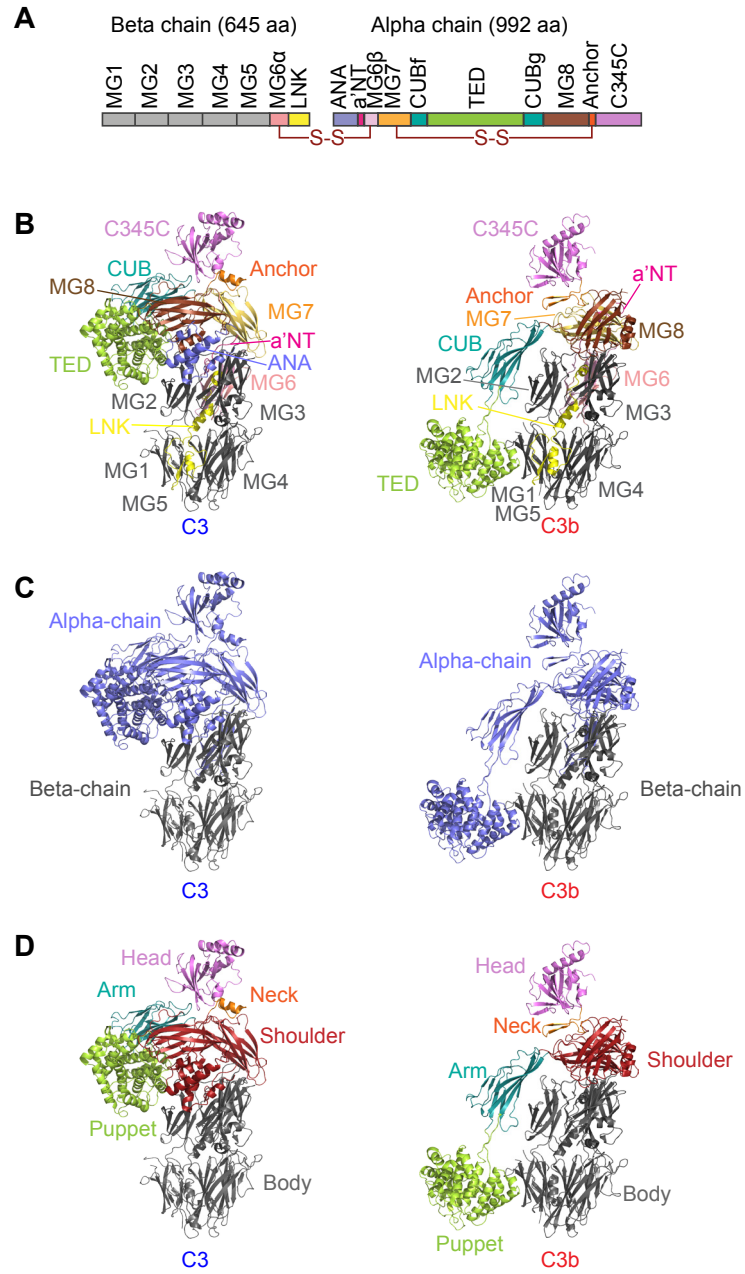
Supplemental Information

Structure of complement C3(H₂O) revealed by quantitative cross-linking/mass spectrometry and modelling

Zhuo A. Chen, Riccardo Pellarin, Lutz Fischer, Andrej Sali, Michael Nilges,
Paul N. Barlow*, Juri Rappsilber*

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Supplemental Figure 1



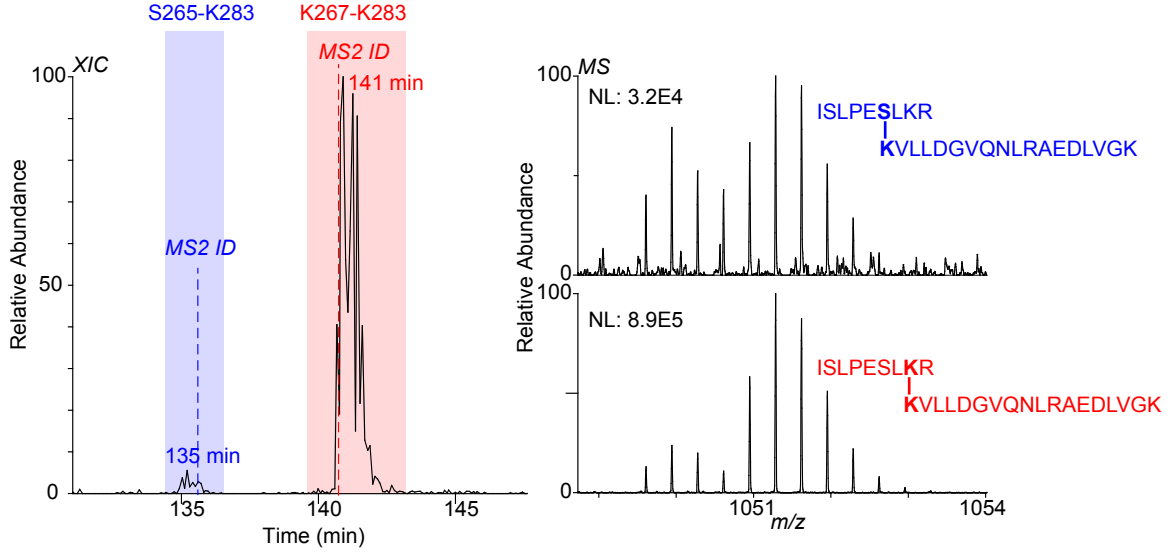
Supplemental Figure 1: Domain arrangements of C3 and C3b in the crystal structures

(A) Summary of the multiple domains of C3 (24). **(B)** Domains of C3 and C3b are coloured and labelled in the crystal structures of C3 (PDB|2A73) and C3b (PDB|2I07) (the same PDB entries are used for C and D). **(C)** The α -chain and the β -chain of C3/C3b are coloured and labelled in the crystal structures of C3 and C3b **(D)** The crystal structures of C3 and C3b are coloured and labelled according to the metaphor of a puppeteer (1).

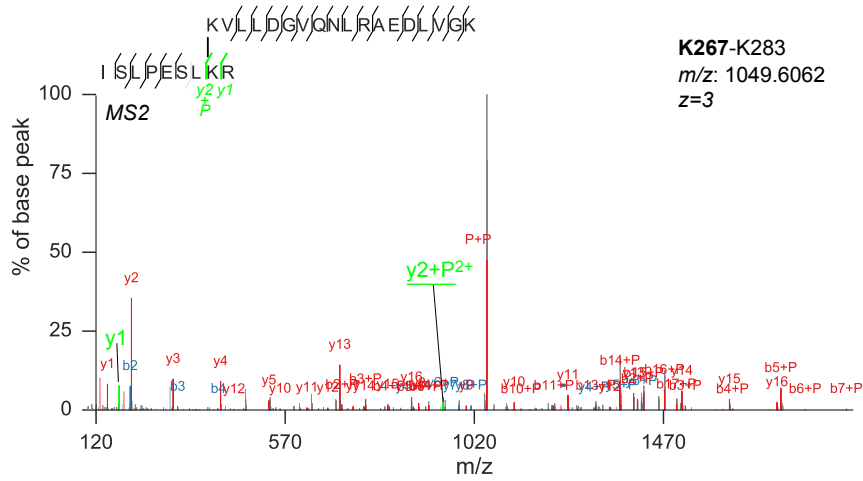
1. Janssen, B. J., Christodoulidou, A., McCarthy, A., Lambris, J. D., and Gros, P. (2006) Structure of C3b reveals conformational changes that underlie complement activity. *Nature* 444, 213-216

Supplemental Figure S2

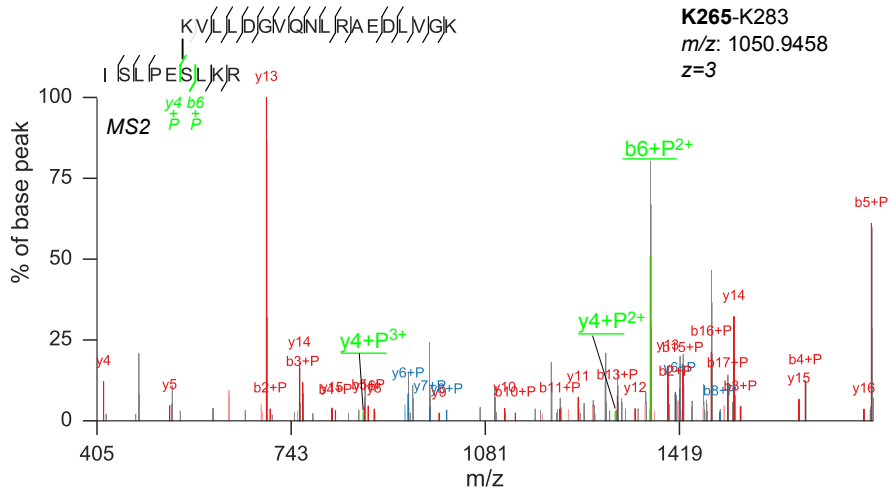
A



B



C

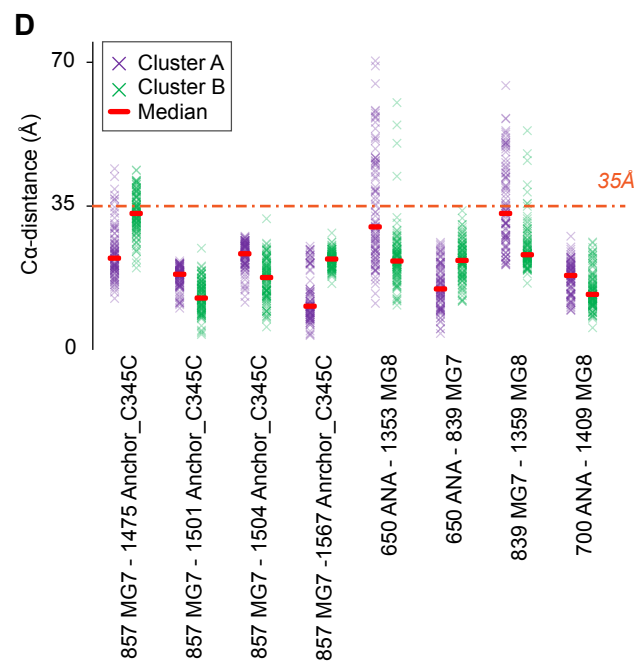
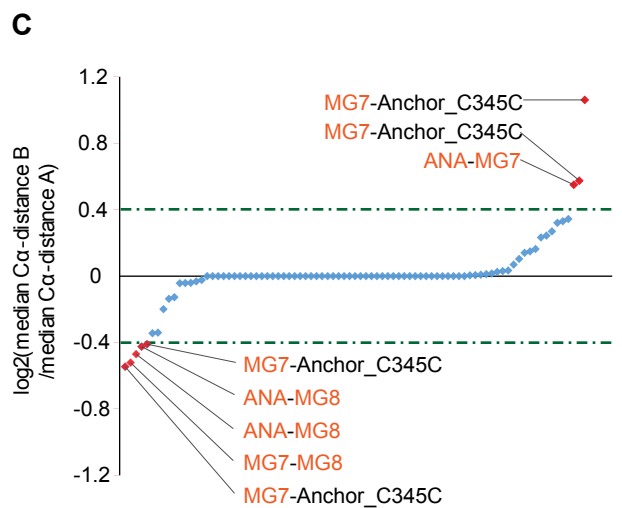
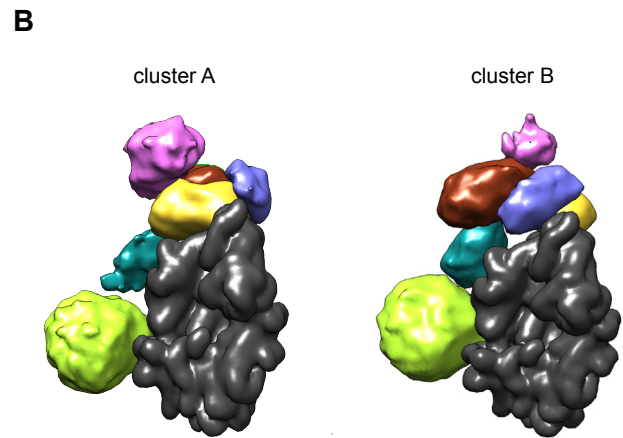
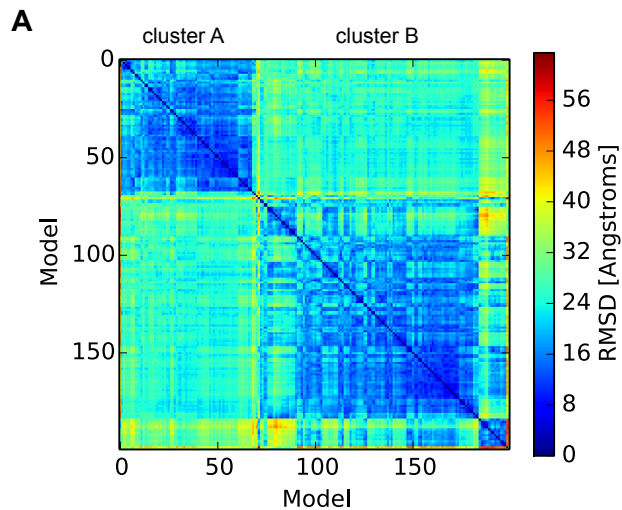


Supplemental Fig S2. Assessment of cross-link site assignment

Assessment of site assignment of cross-linked peptides is demonstrated using an example.

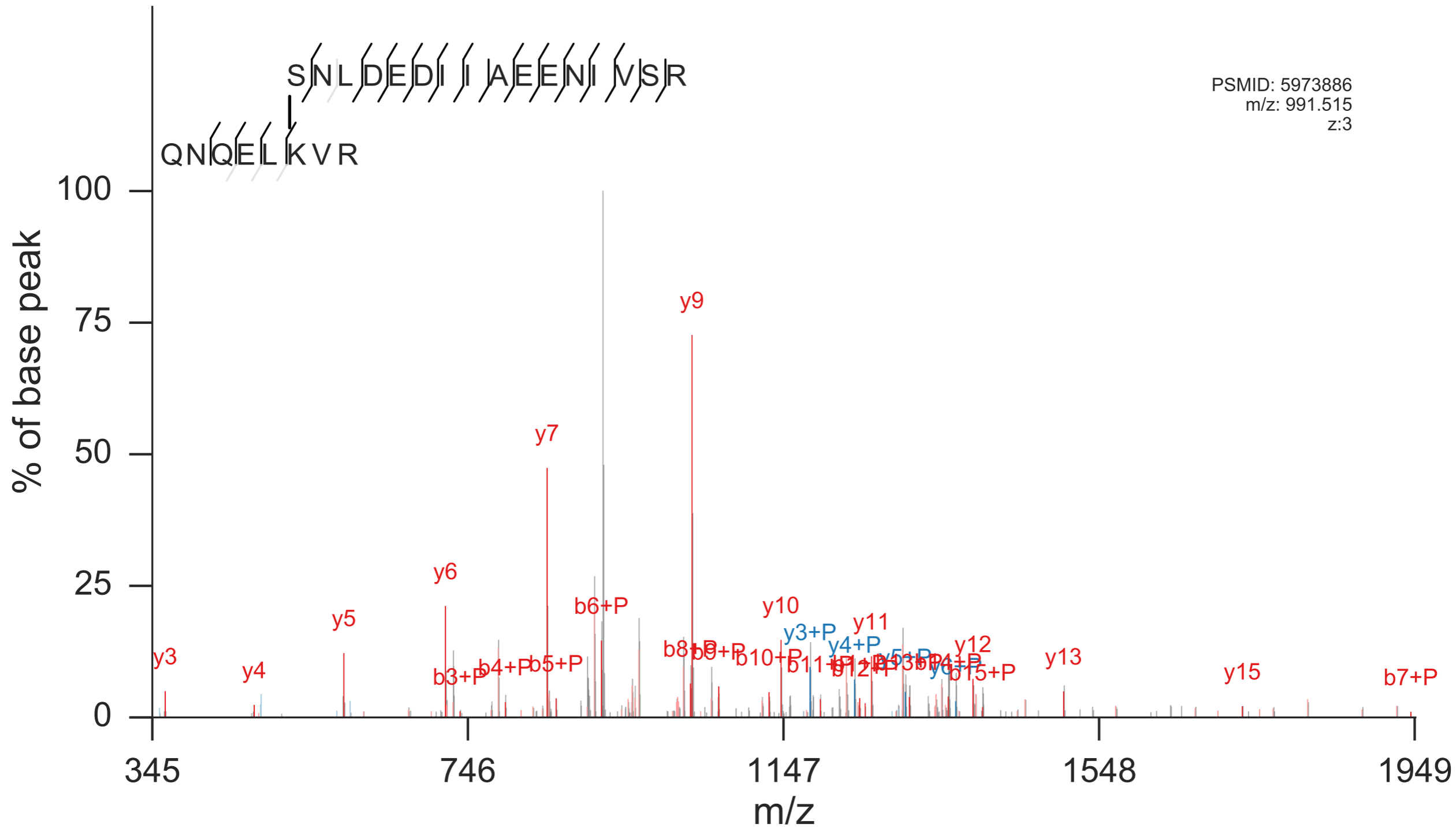
(A) The peptides ISLPESLKR and KVLLDGVQNLRAEDLVGK were found cross-linked in two ways, once linking 267K and 283K and once linking 265S and 283K. The 267K – 283K linked peptide eluted with a retention time of 141 minute from our HPLC system and was assigned this linkage based on back-bone fragments y1 and y2+P ions that flank 267K (annotated spectrum shown in (B)). 265S – 283K is placed in close sequence proximity to 267K – 283K. Given the much higher reactivity of NHS-esters to K when compared to S, special caution needed to be employed when calling 265S – 283K. 265S is supported over 267K in the matching fragmentation spectrum by the observation of b6+P and y4+P ions that flank 265S (annotated spectrum shown in (C)). In addition, the 265S – 283K peptide eluted at 135 minute in a baseline separated peak from our HPLC system. Notably, the intensity of this peptide is much lower than that carrying the 267K – 283K link as one might expect considering the reactivity difference of S and K.

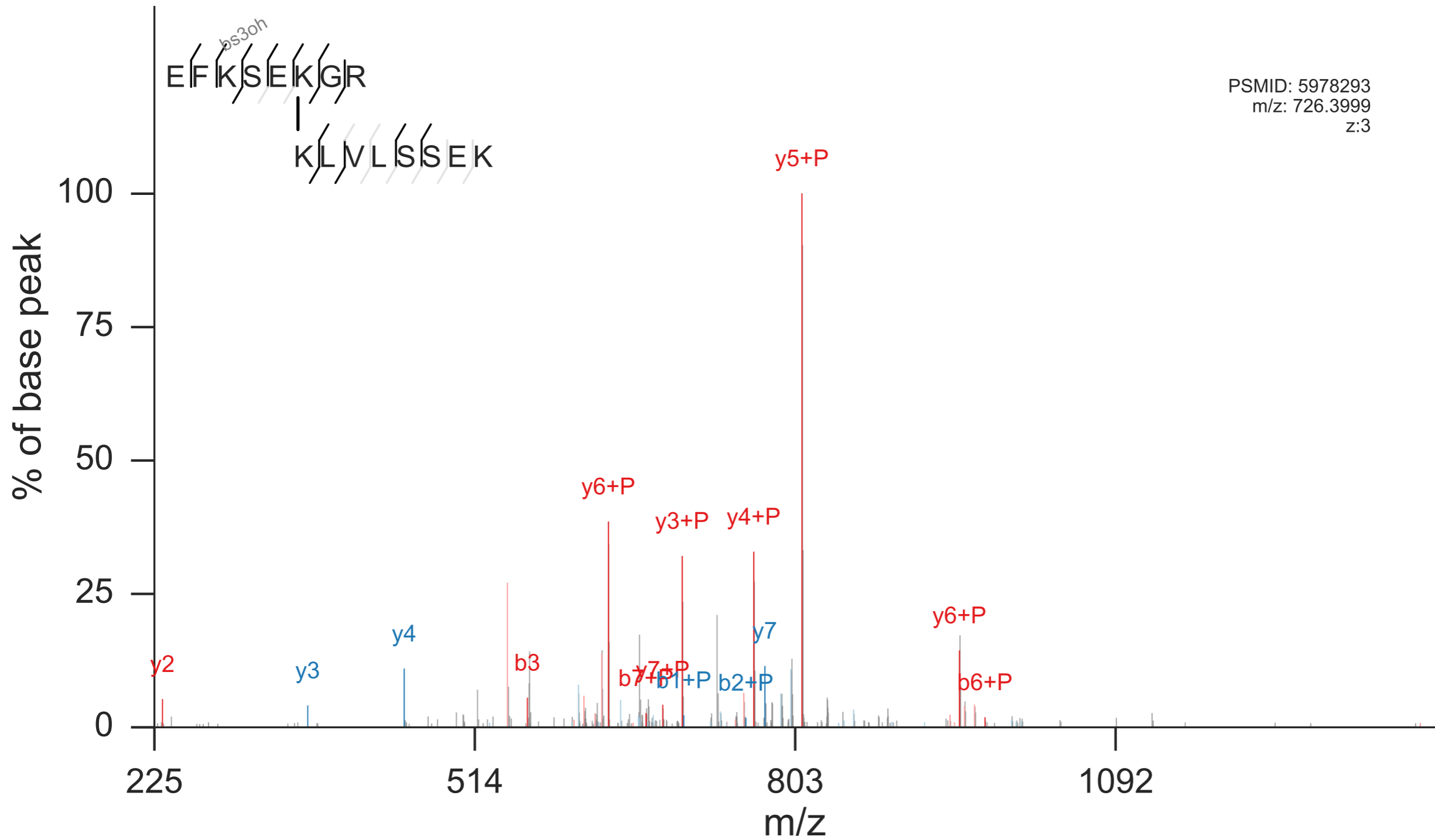
Supplemental Figure S3

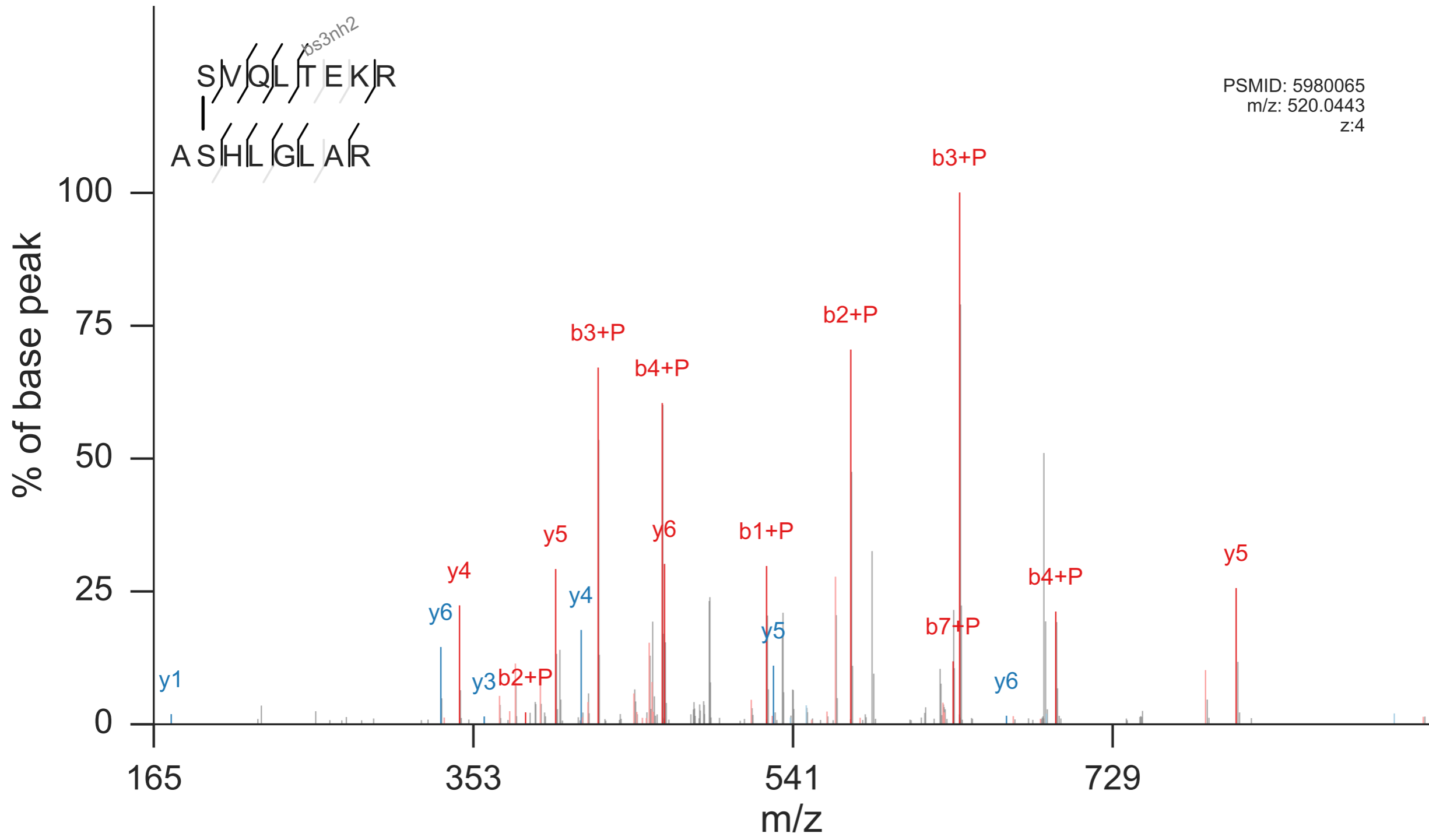


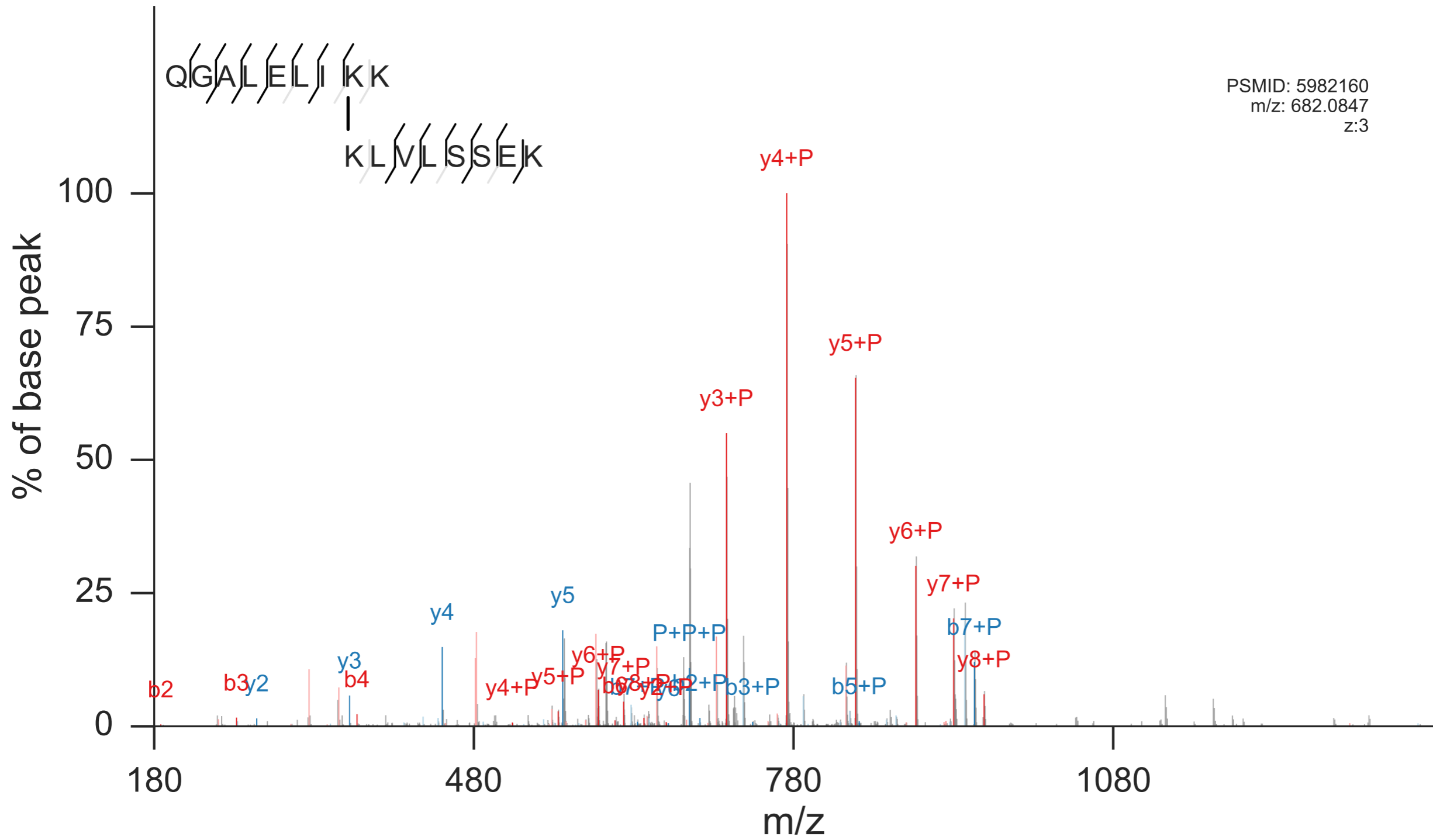
Supplementary Figure 3. Clustering of C3(H₂O) solutions.

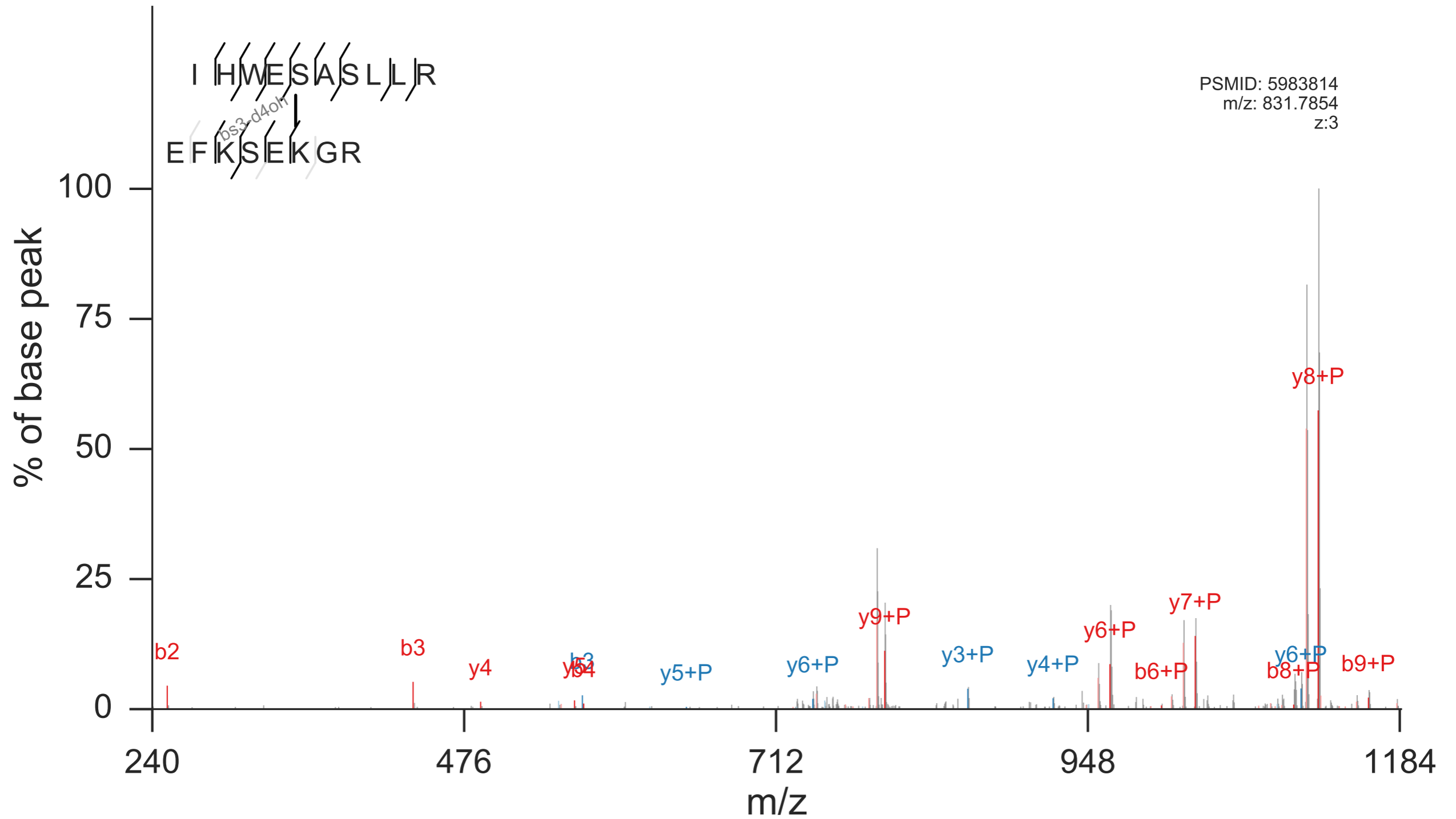
The 200 best scoring models (solutions) were partitioned into clusters, using the k-means clustering method. The two blue blocks of the pair-wise r.m.s.-distance matrix calculated between the 200 models (**A**) suggested the presence of at least two main clusters of similar structures. The resulting clusters (**B**) displayed a different orientation of MG8 (brown density) and ANA (violet density), with respect to the other domains. A comparison of the distances between cross-linked residues, in cluster A *versus* cluster B, is shown in (**C**). For each cross-link (displayed along the *x*-axis) the ratio (for cluster A *versus* cluster B) of median C α -C α distances is shown in the *y*-axis (log scale). Distances that differ most between the two clusters (red) all involve domains at the “shoulder” region of the molecule (ANA, MG7 and MG8). (**D**) For those cross-linked pairs of residues for which mean C α -C α distances differed between clusters A and B (highlighted in red in (**C**)), the C α -C α distance for every solution (shown as crosses, purple for cluster A and green for cluster B) are plotted. The median of the distribution is represented by a red line. Cluster B show better satisfaction on residue proximities defined by cross-links.

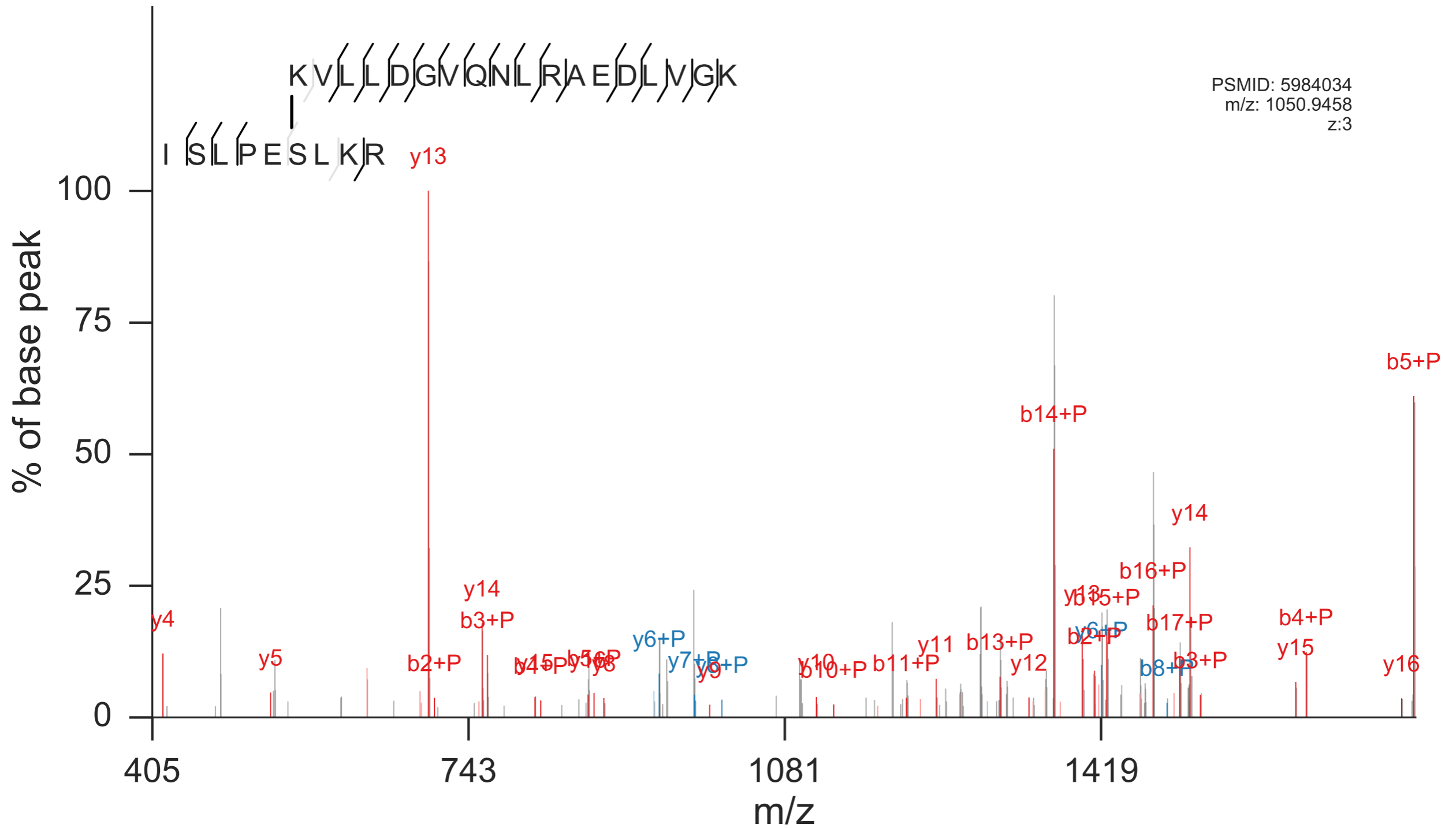


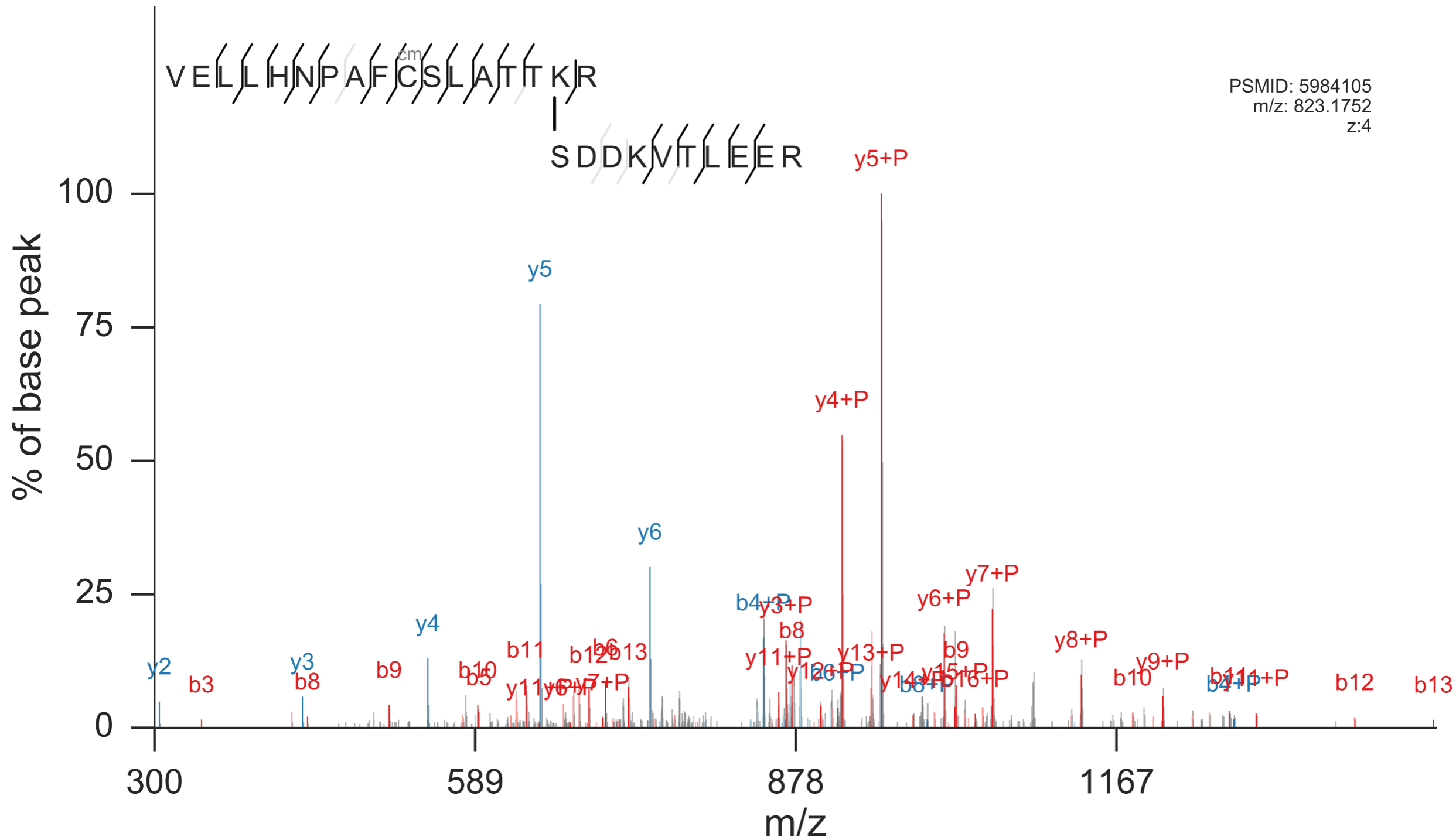


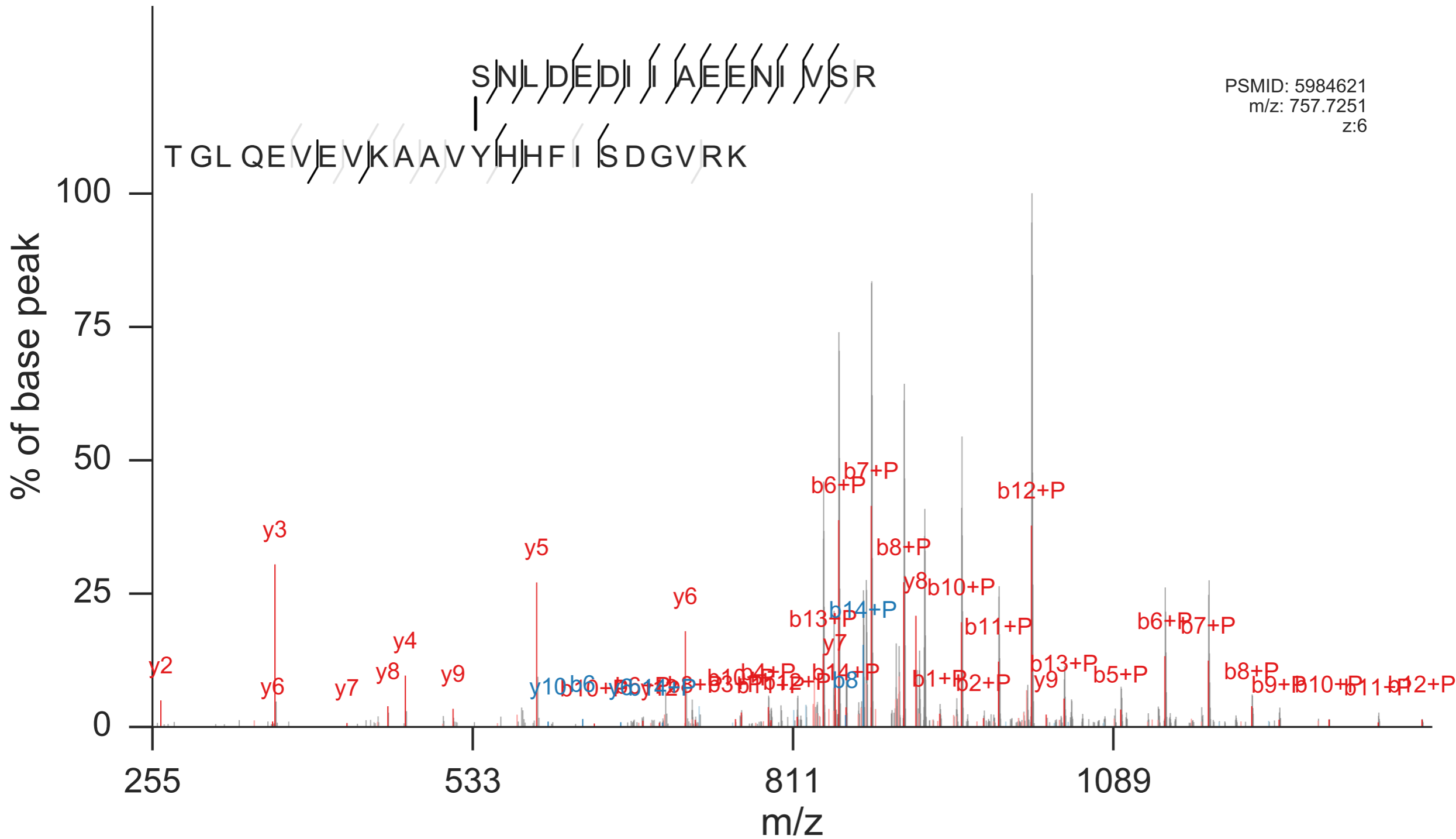


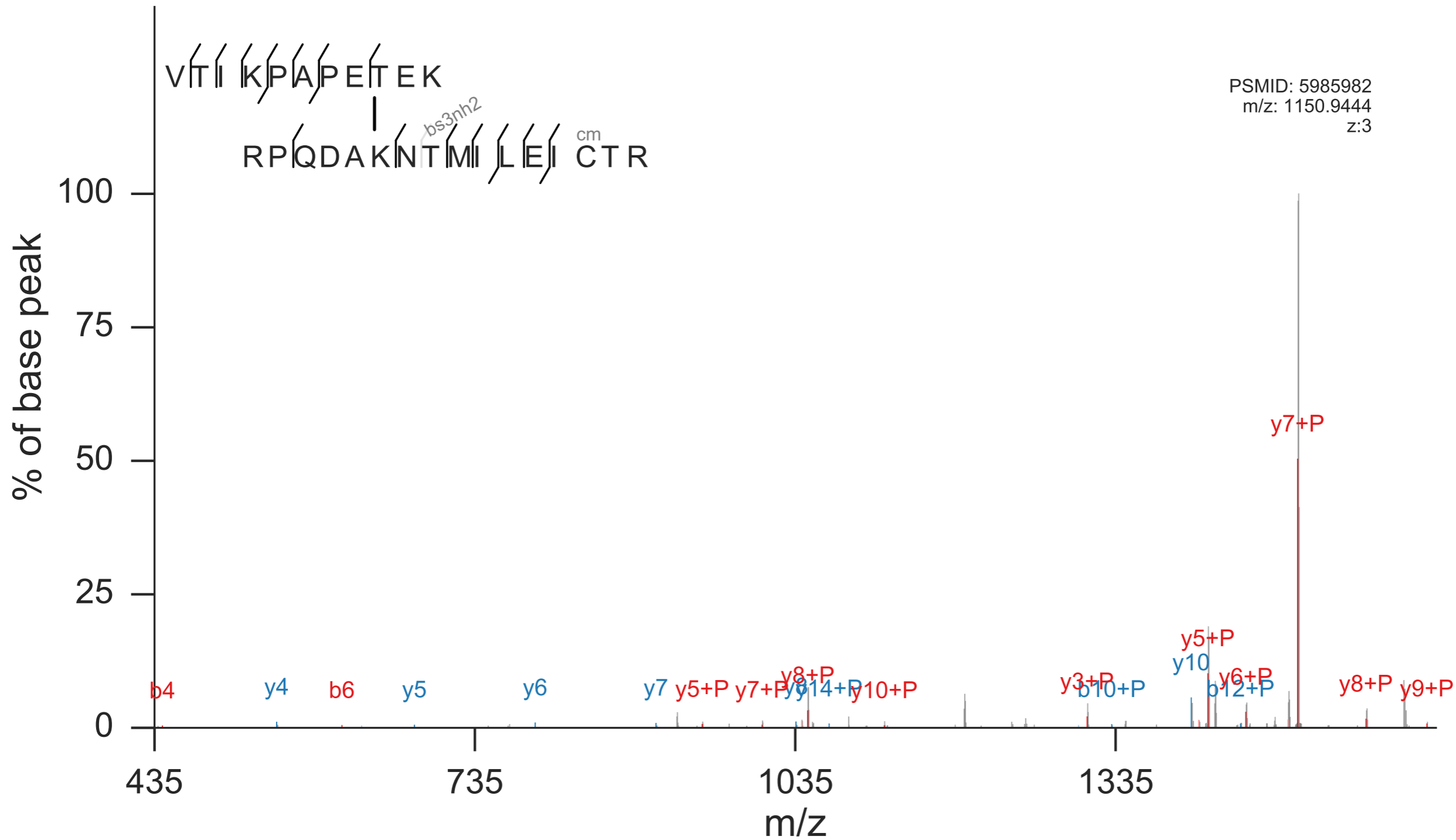


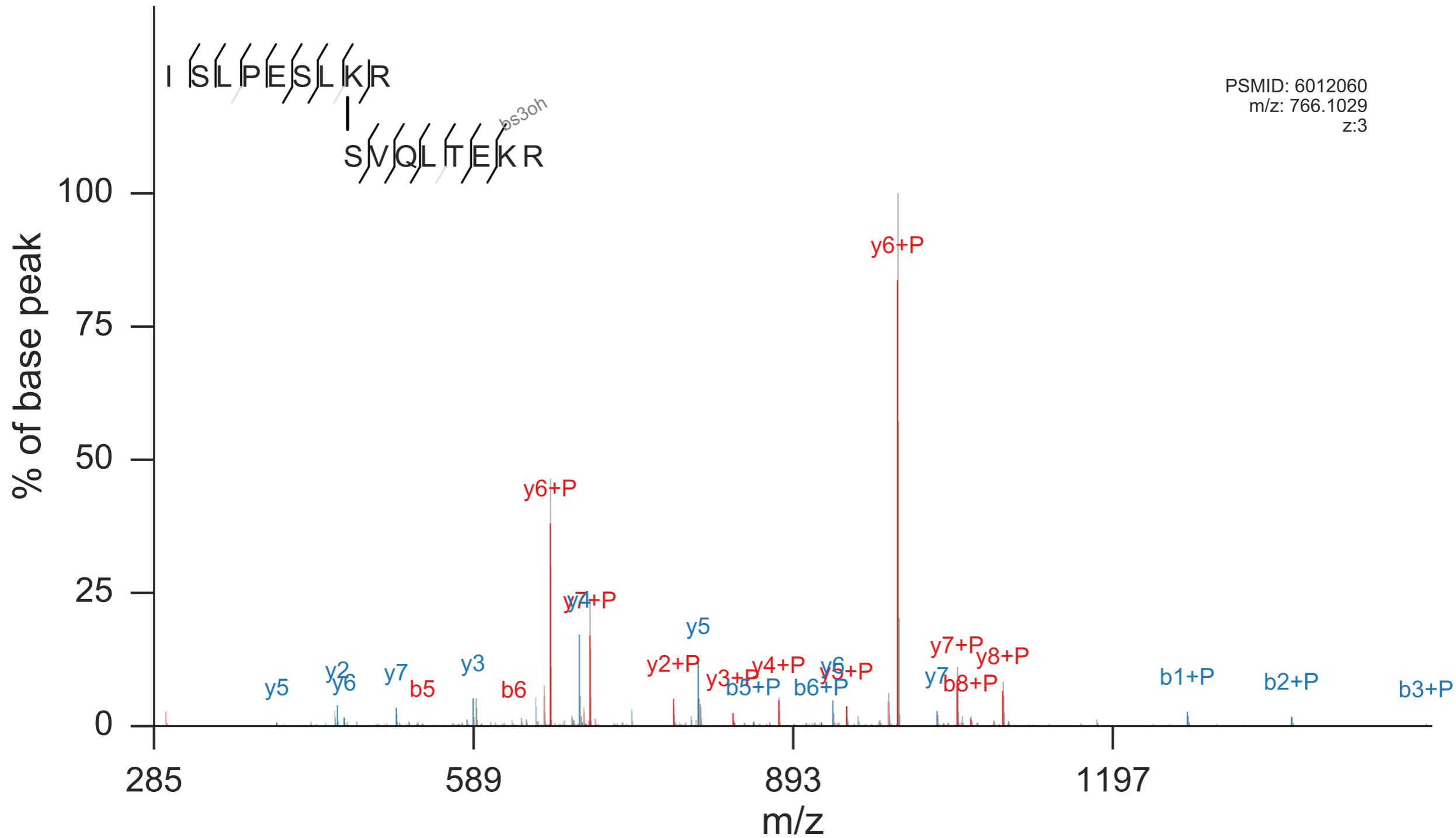


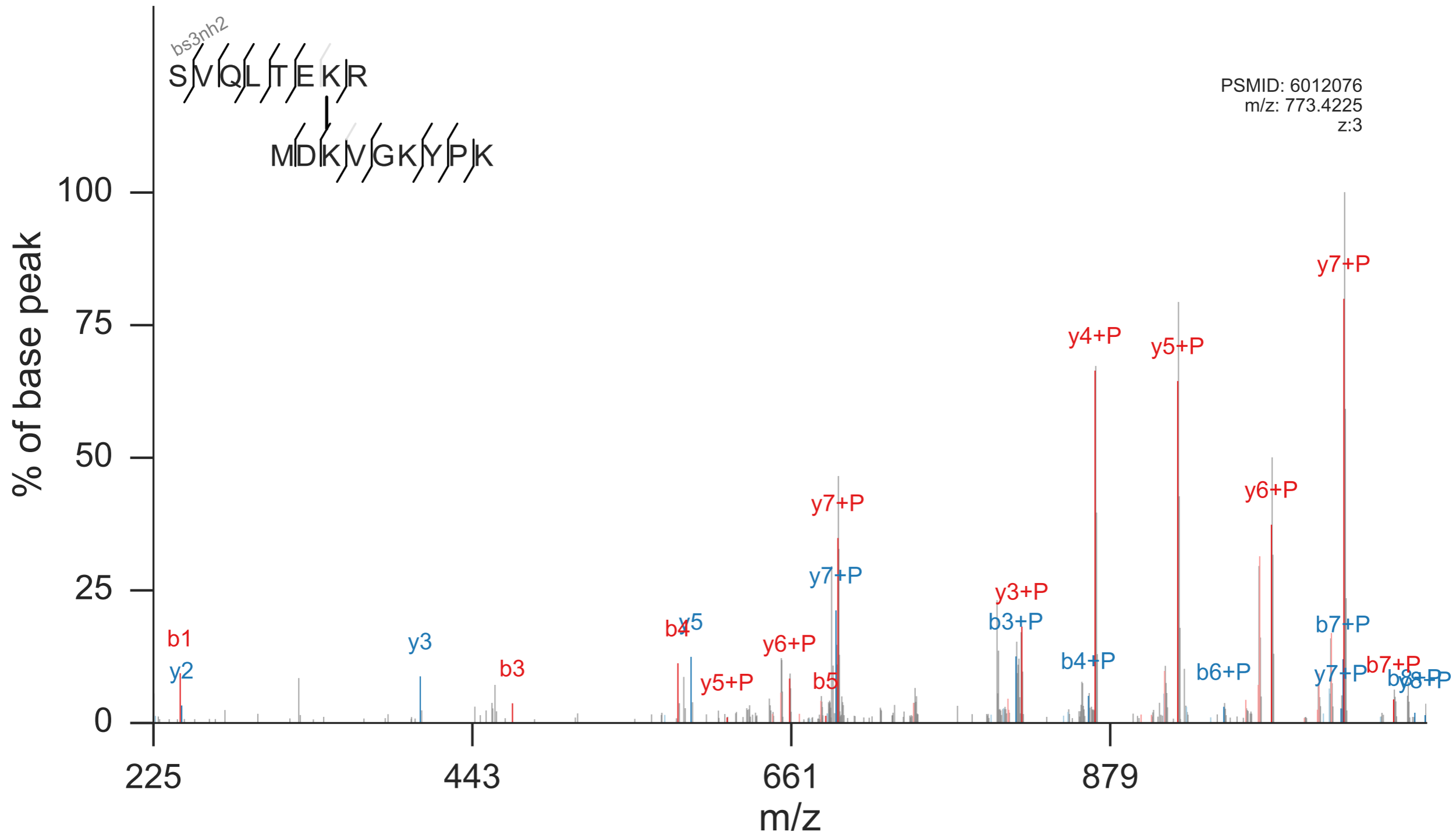


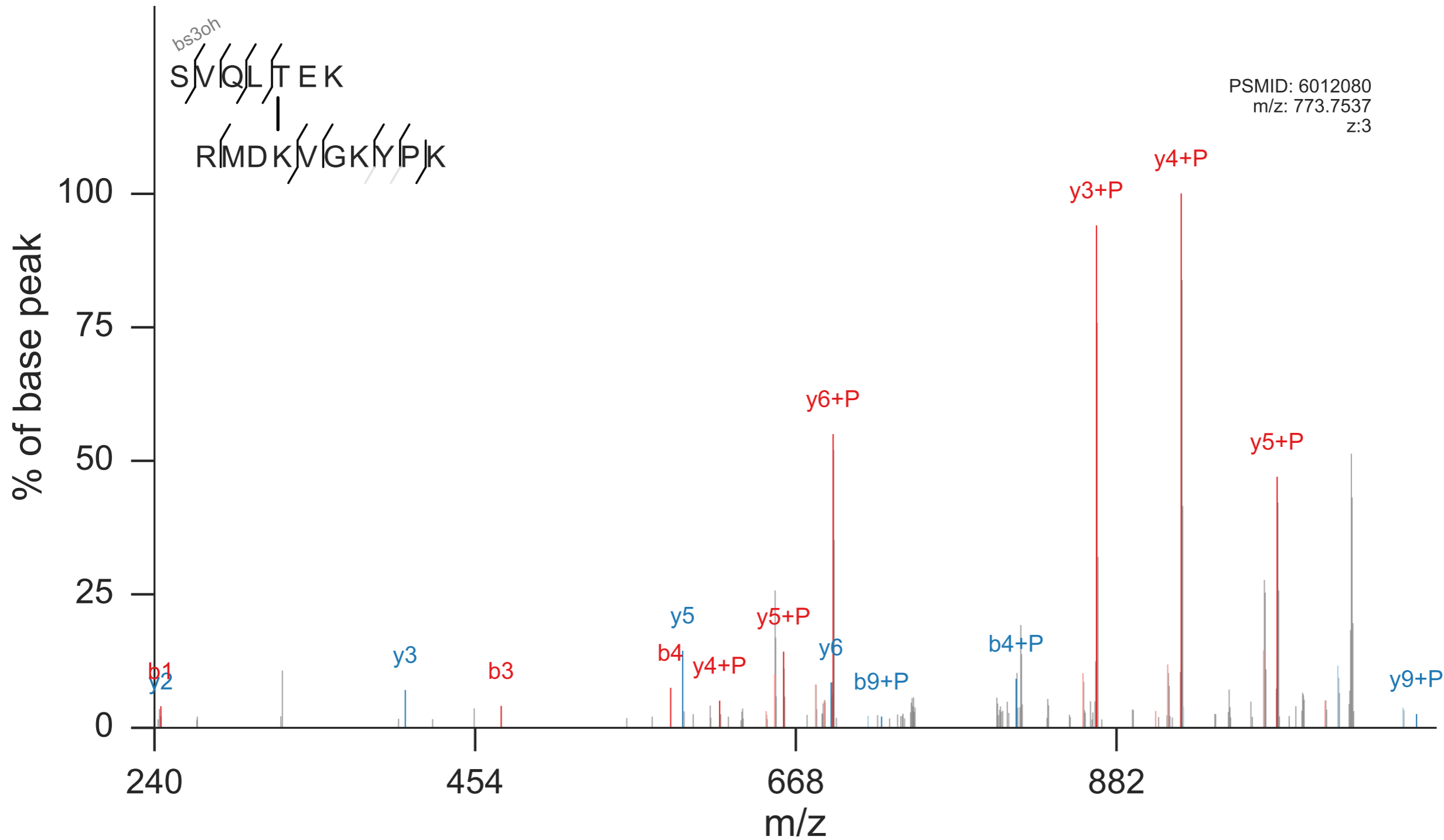


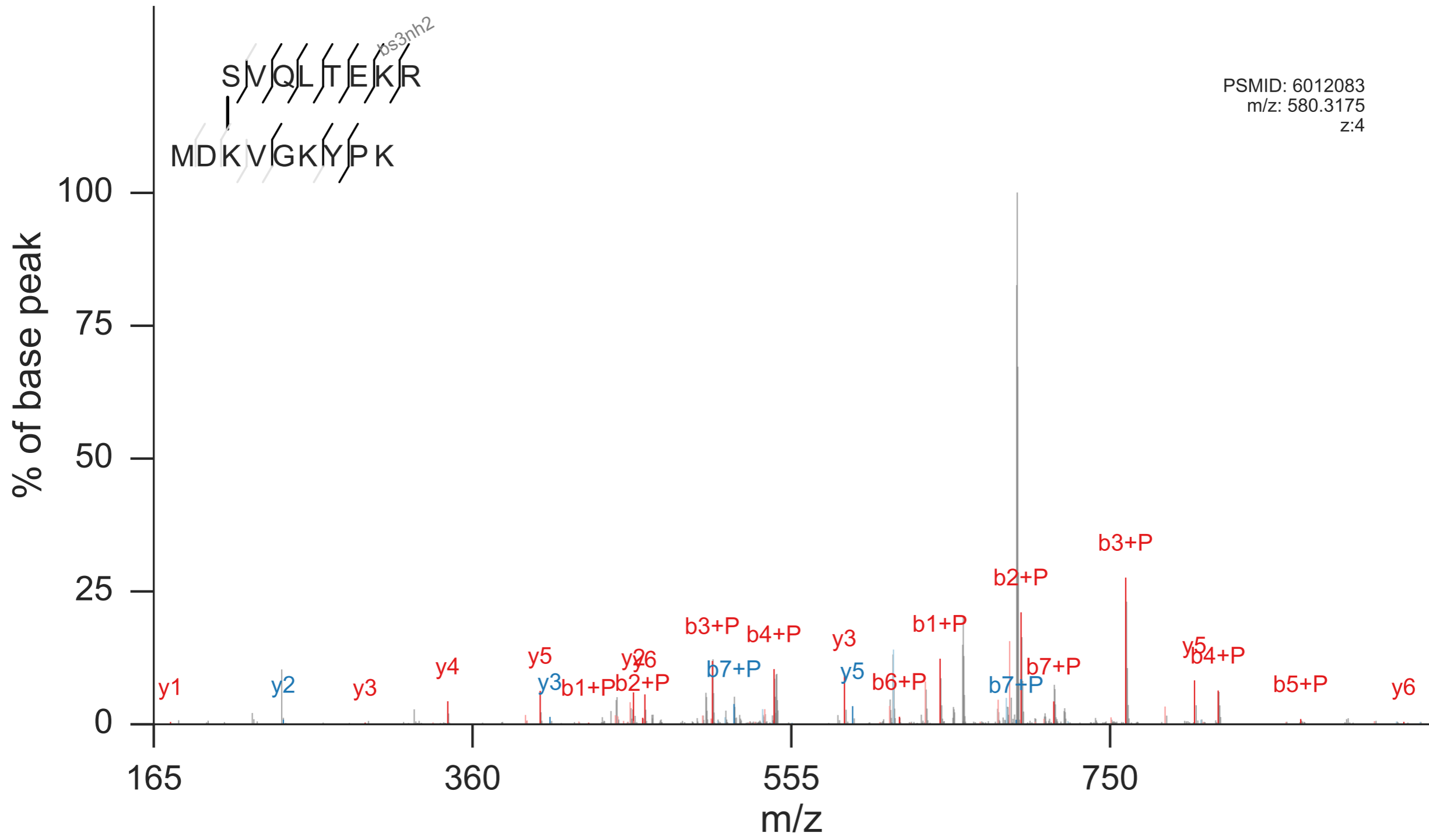


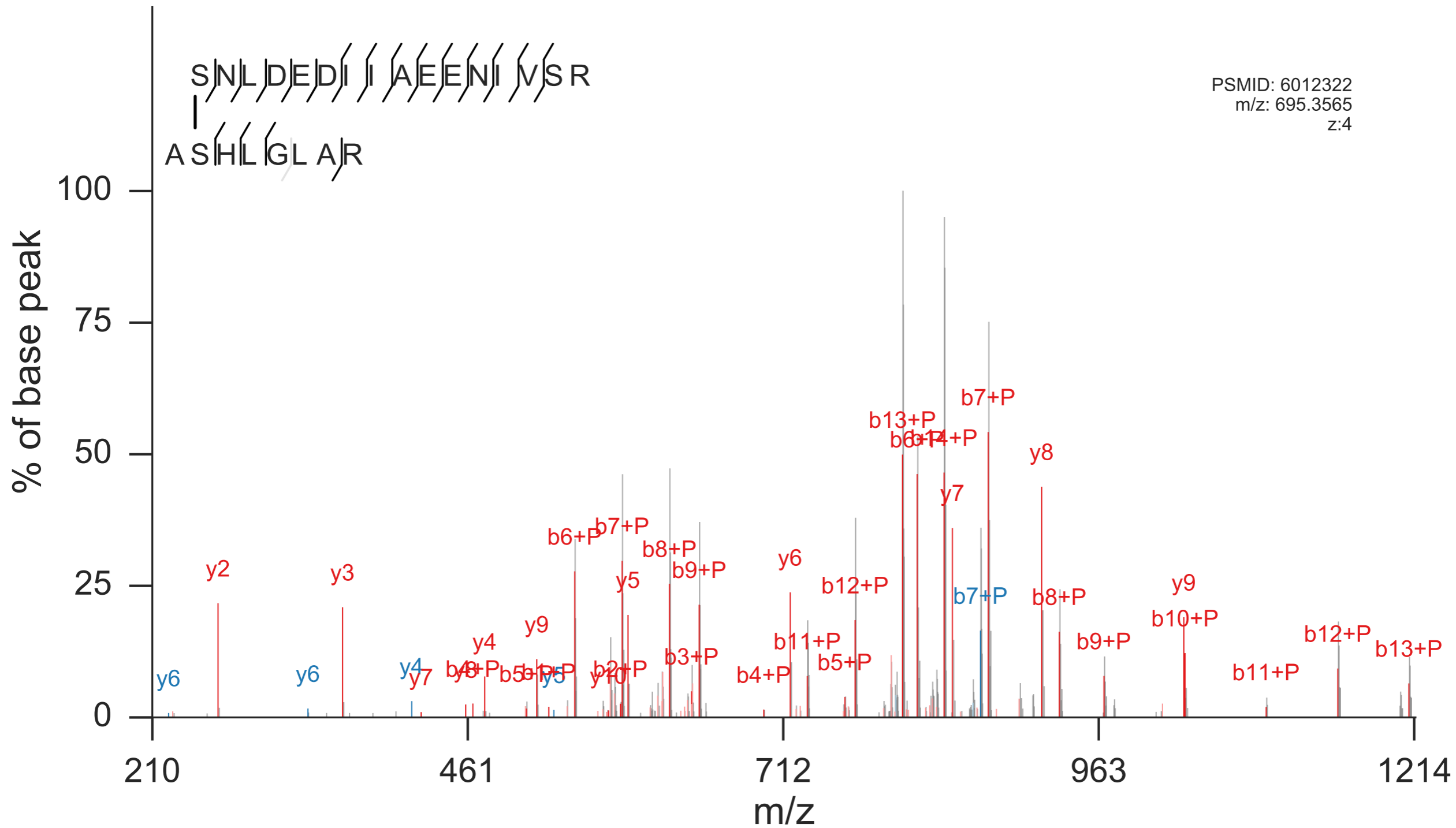


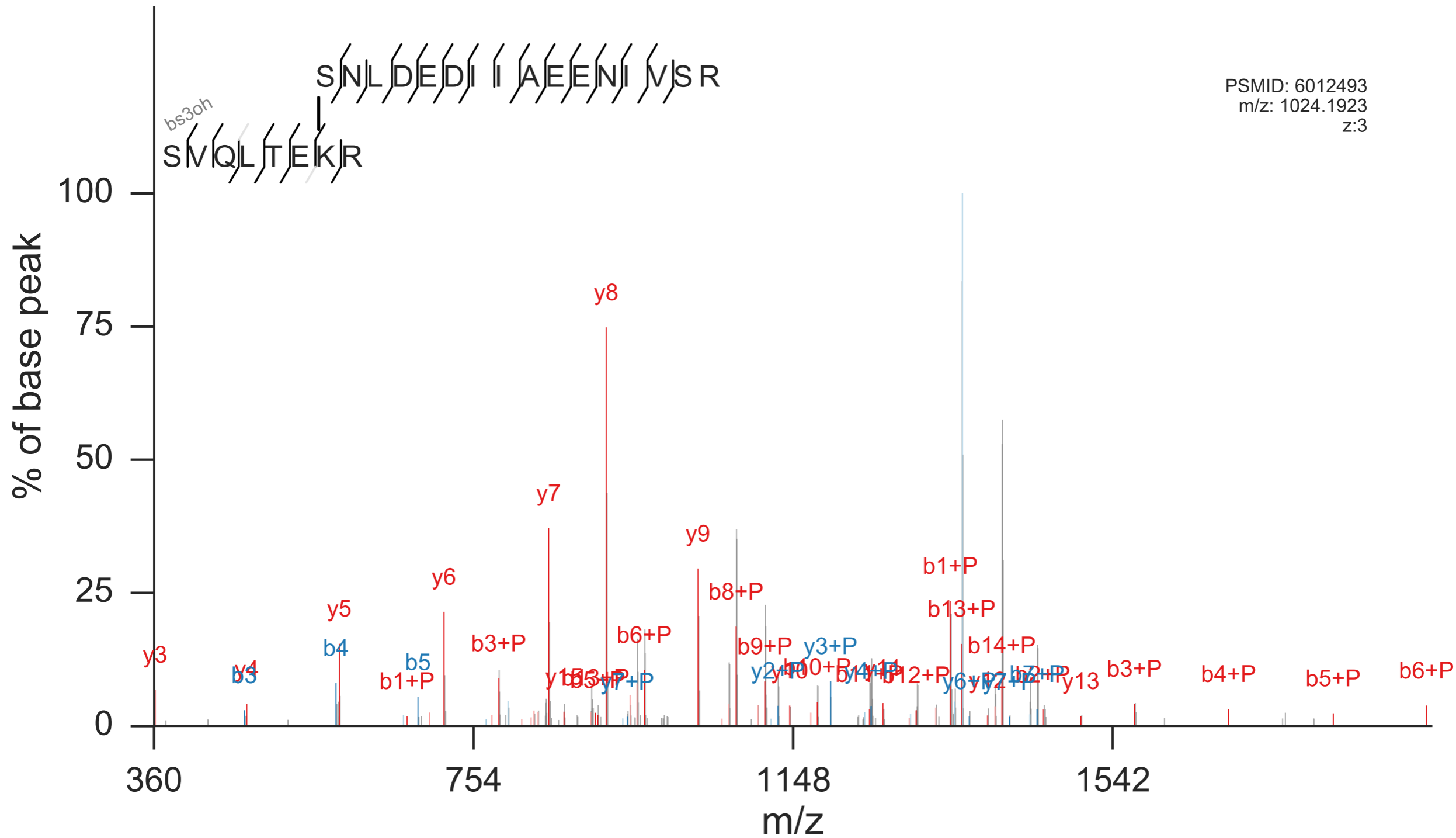


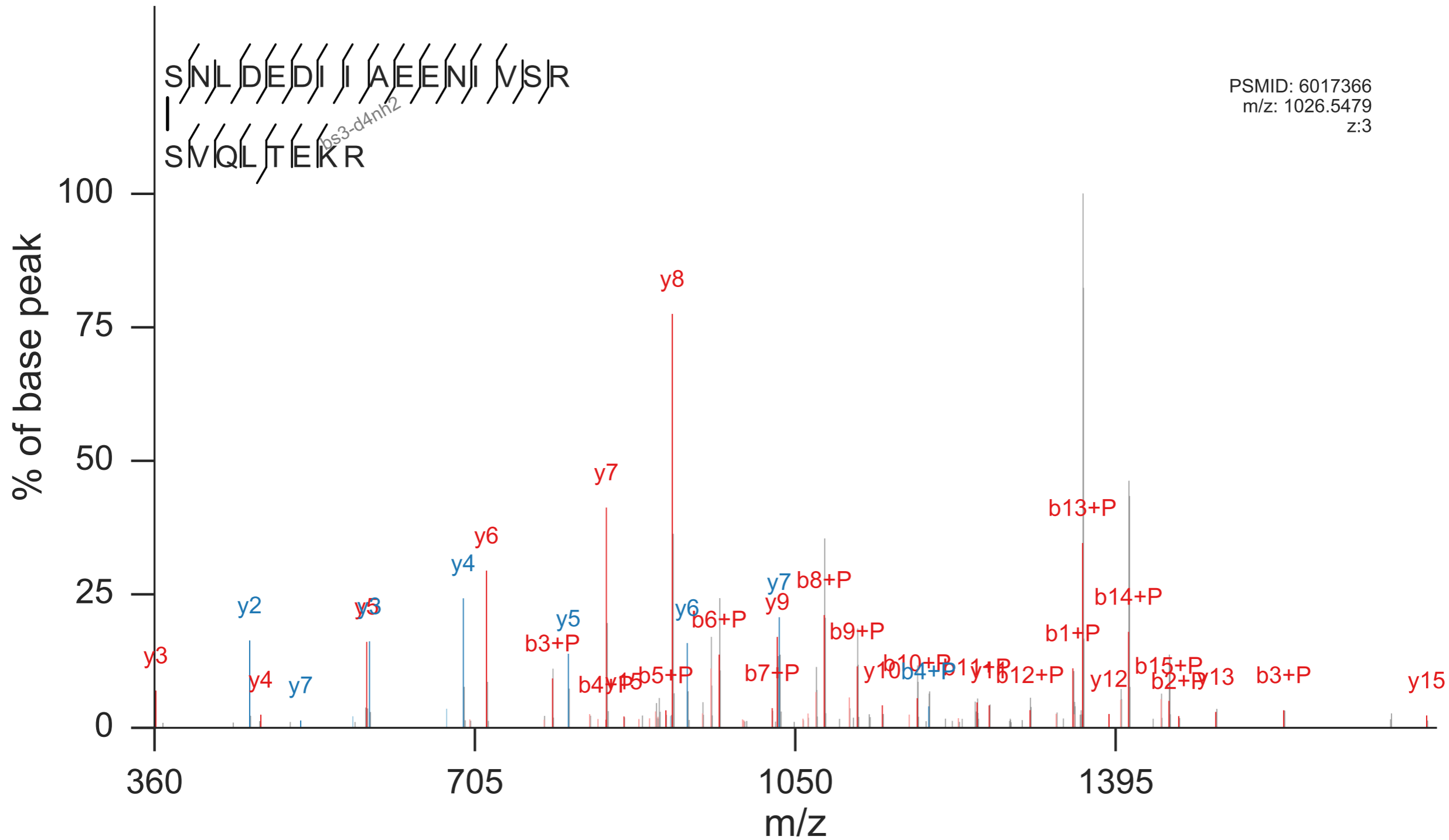


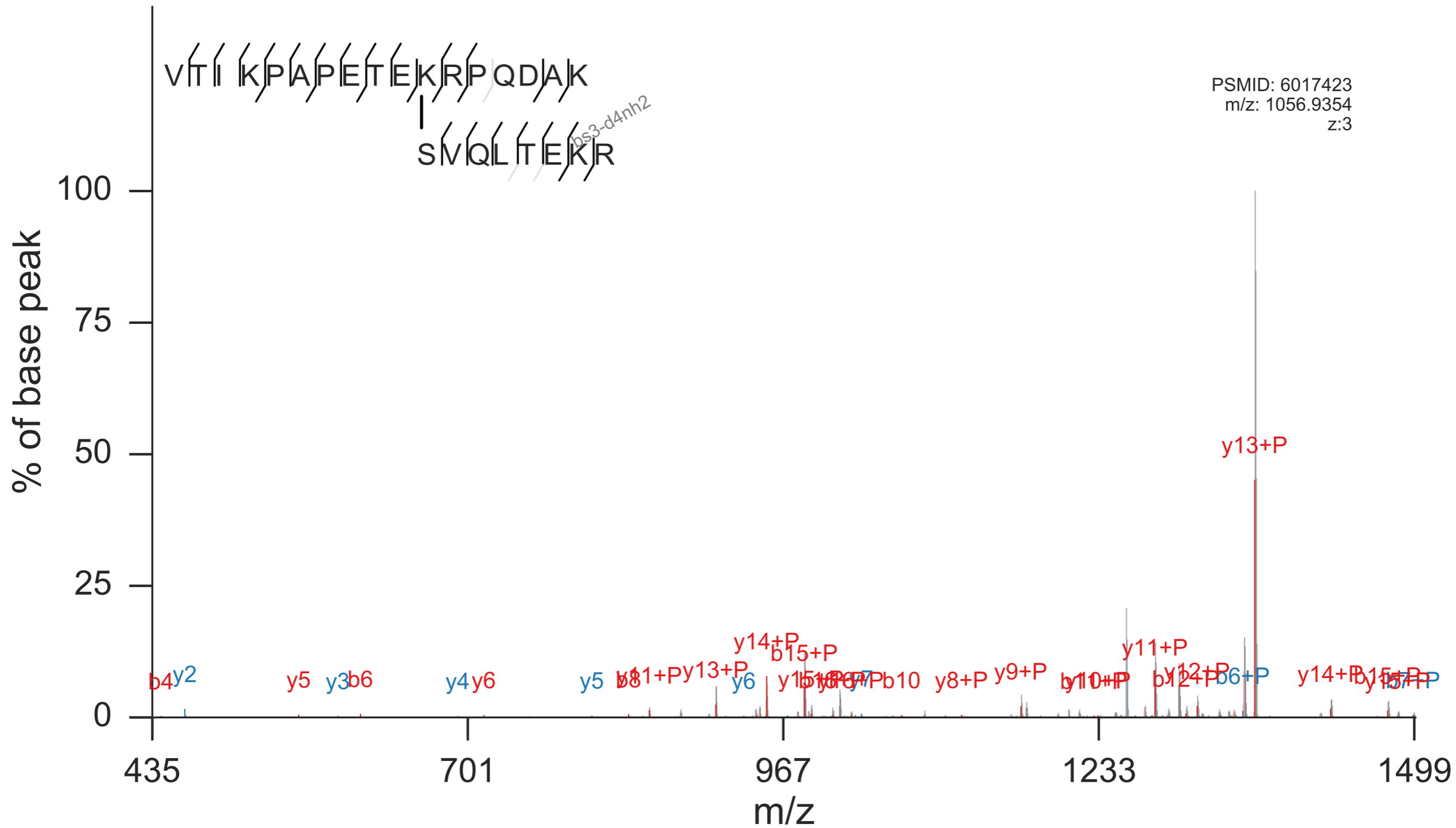


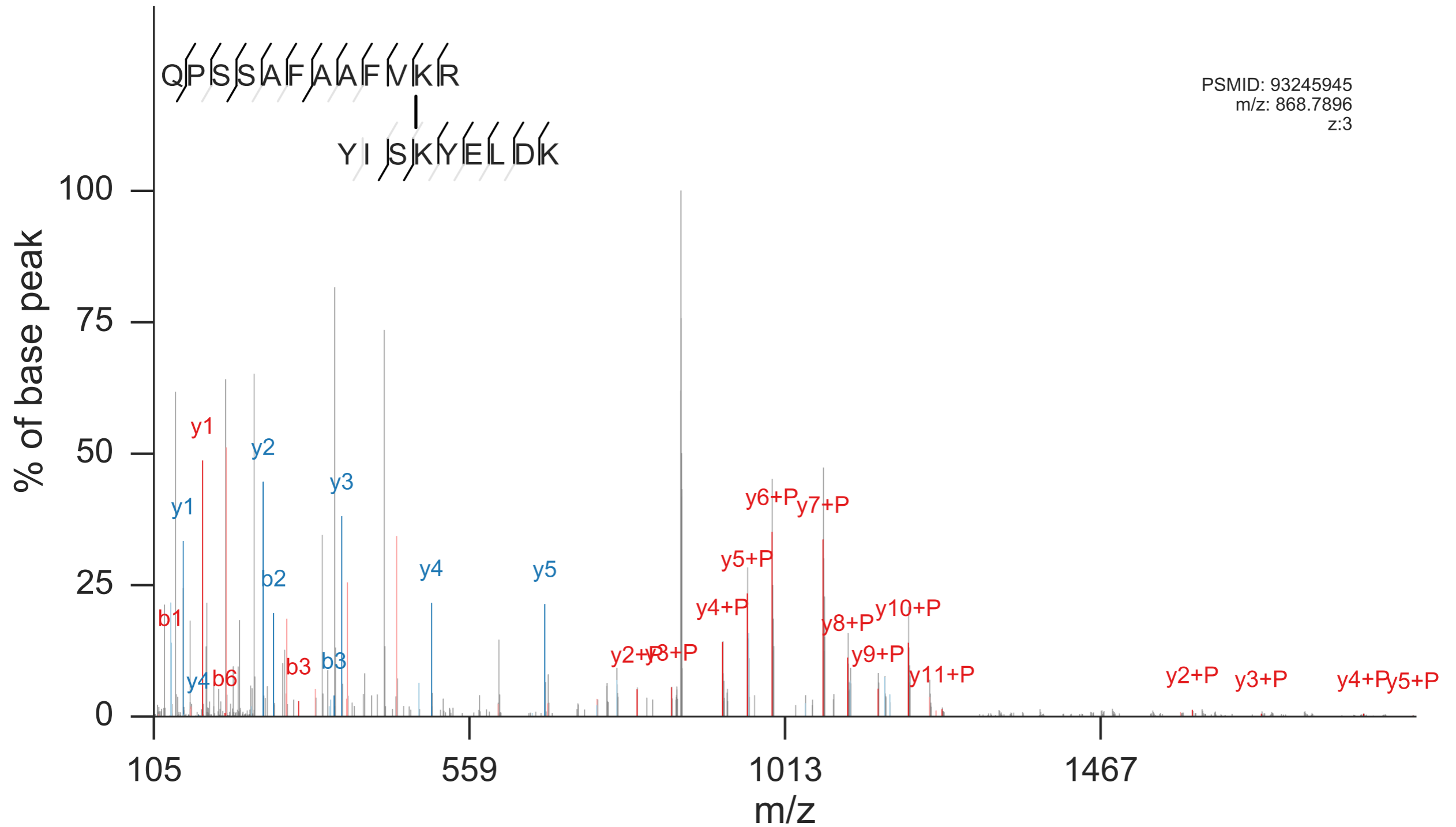


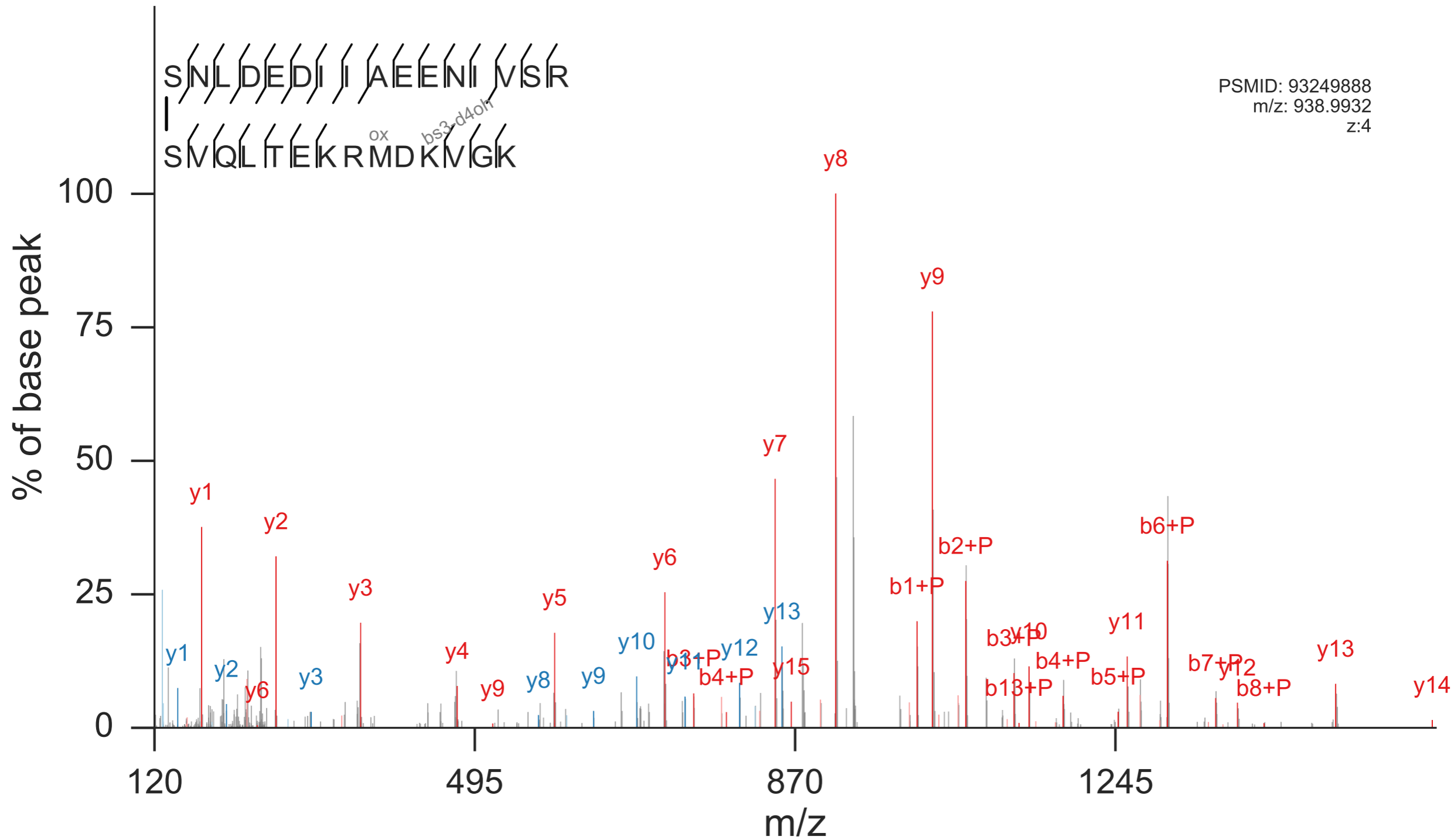


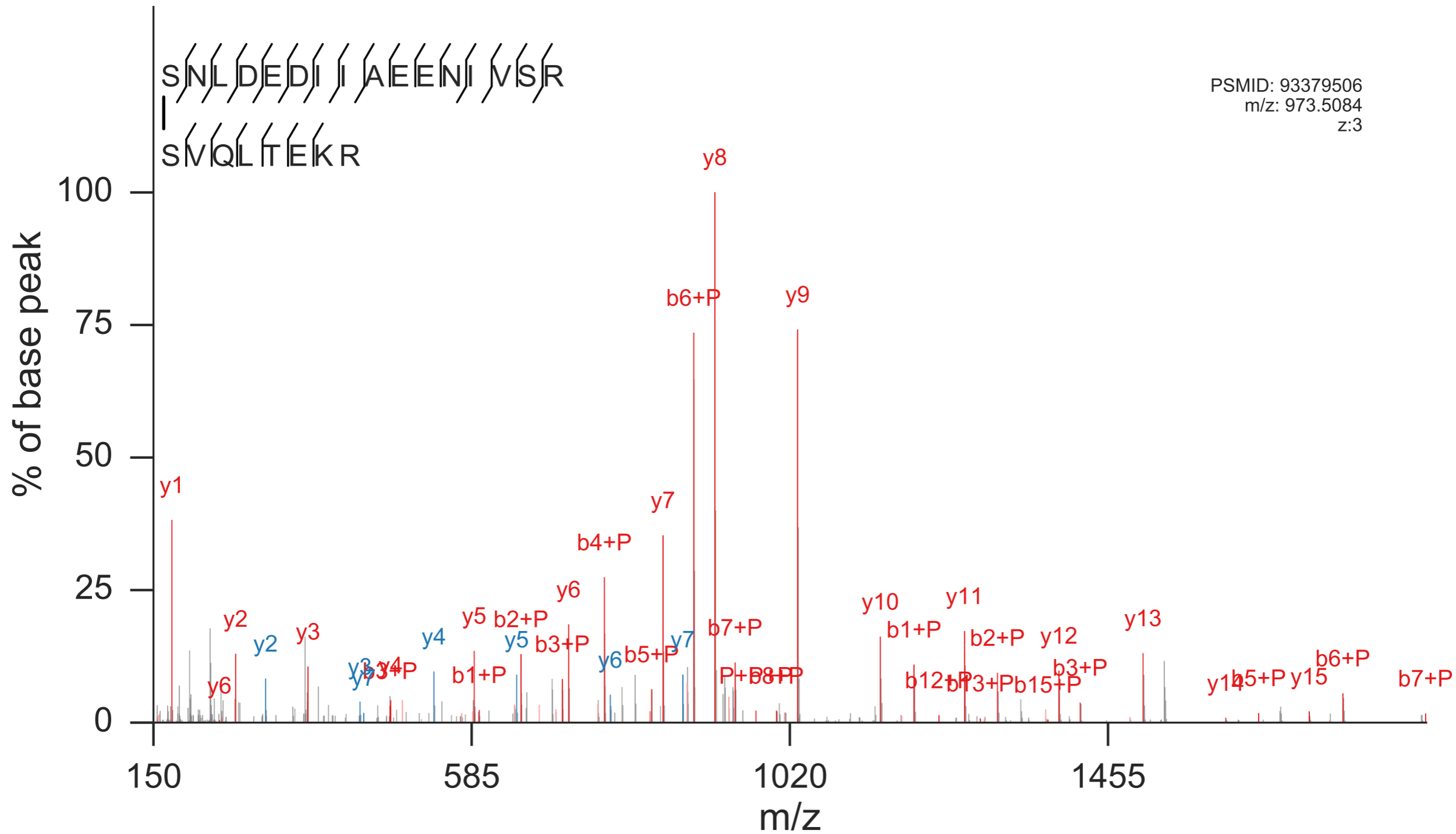


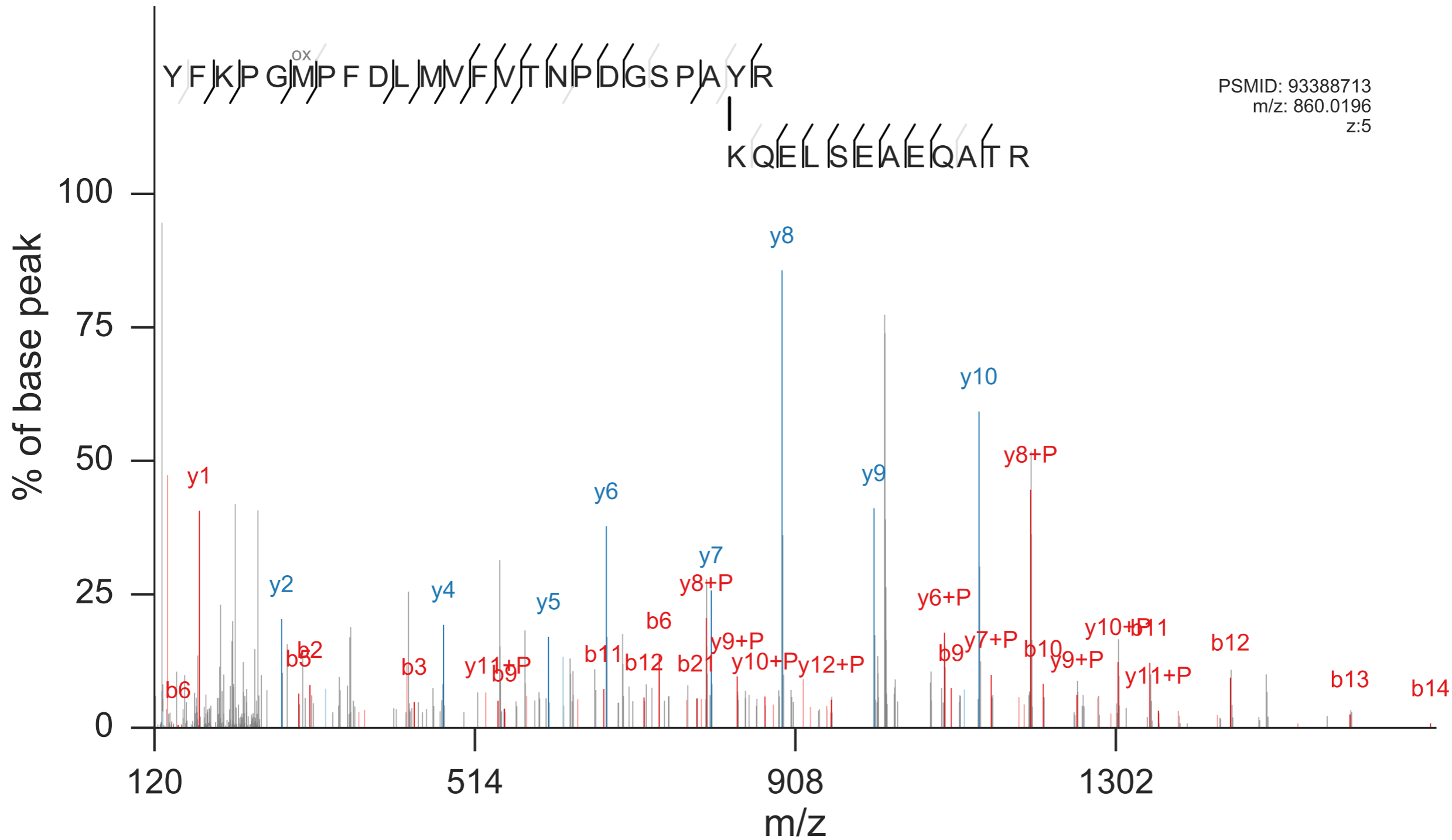


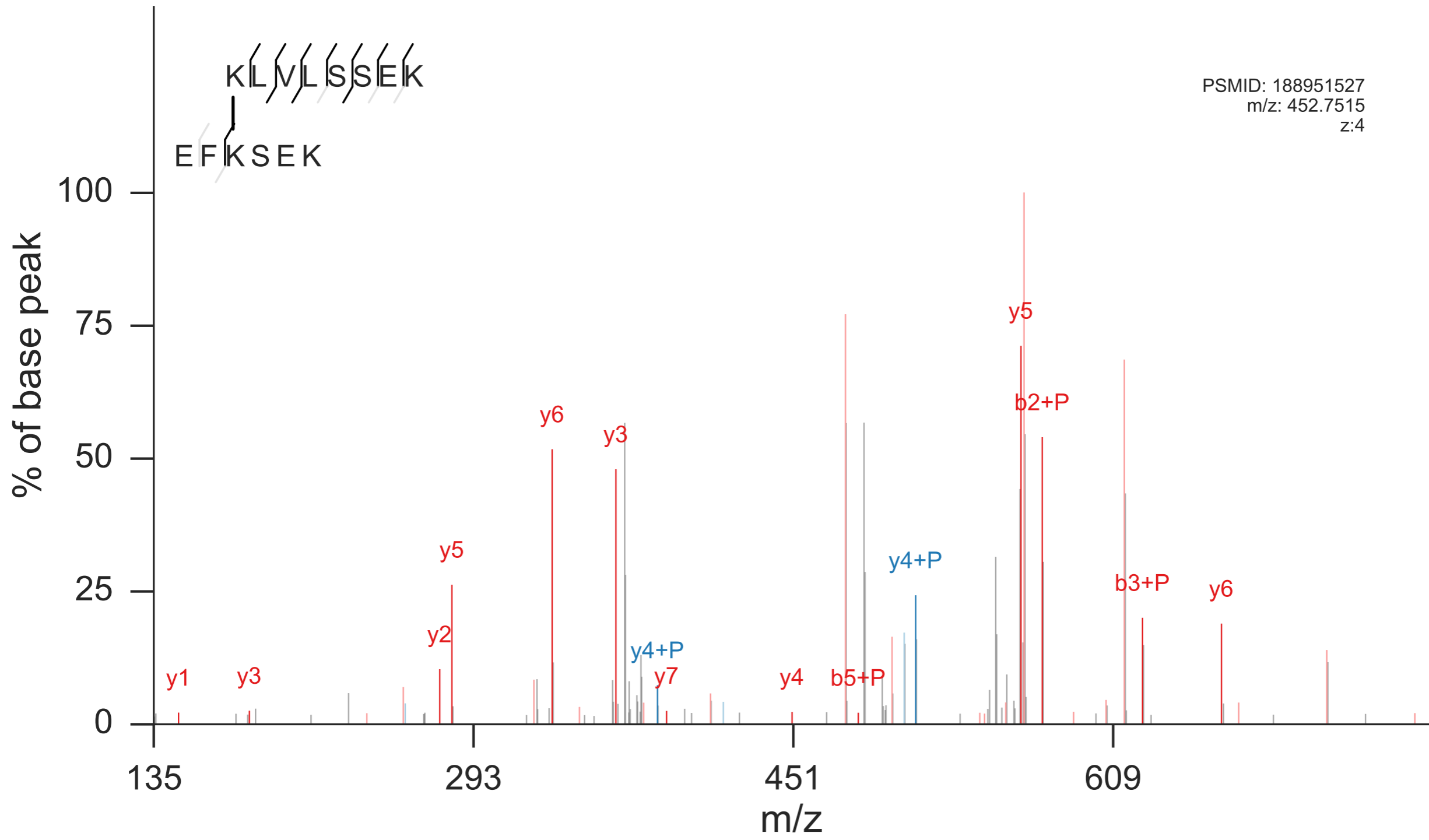


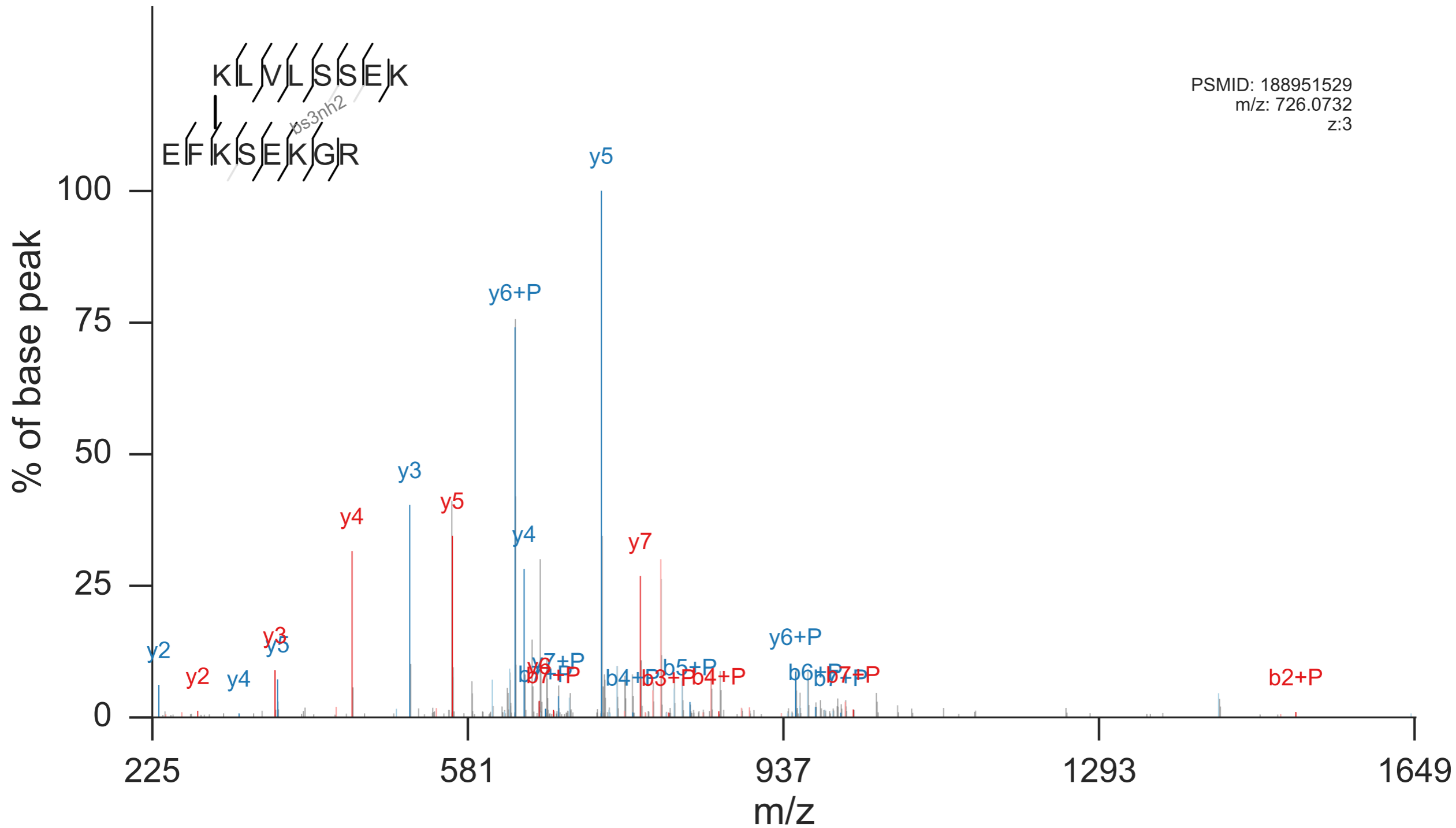


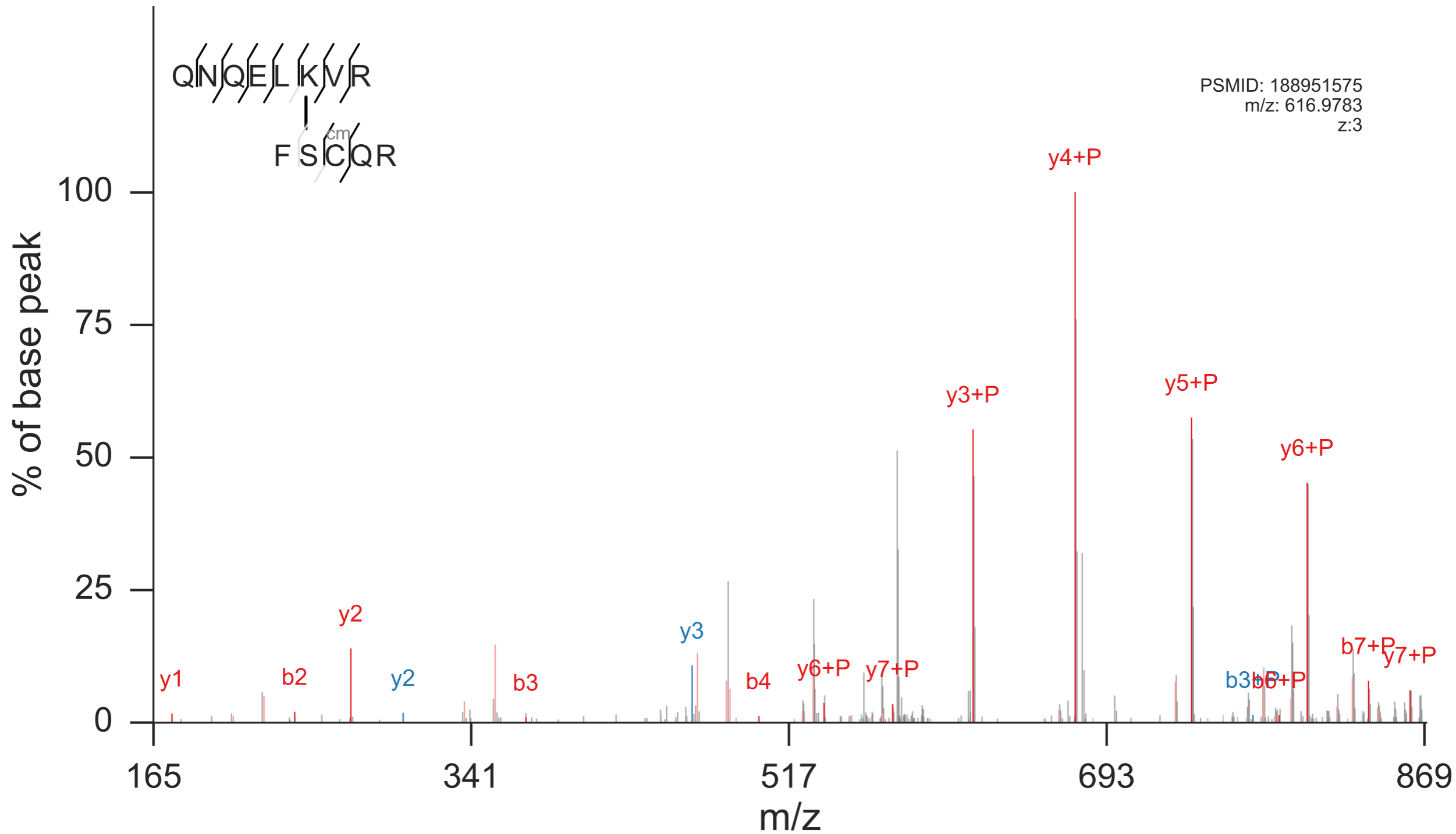


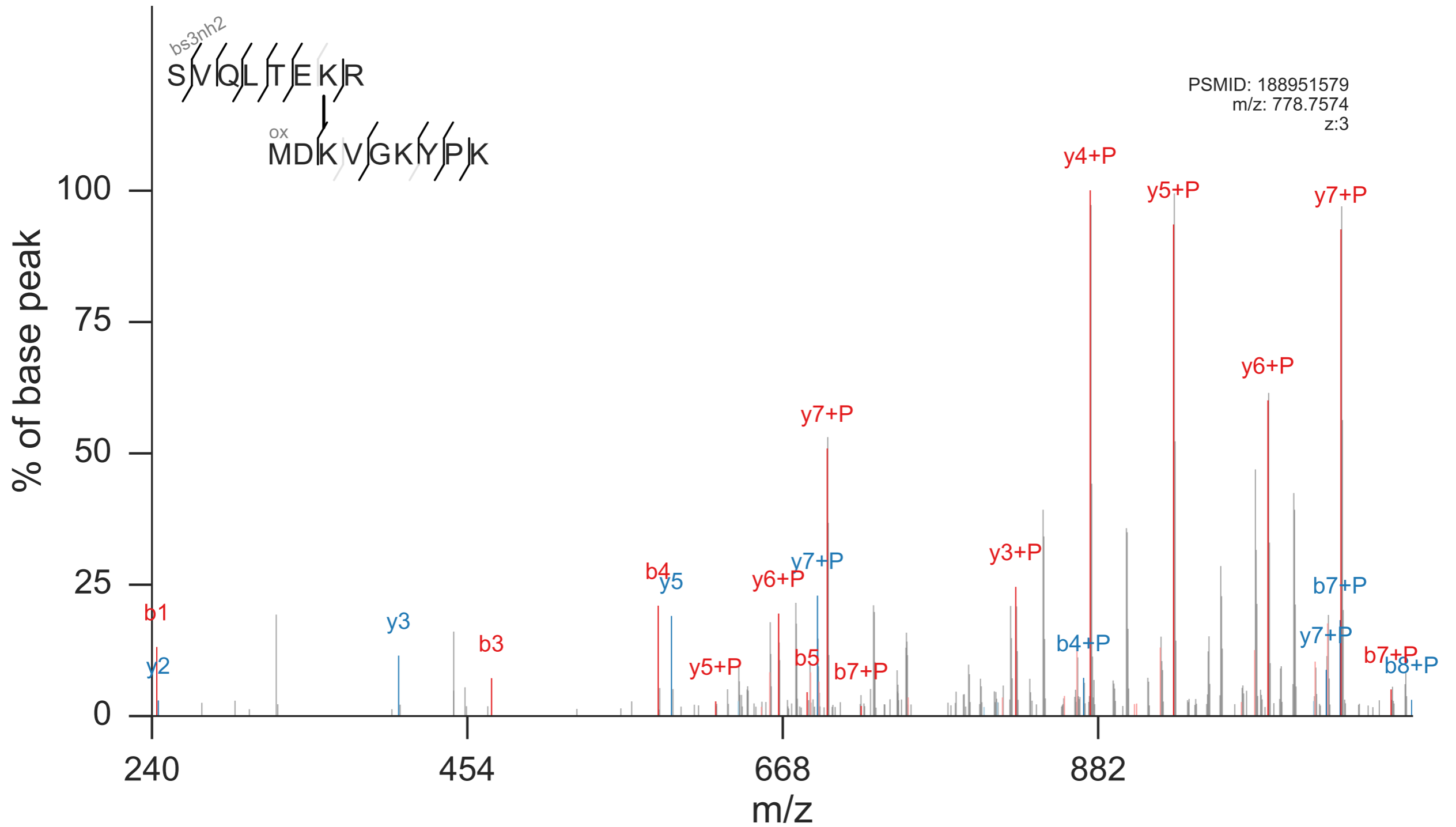


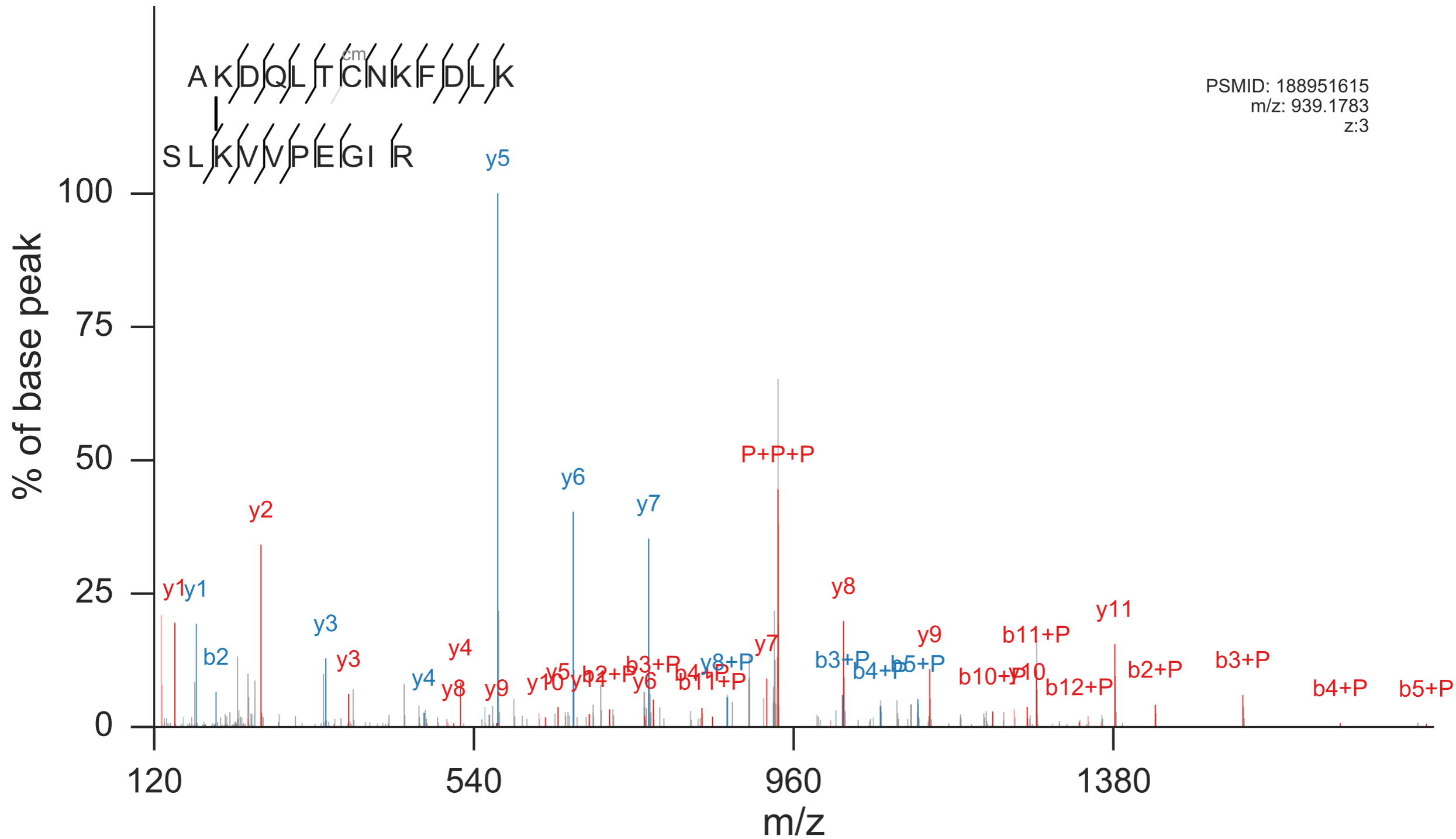


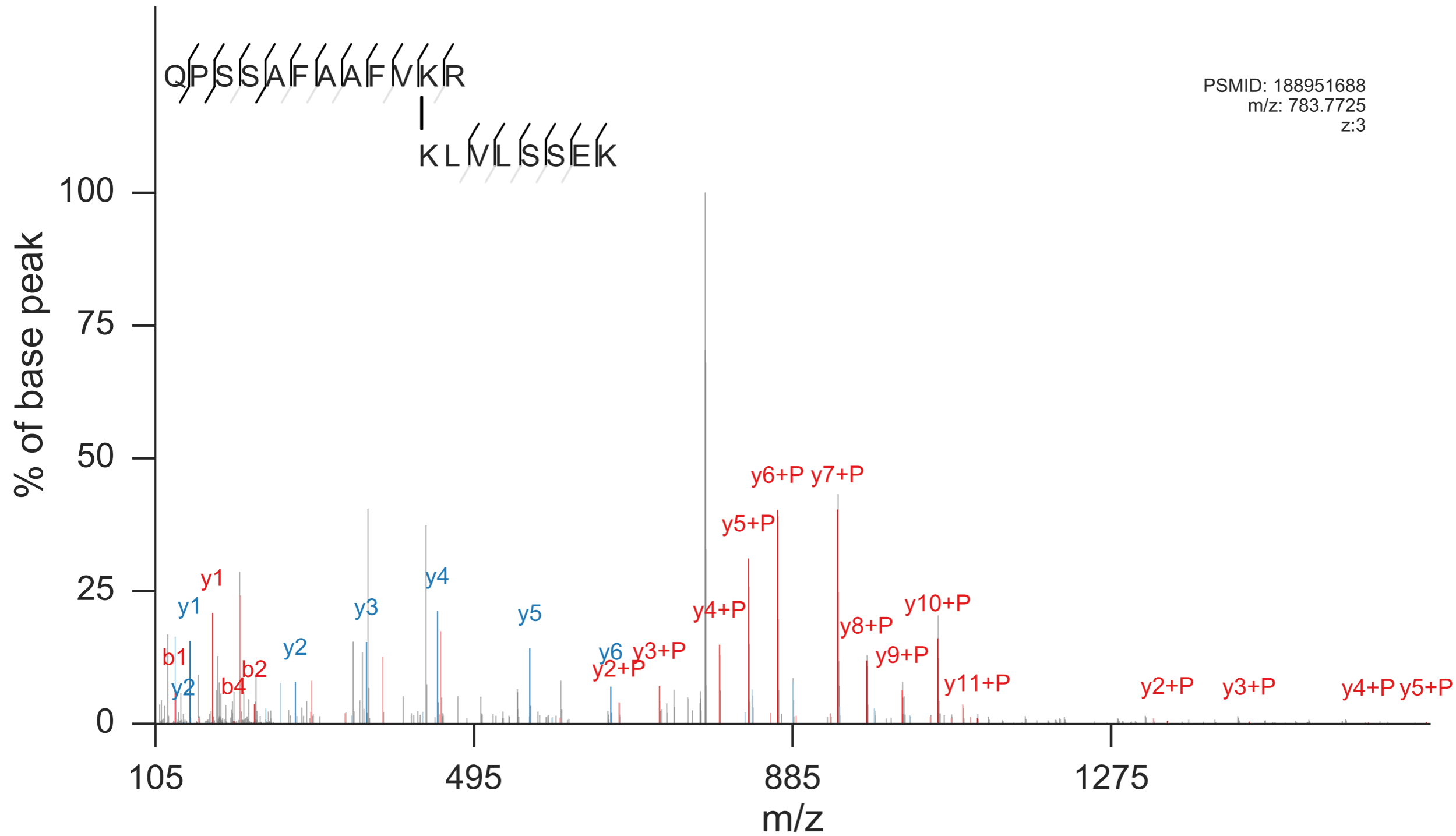


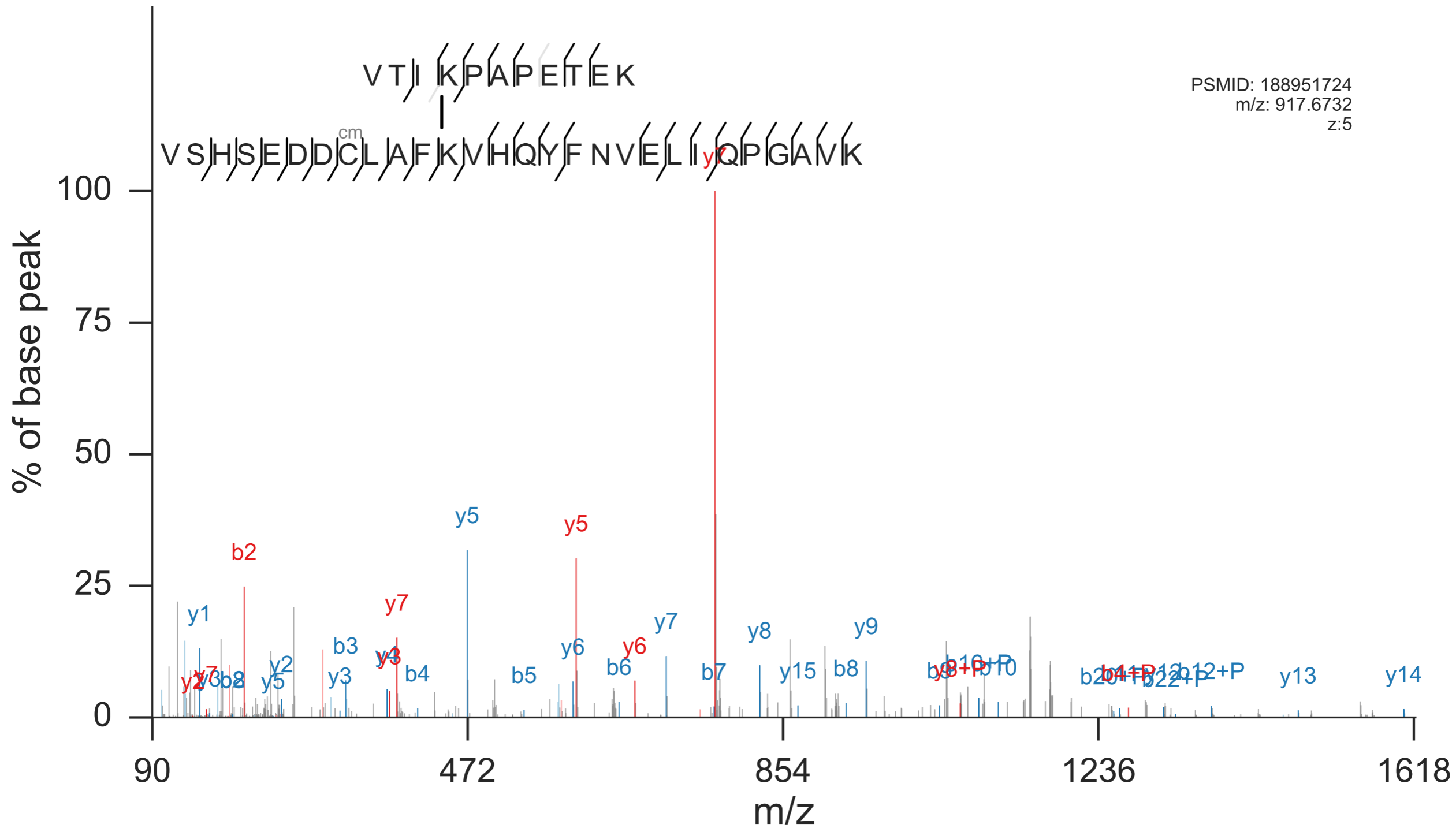


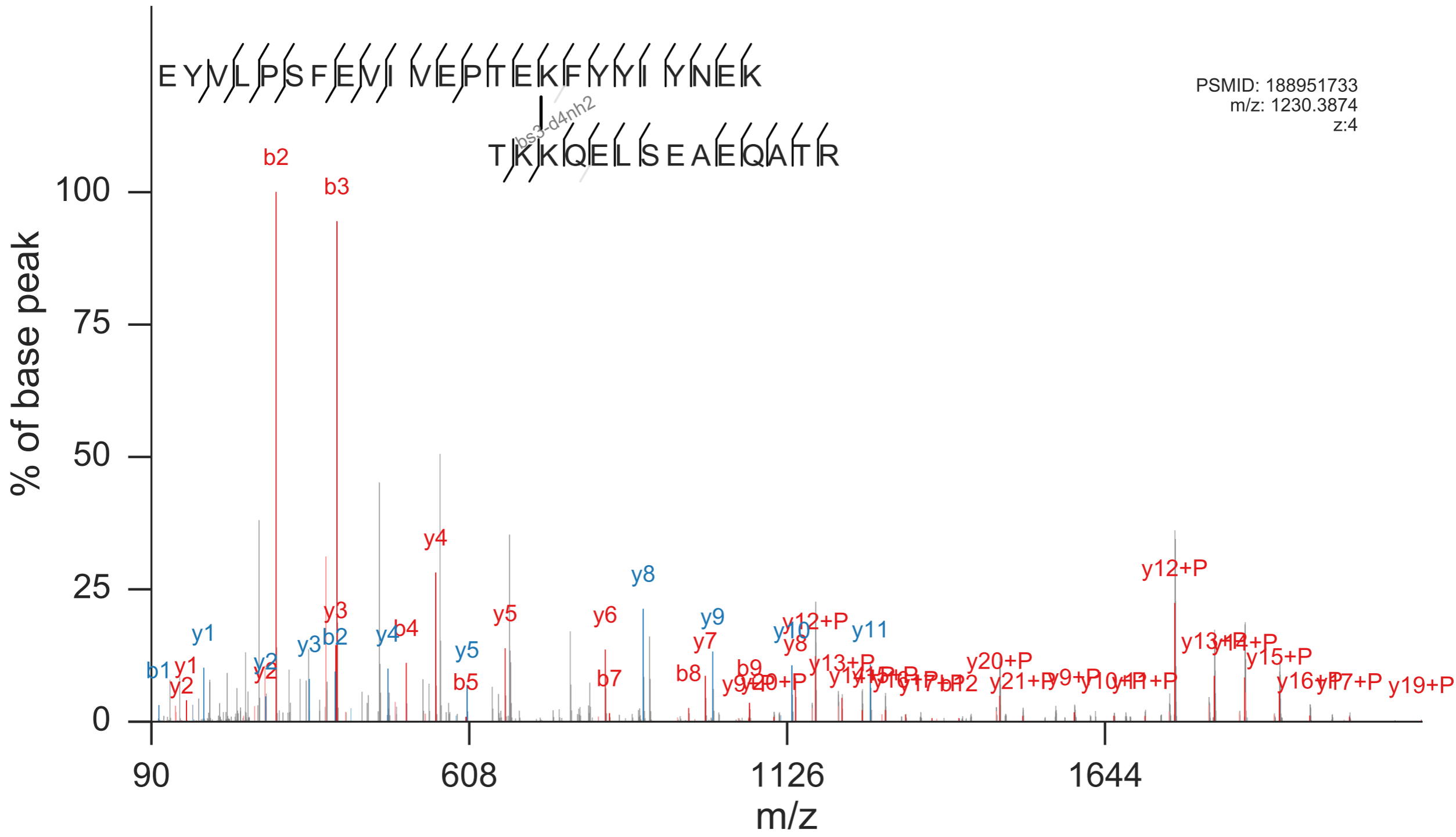


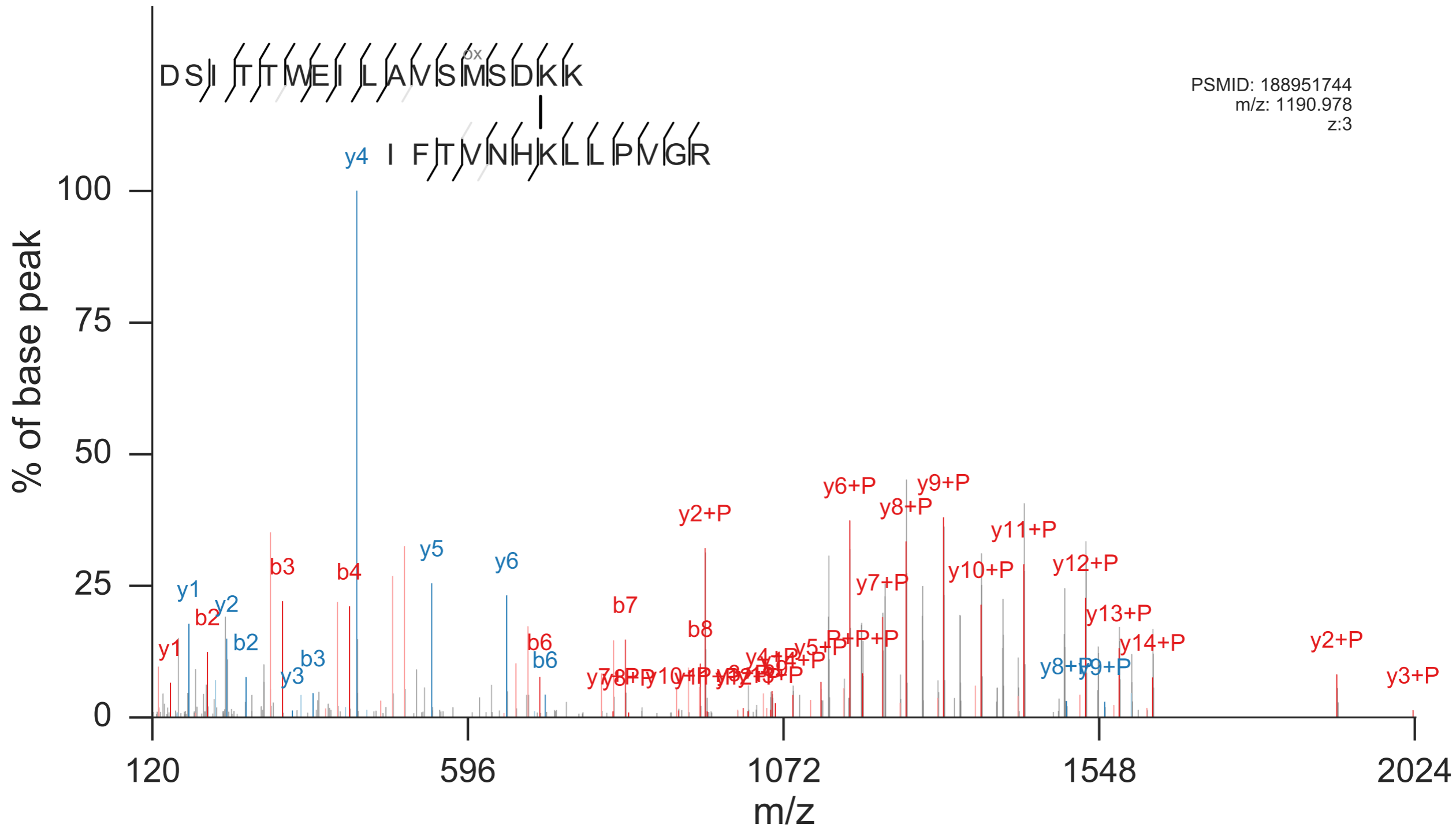


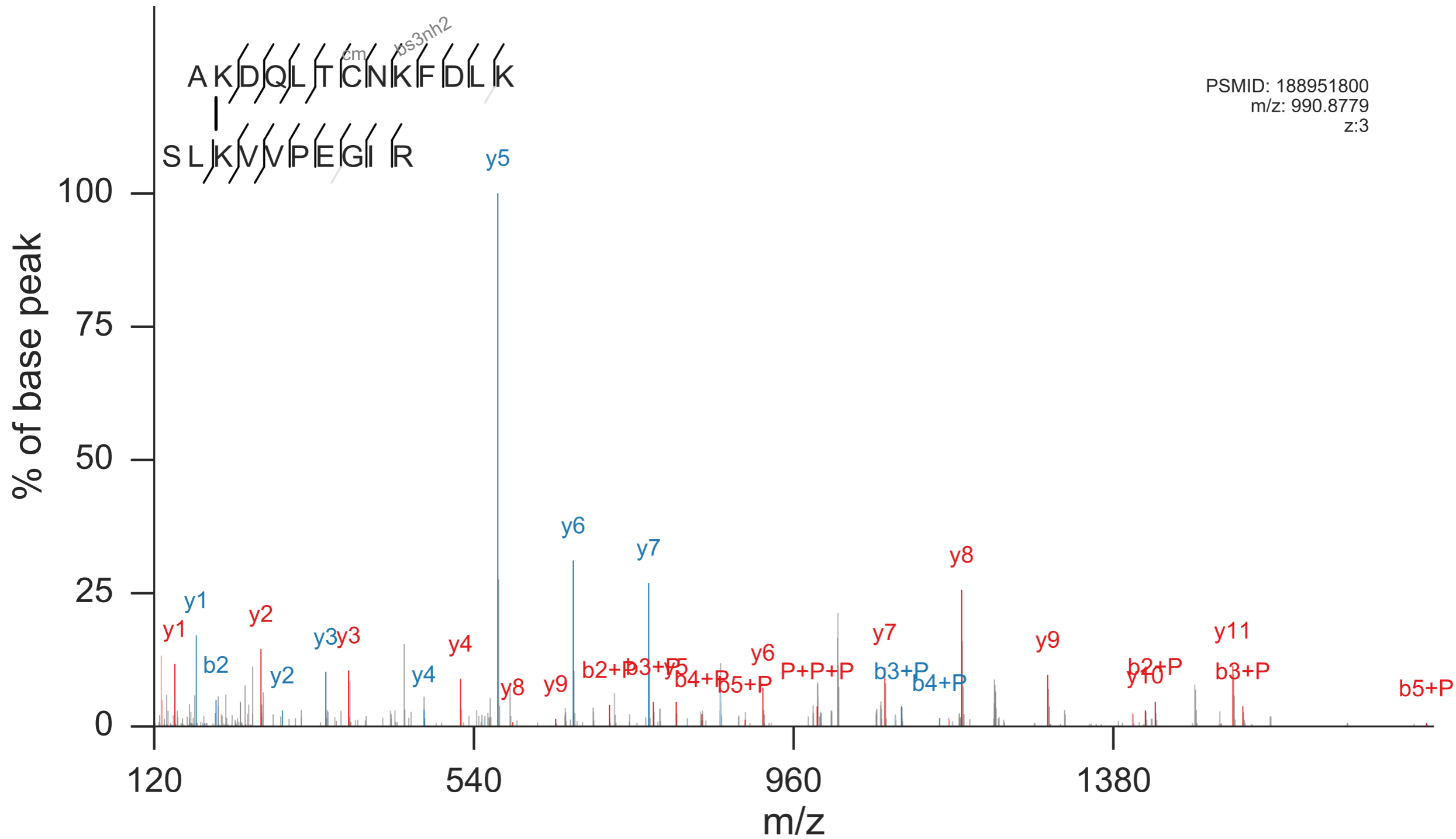


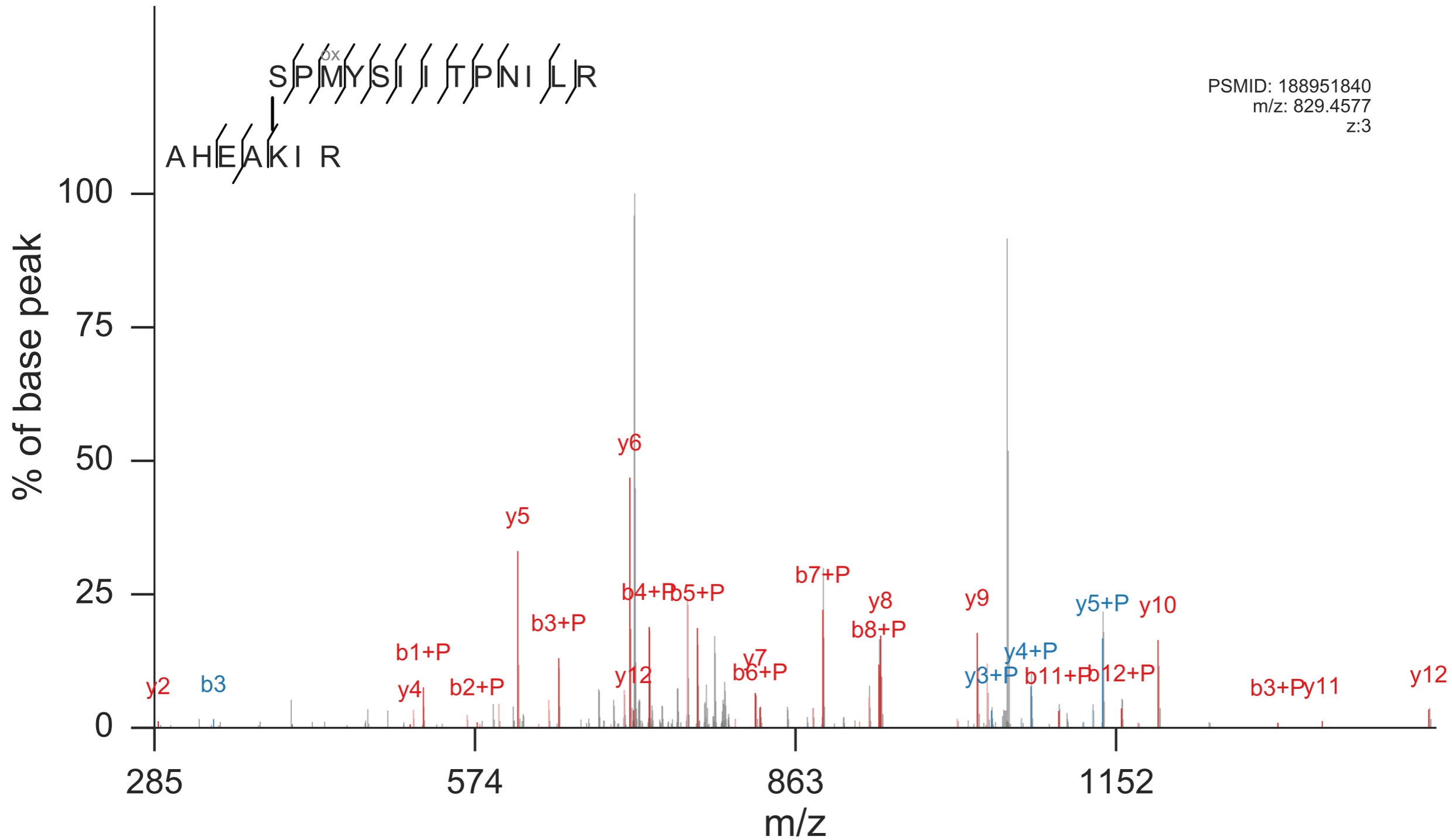


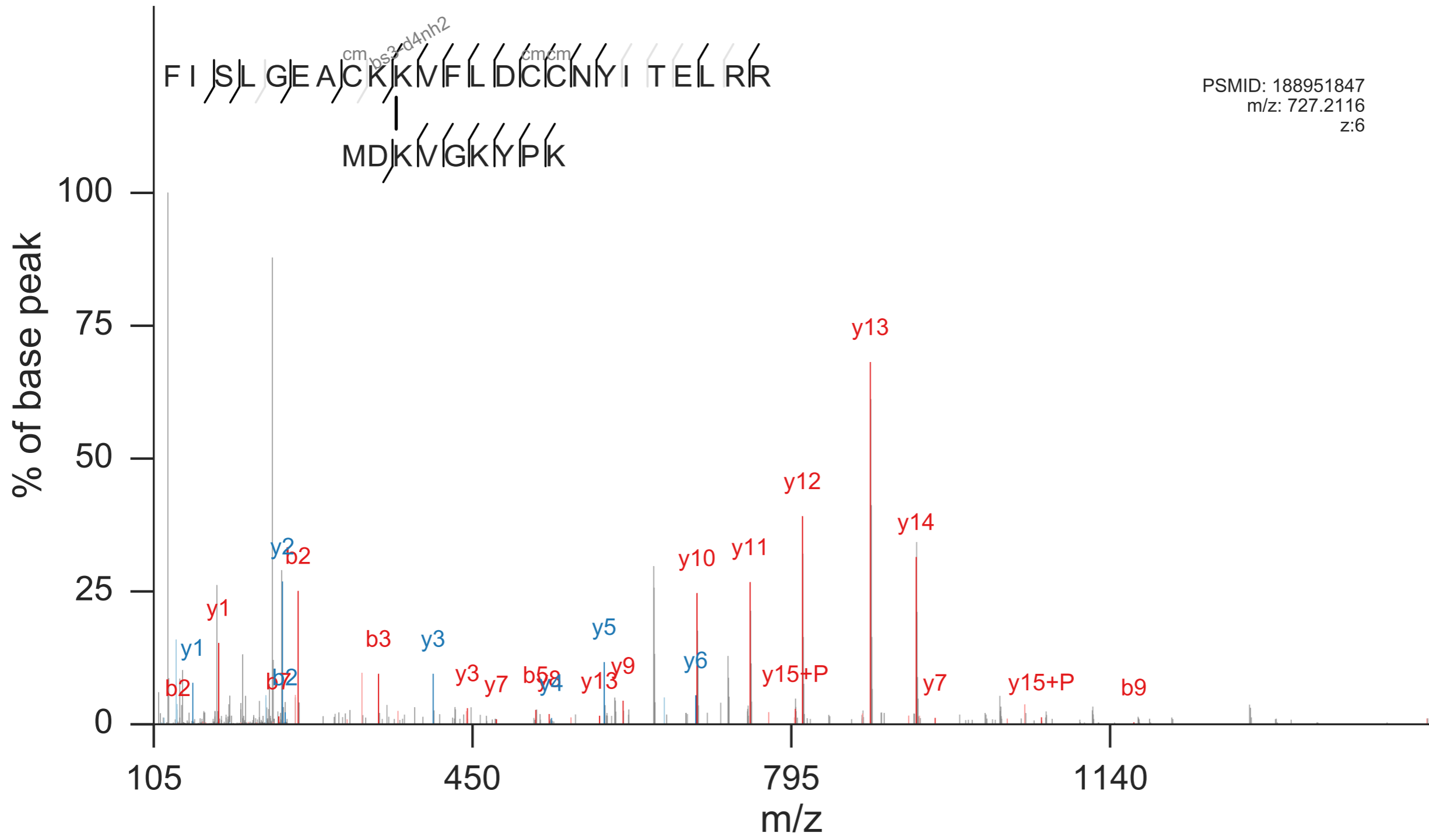


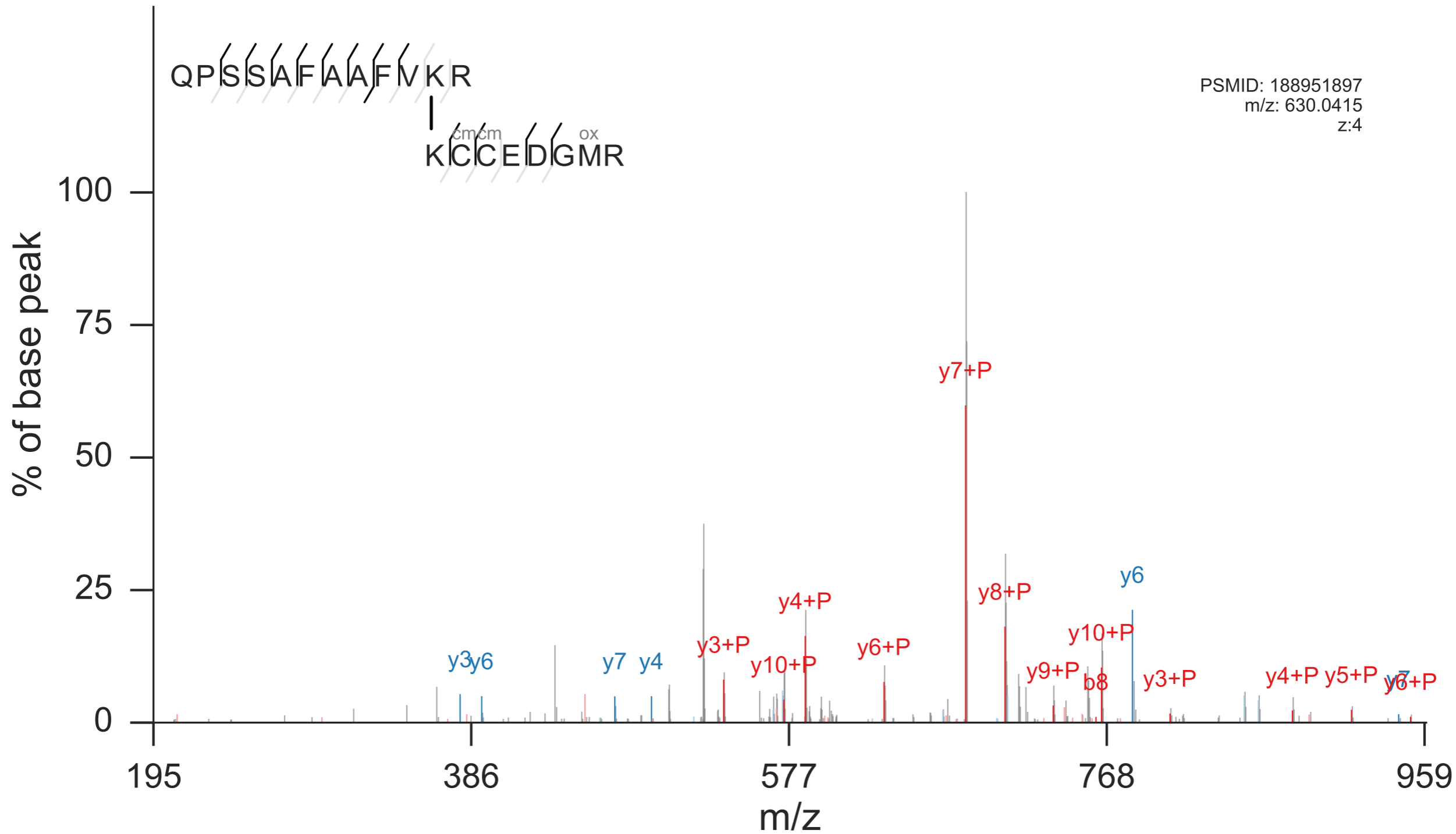


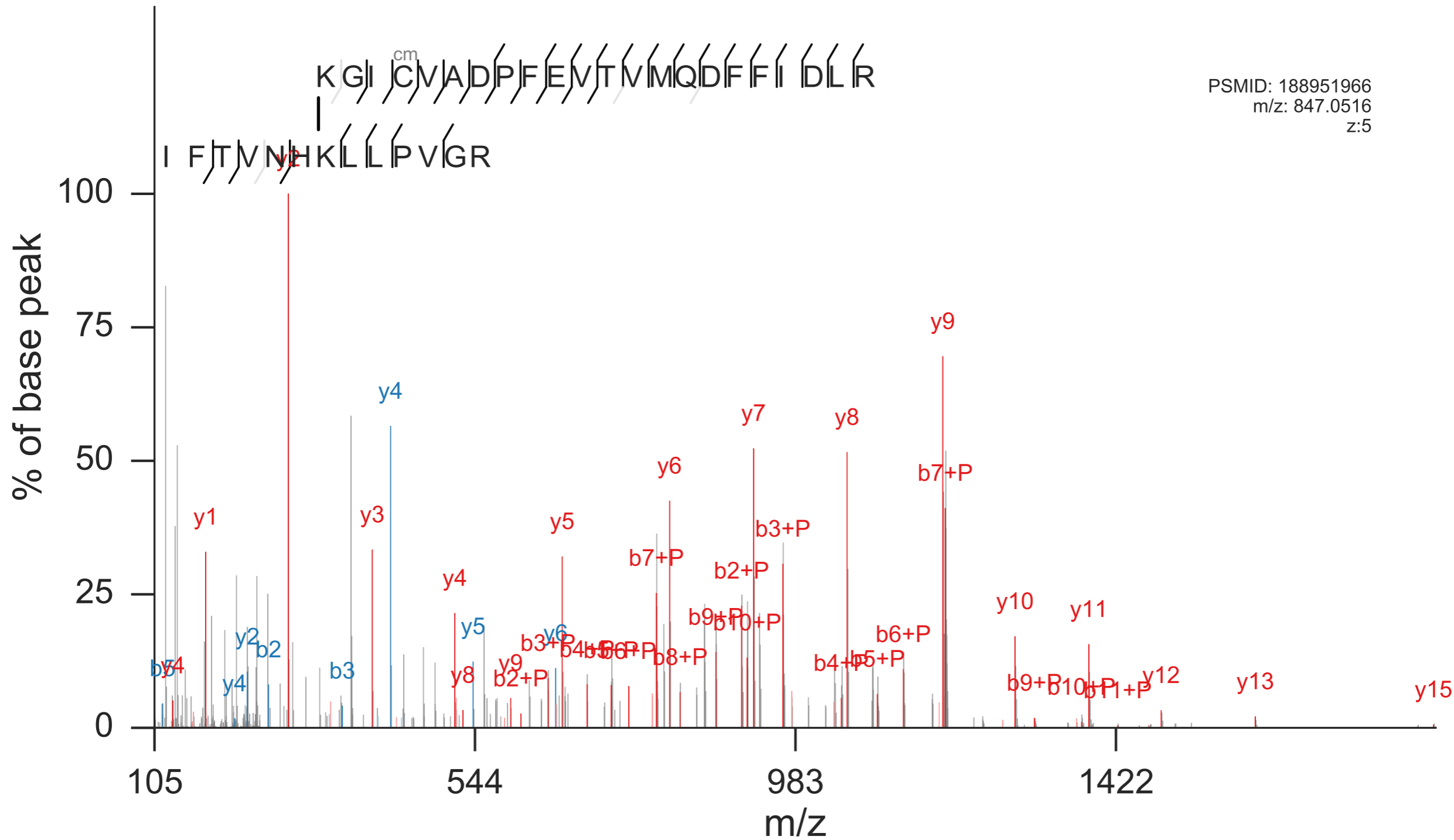


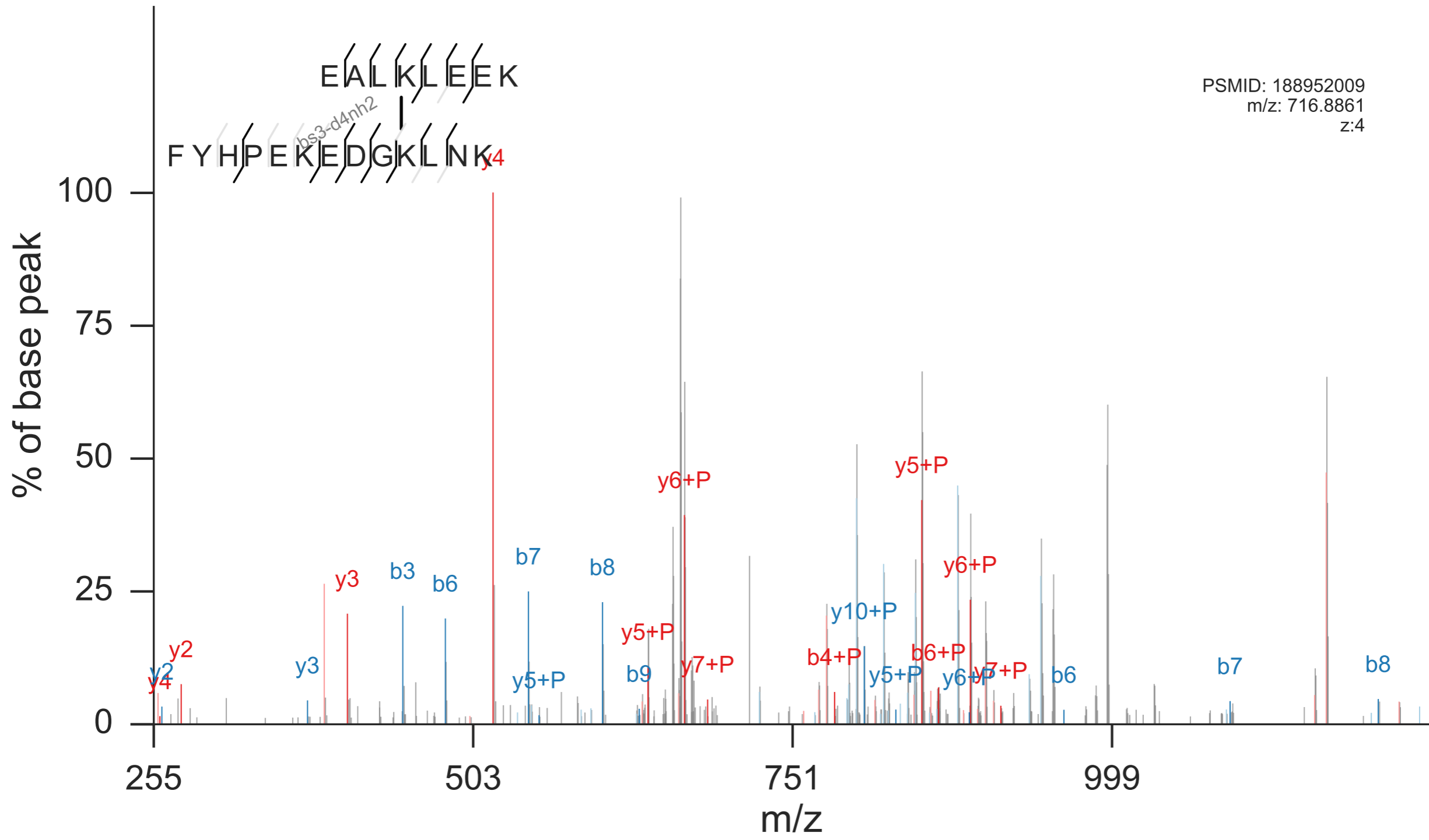


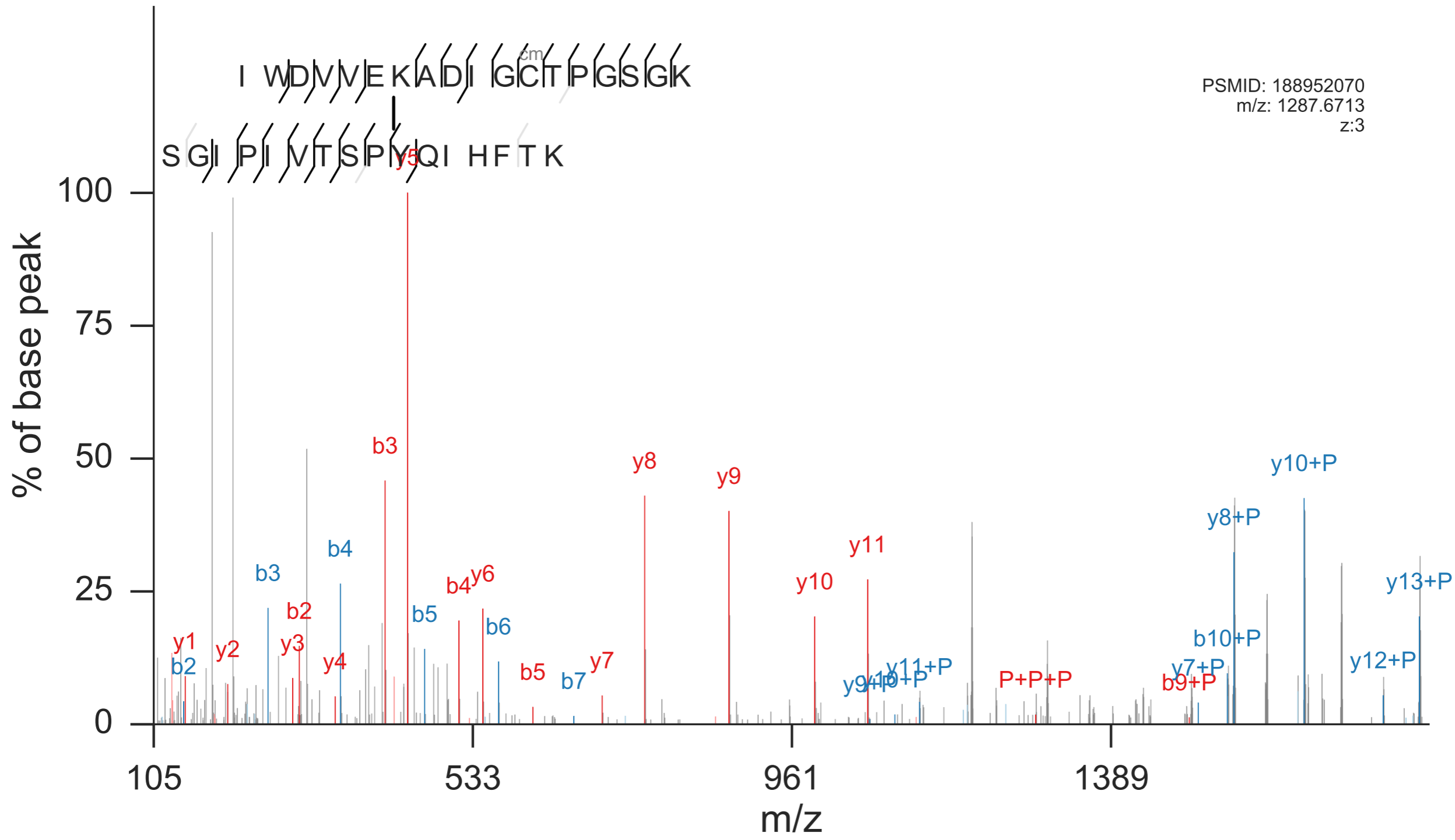


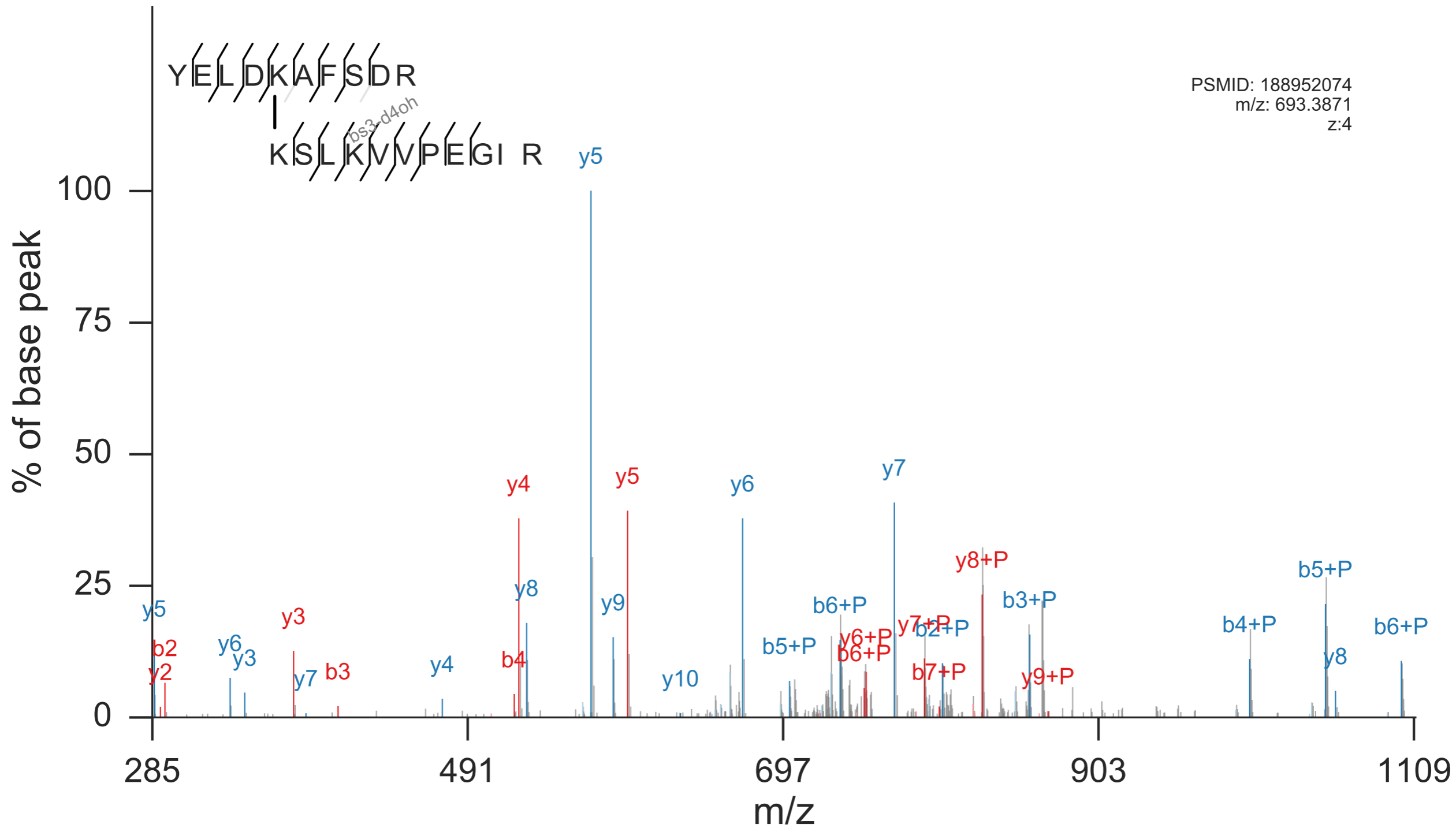


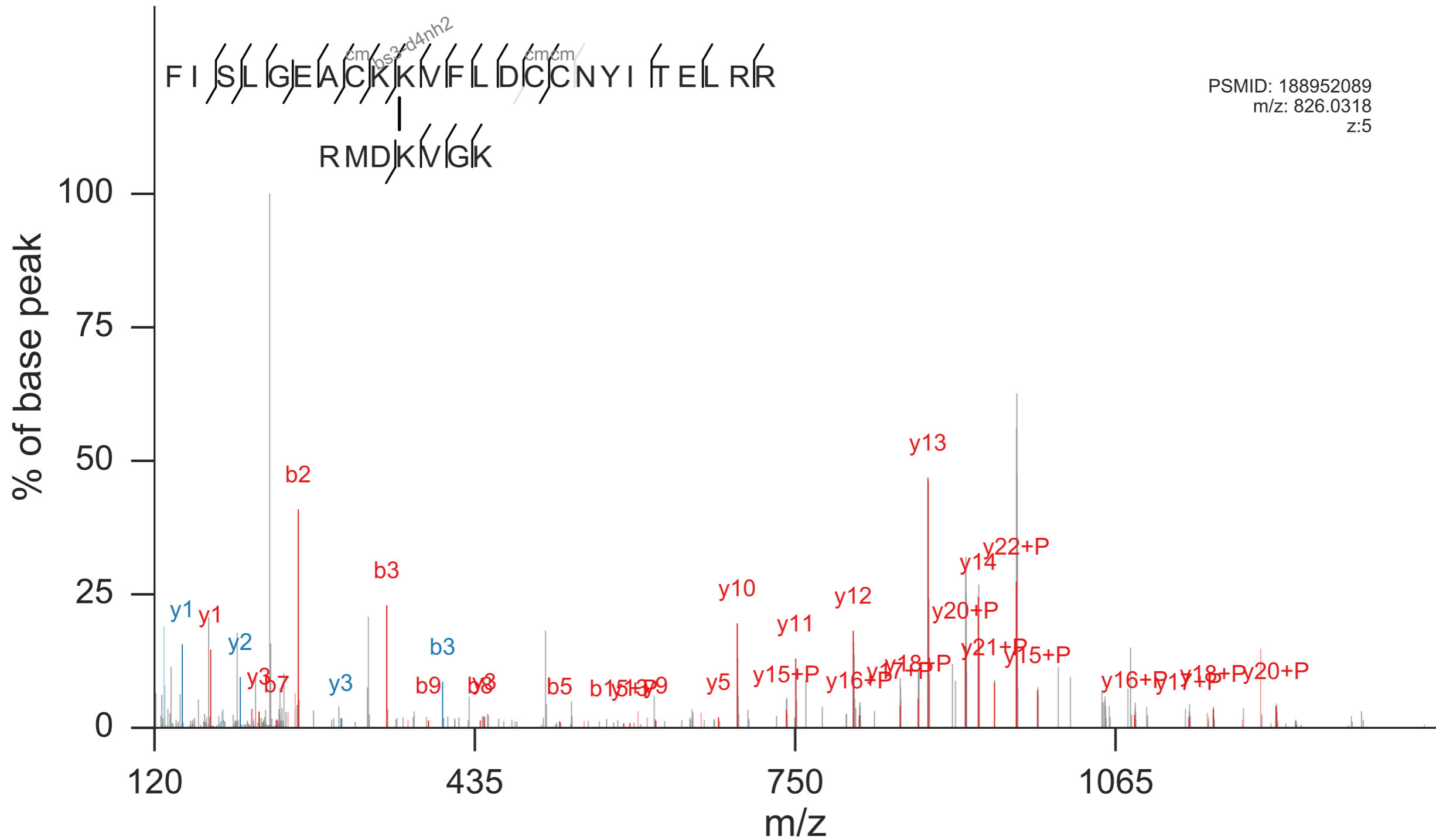


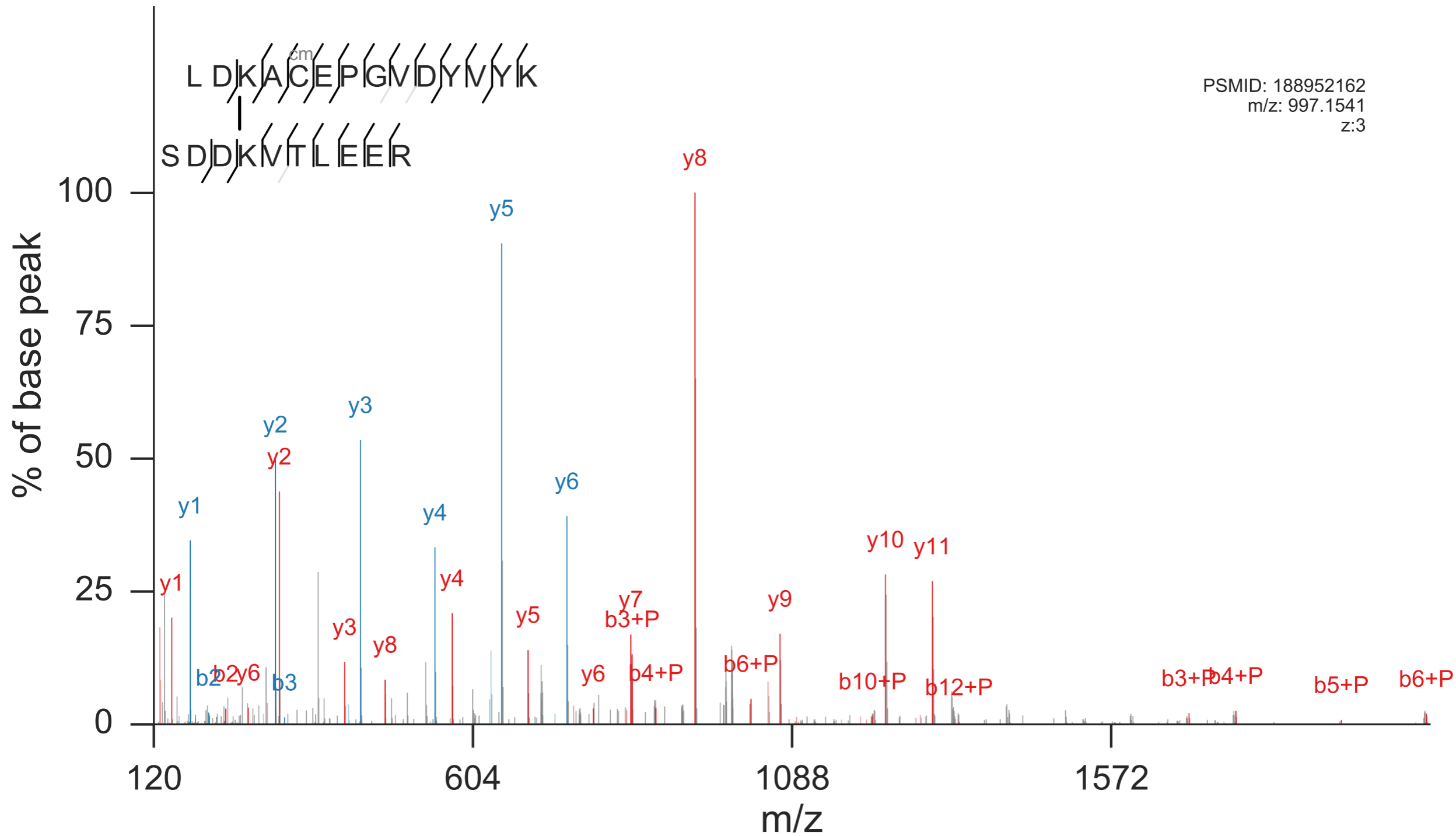


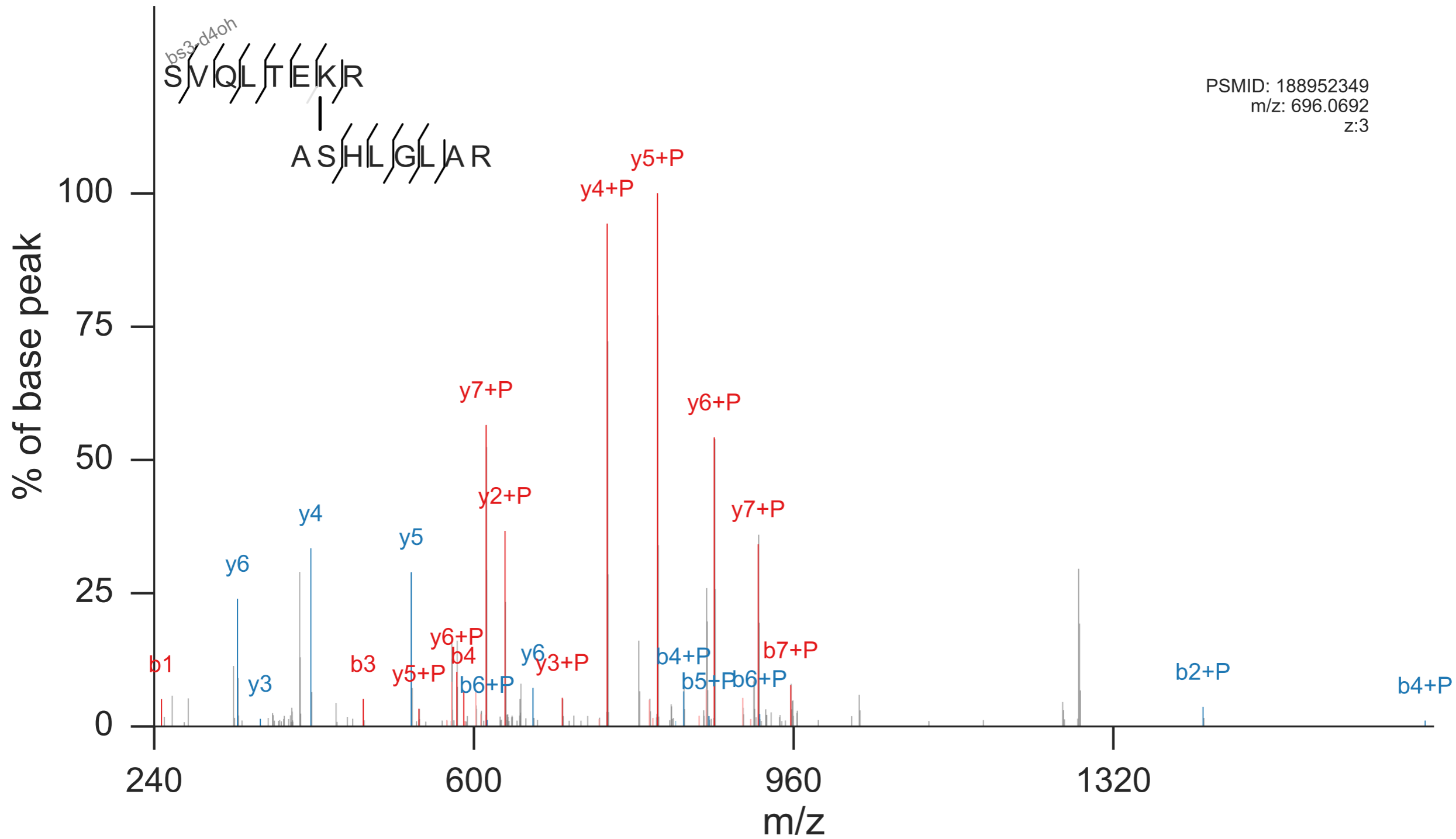


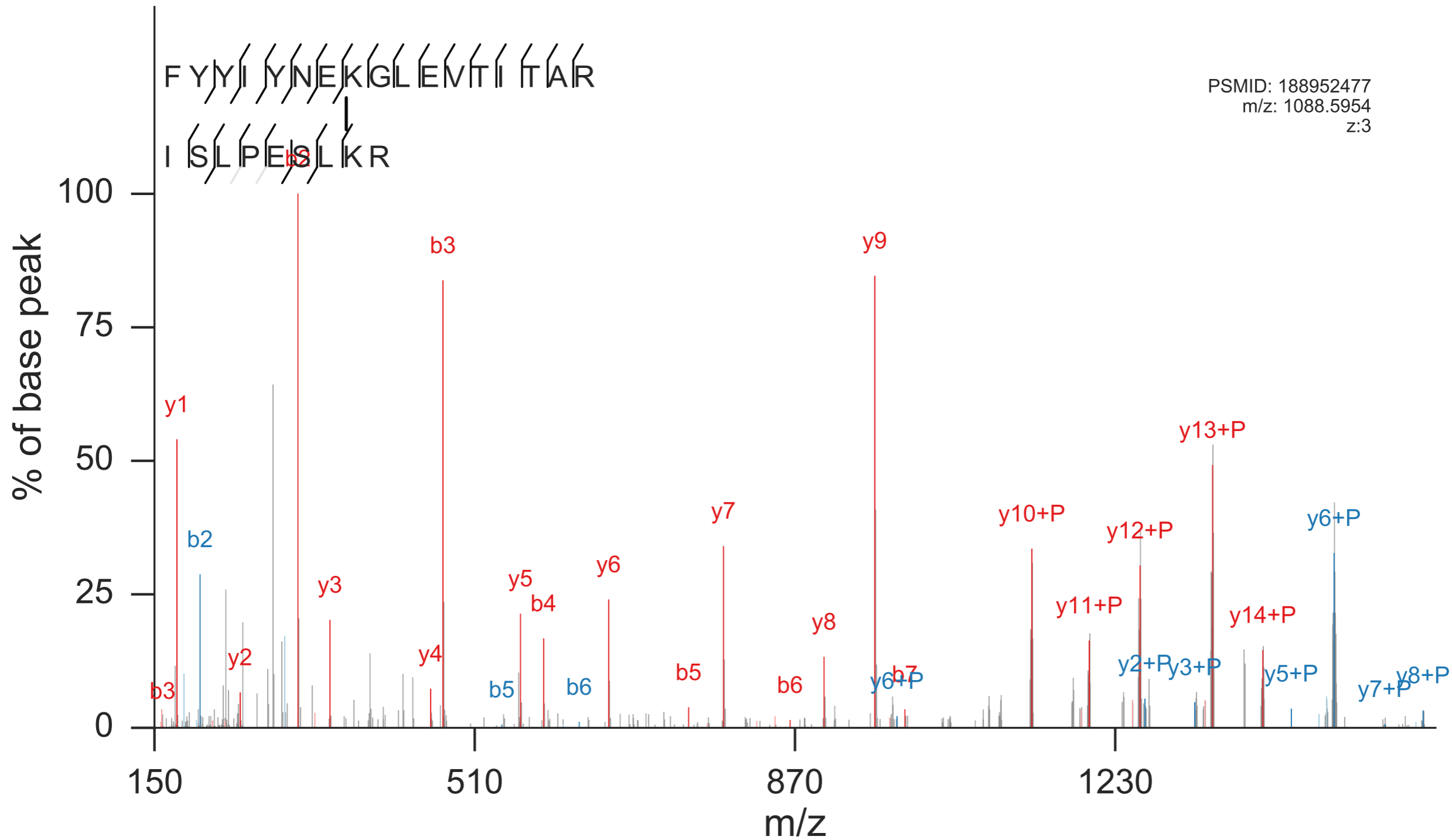


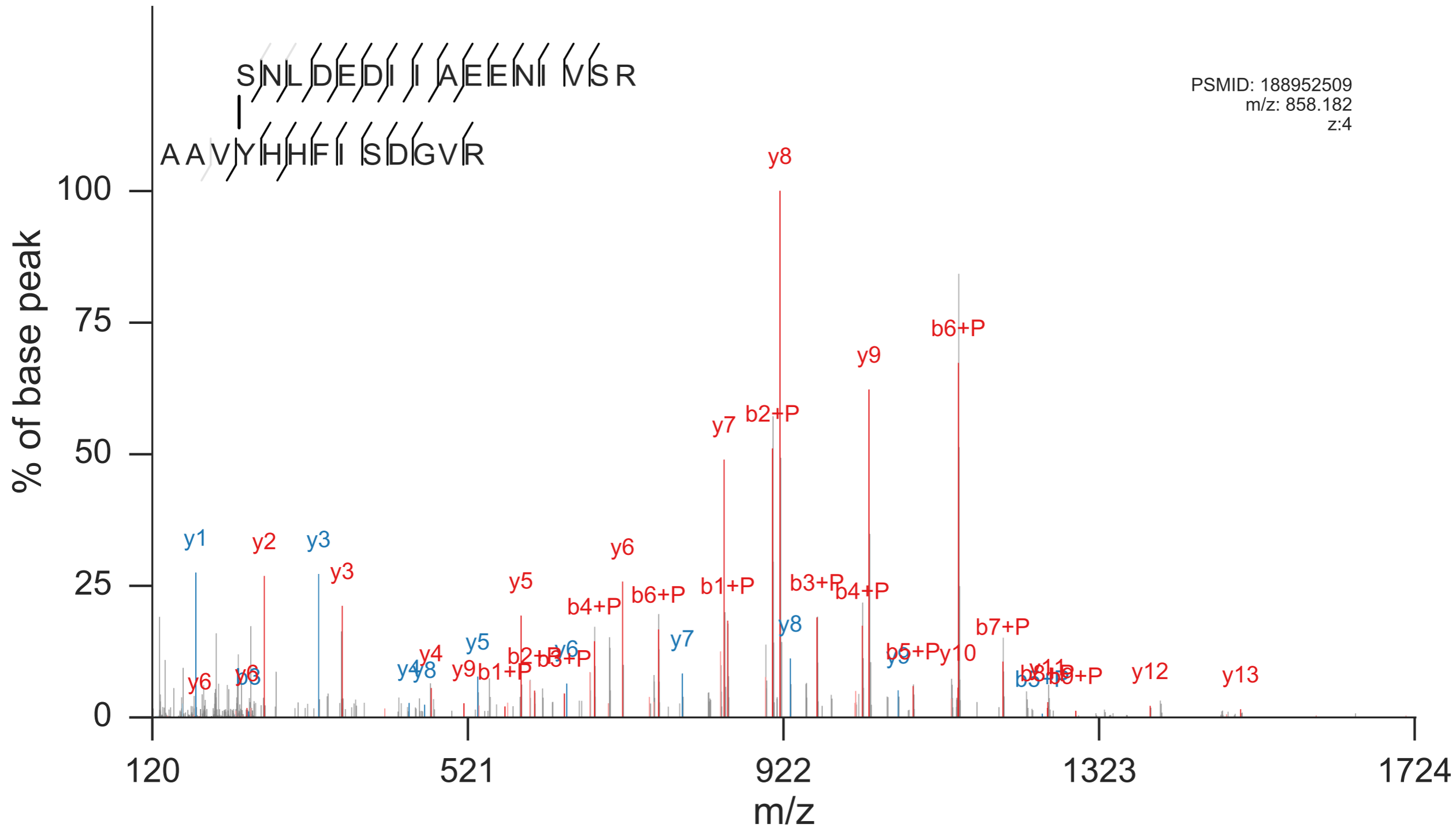


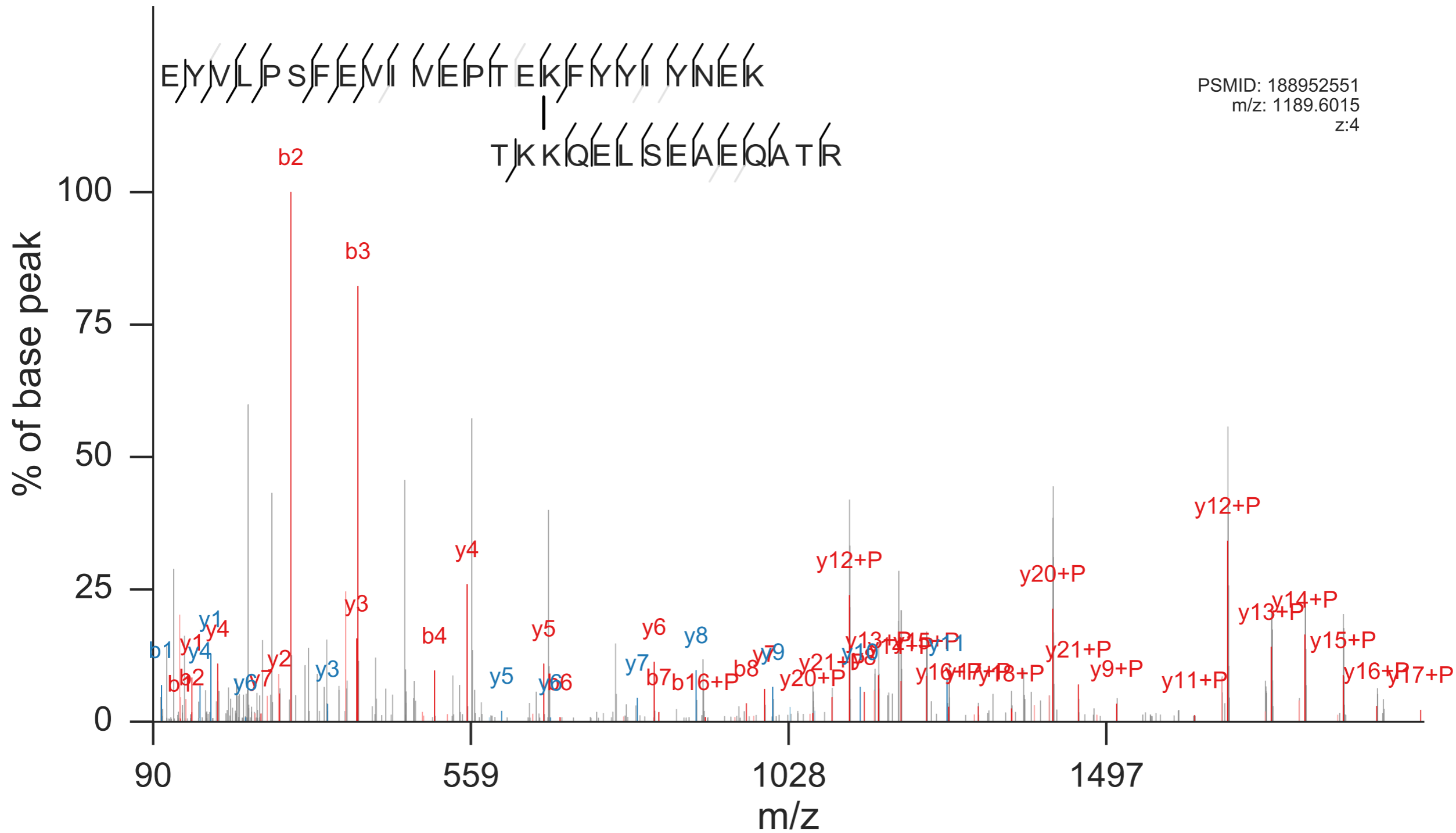


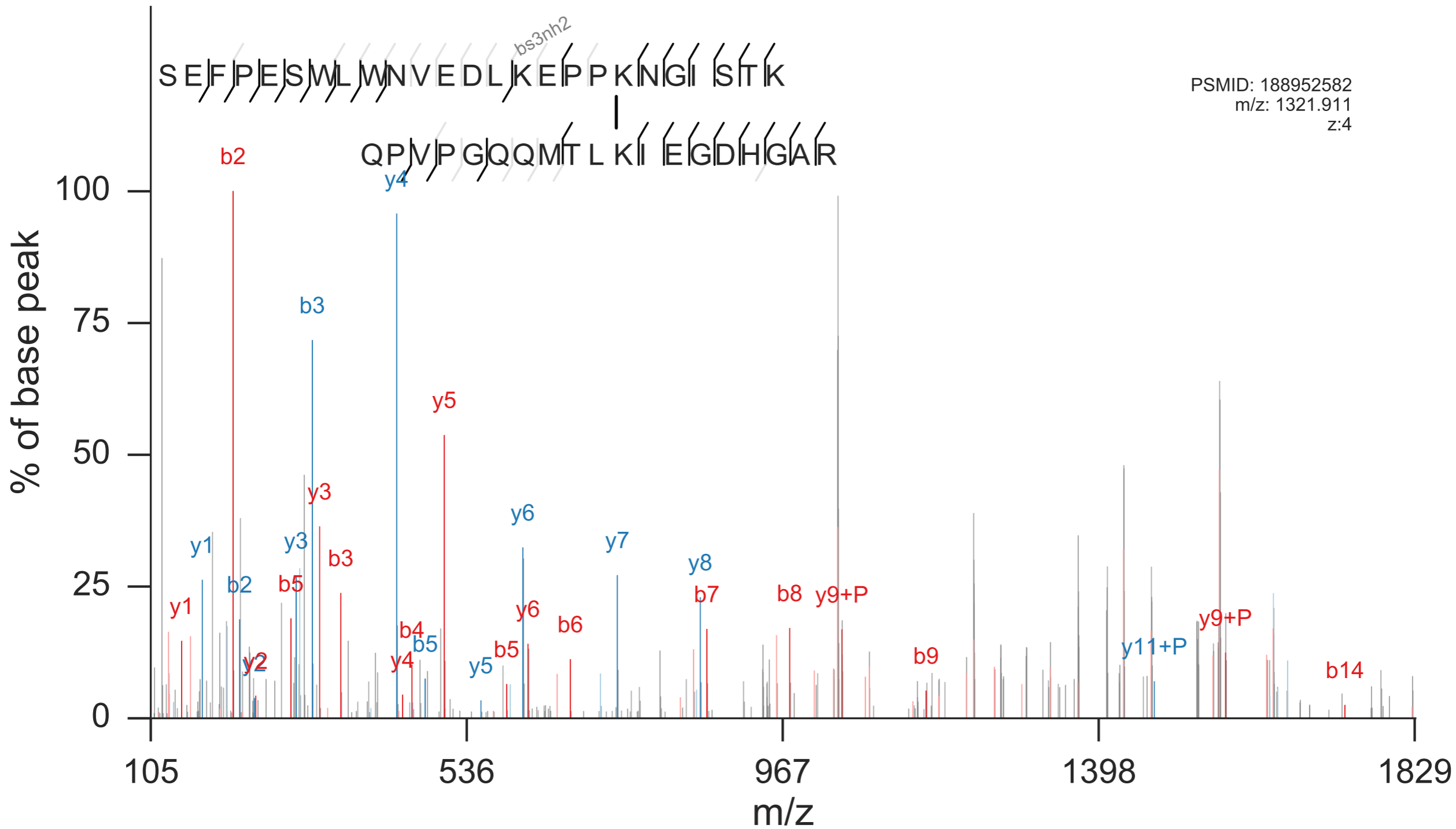


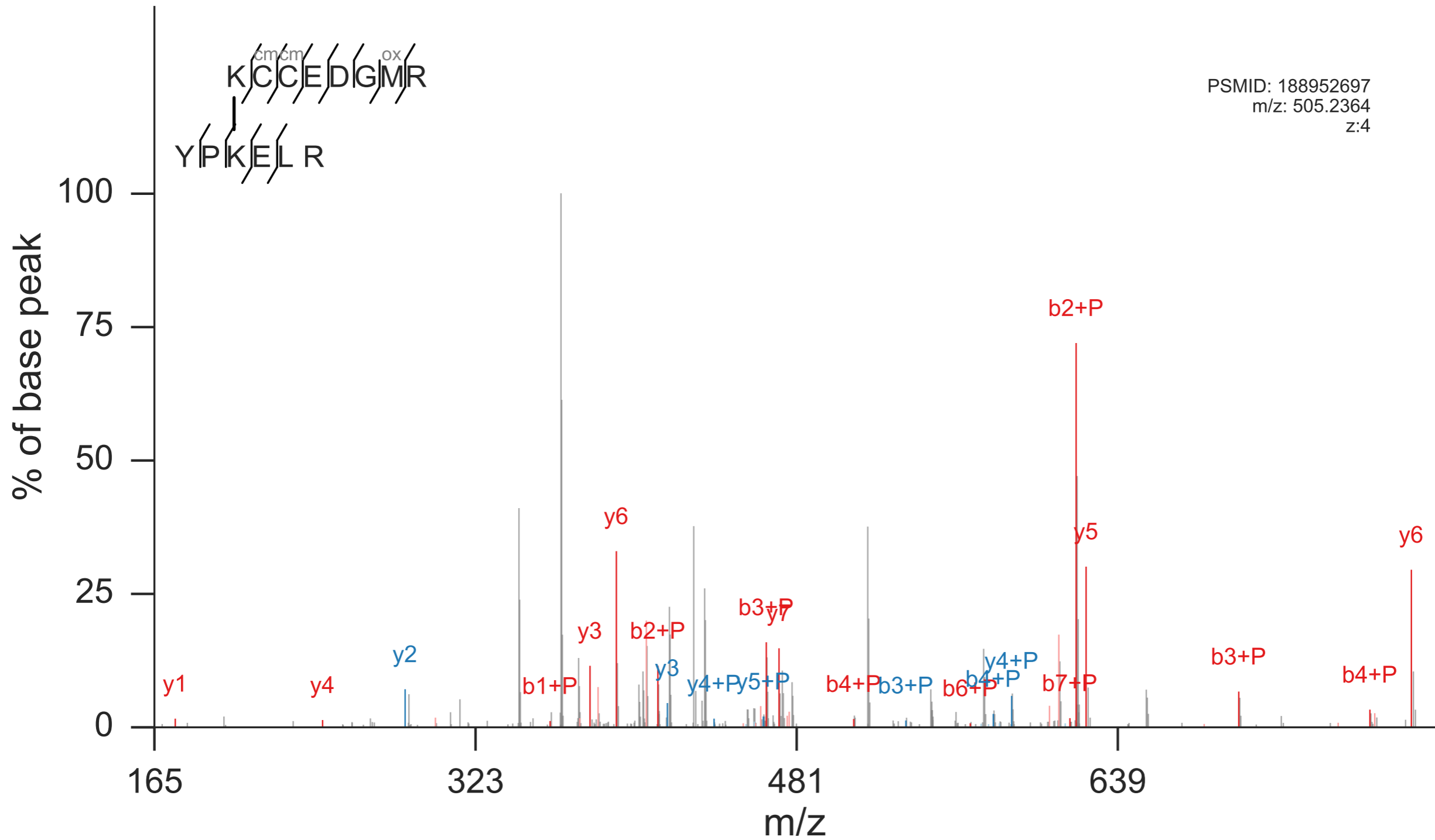


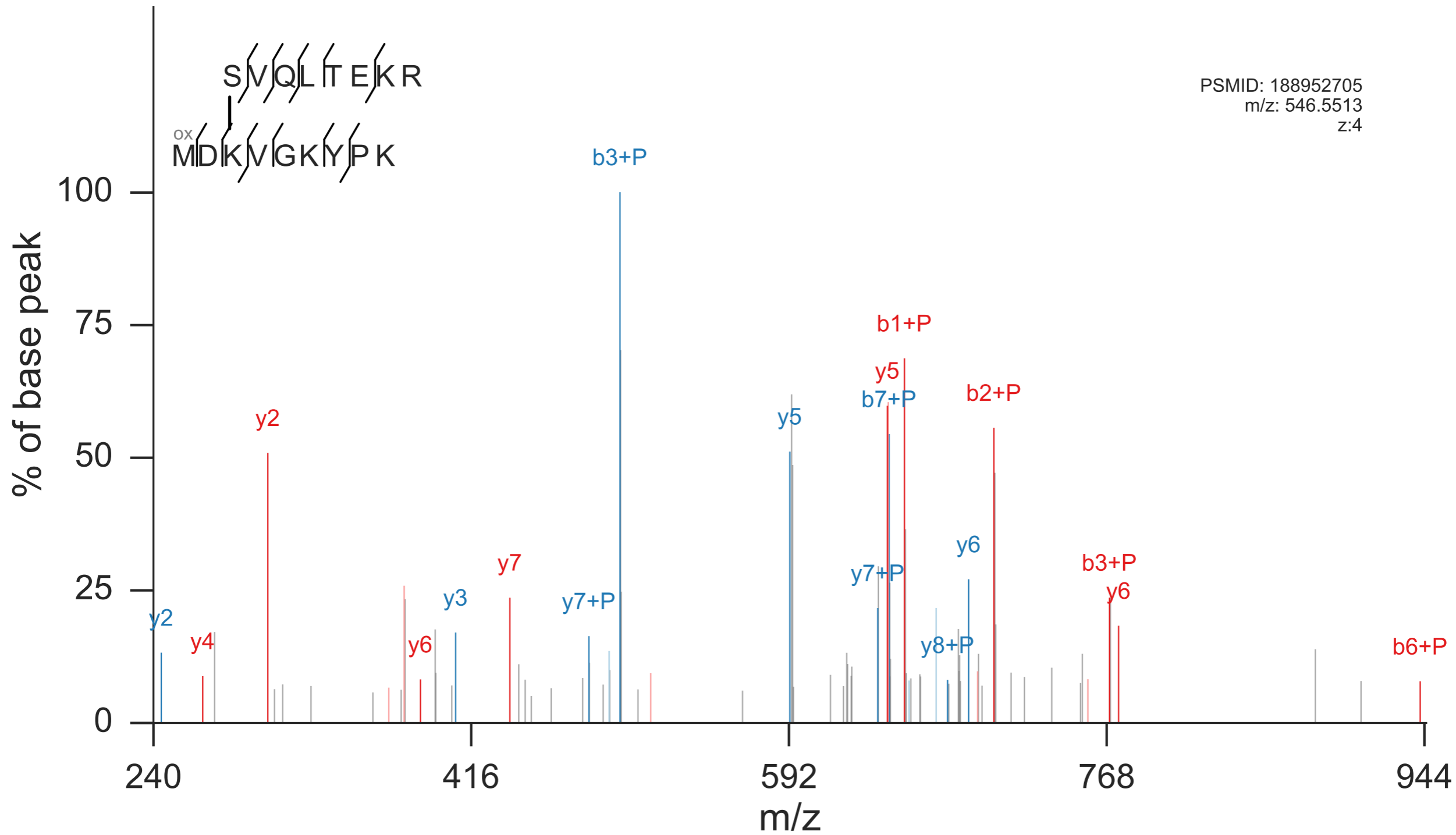


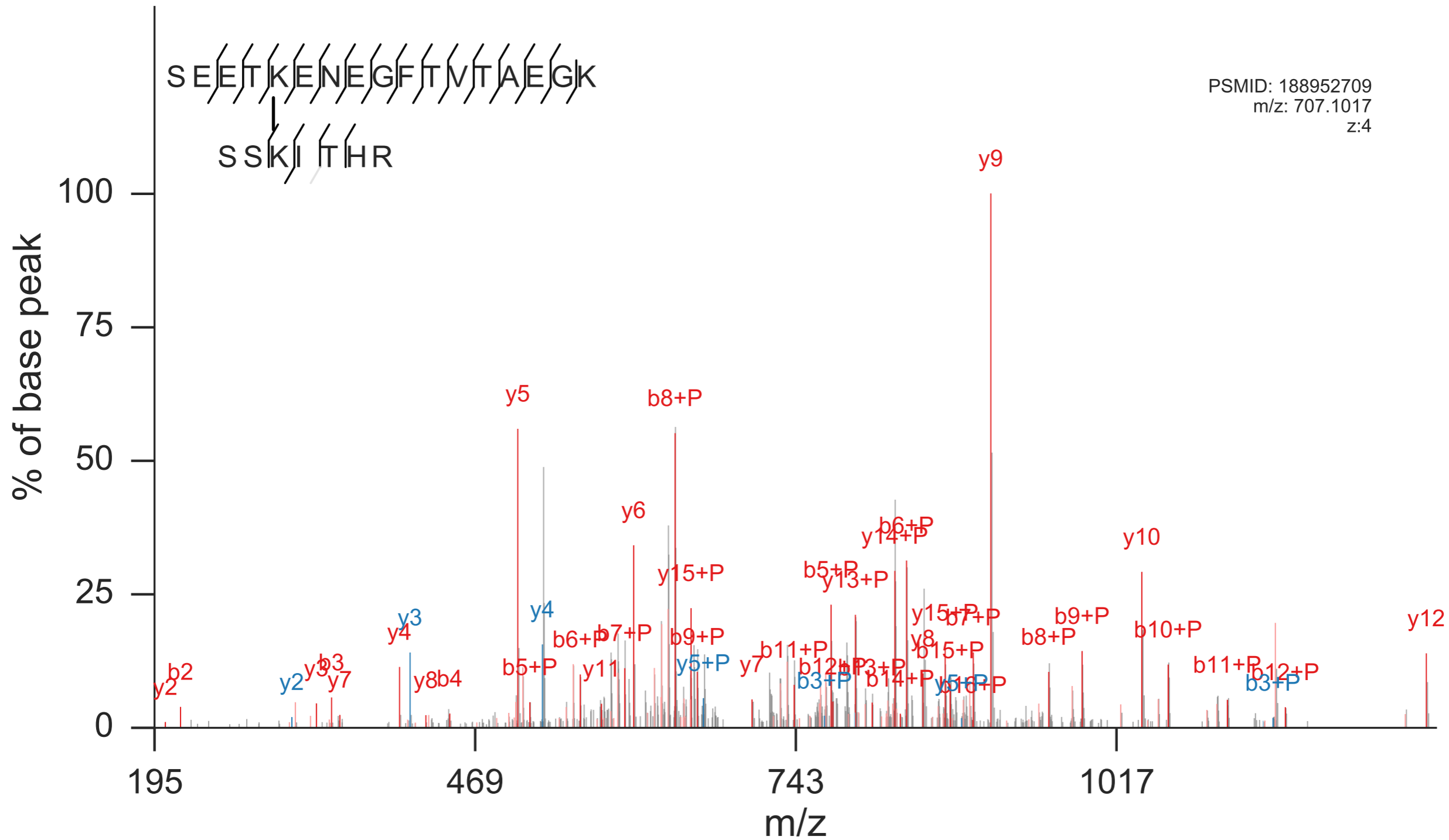


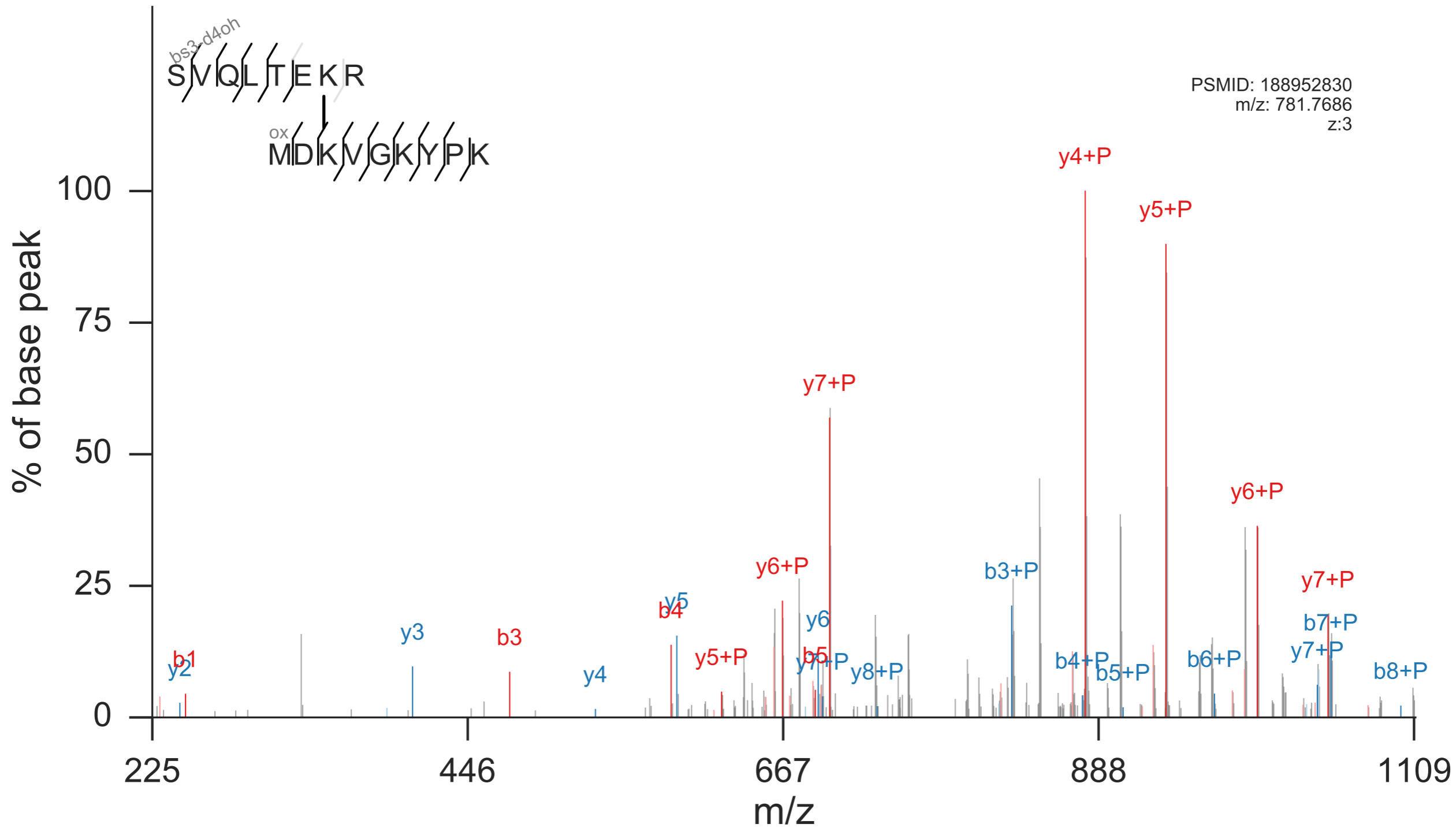


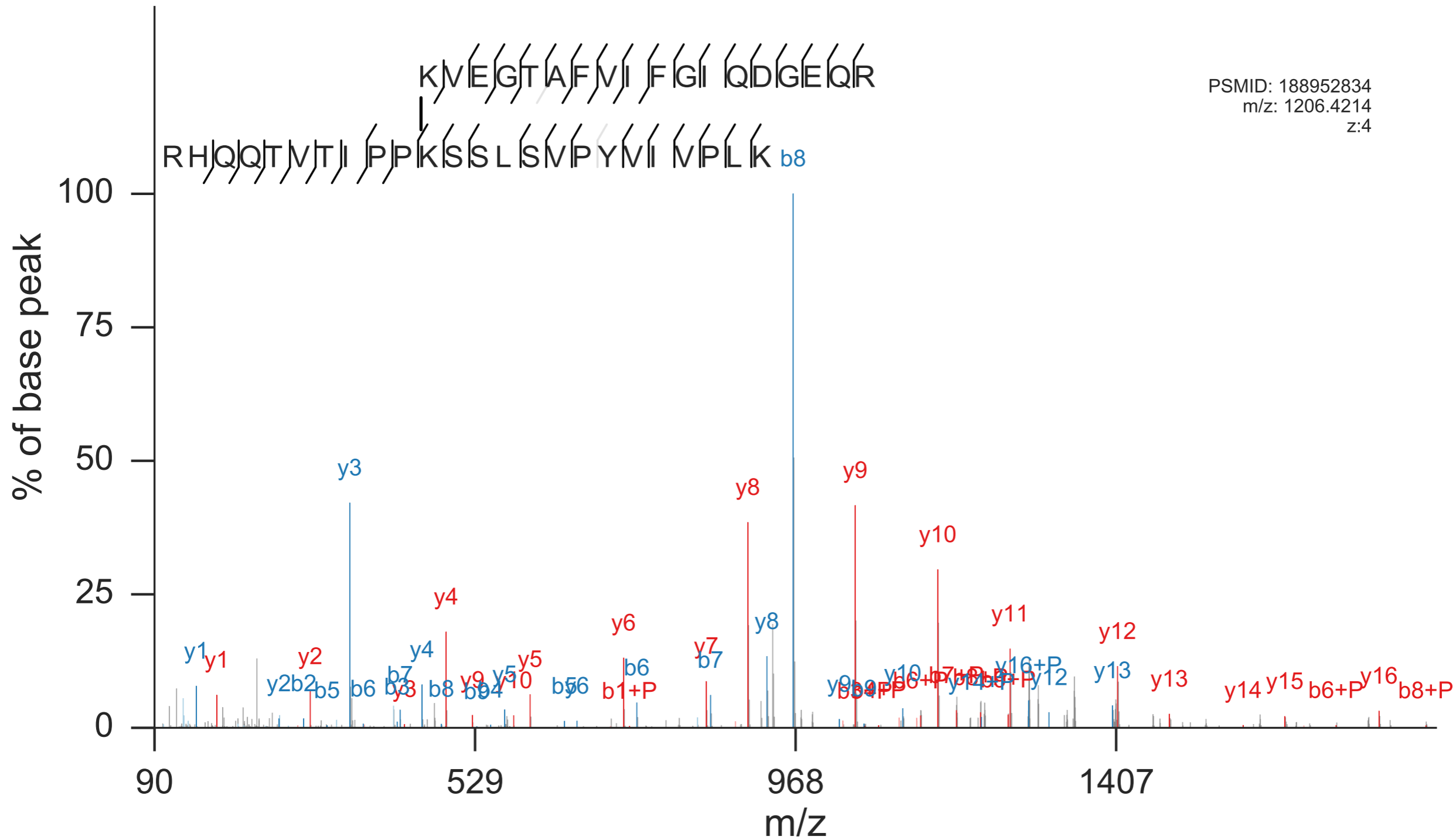


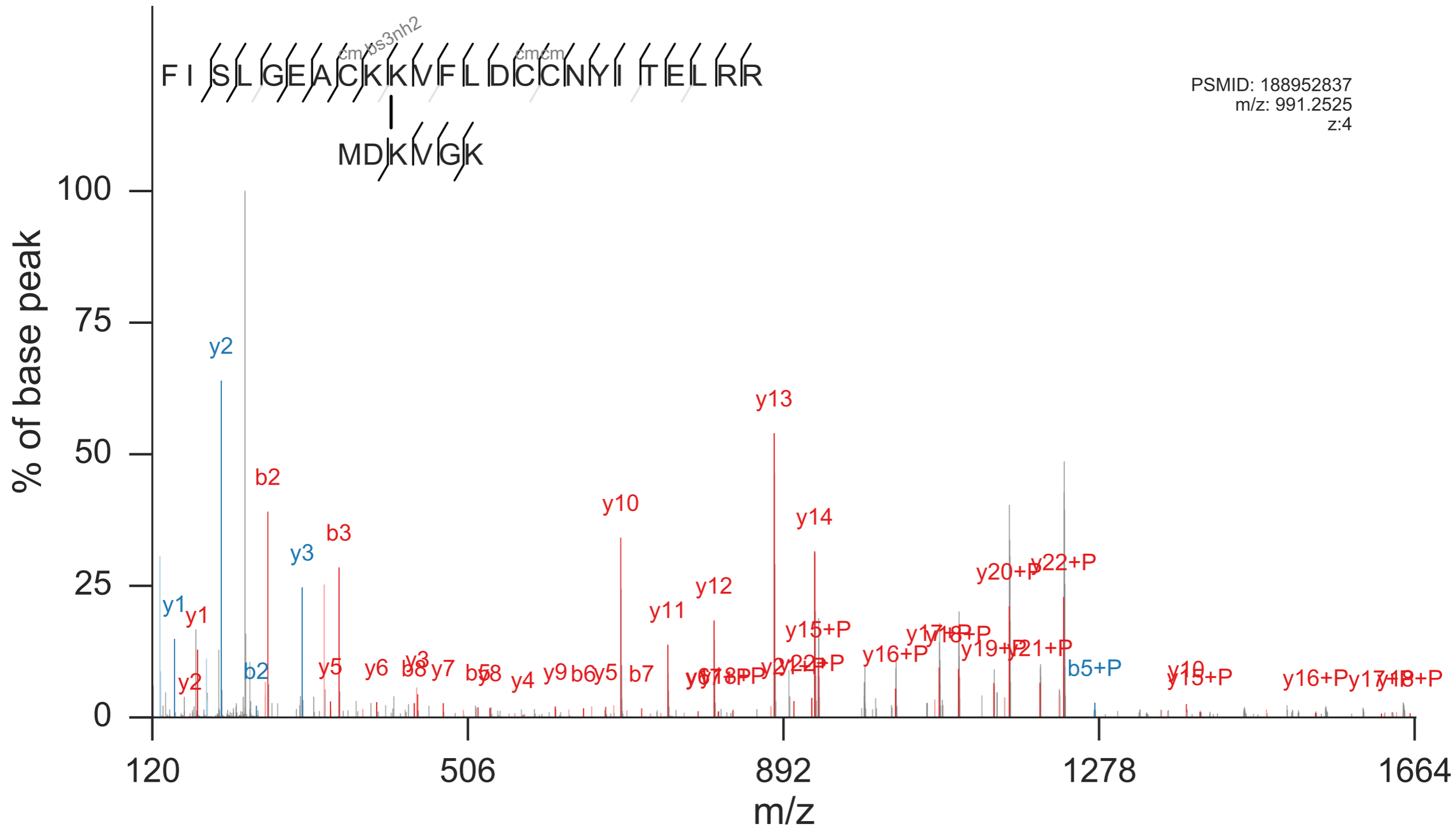


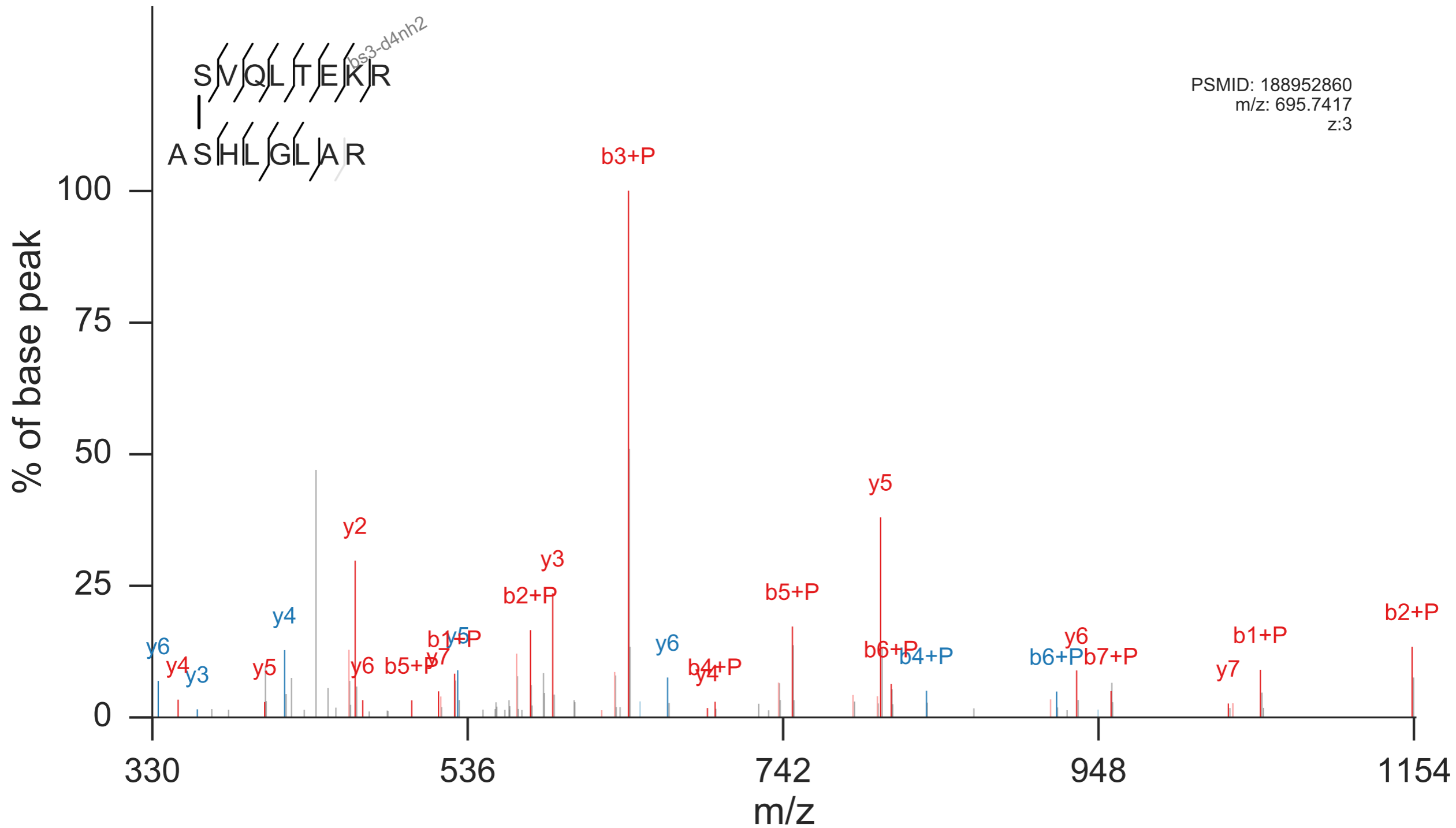


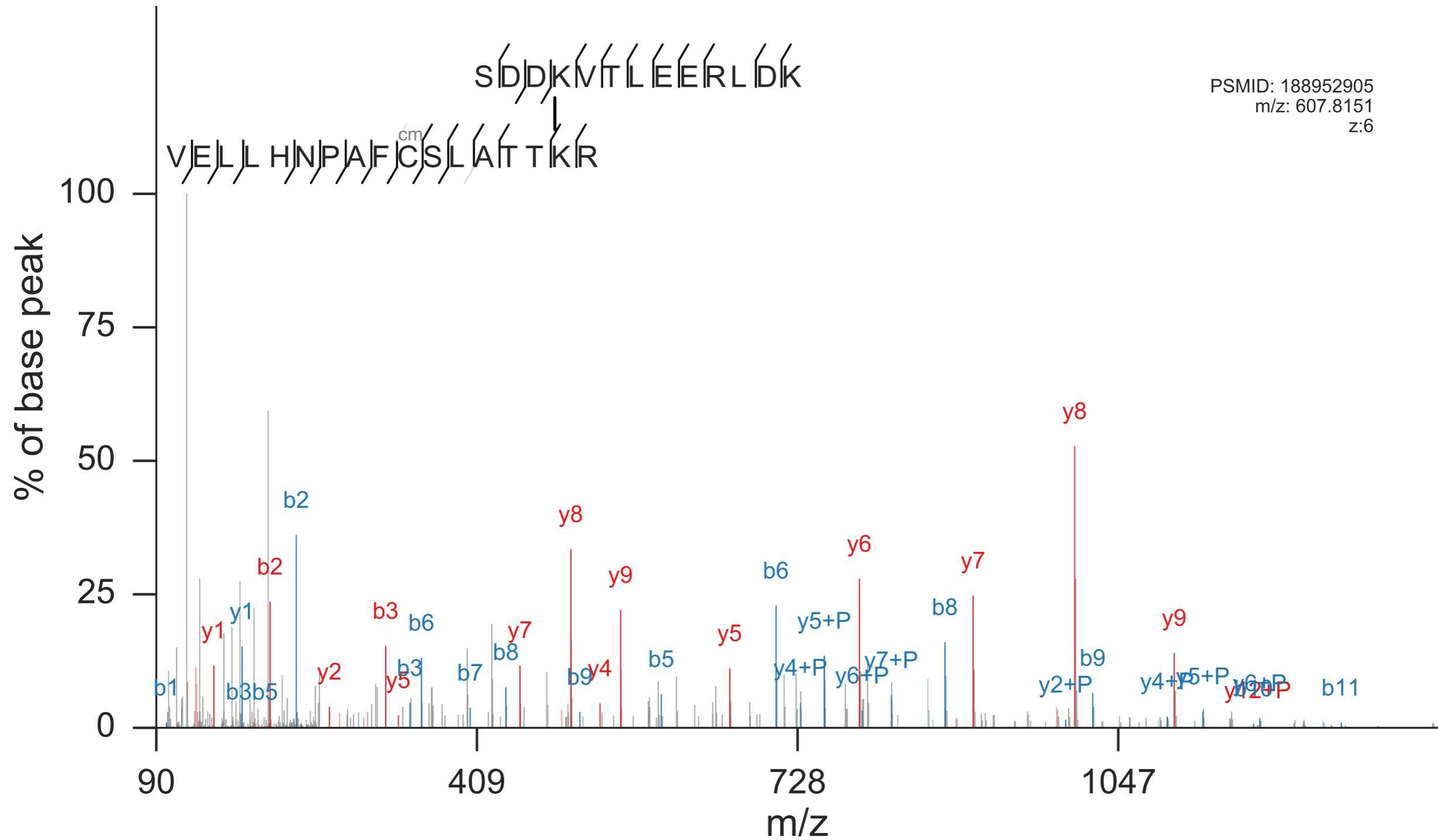


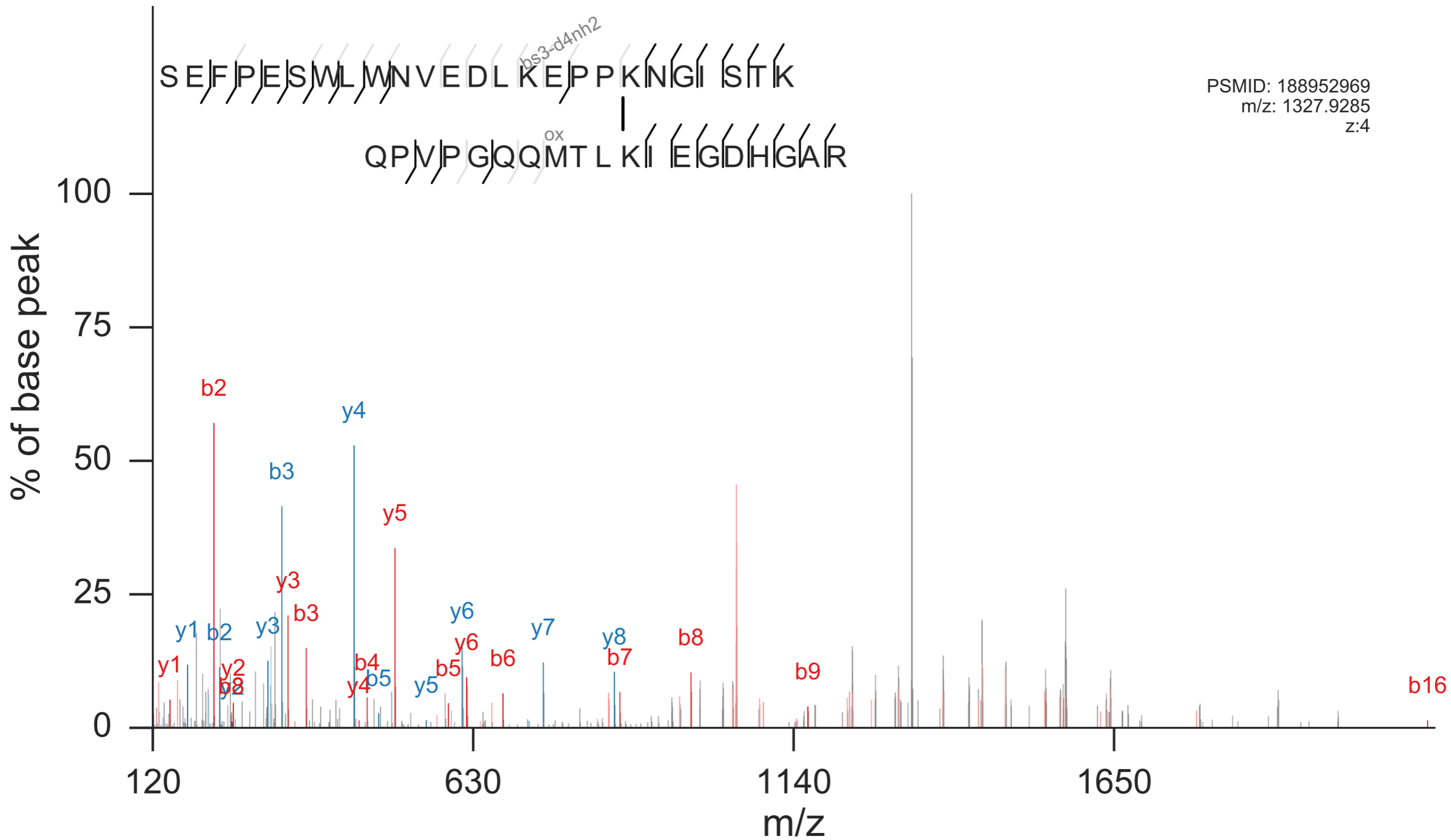


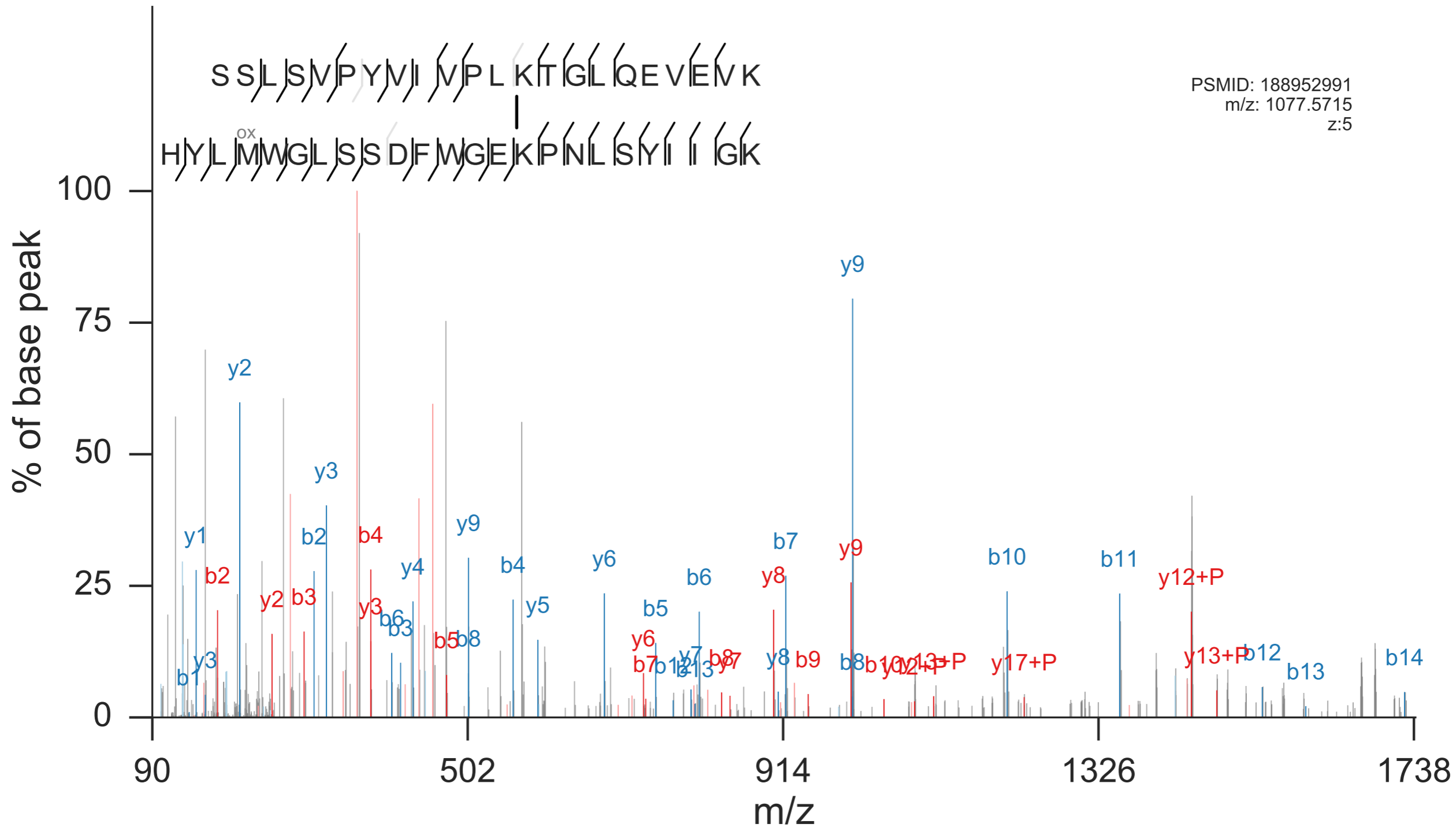


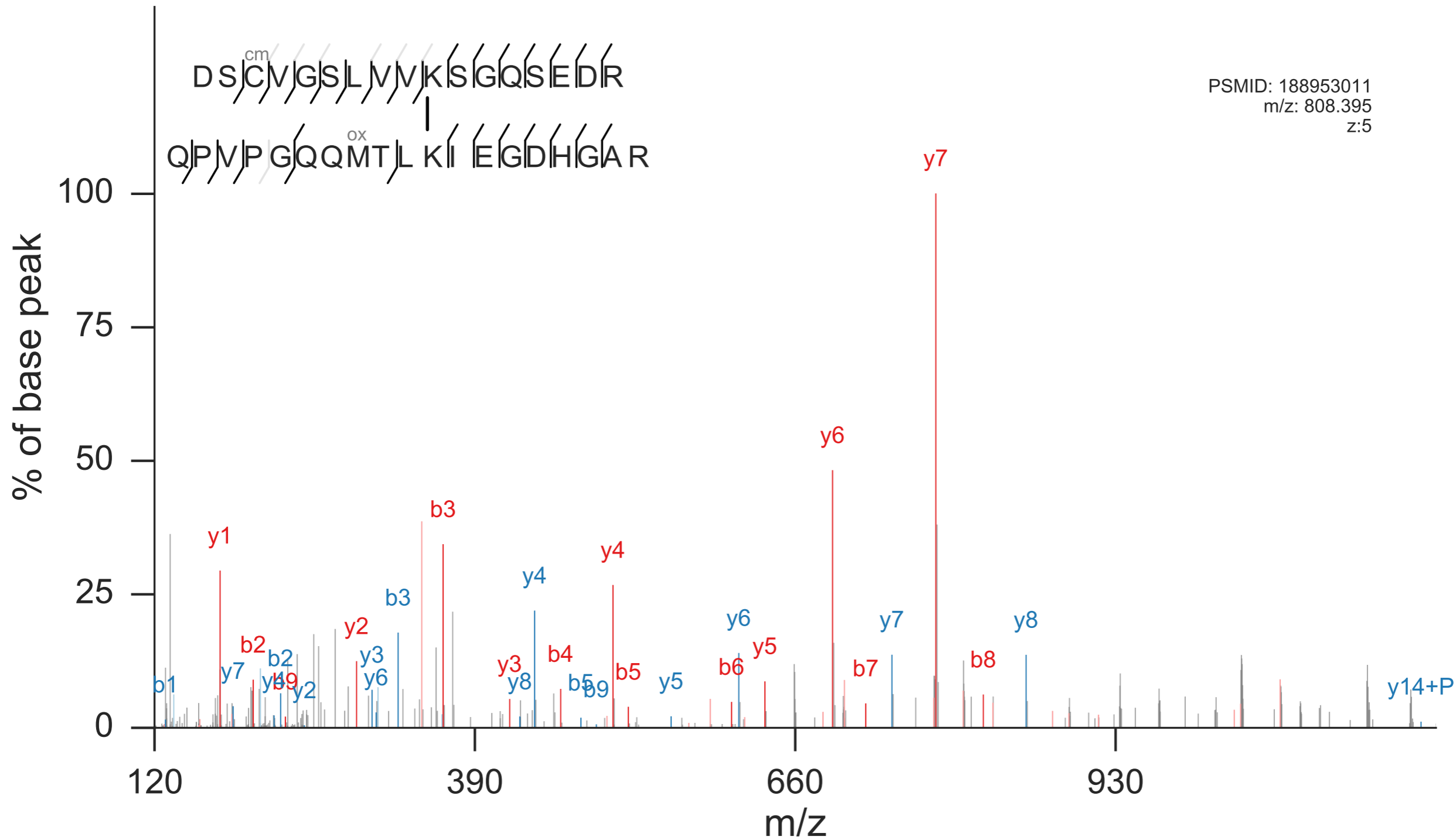


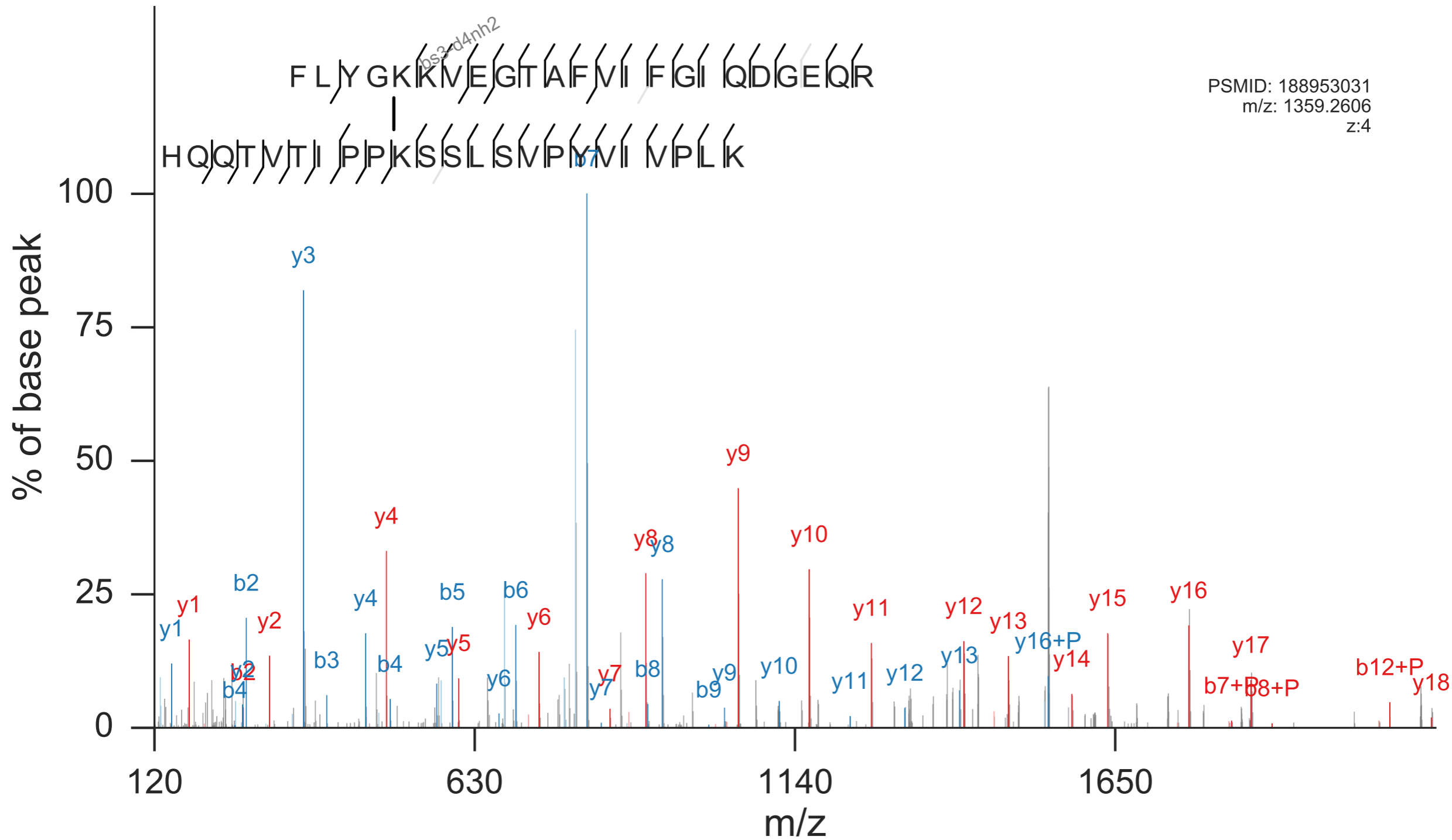


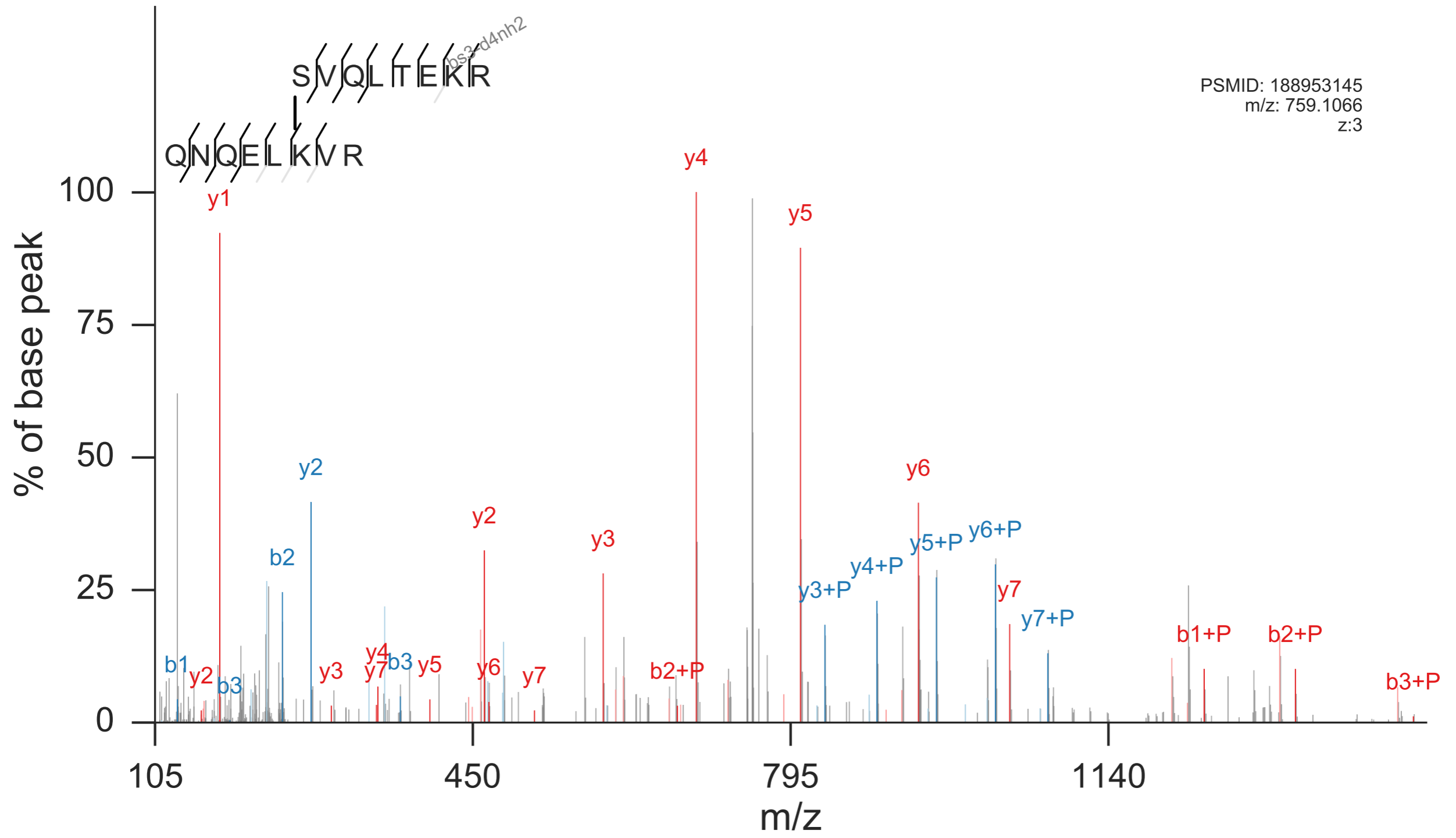


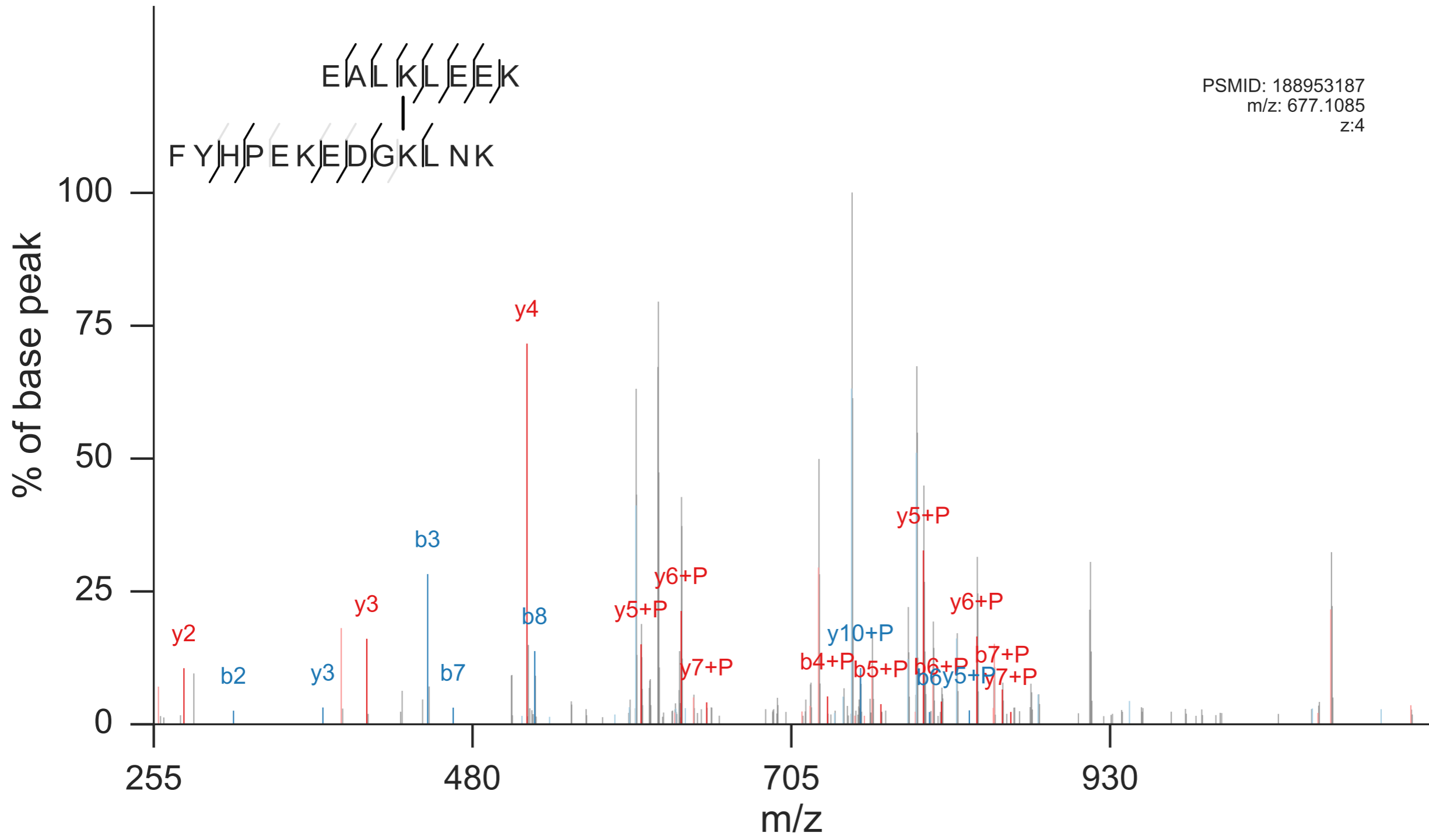


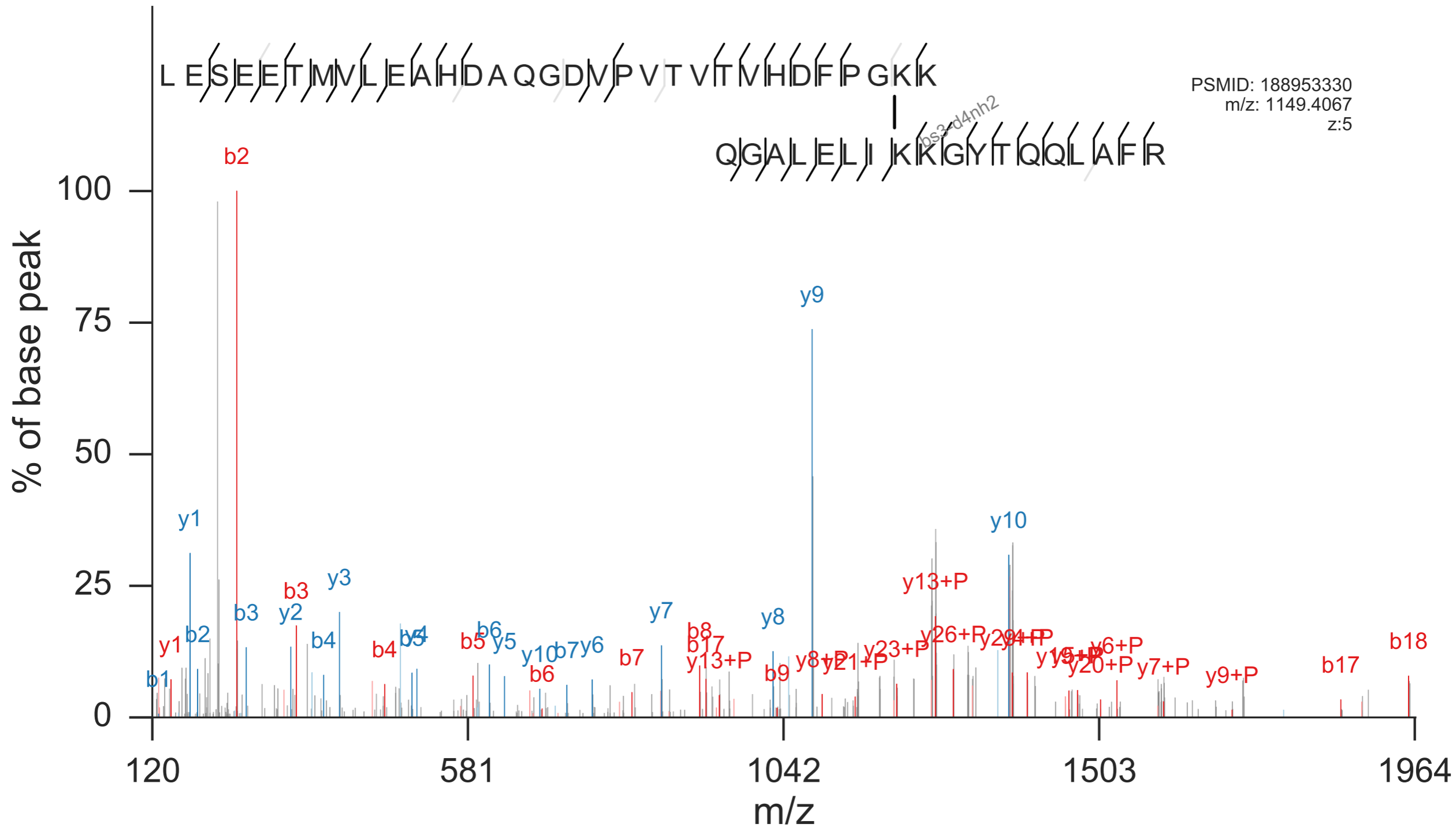


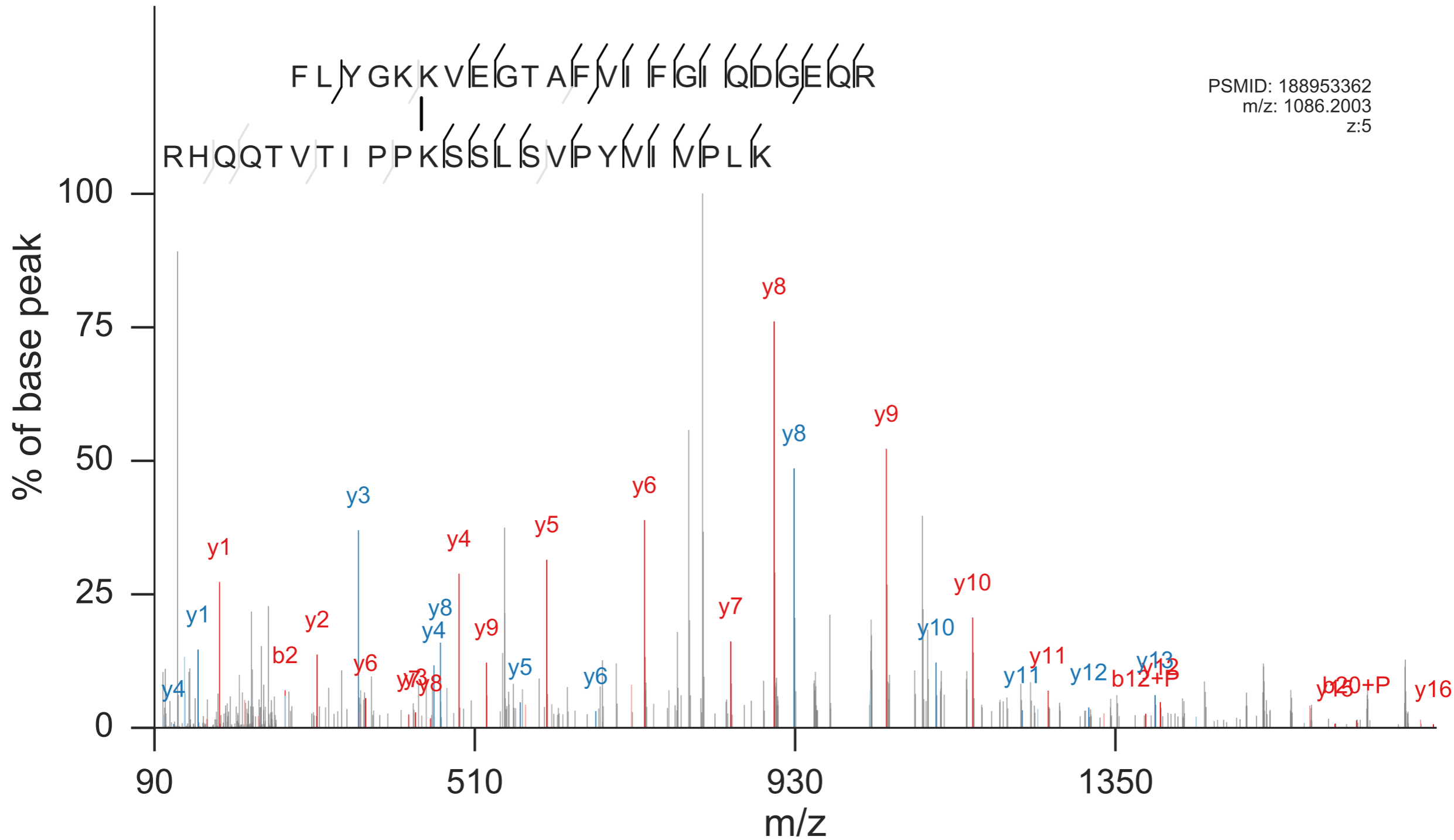


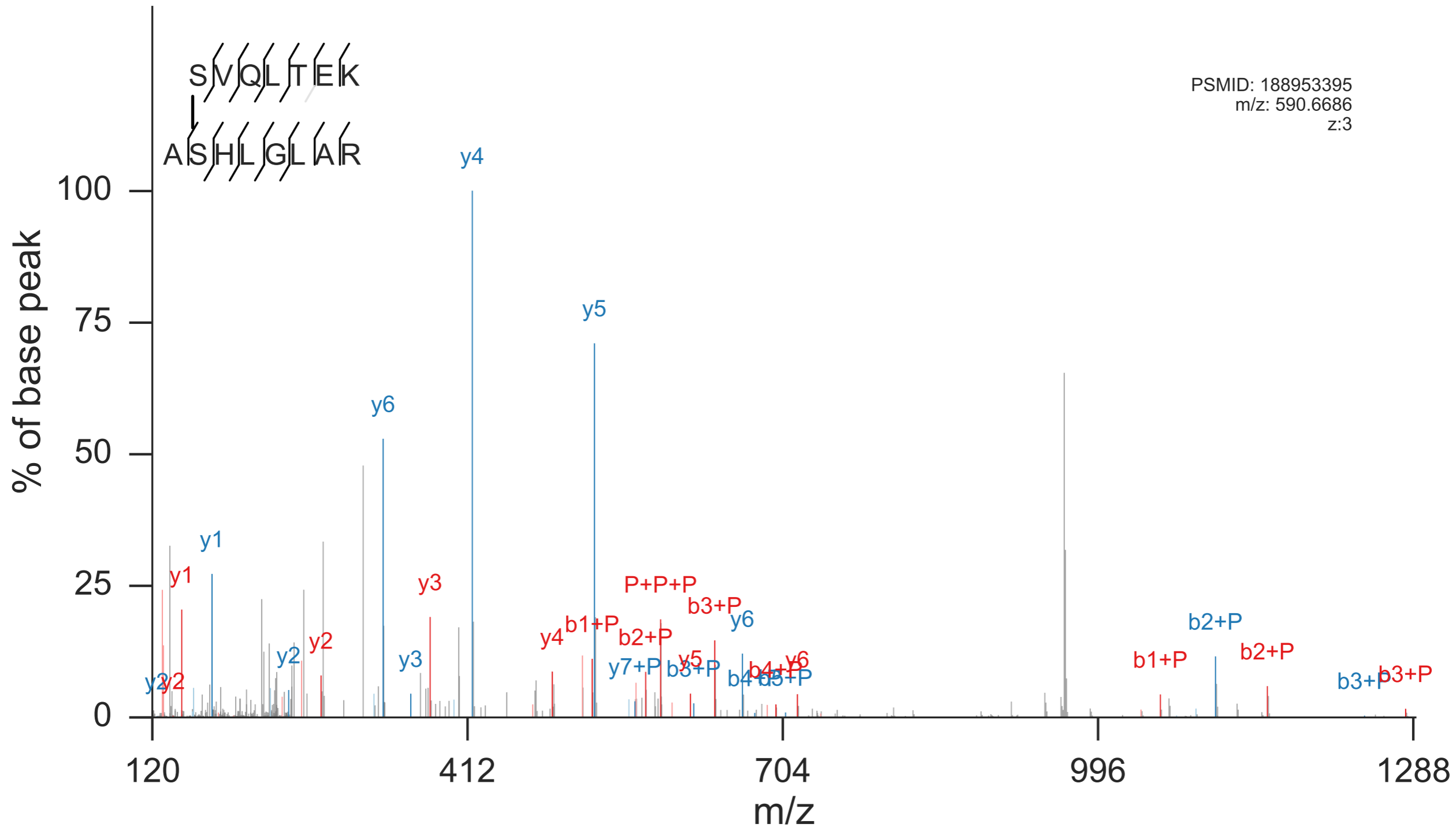


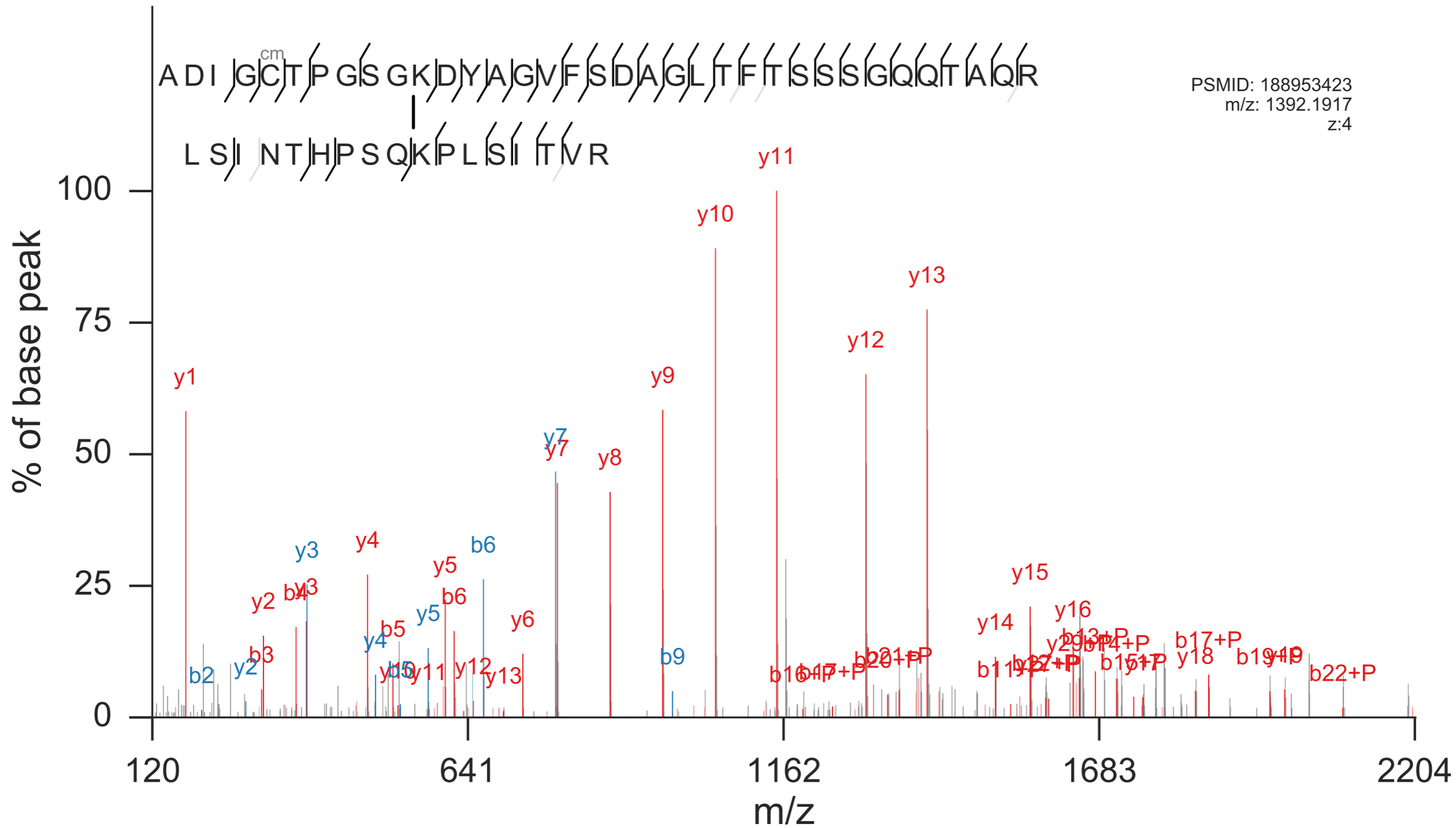


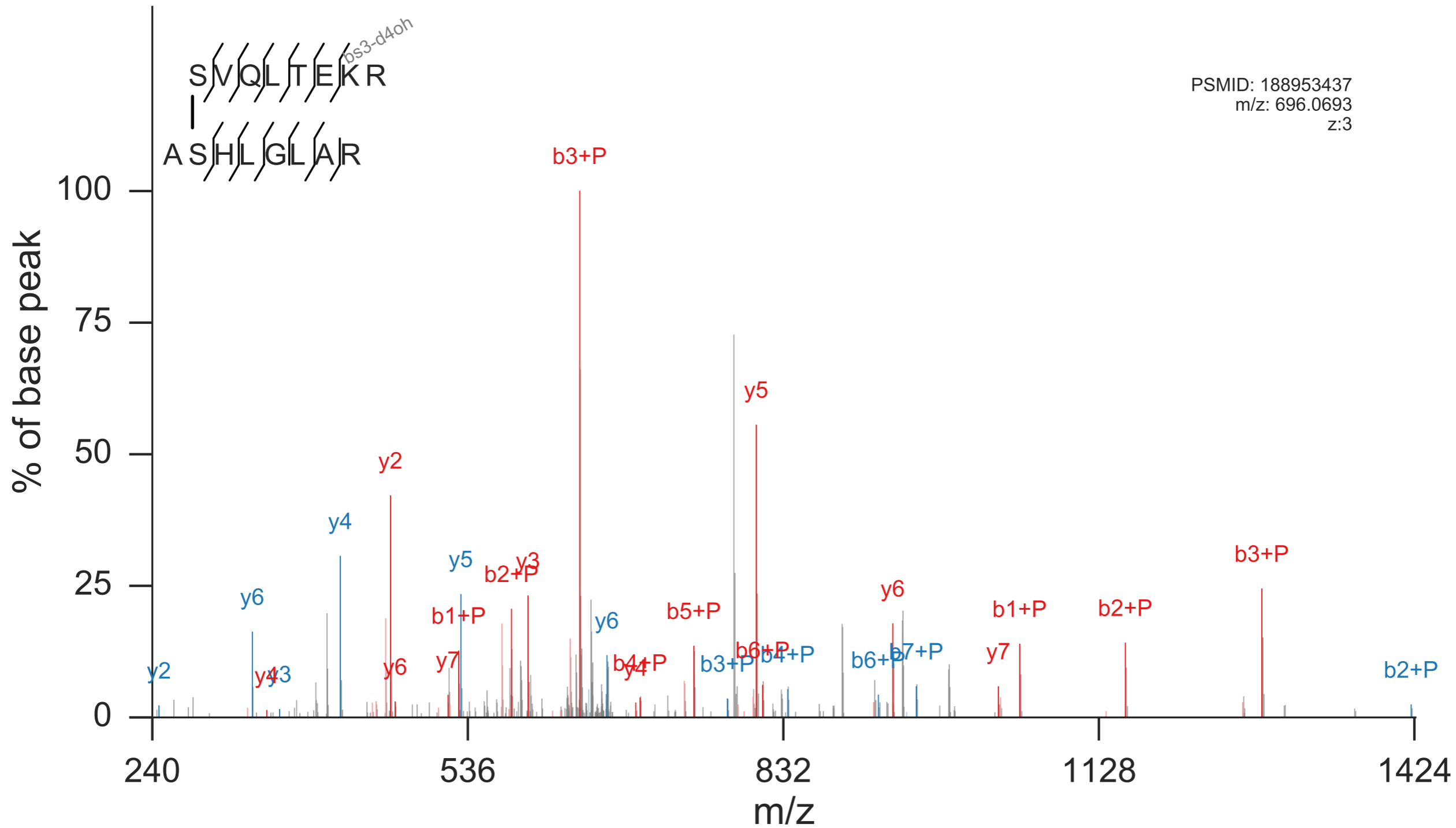


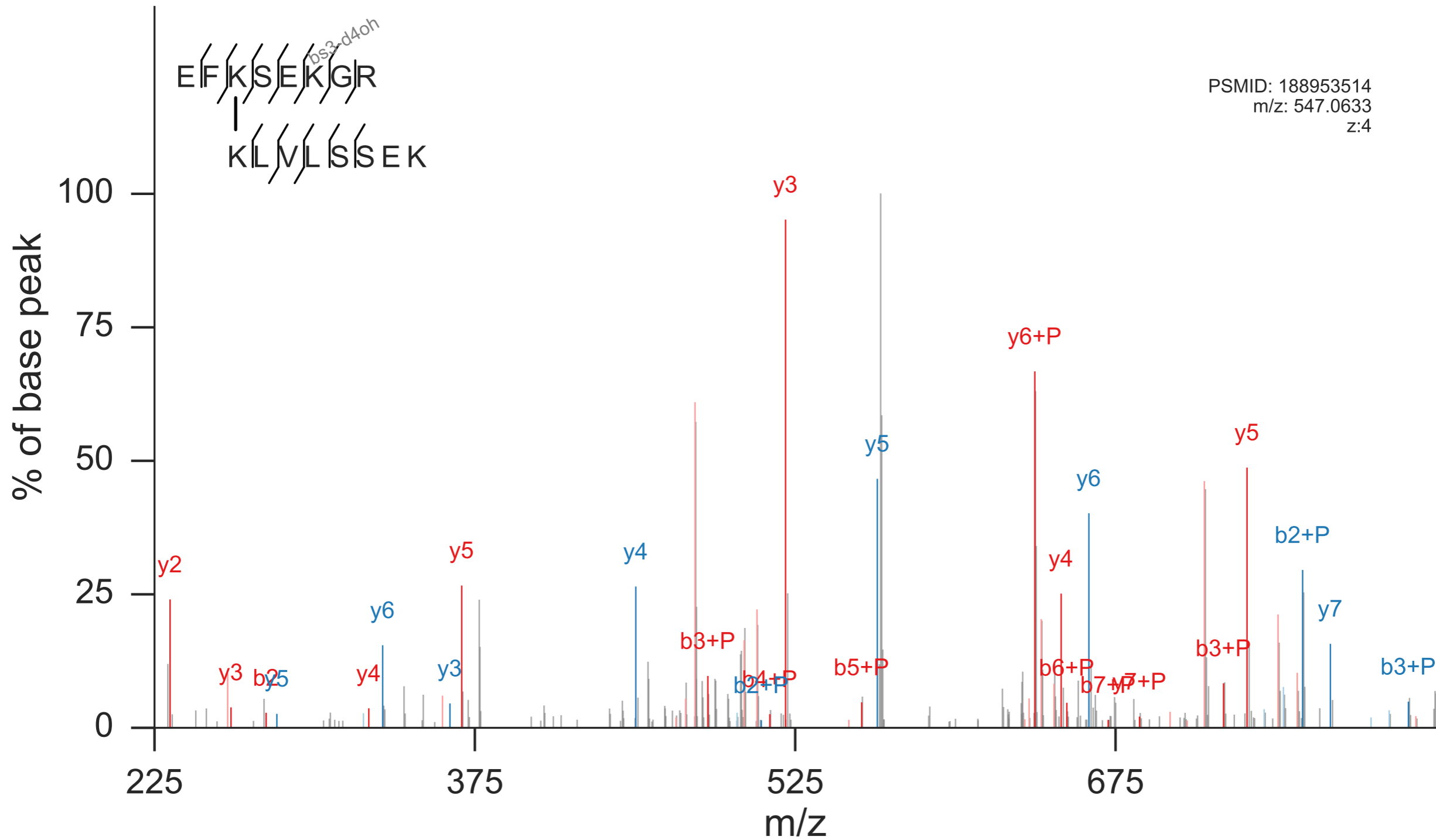


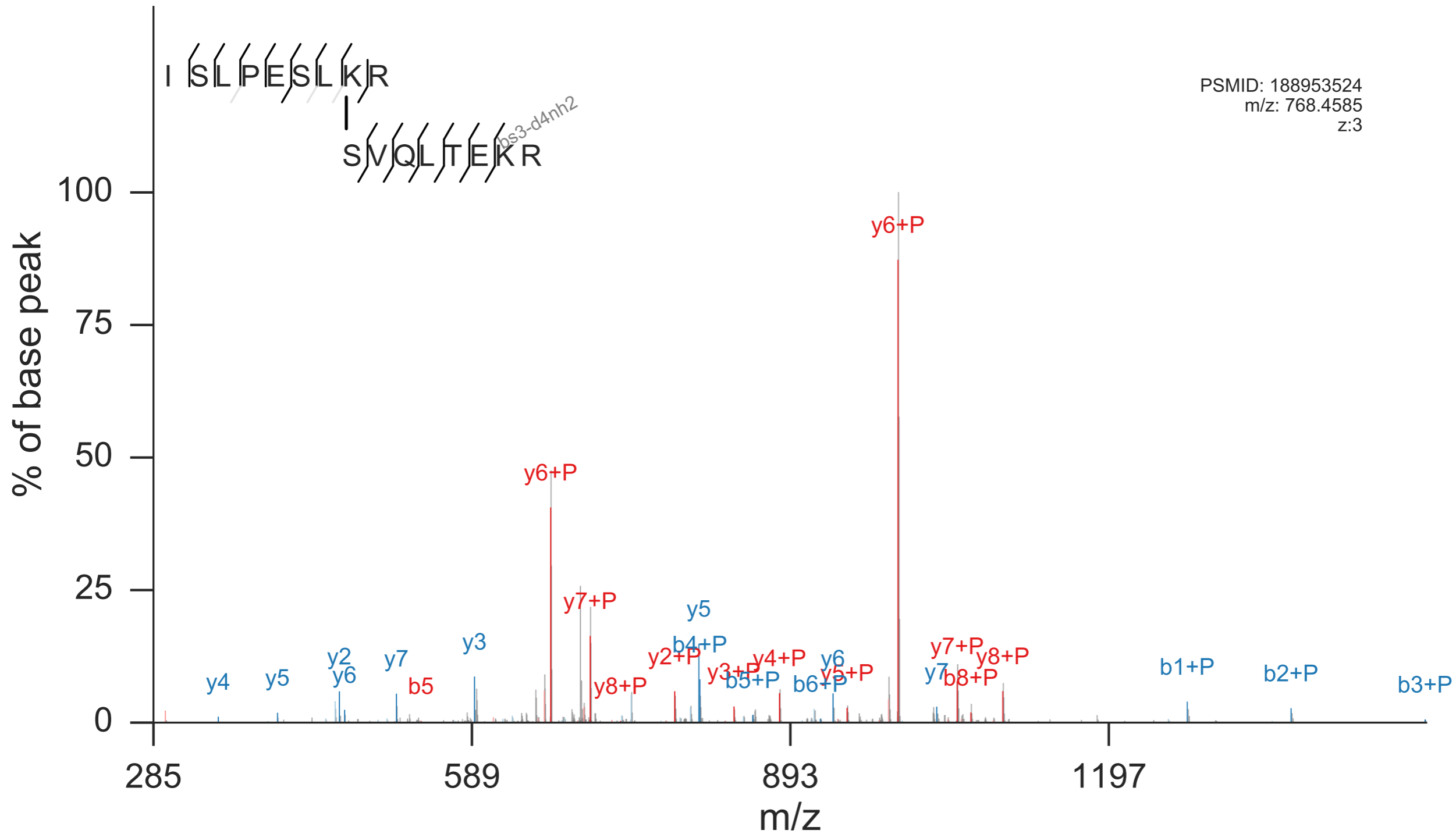


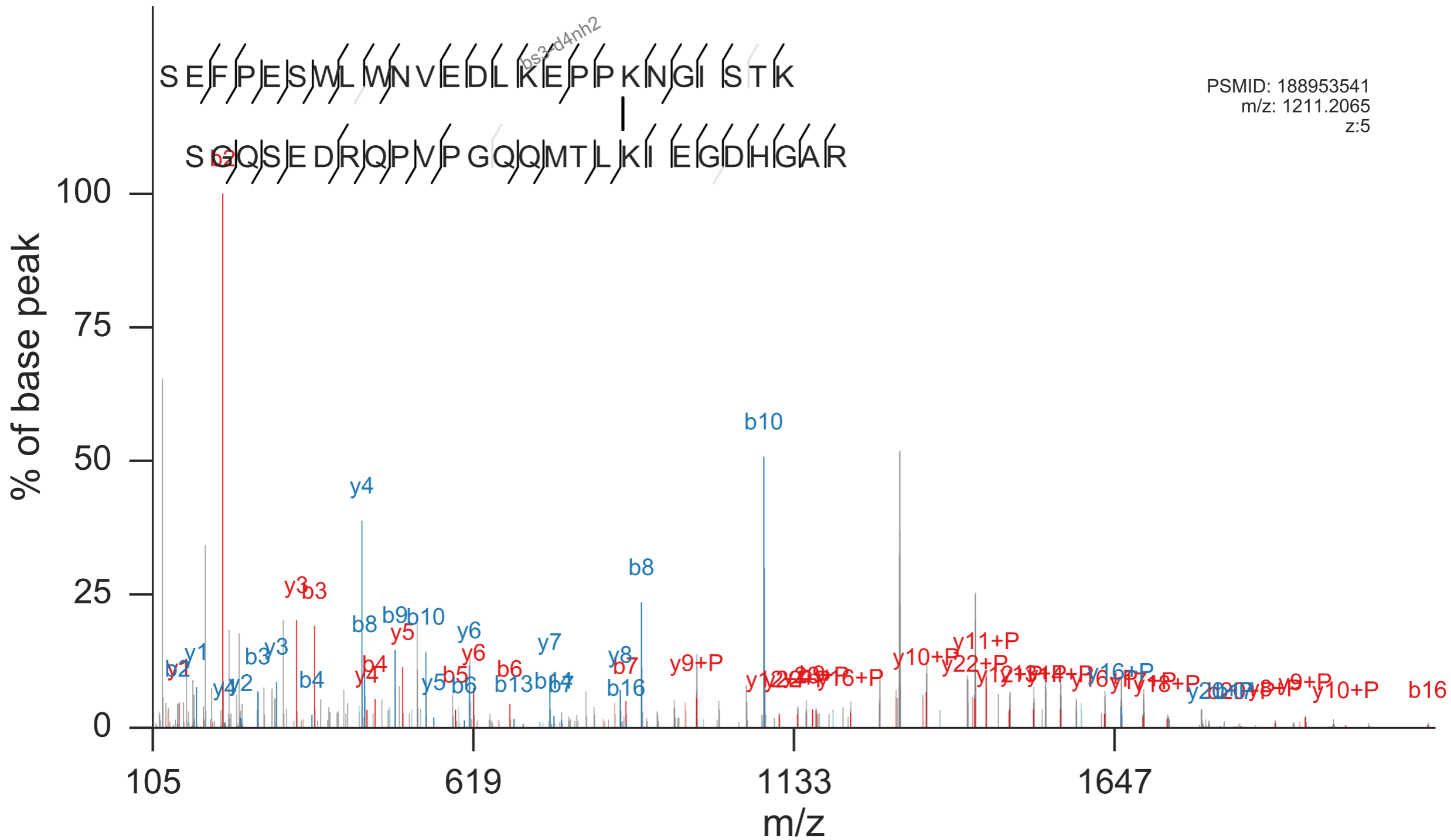


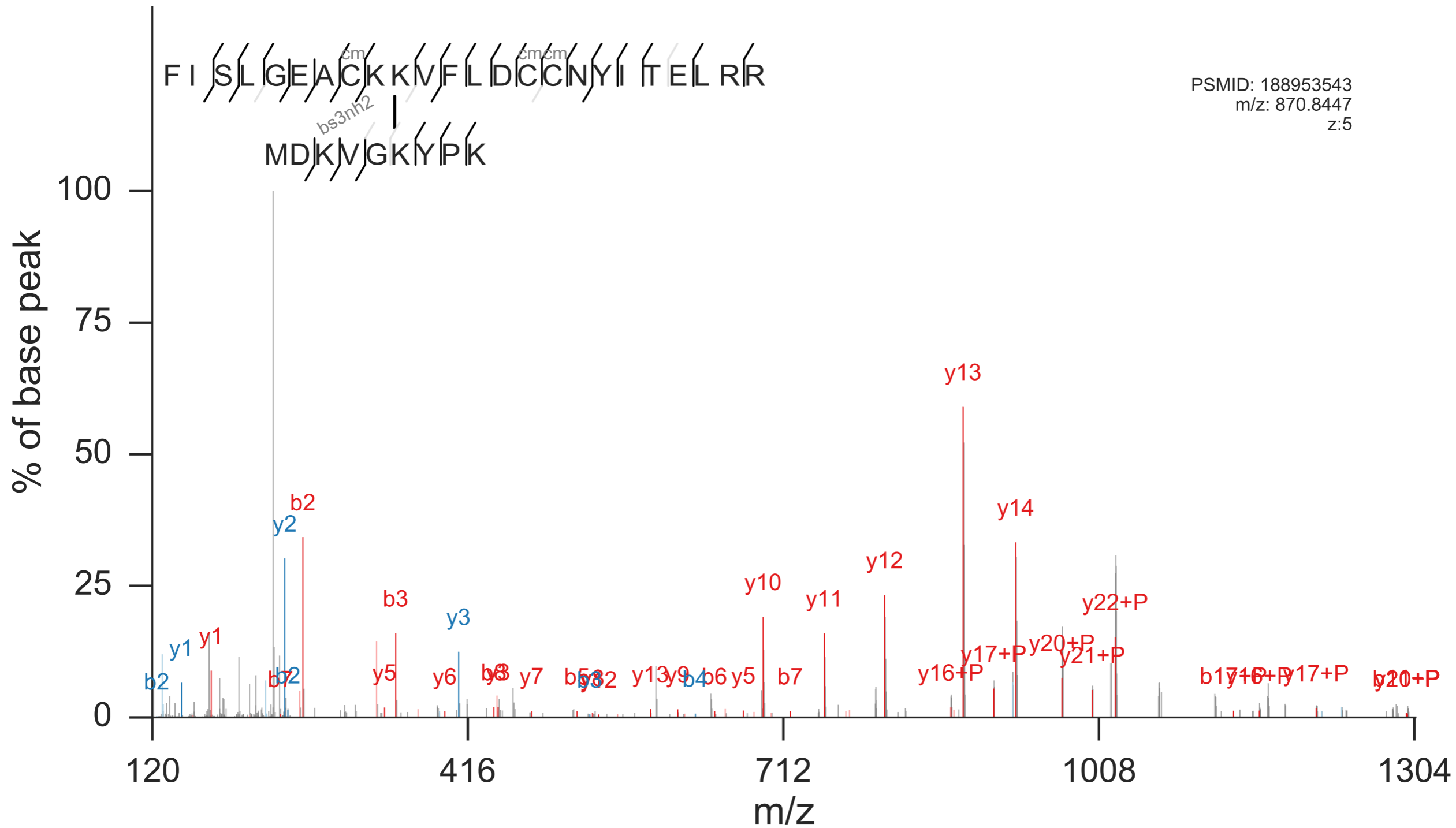


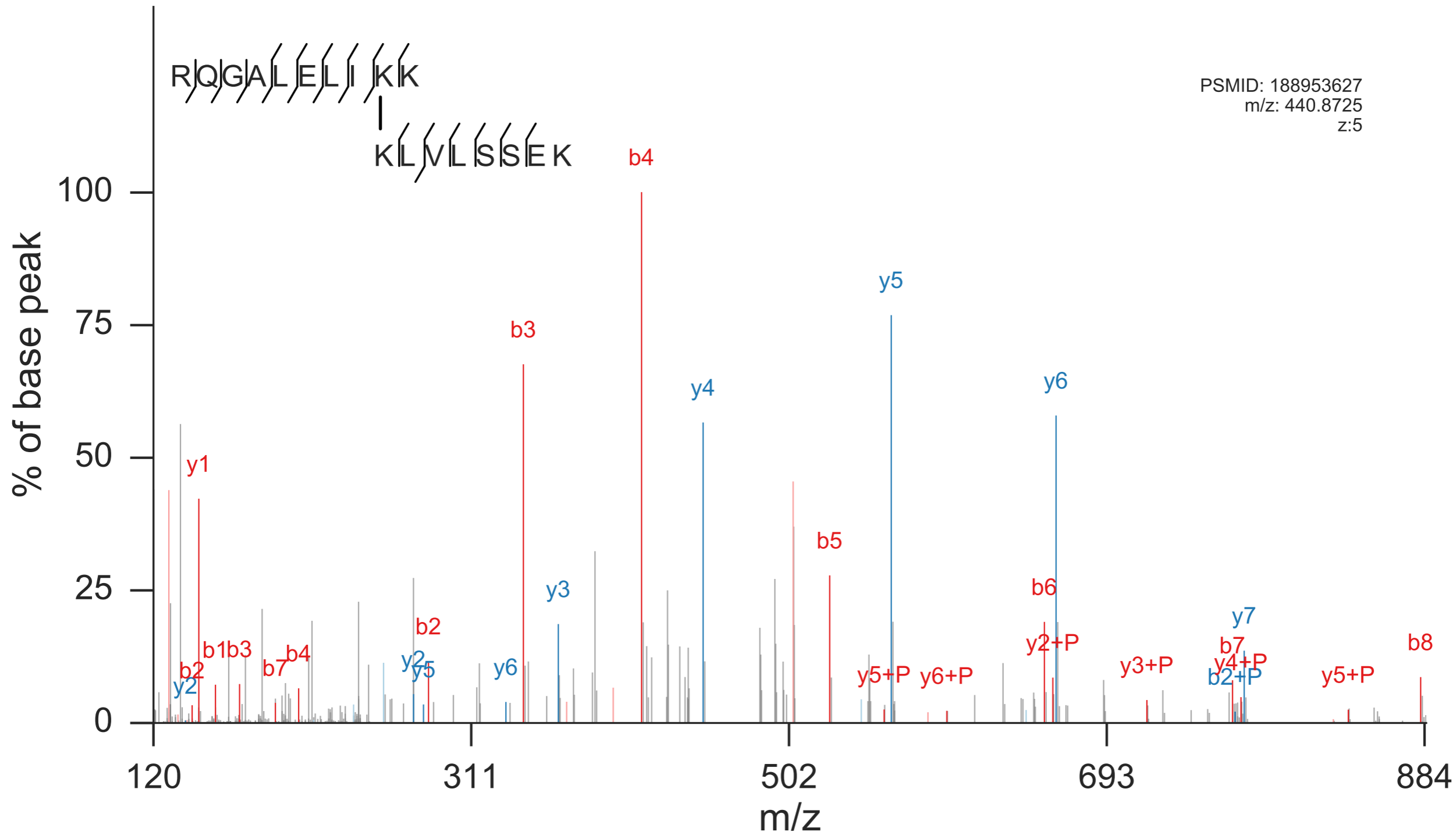


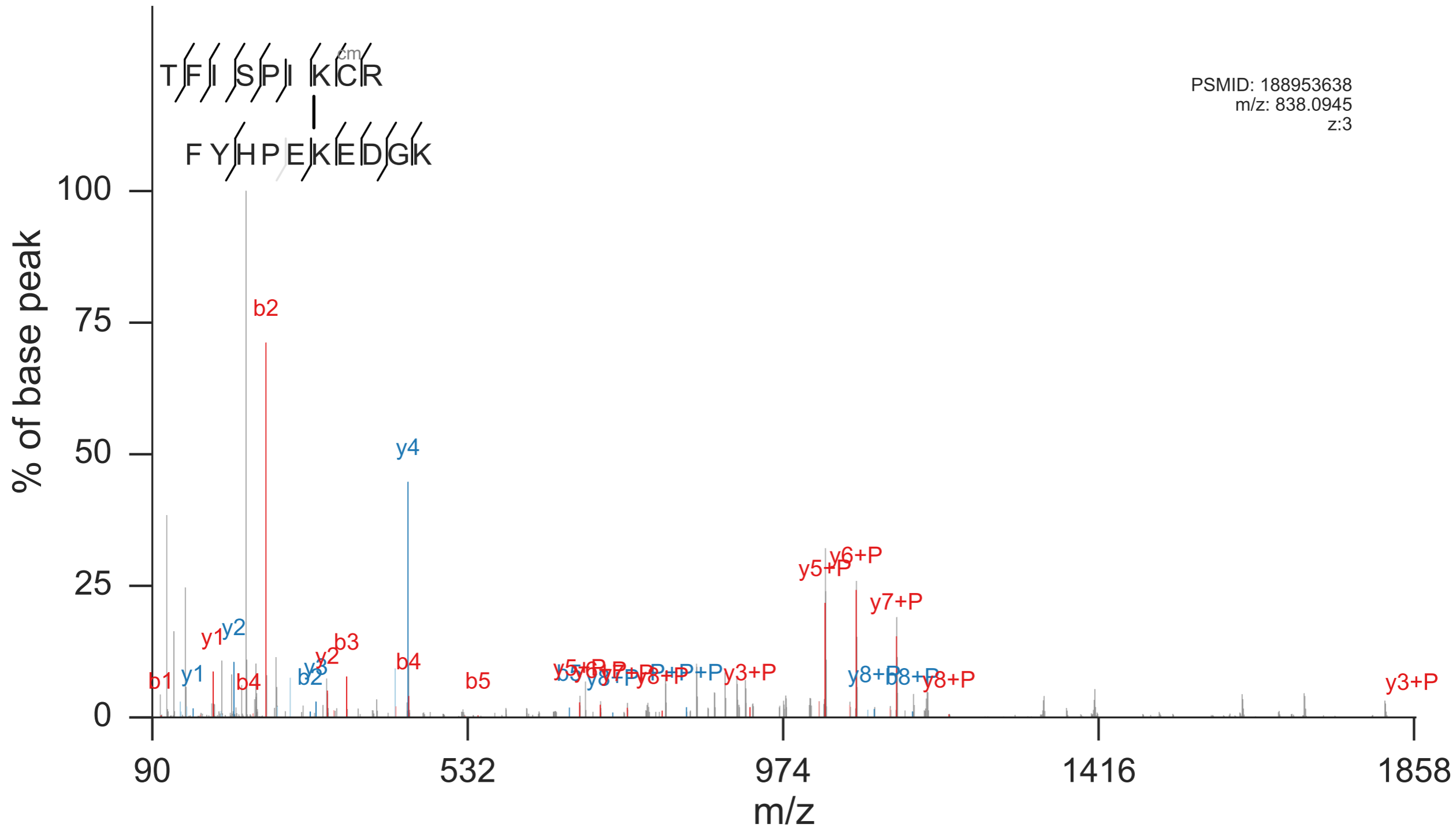


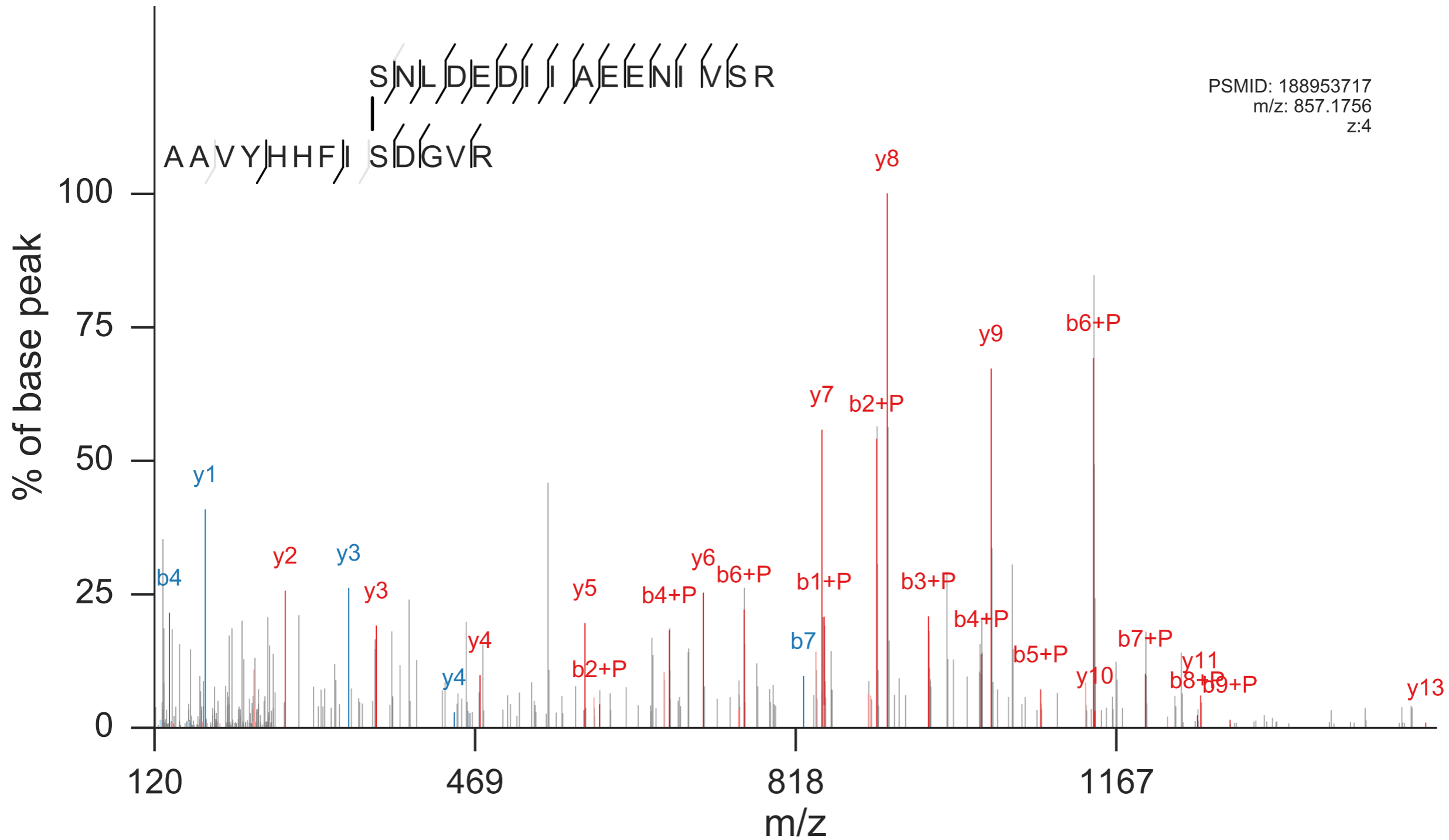


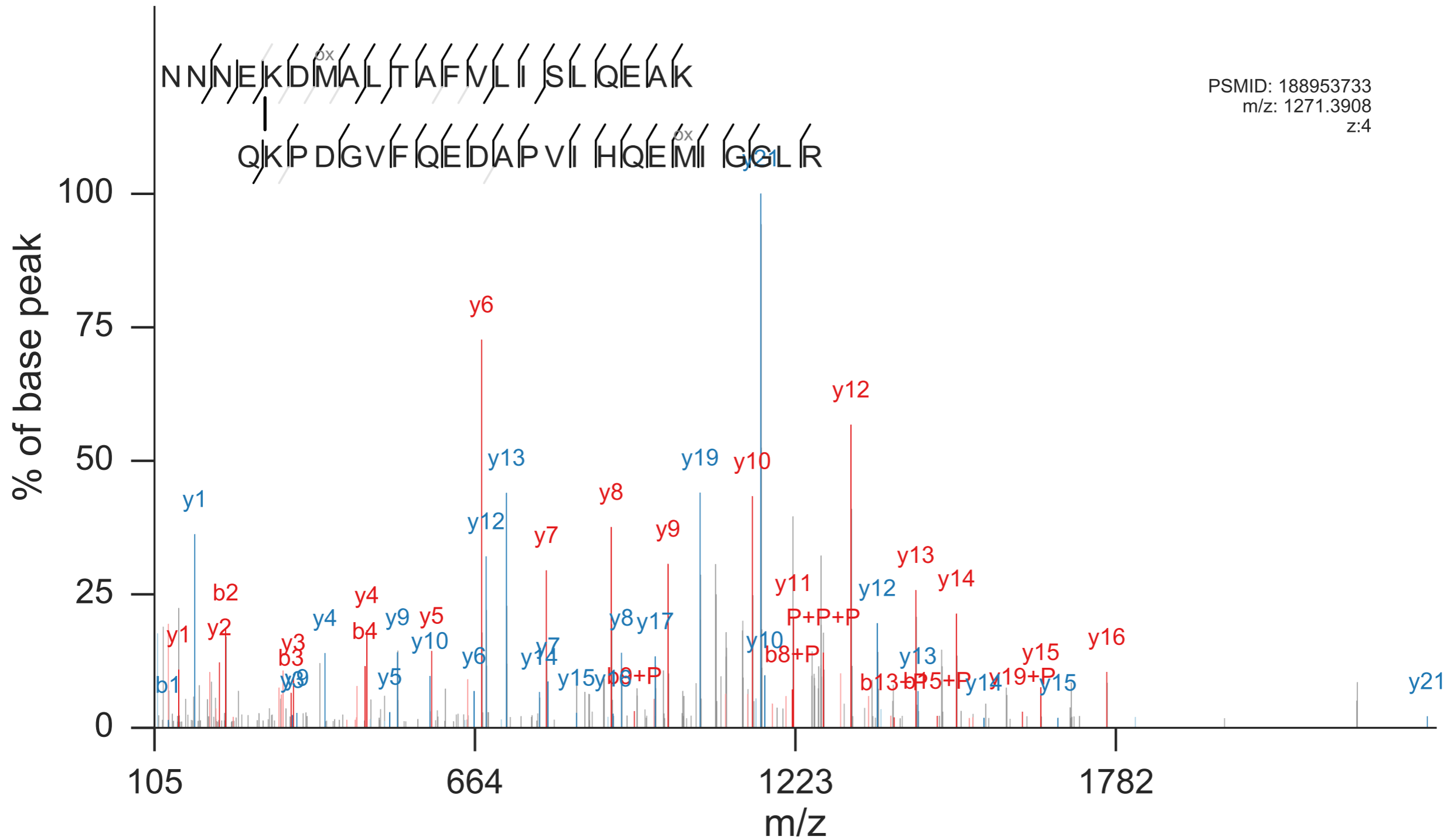


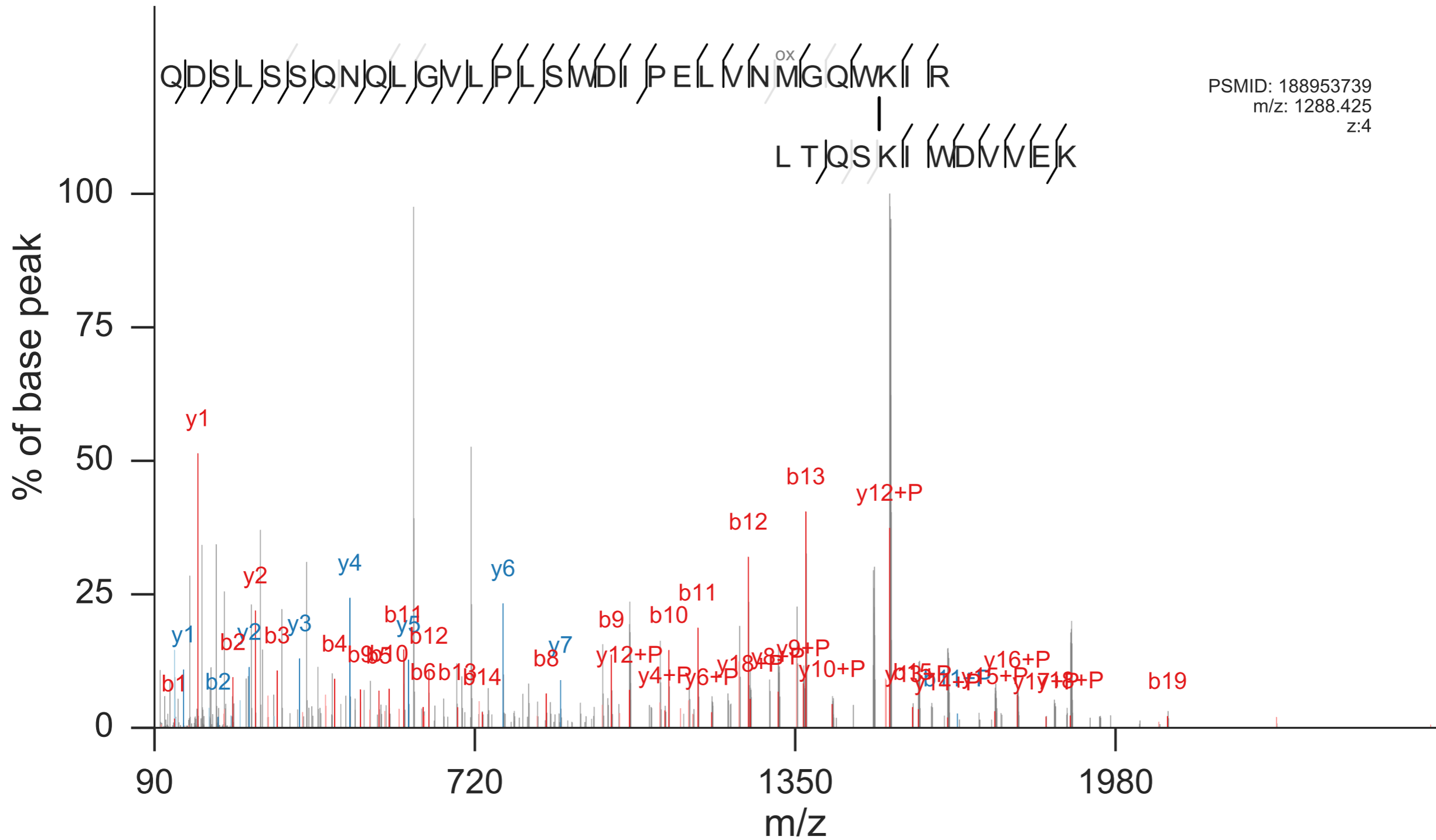








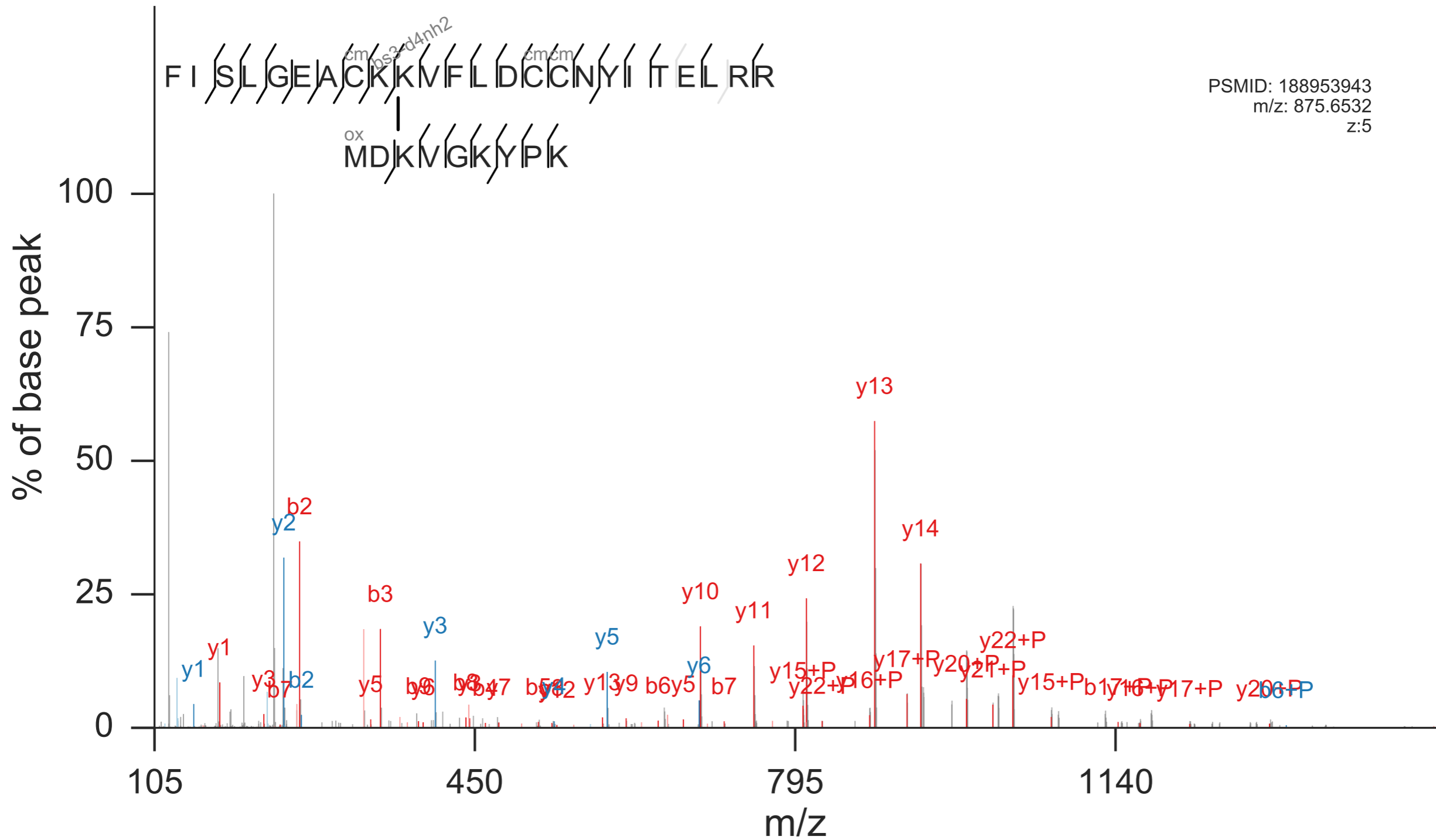


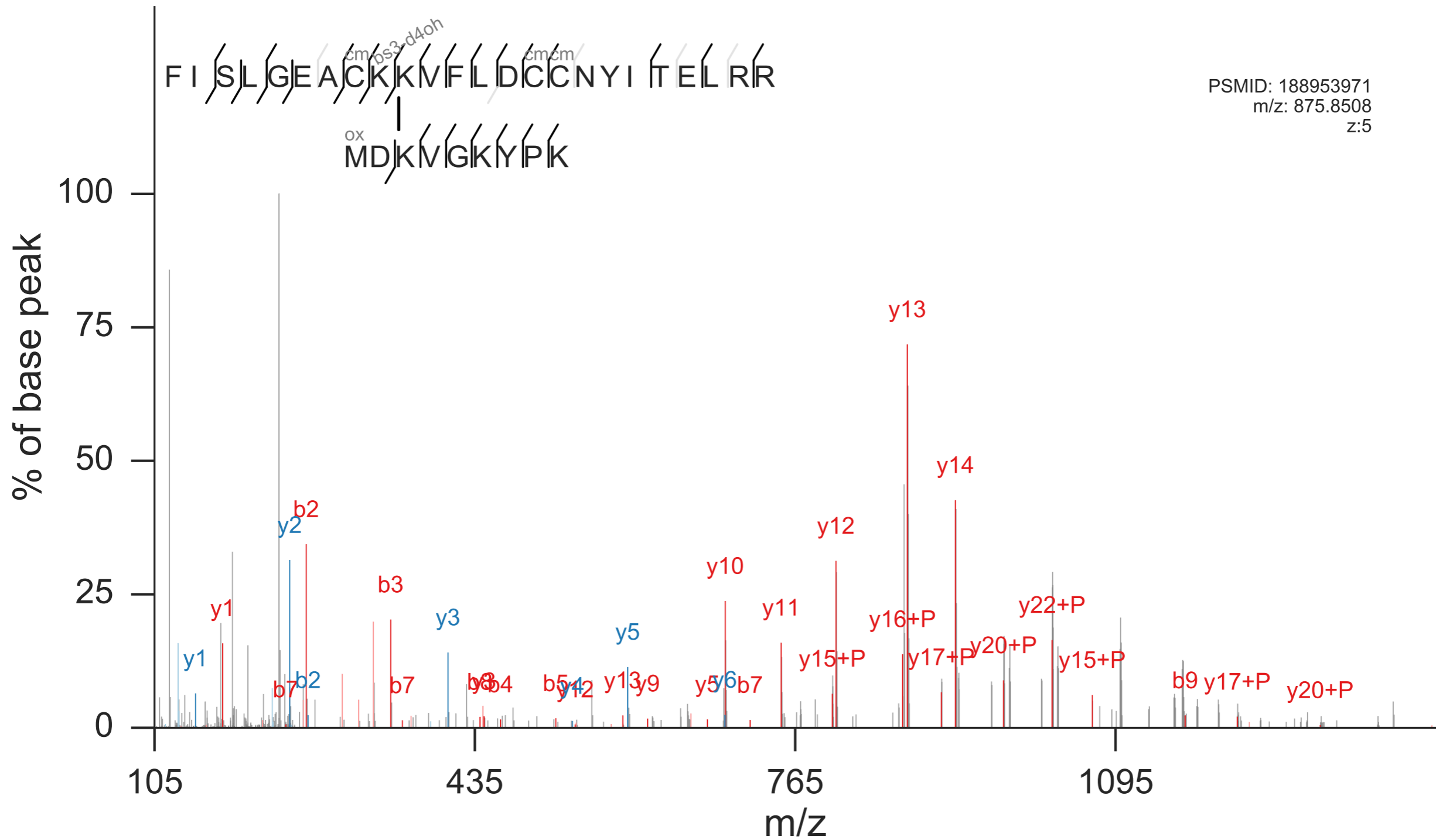


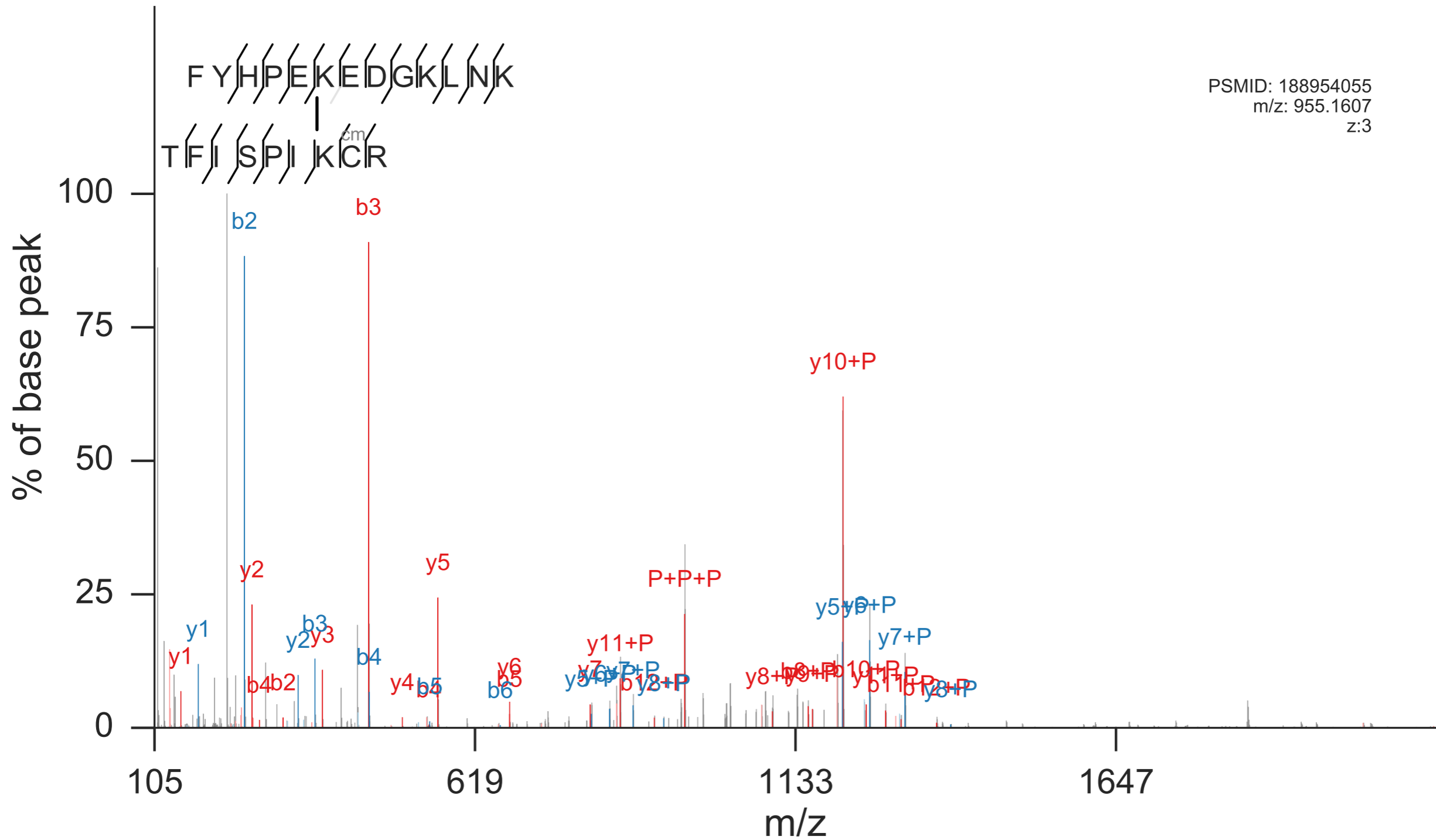
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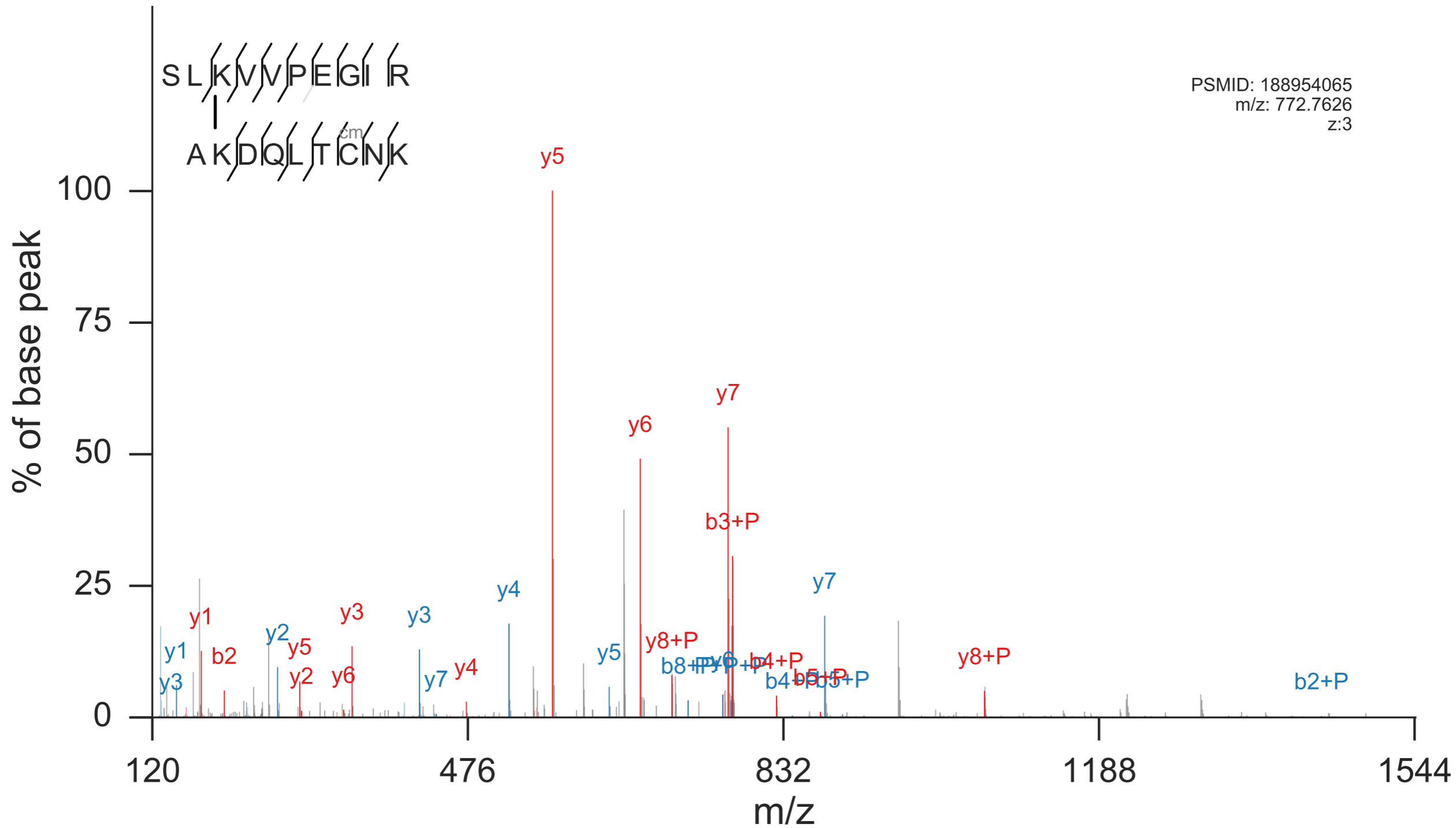
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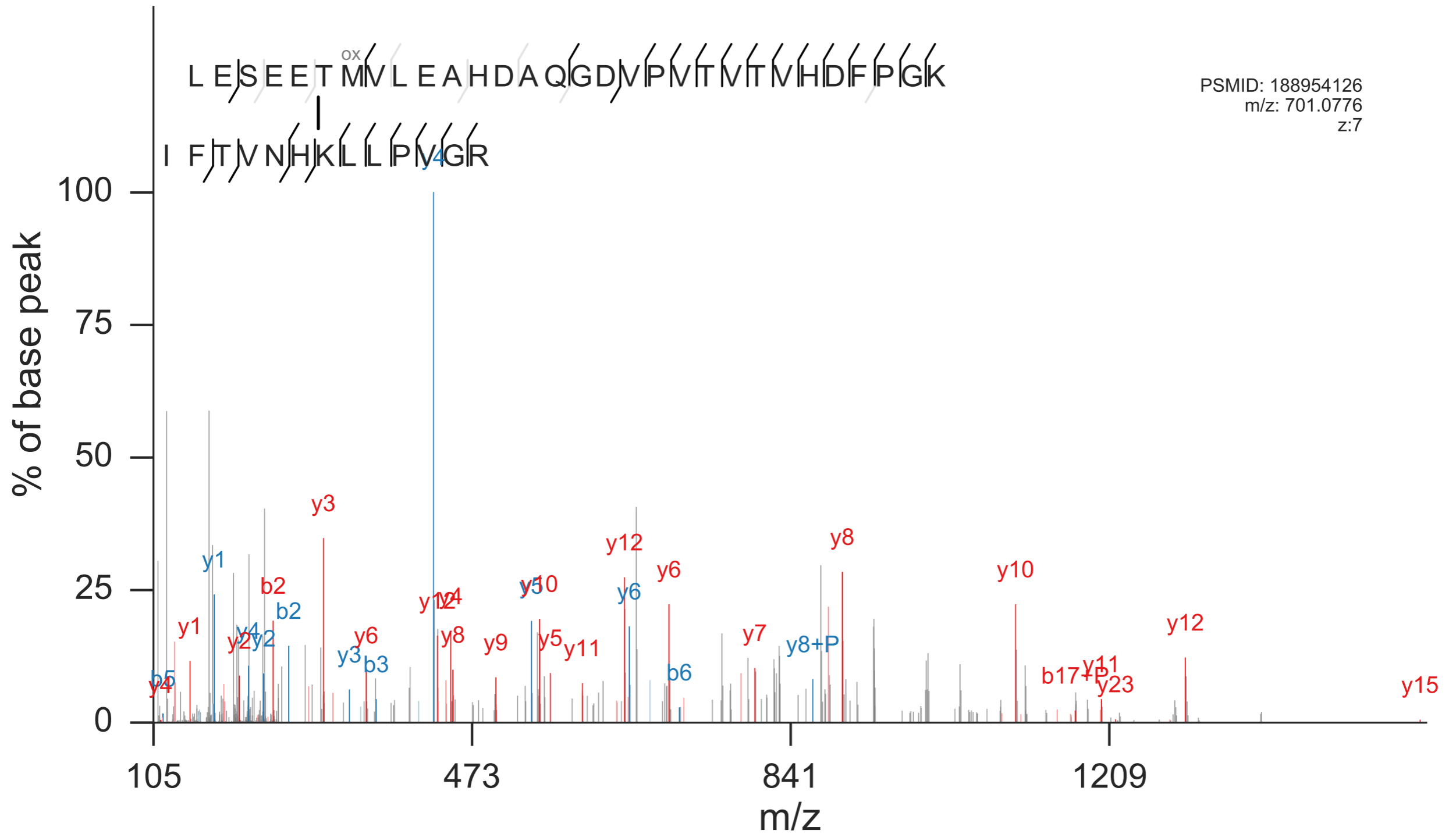
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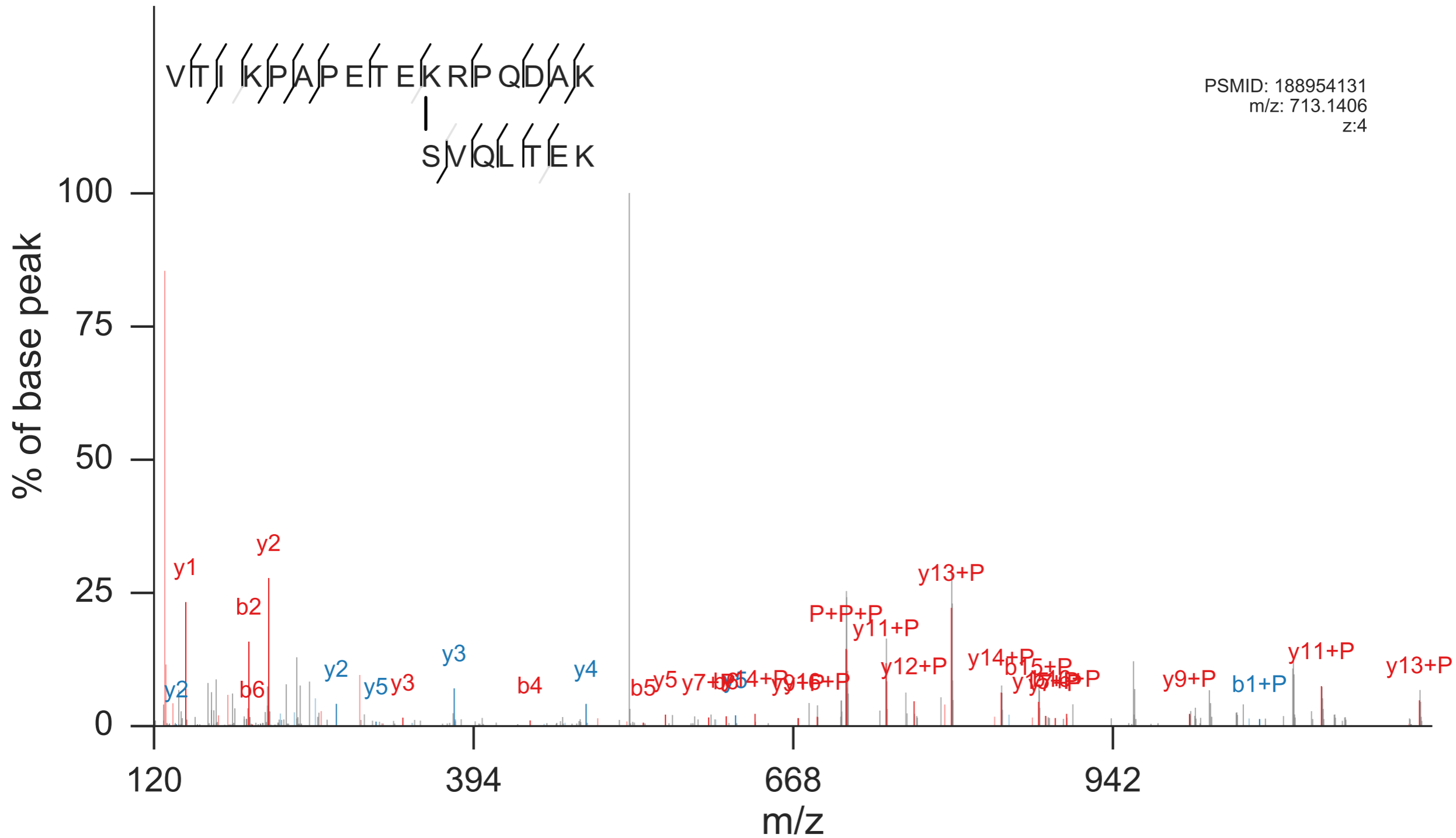


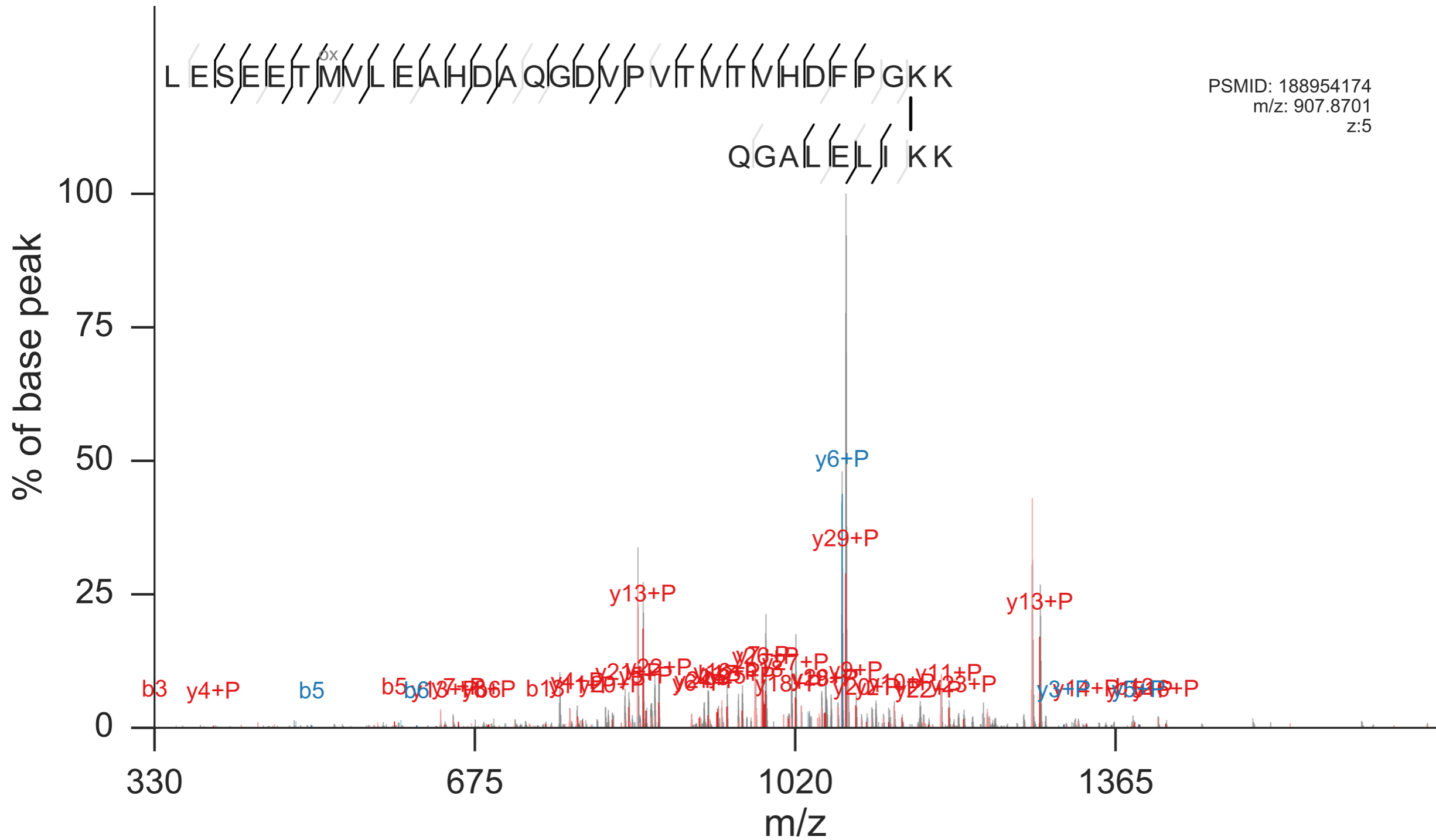


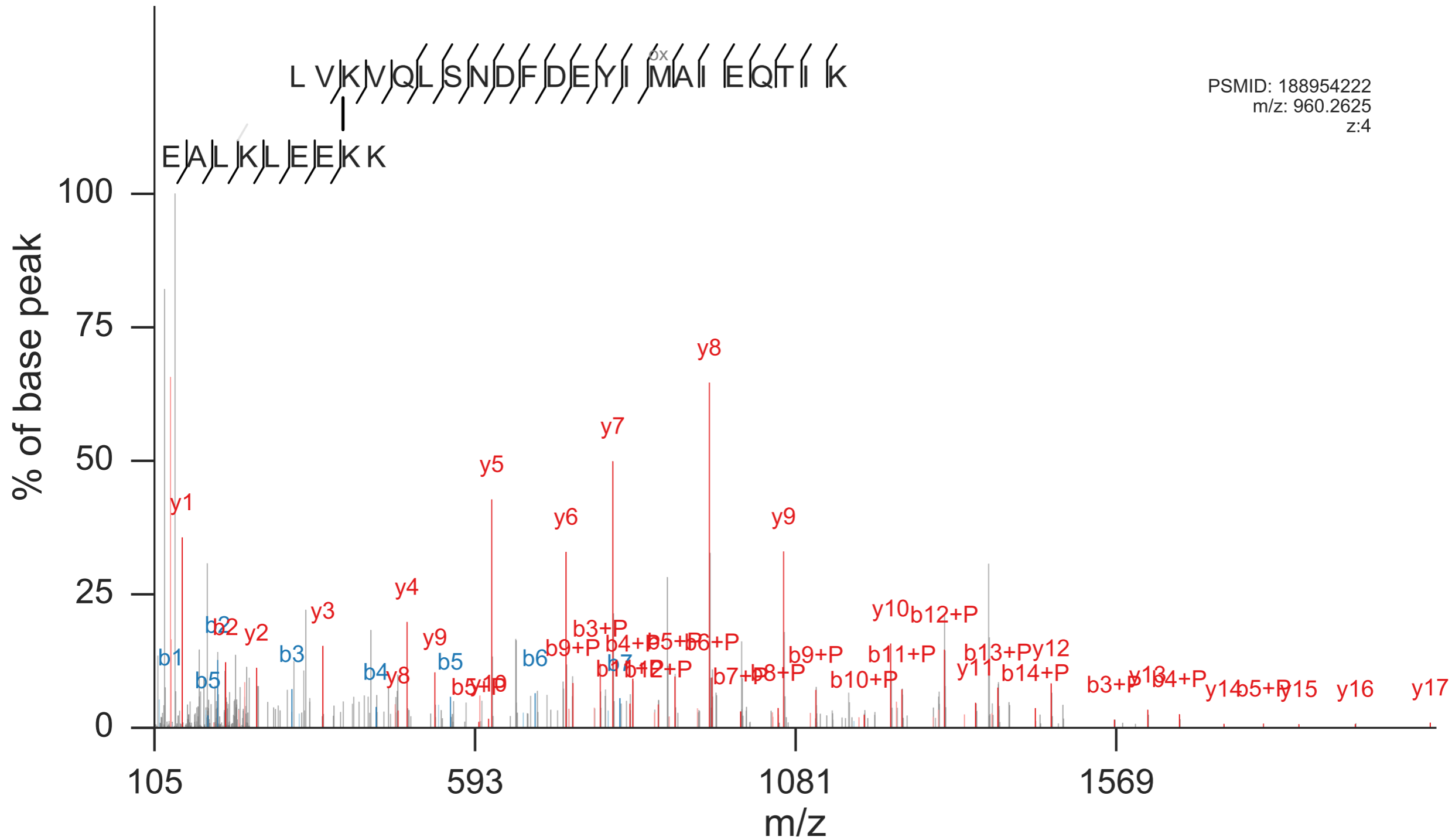


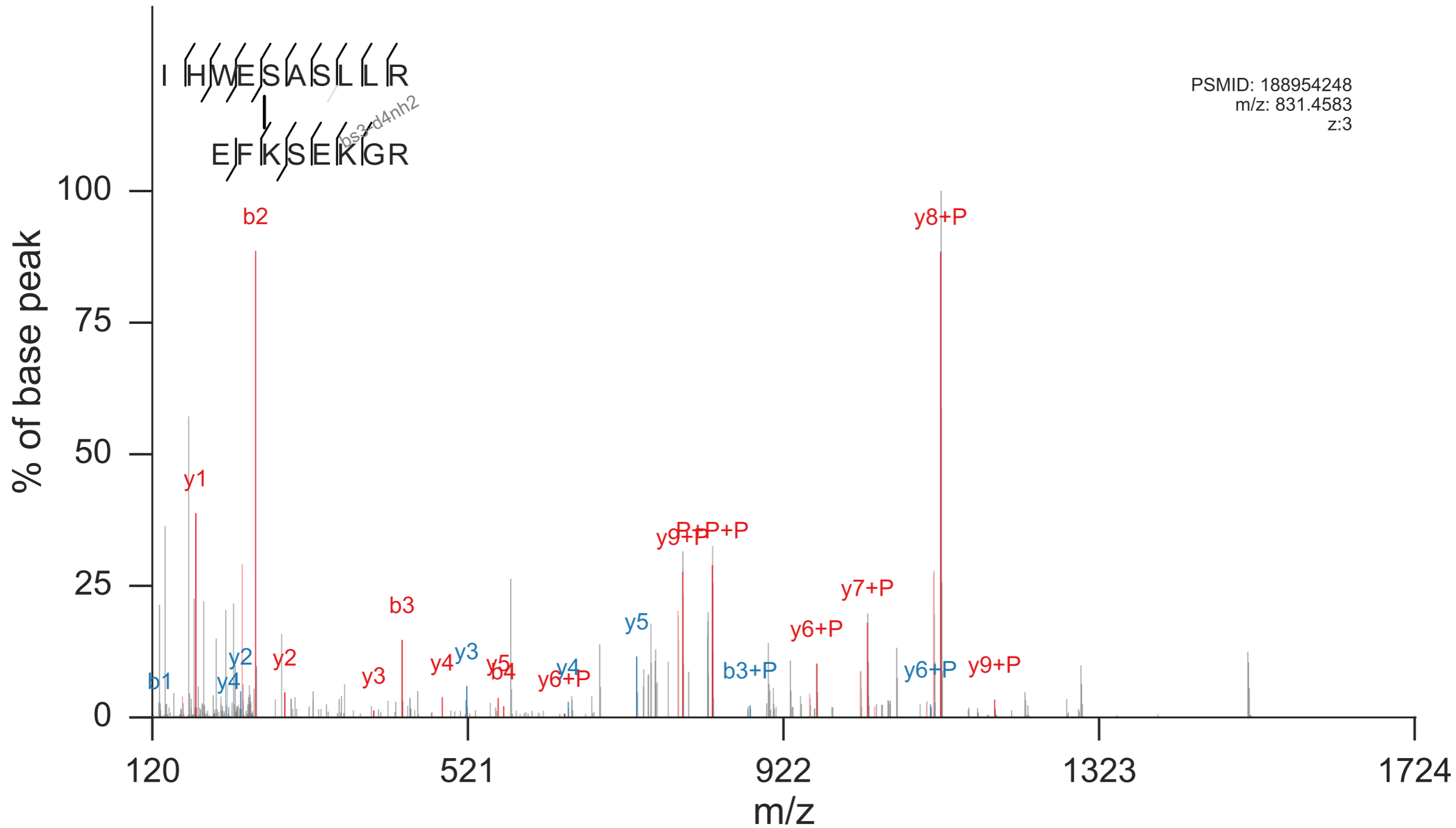


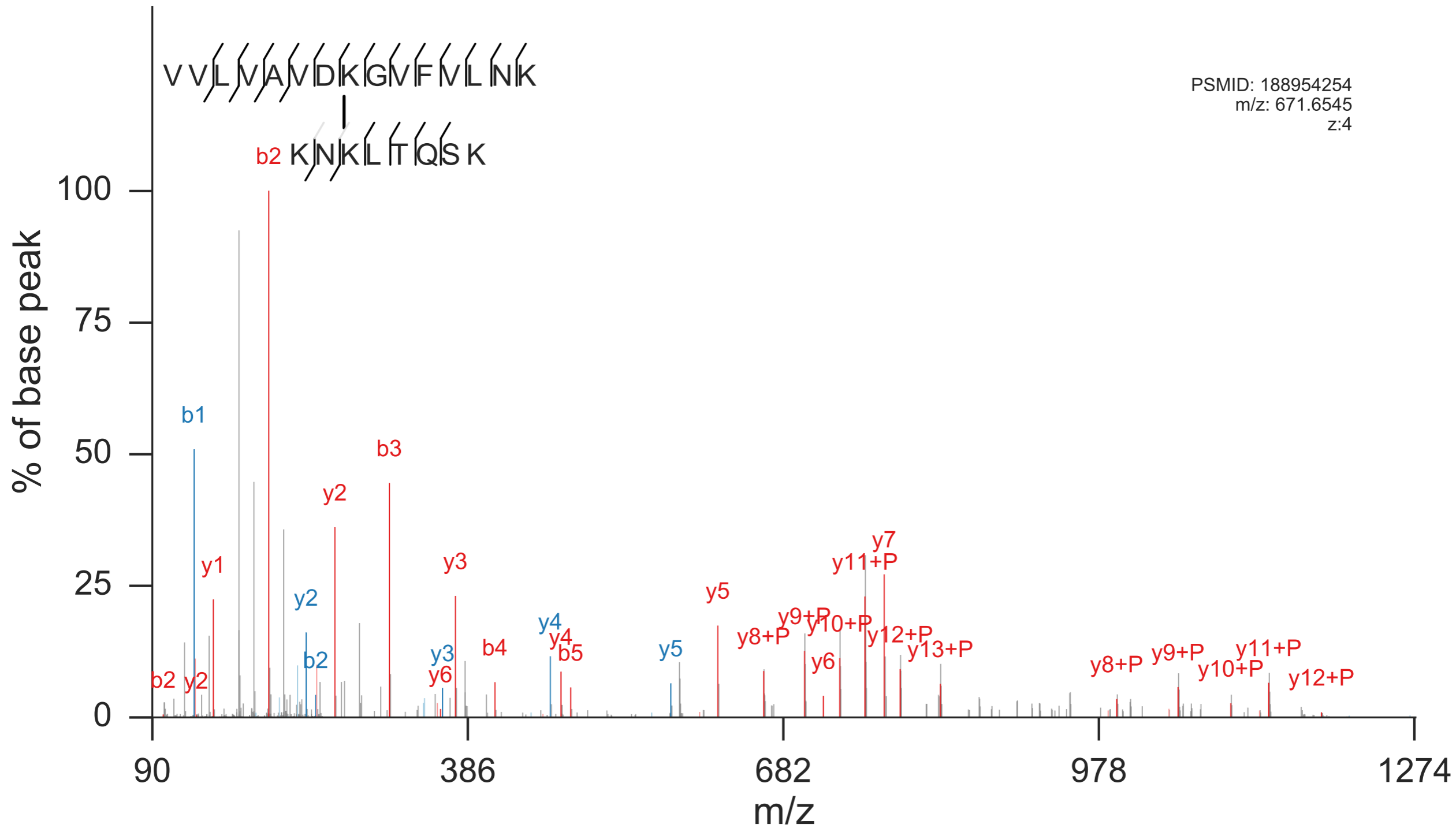


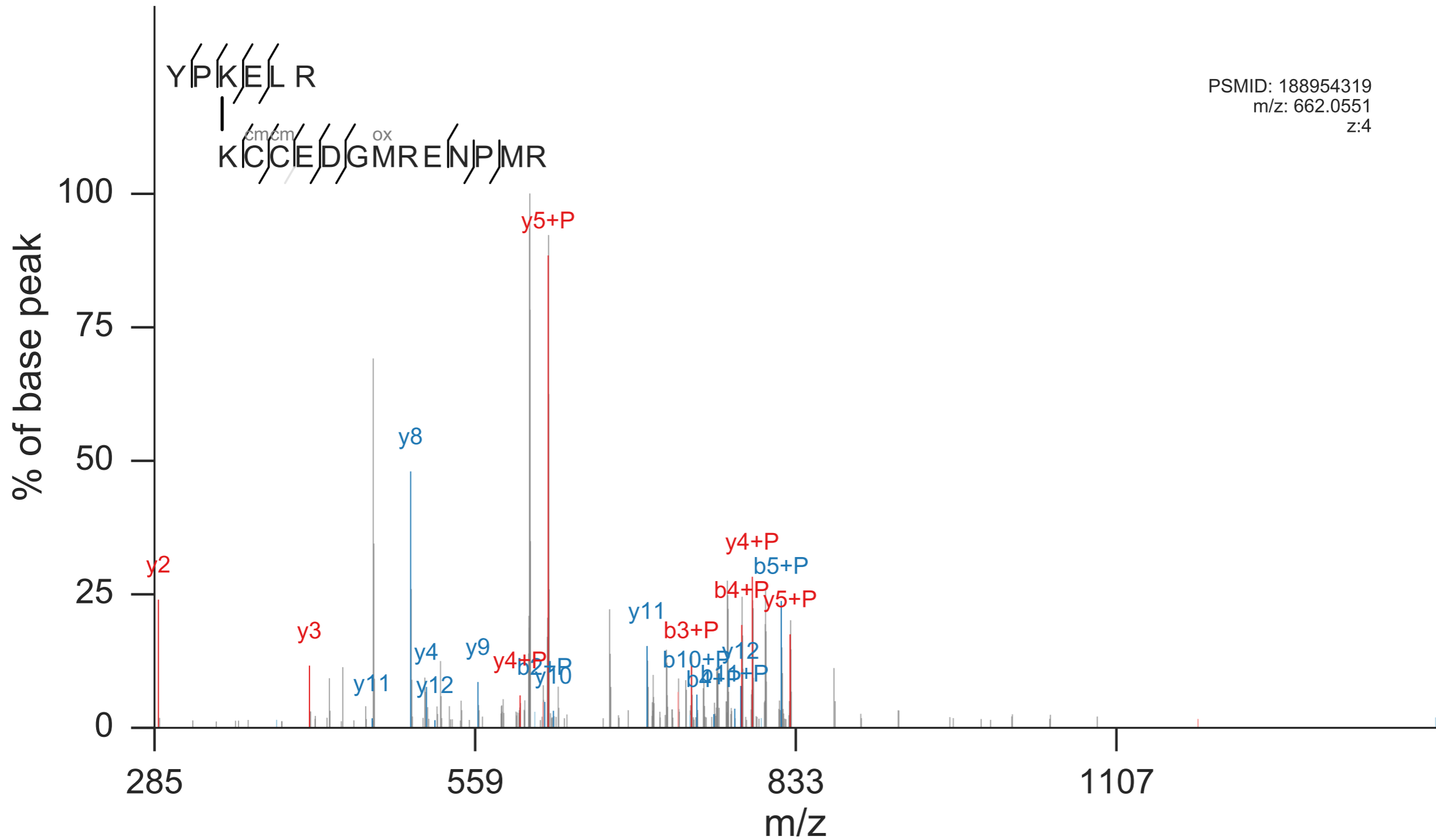


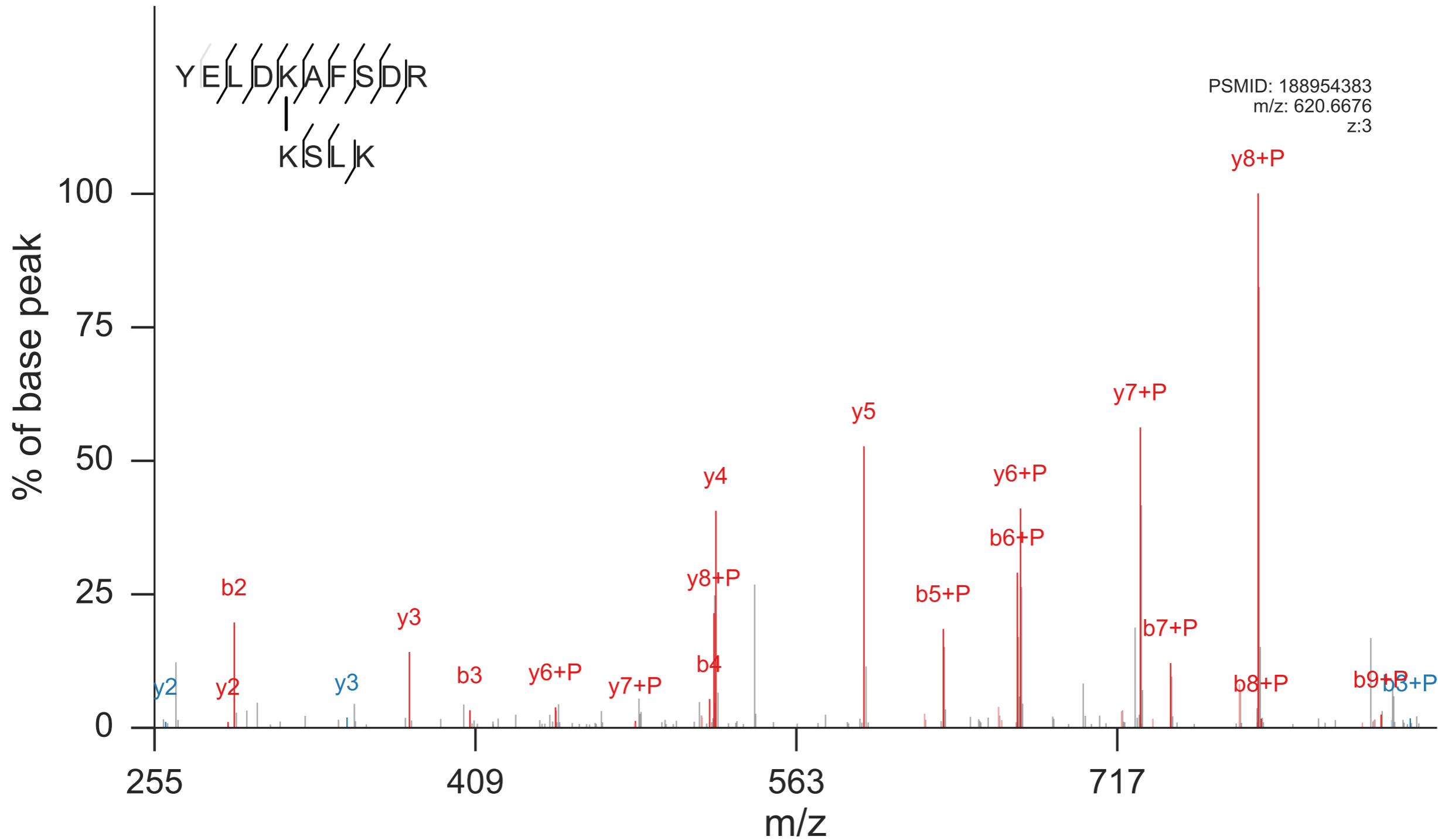


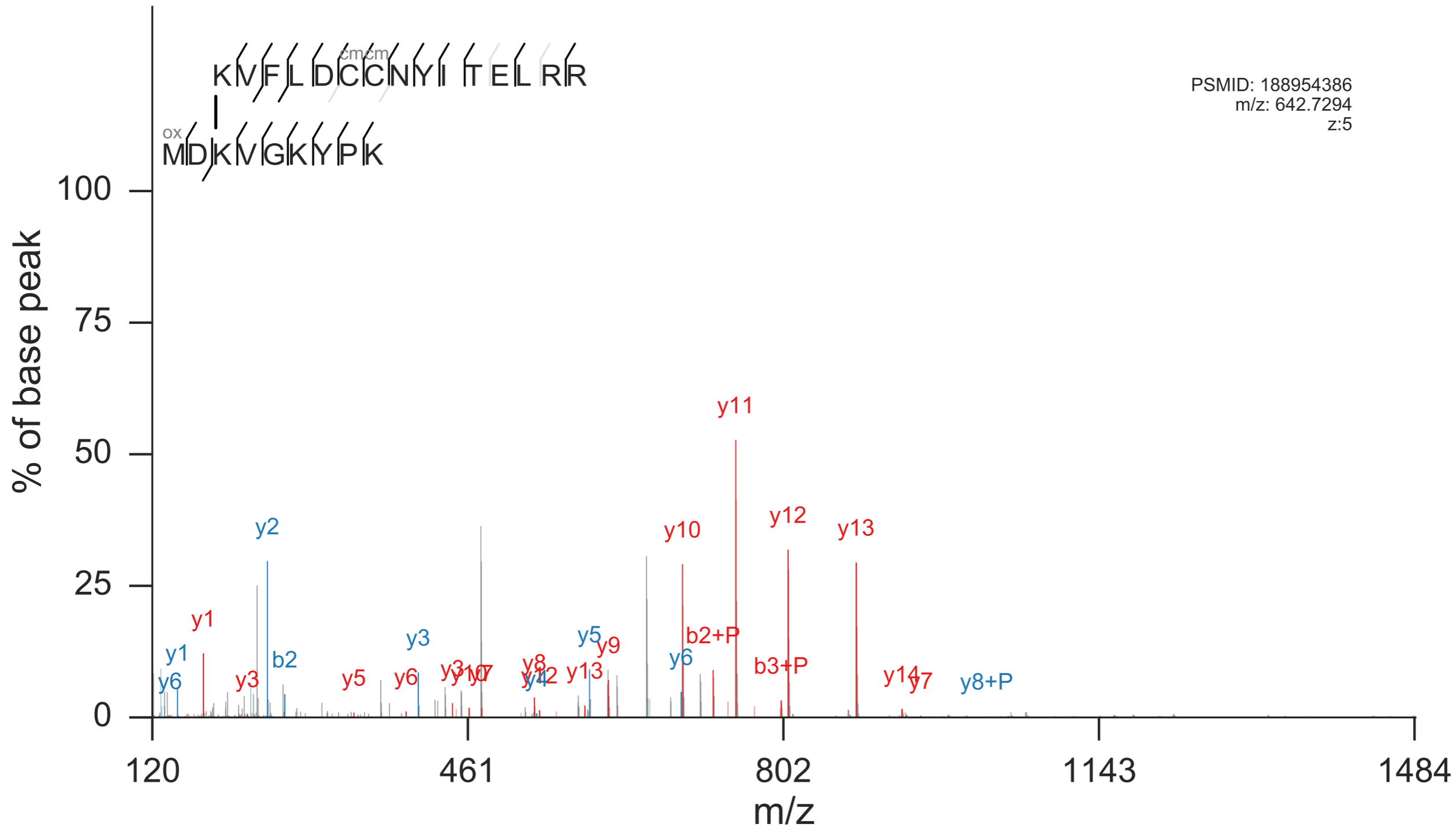


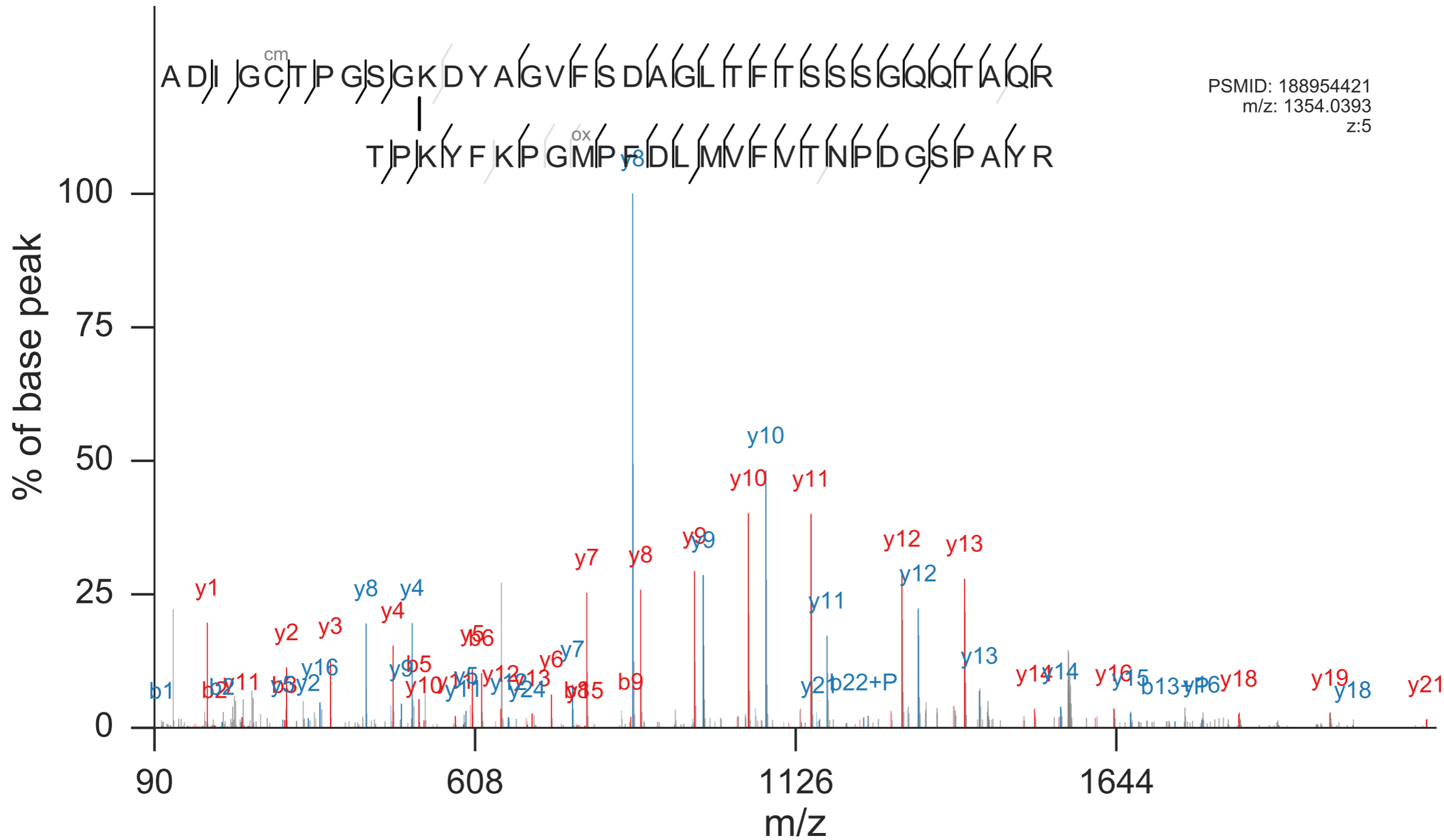


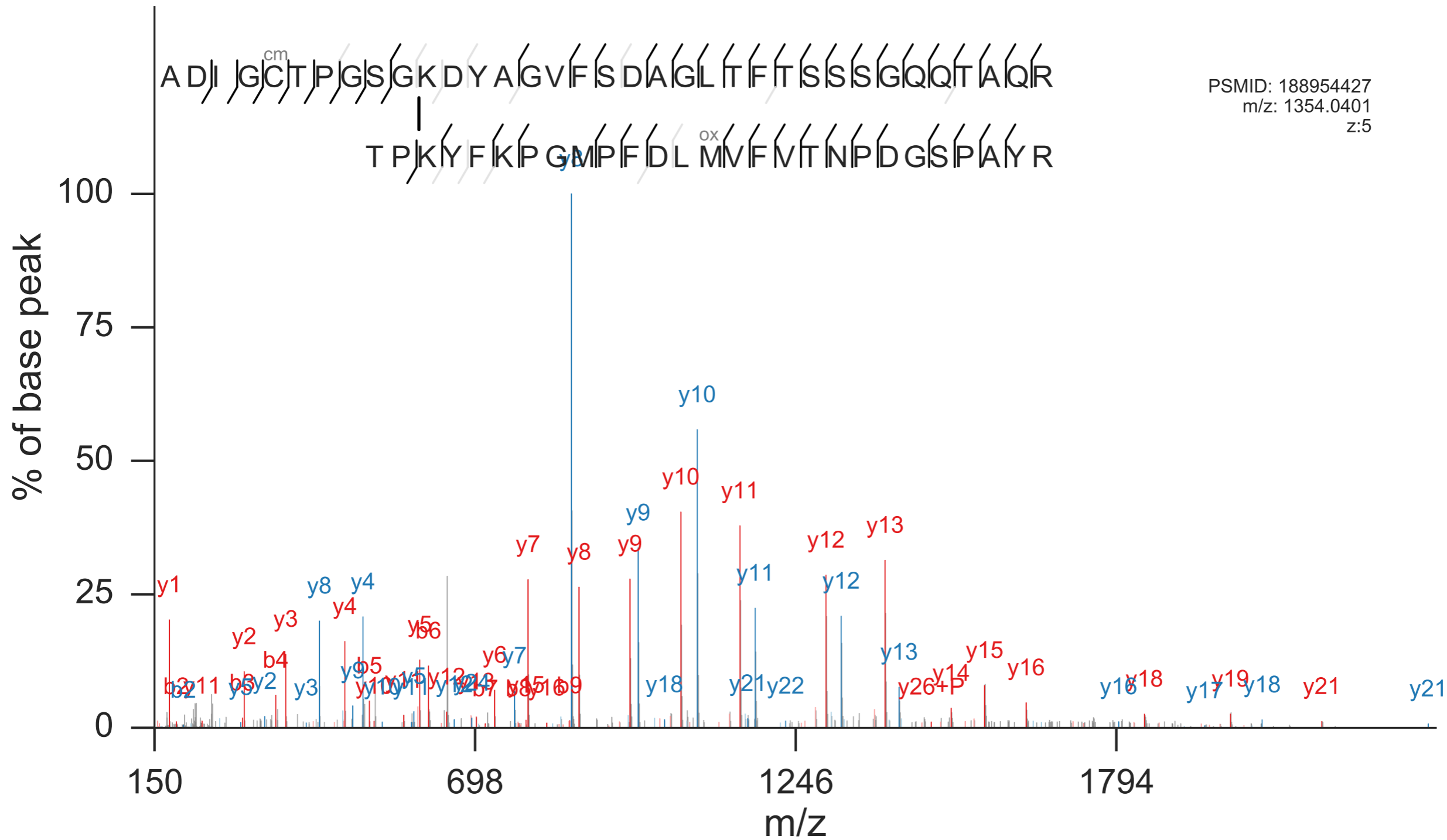


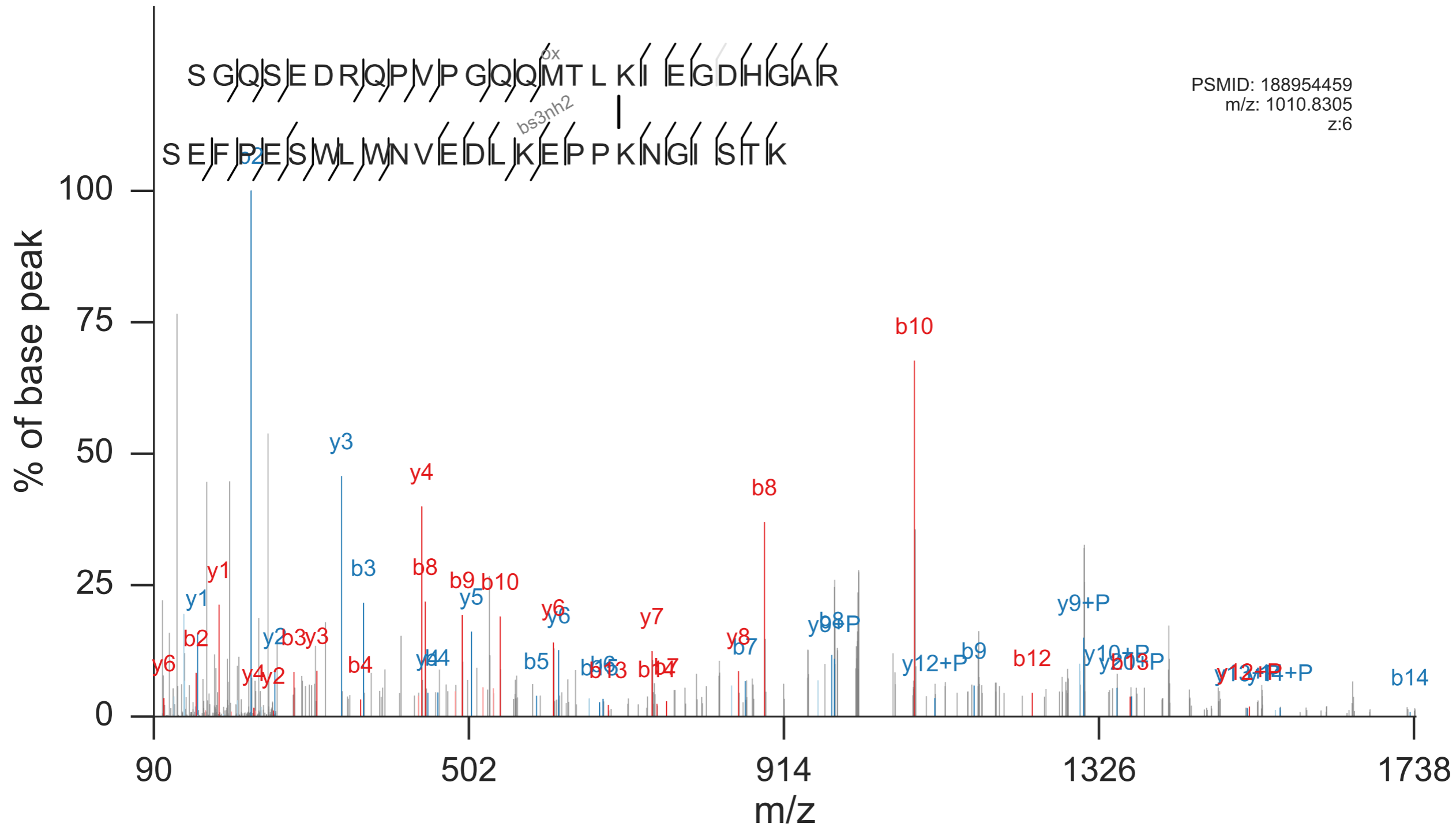


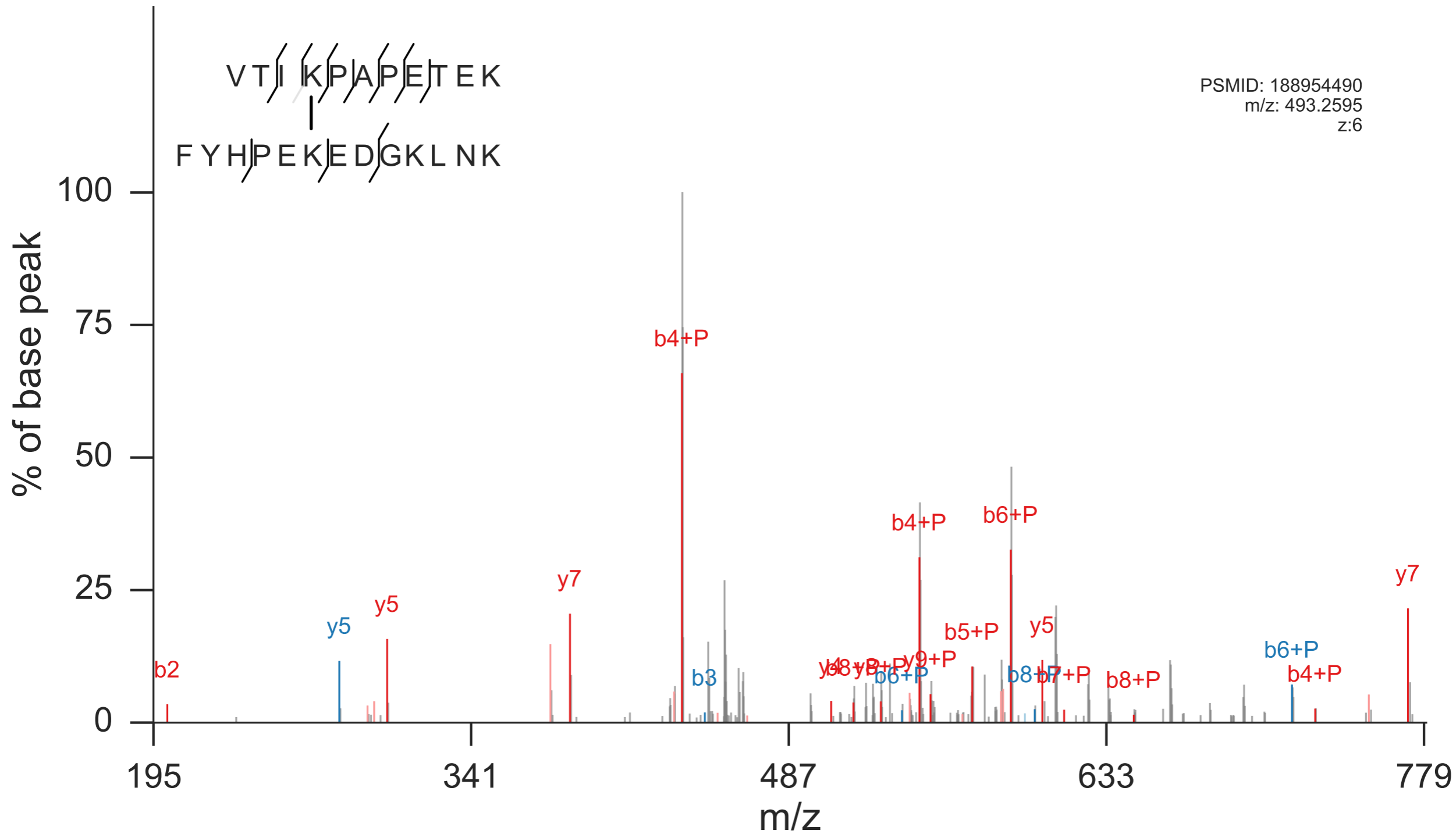


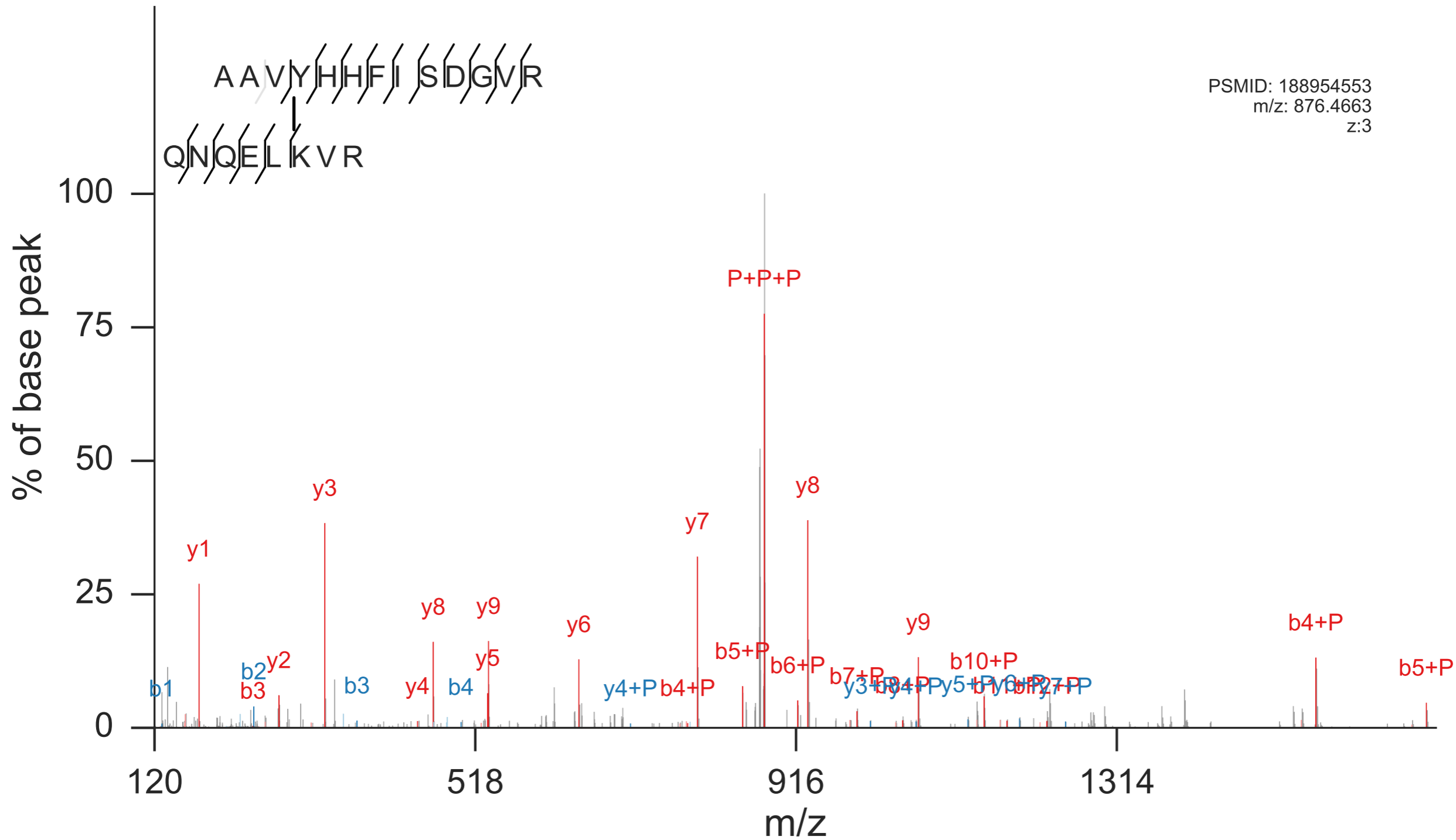


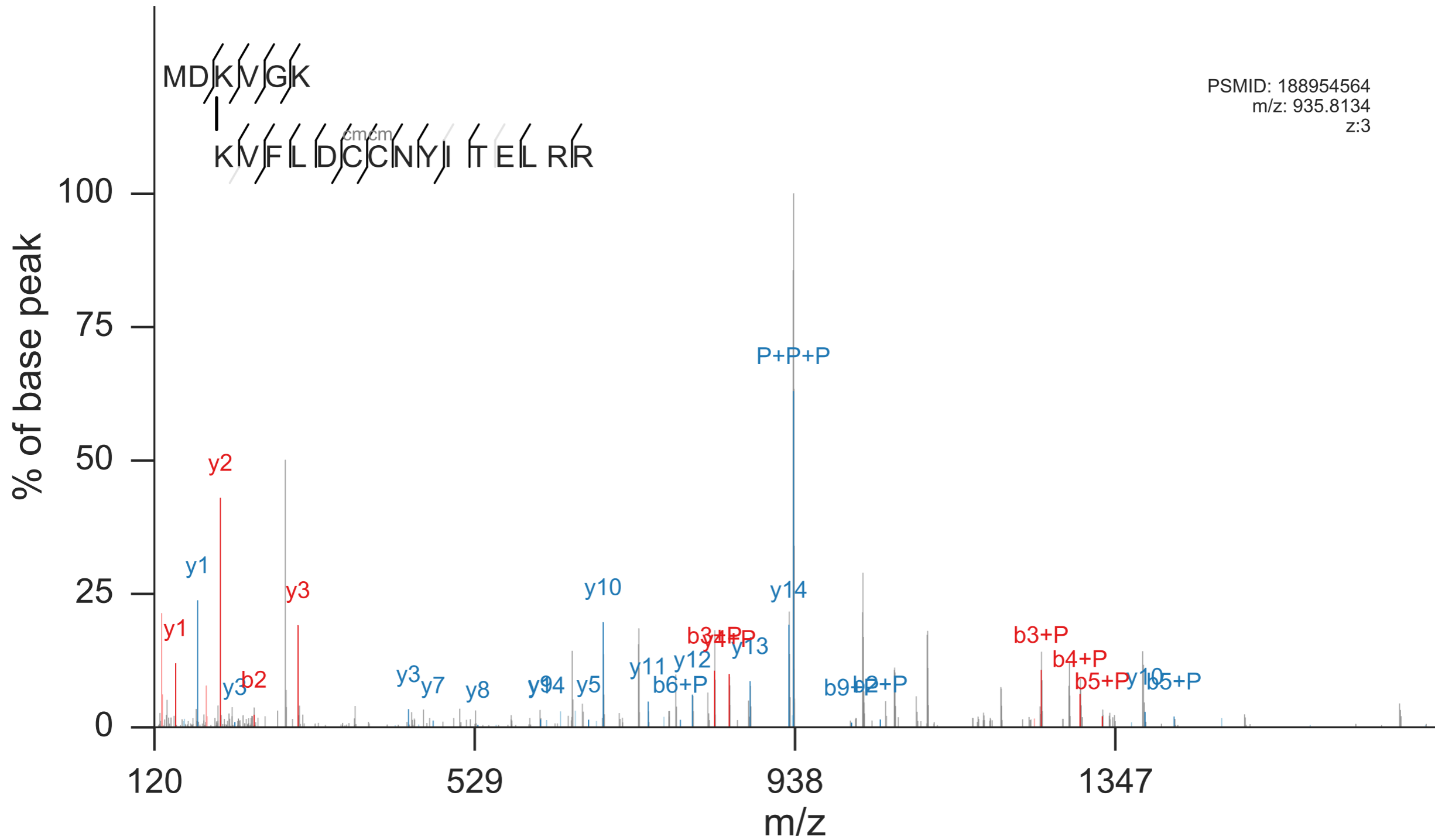


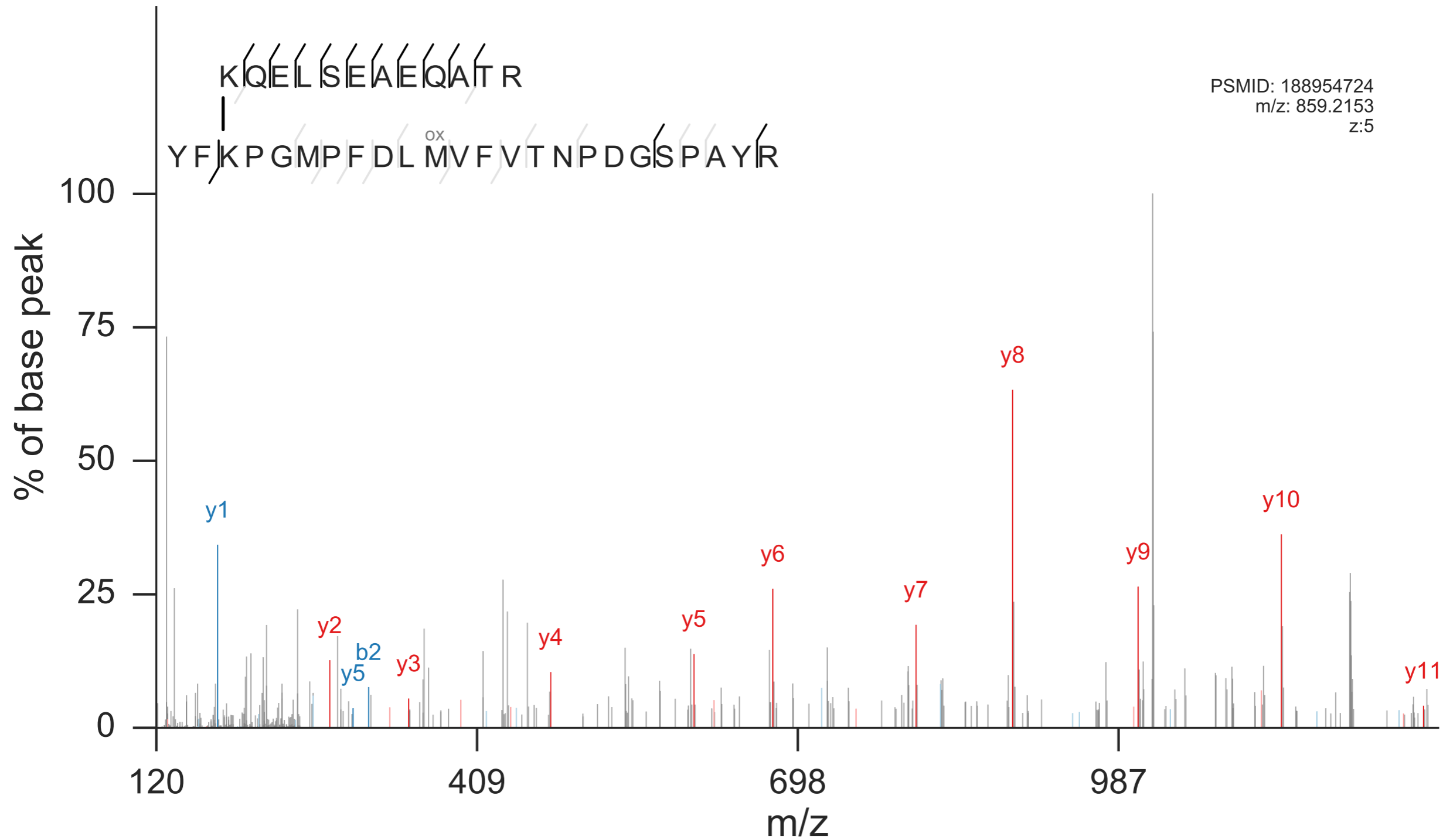


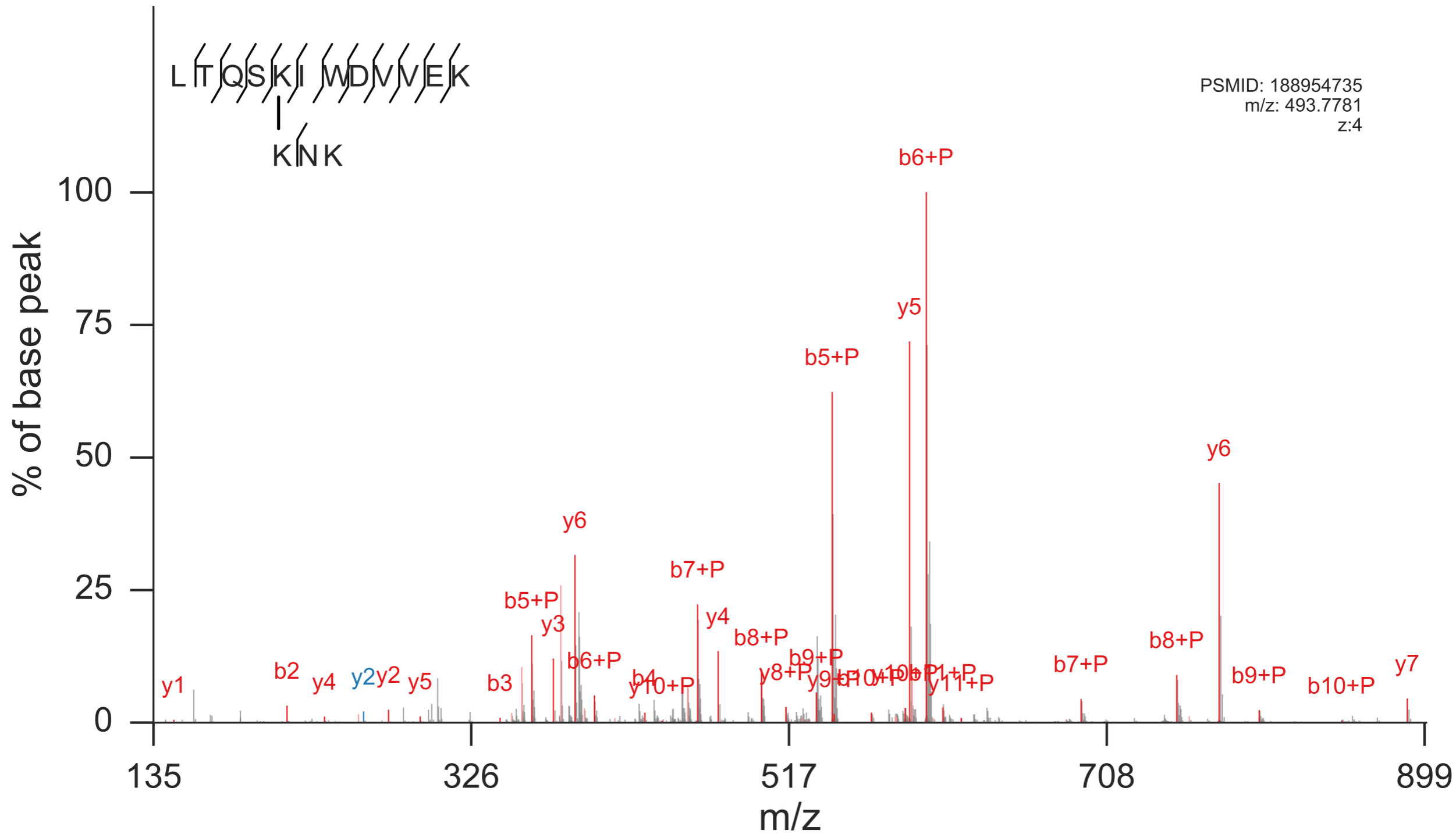


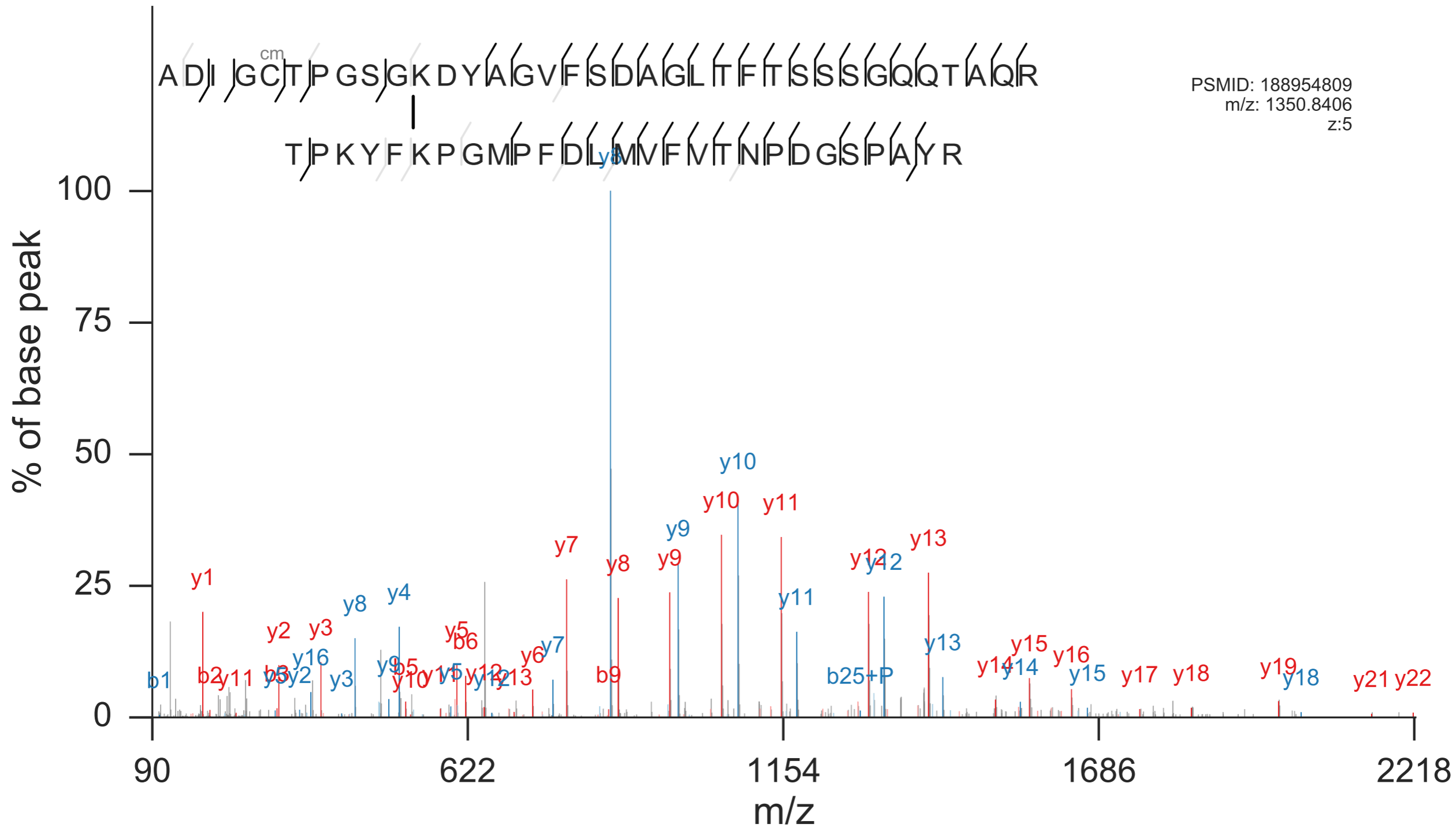


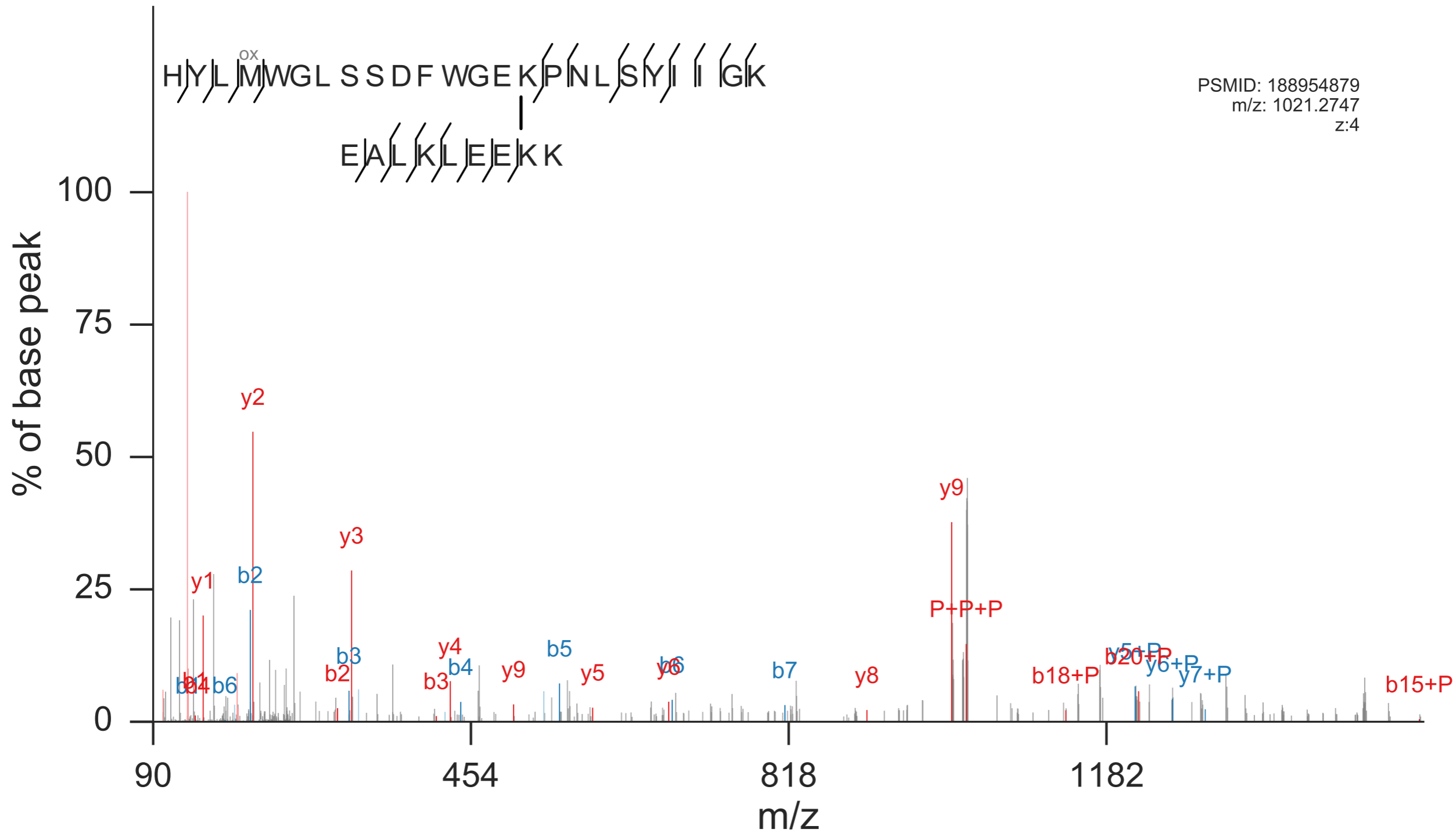


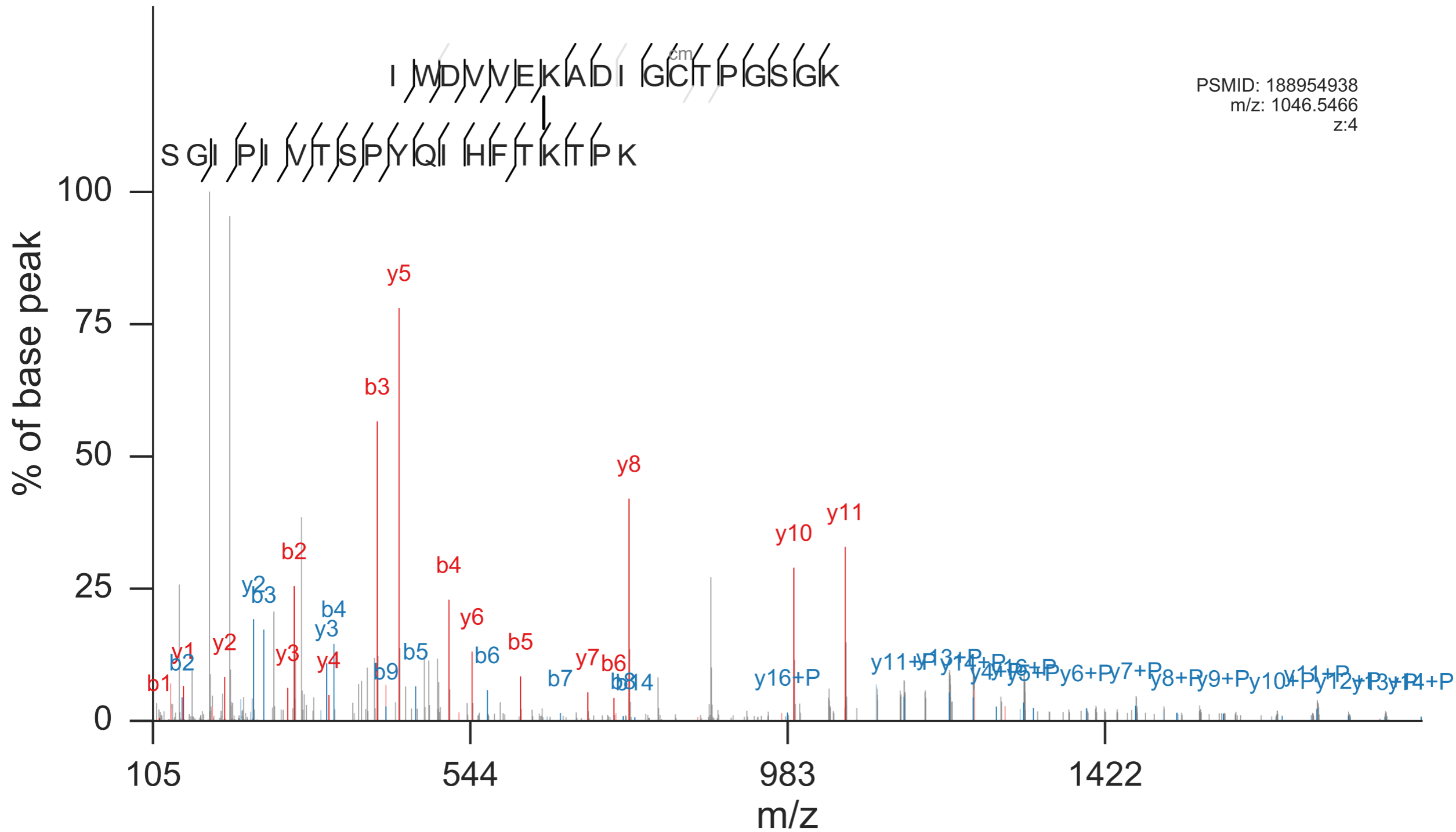


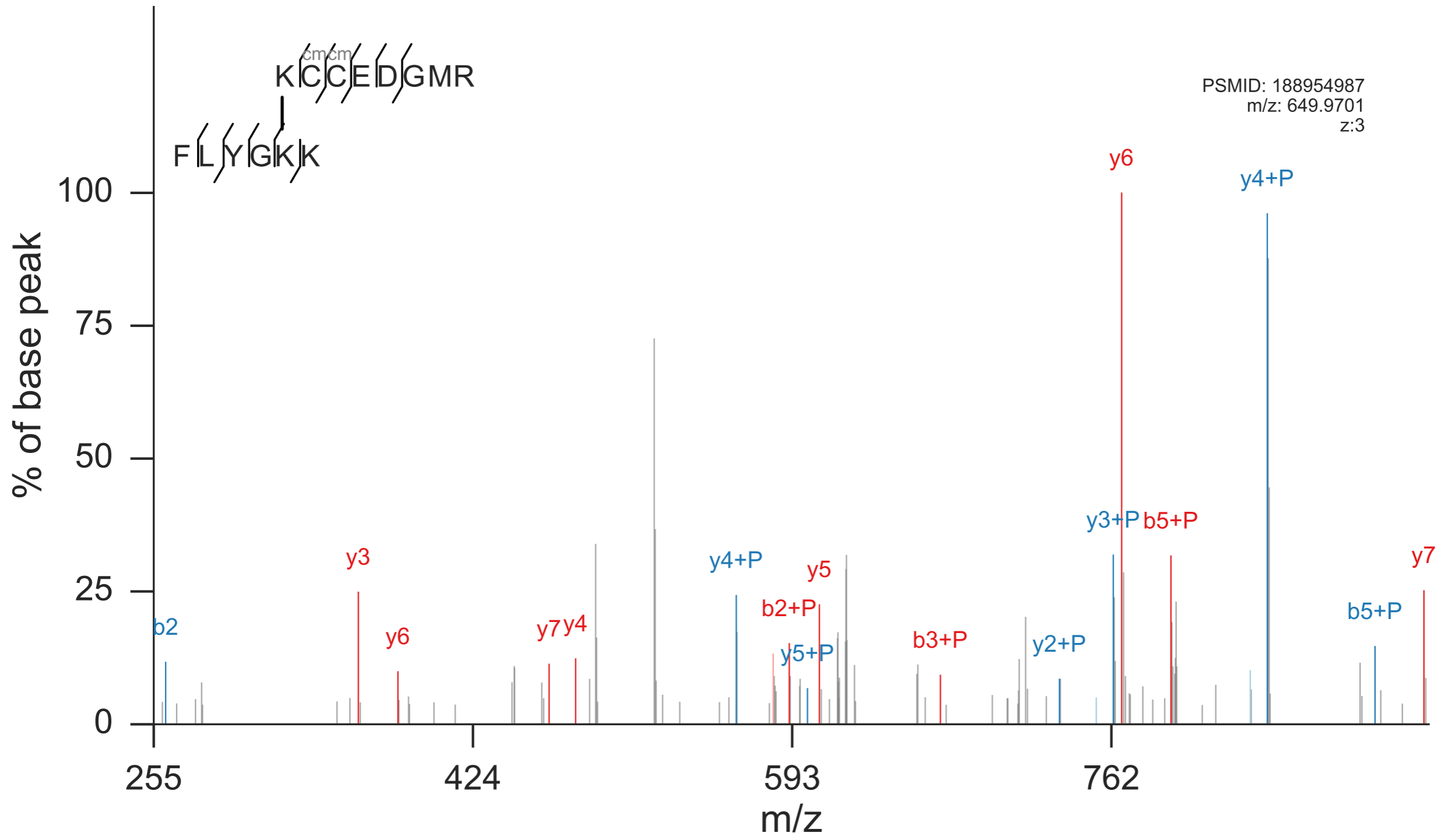


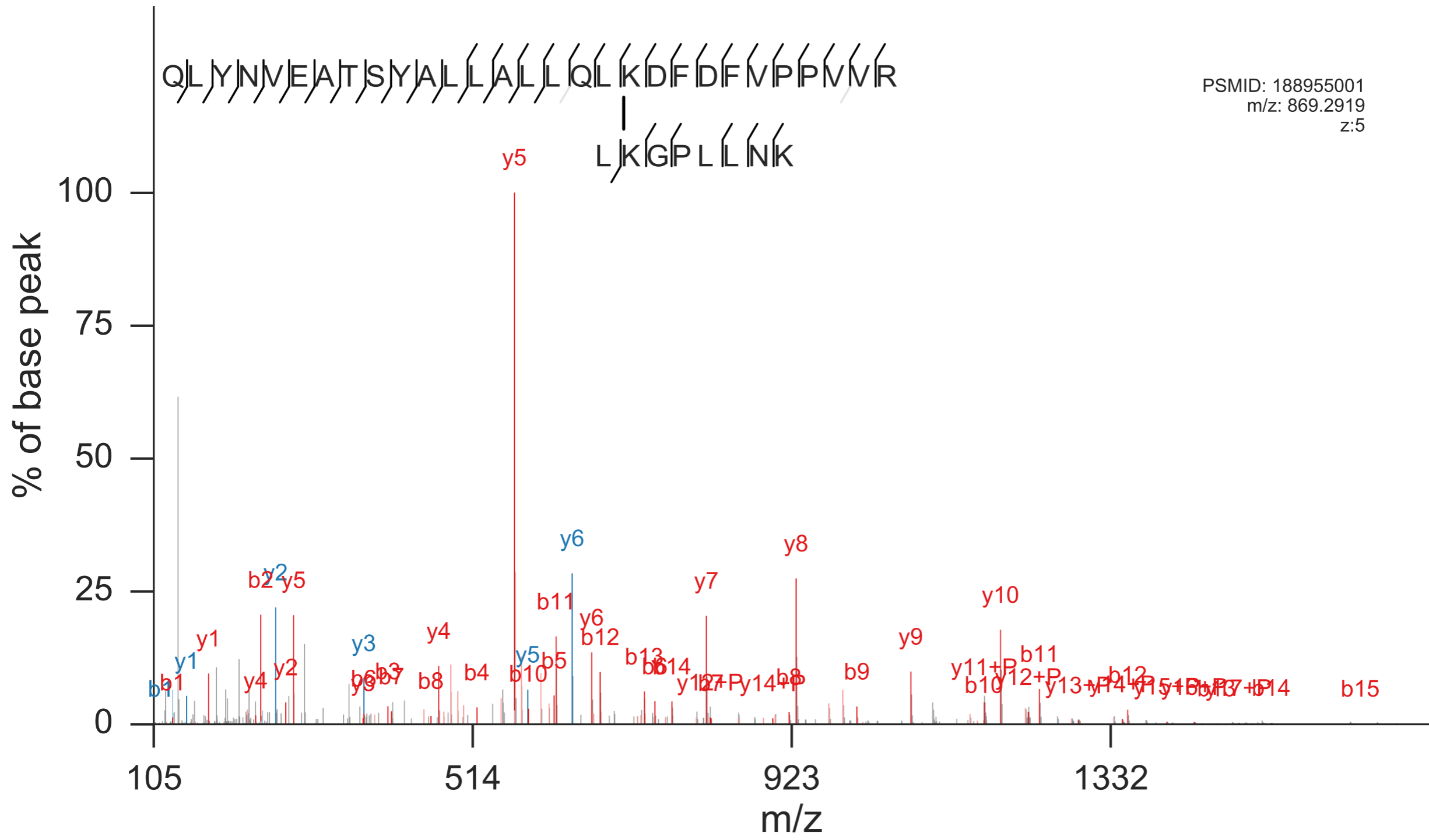


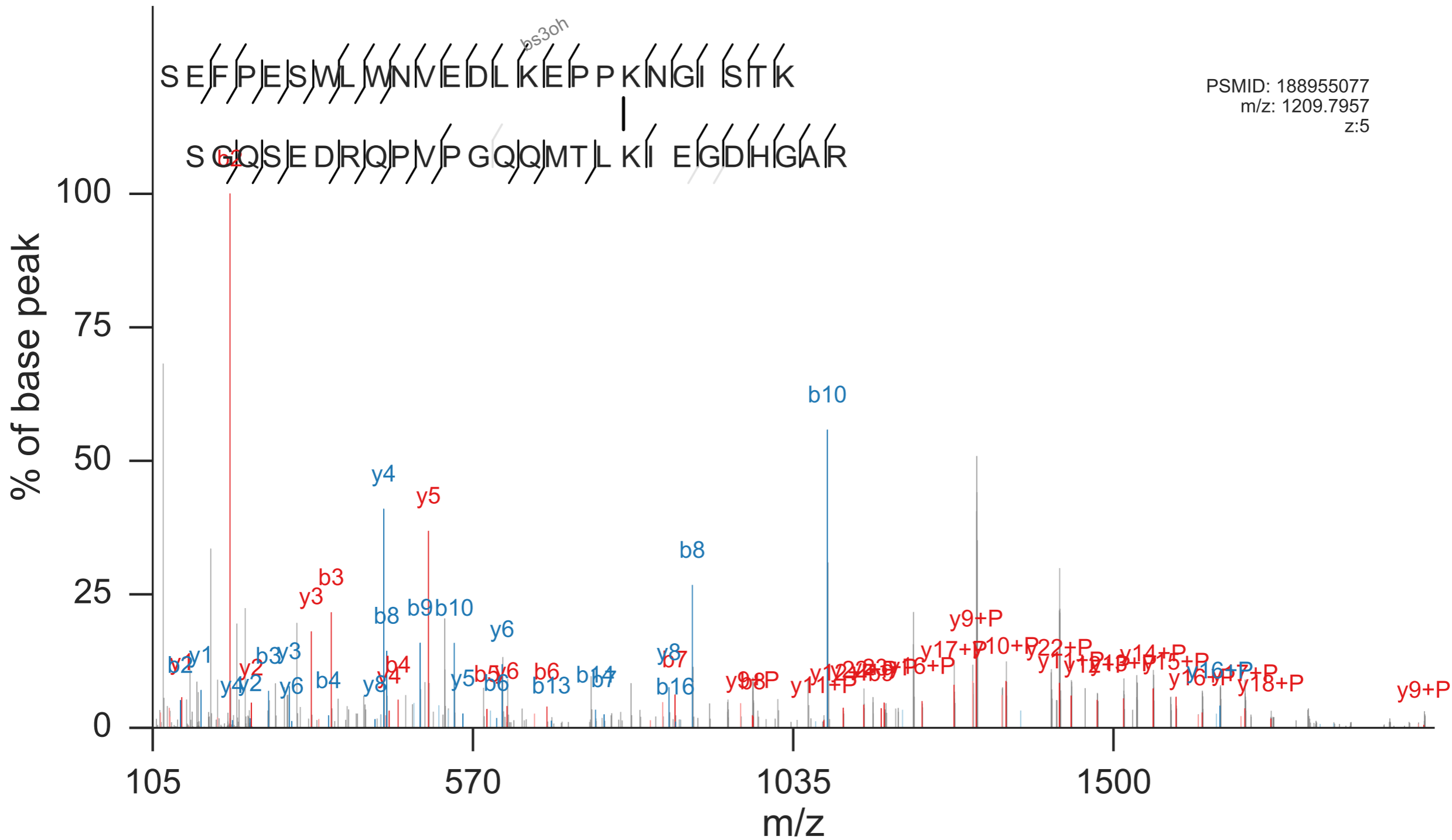


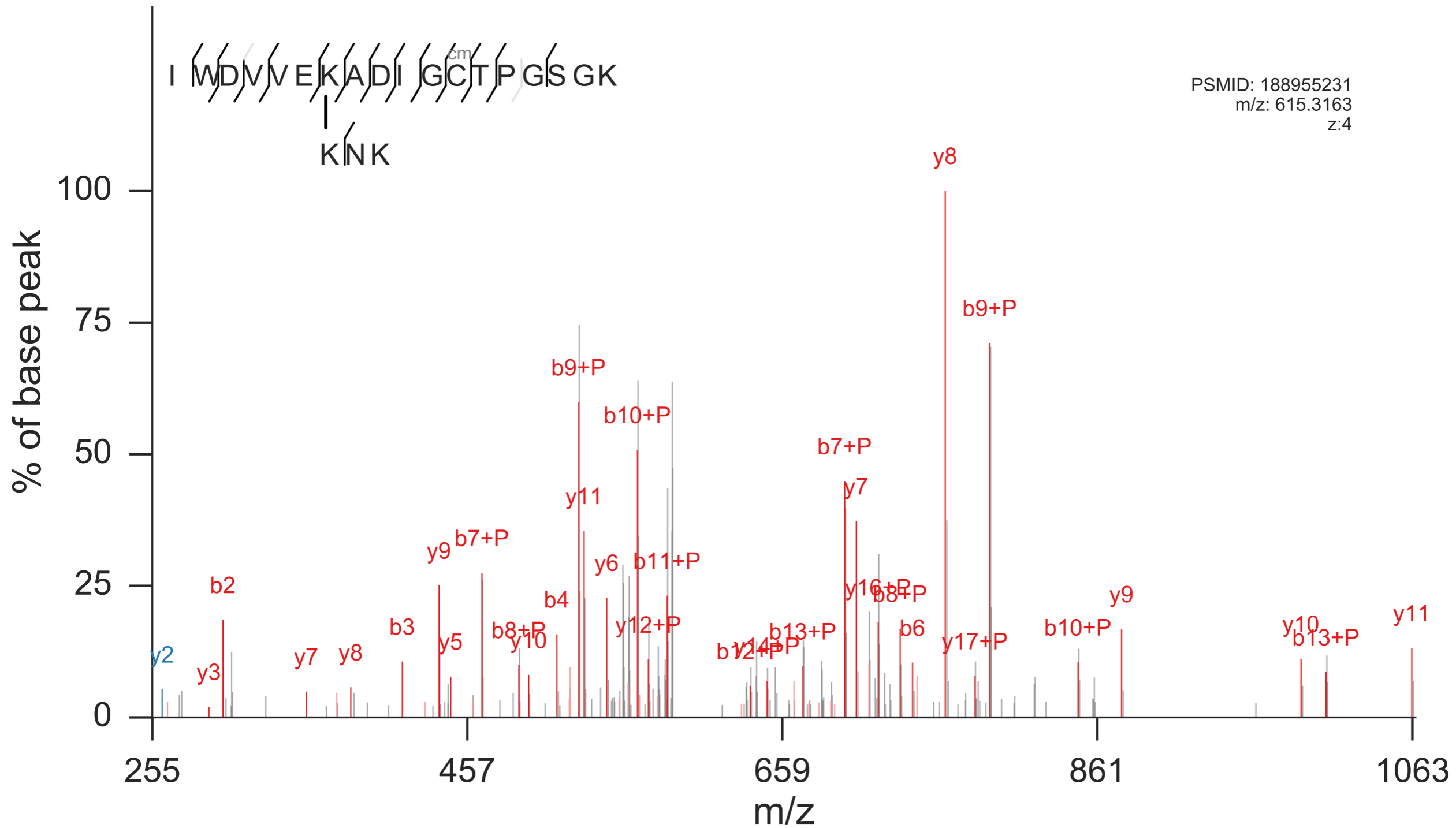


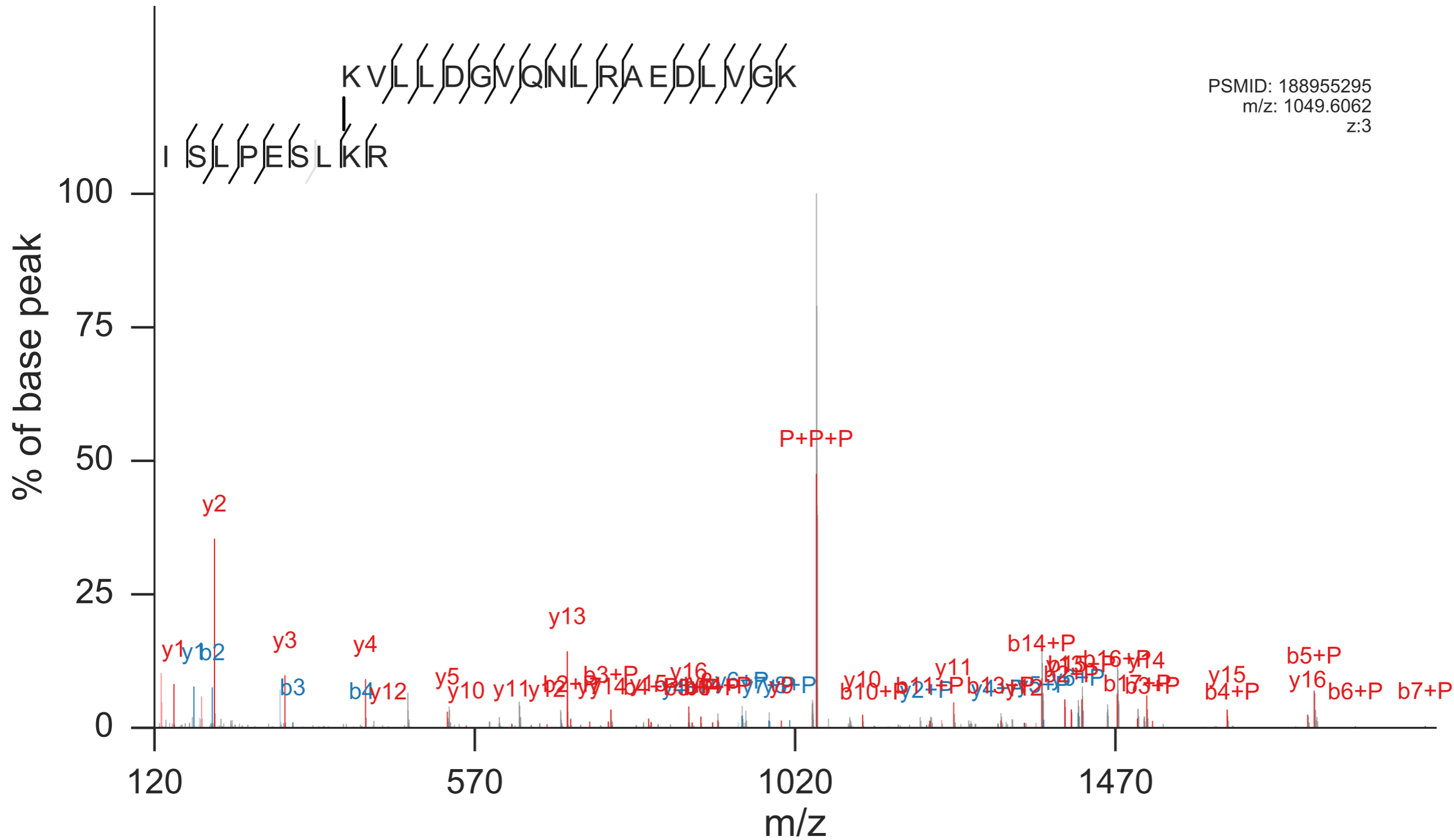


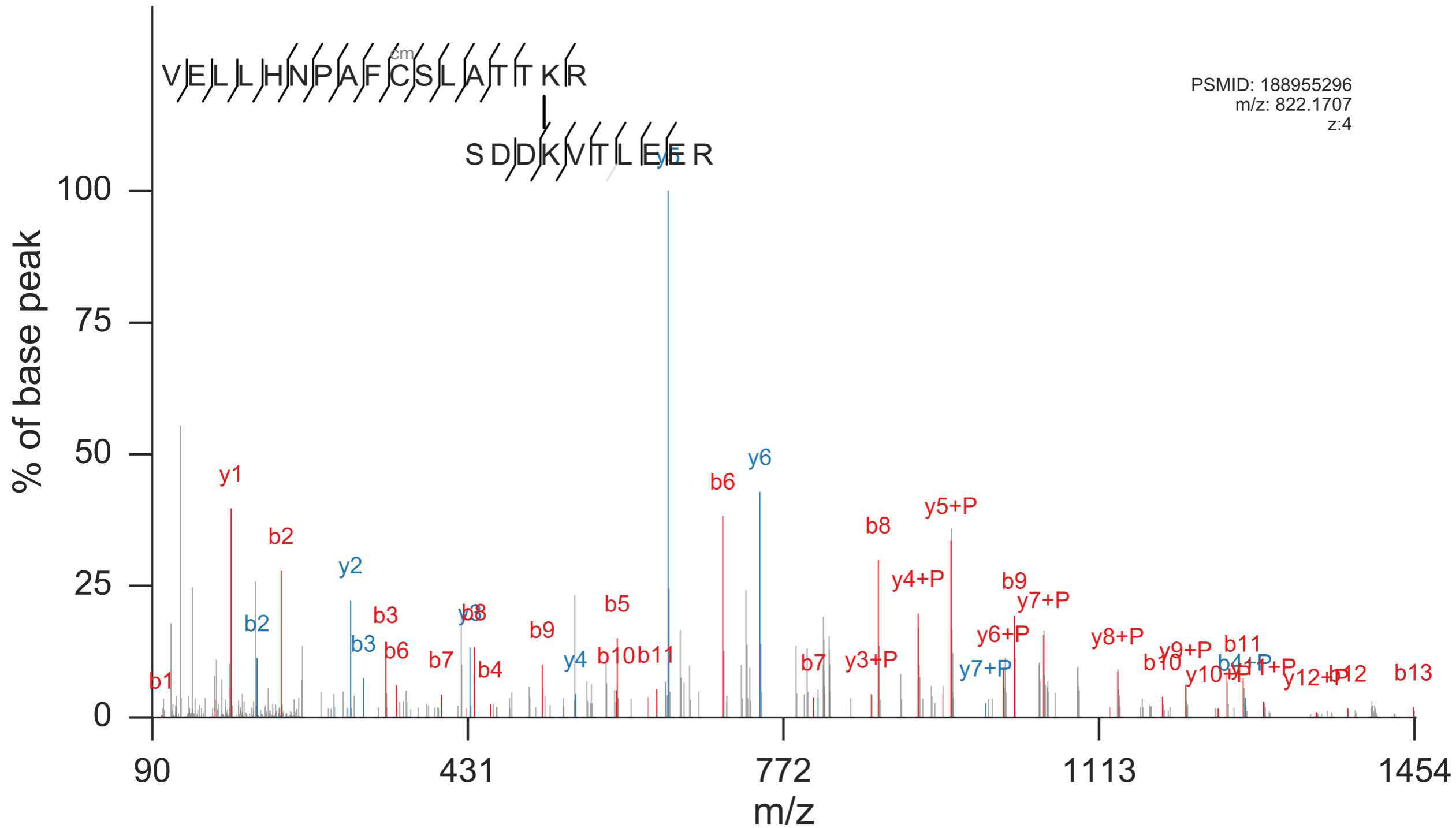


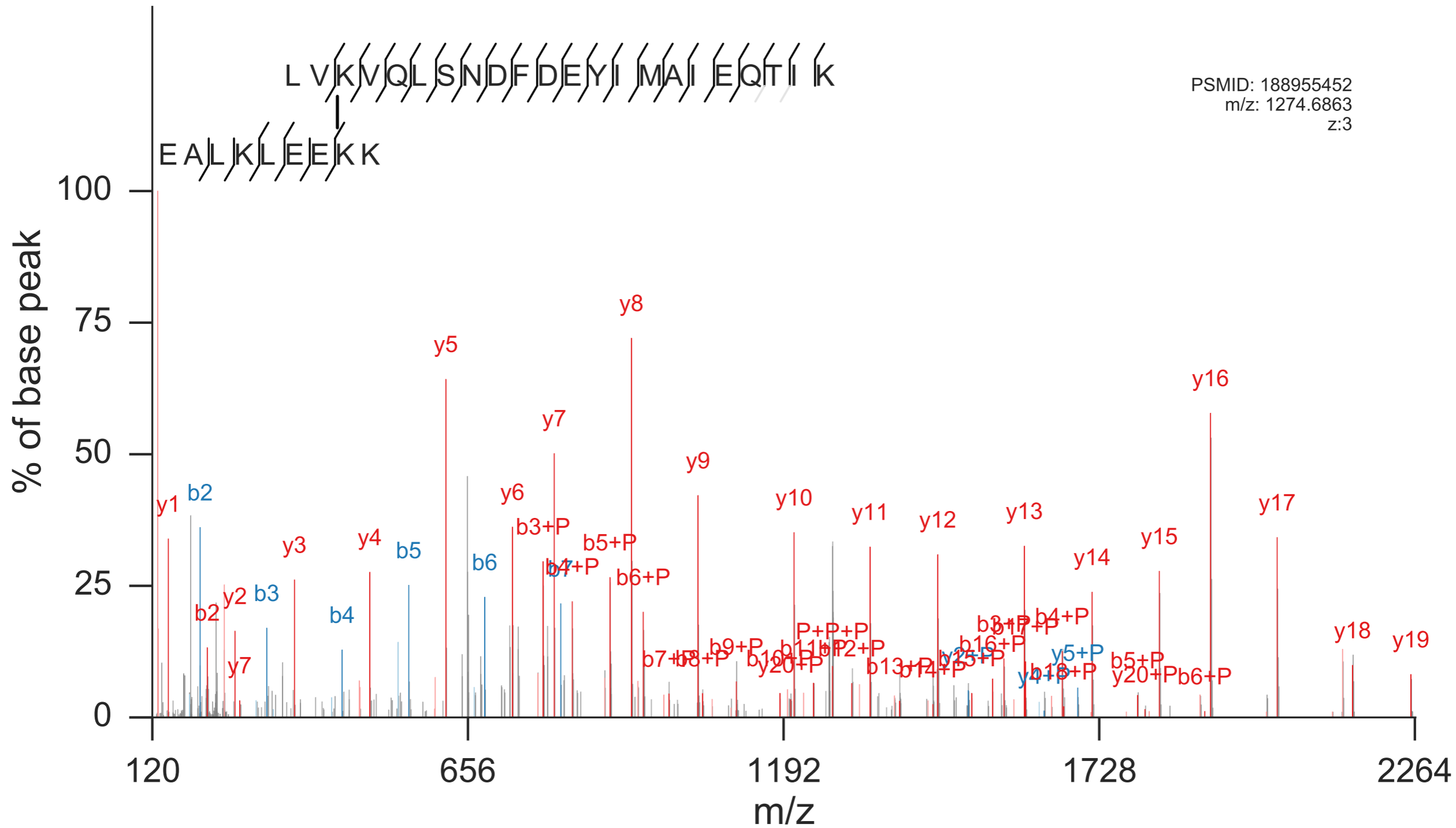


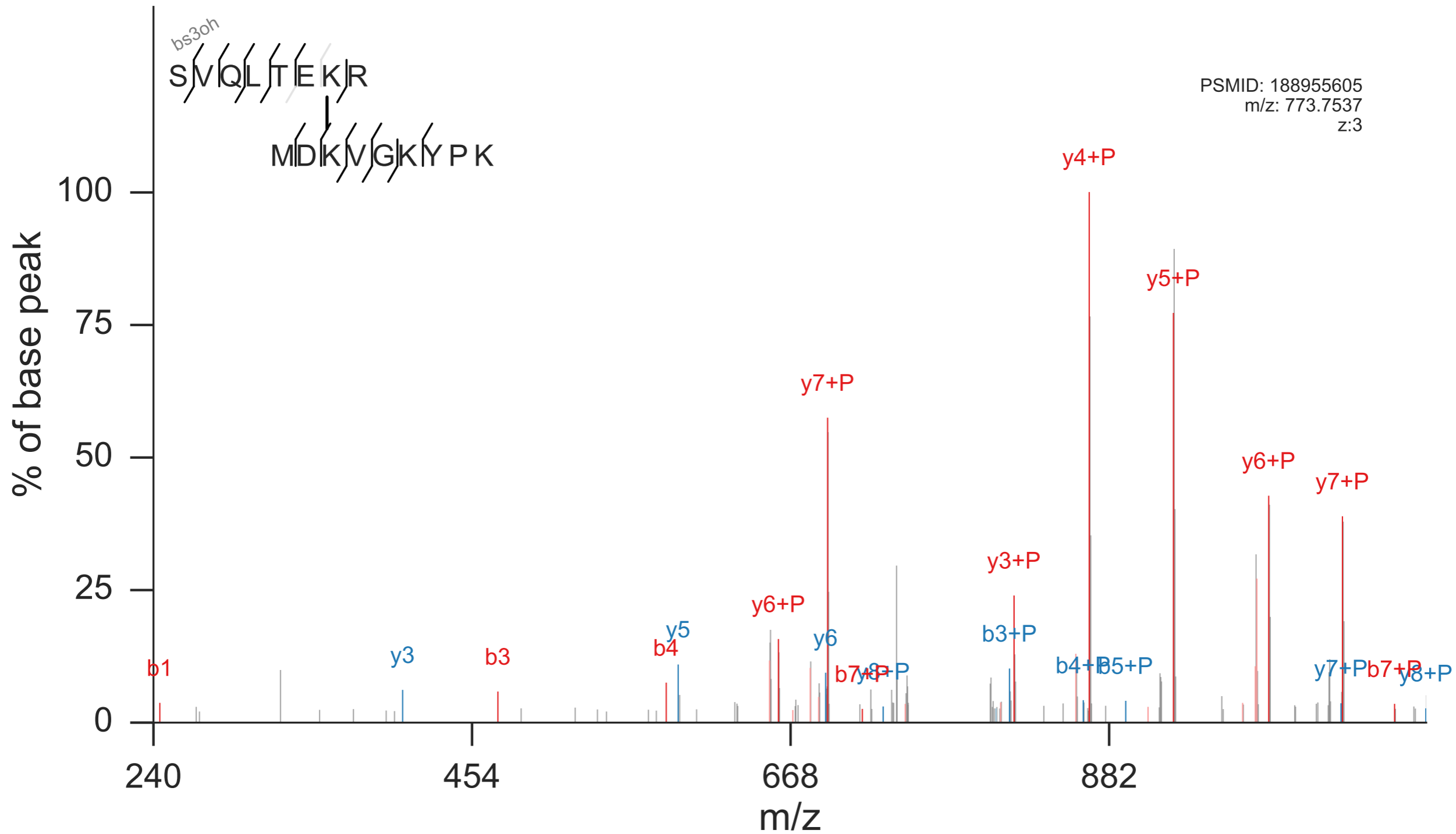


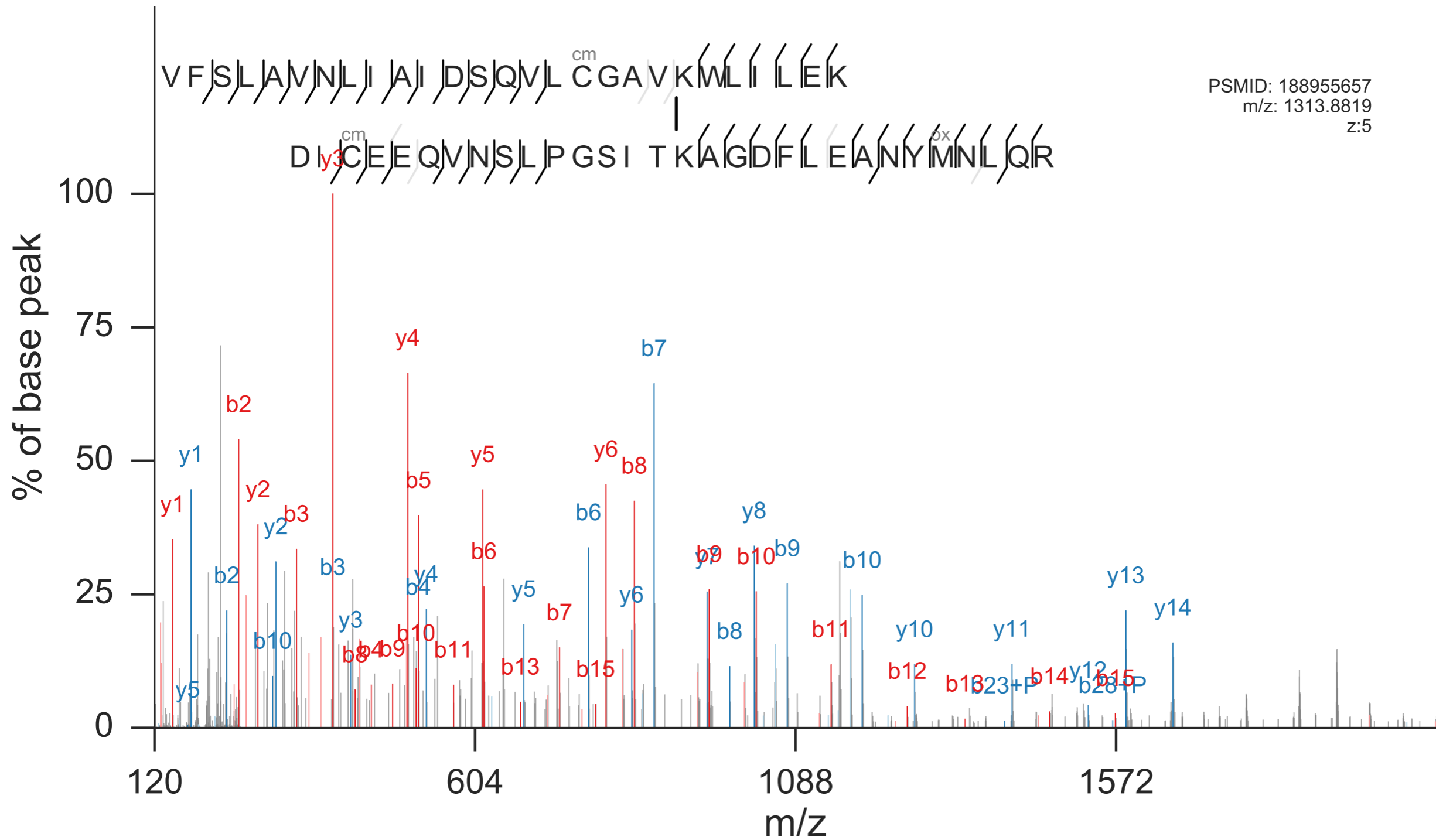


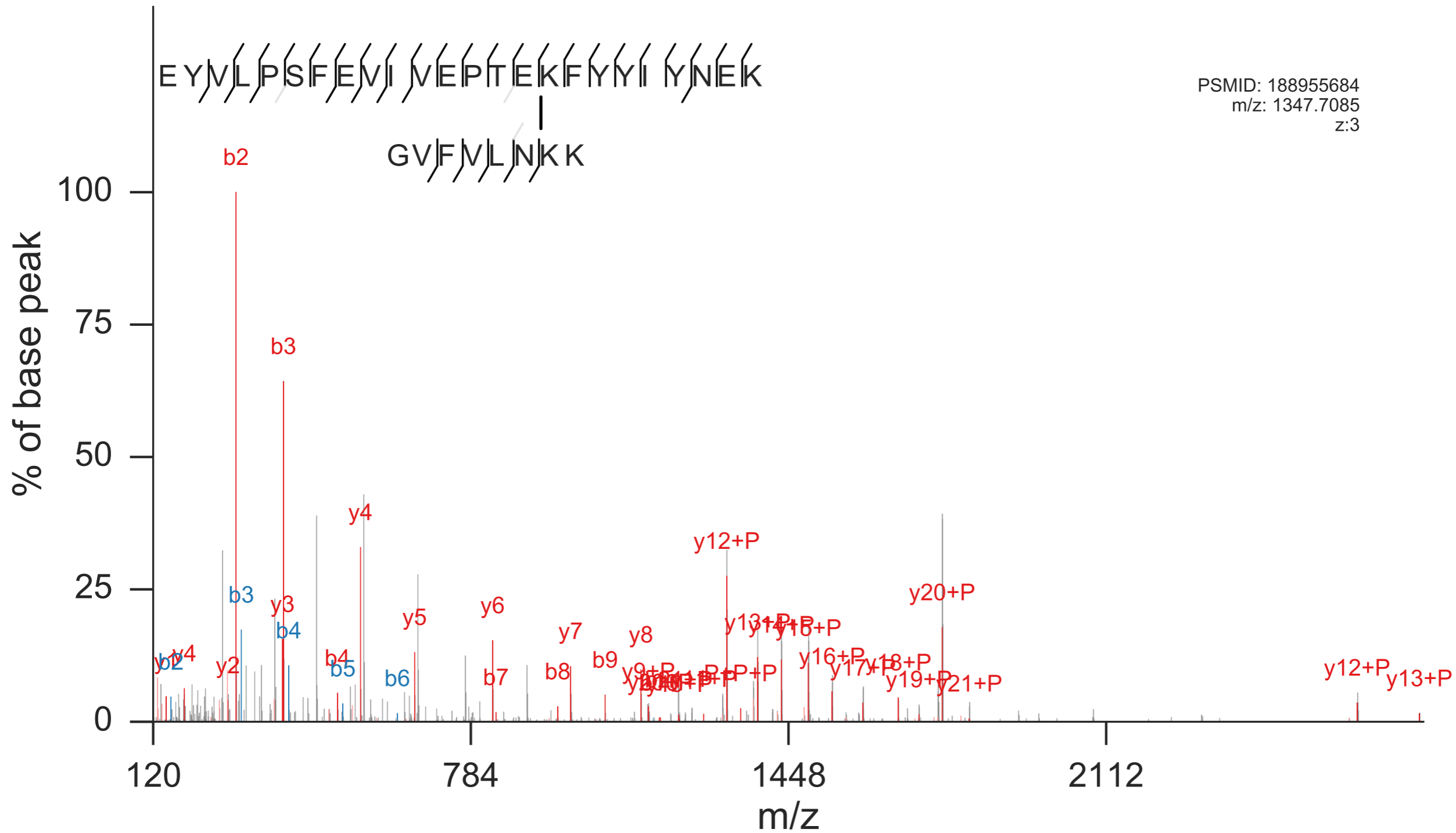


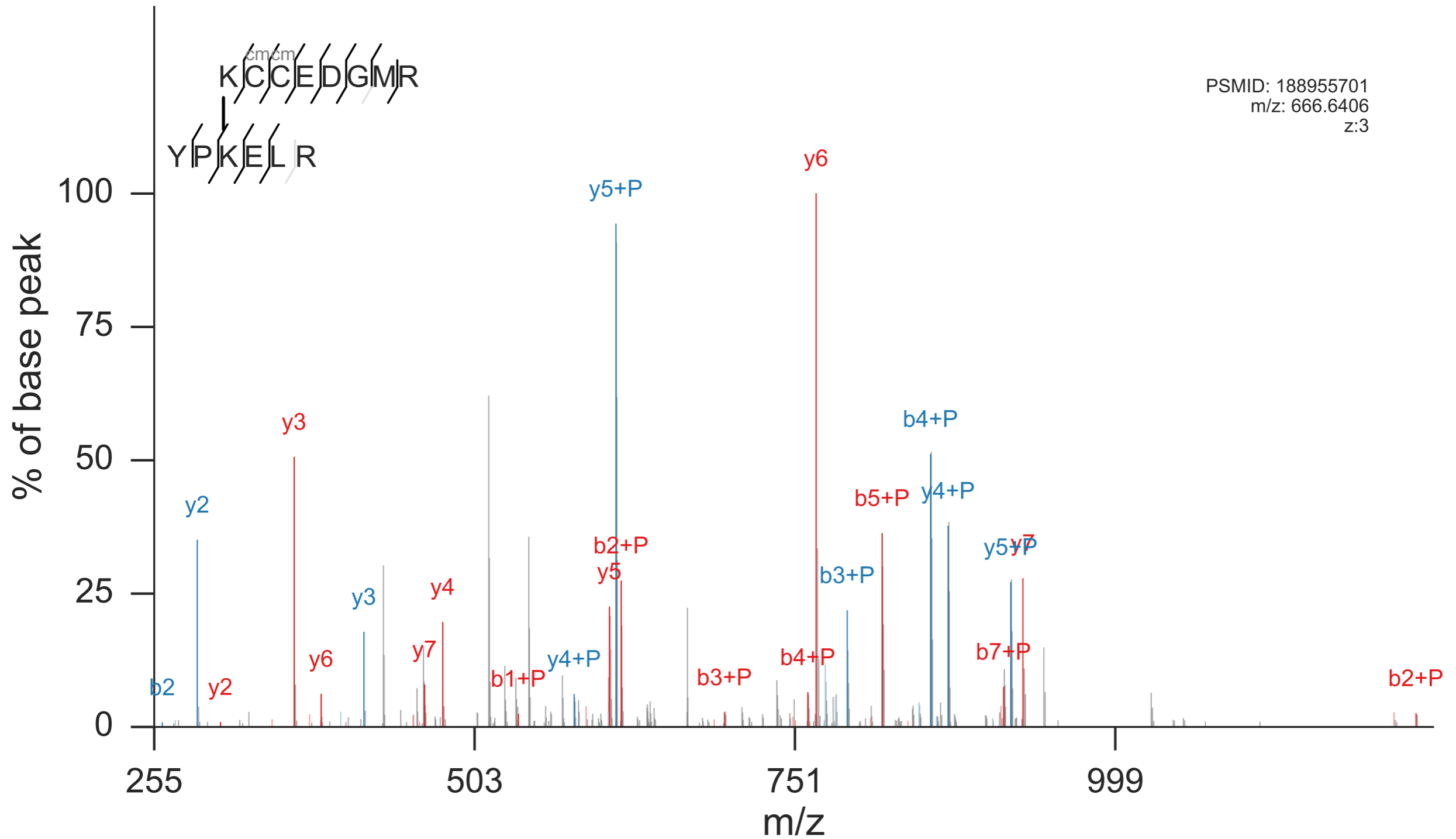


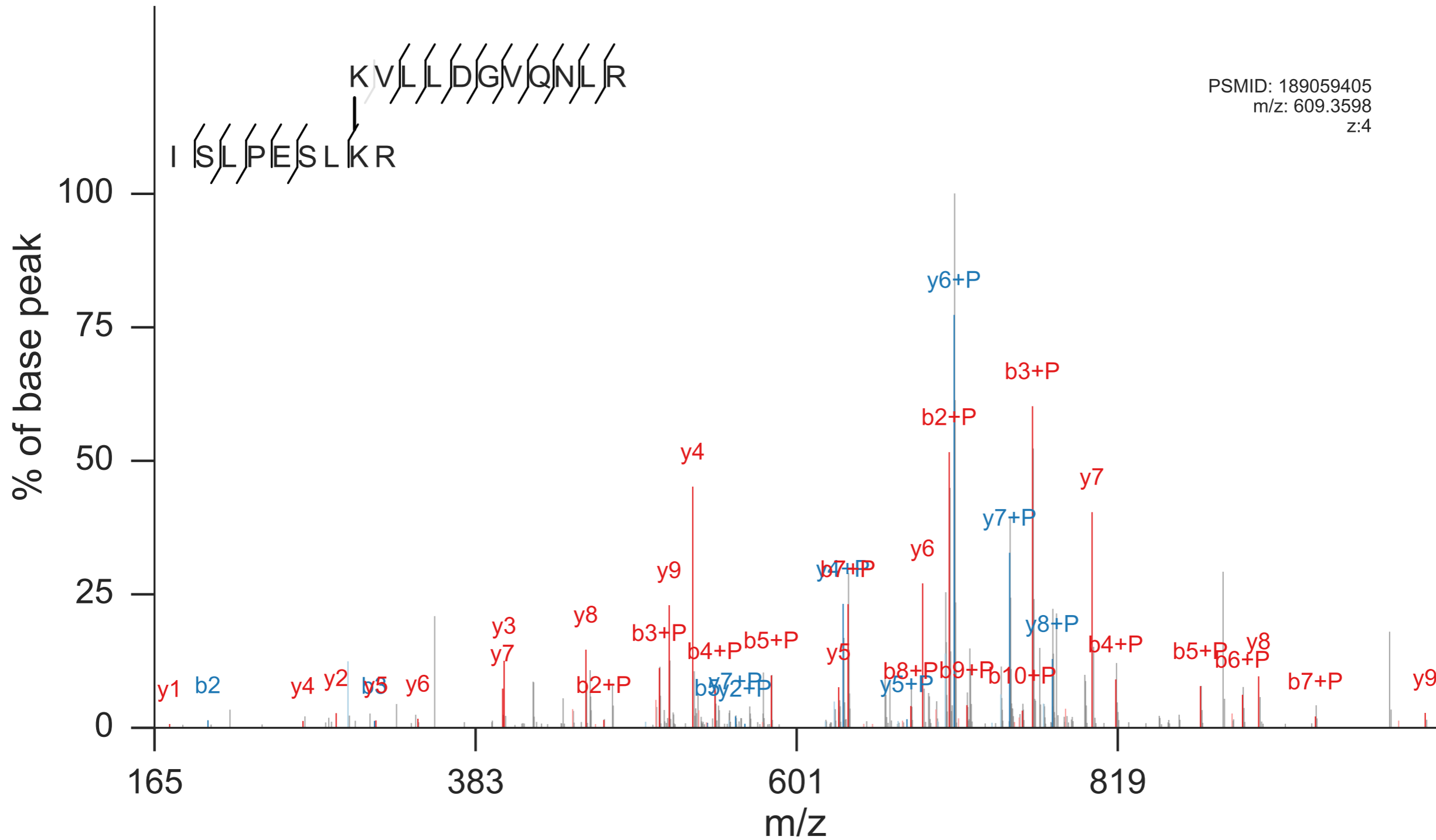


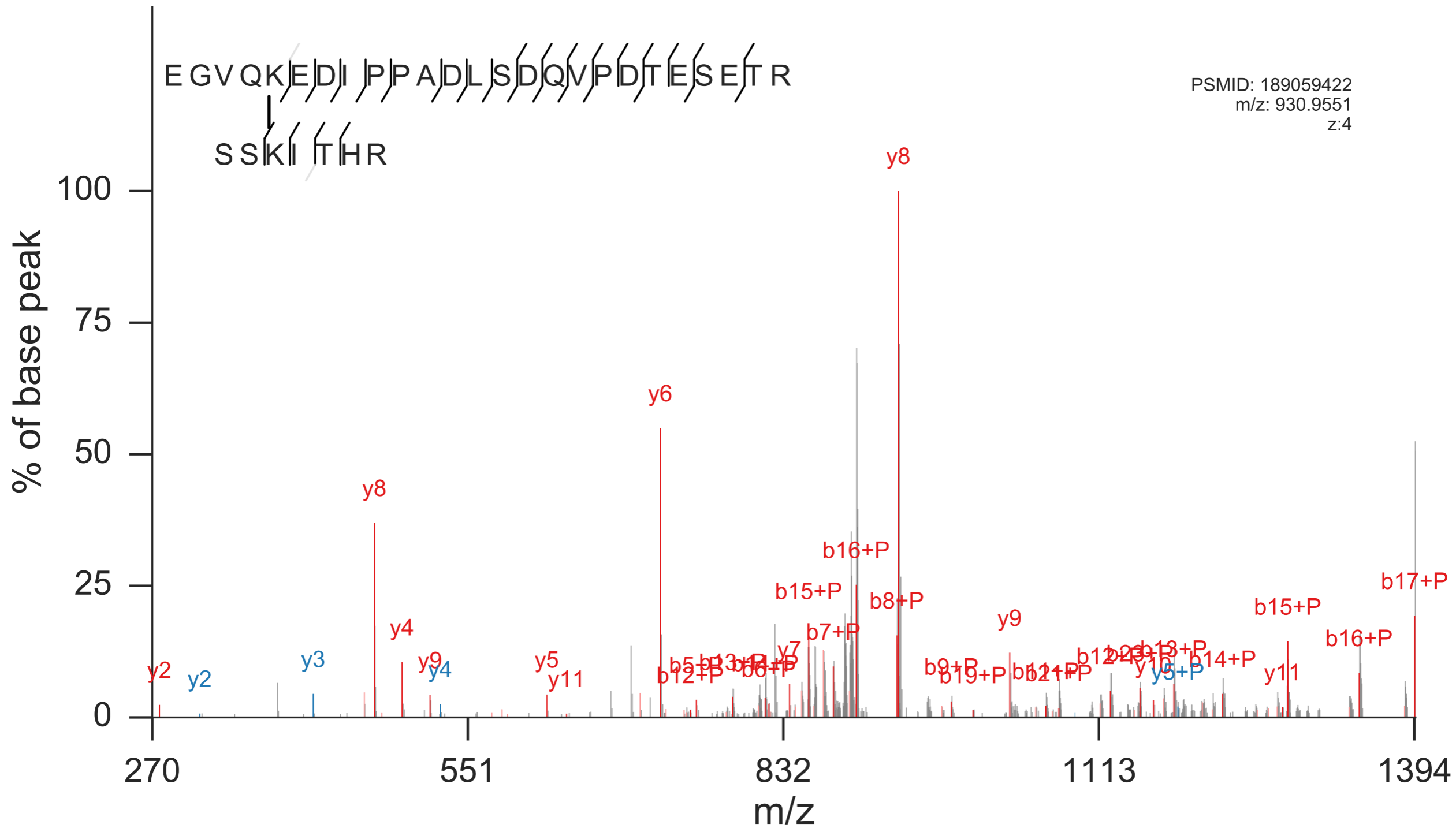


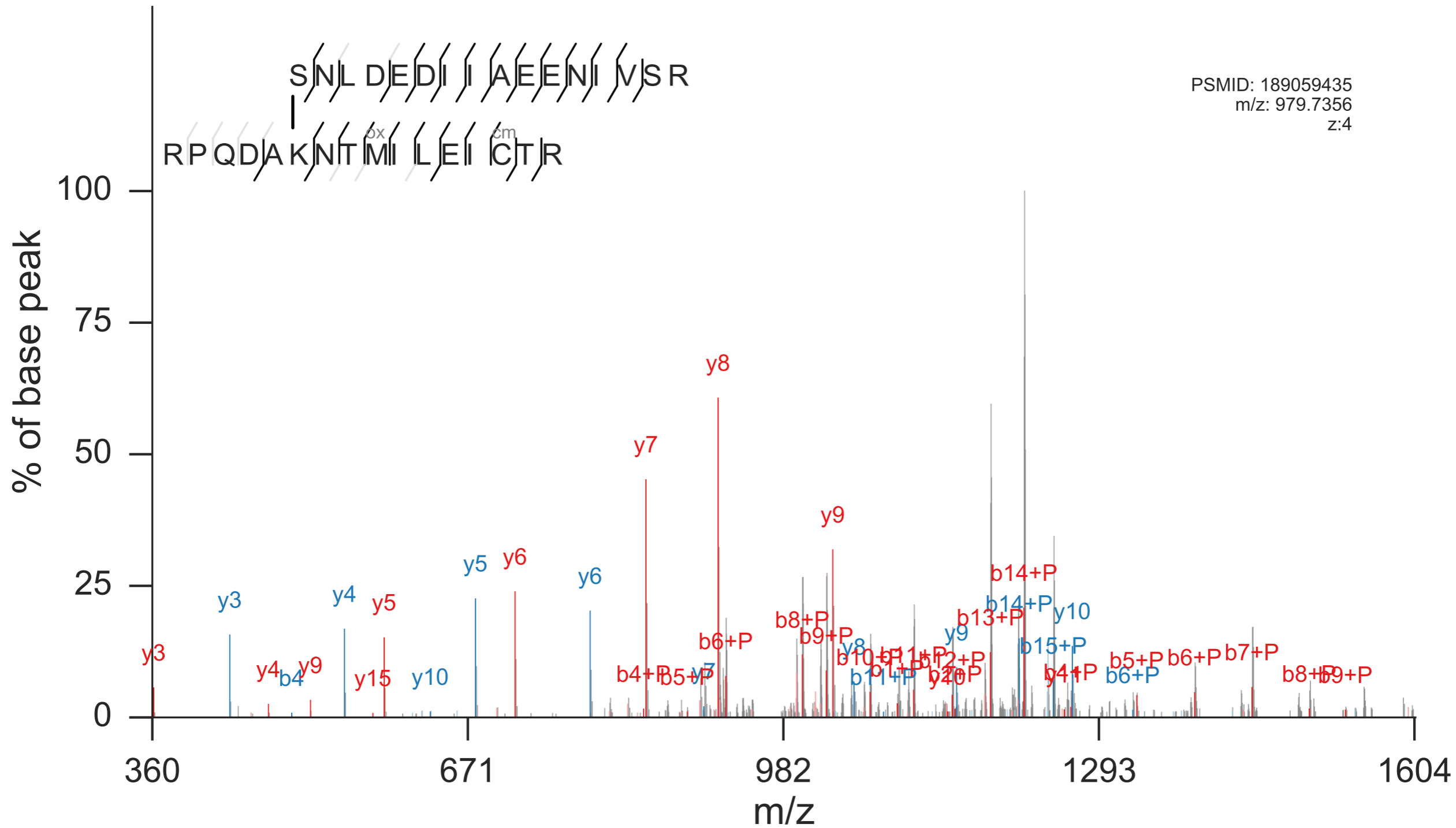


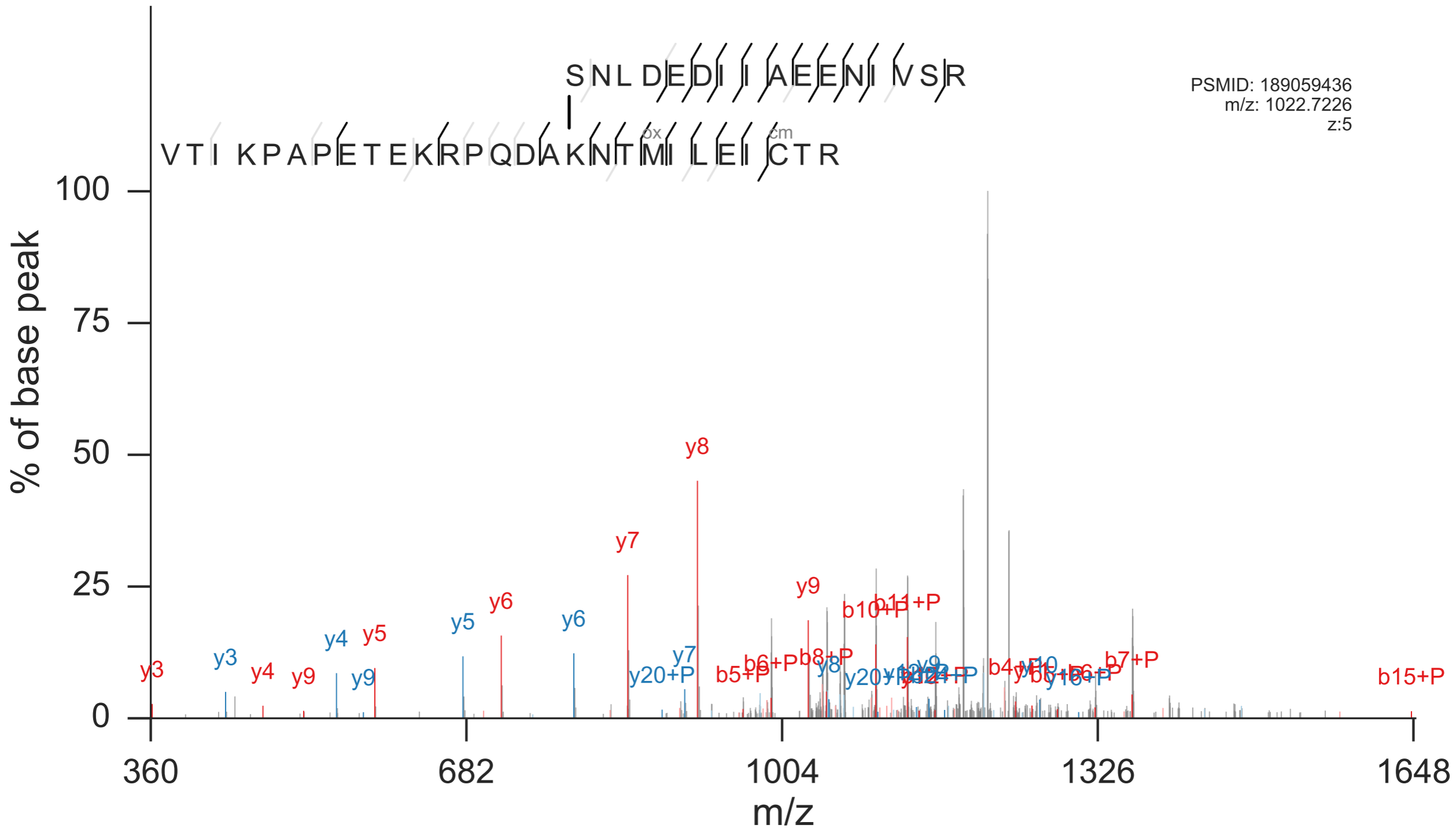


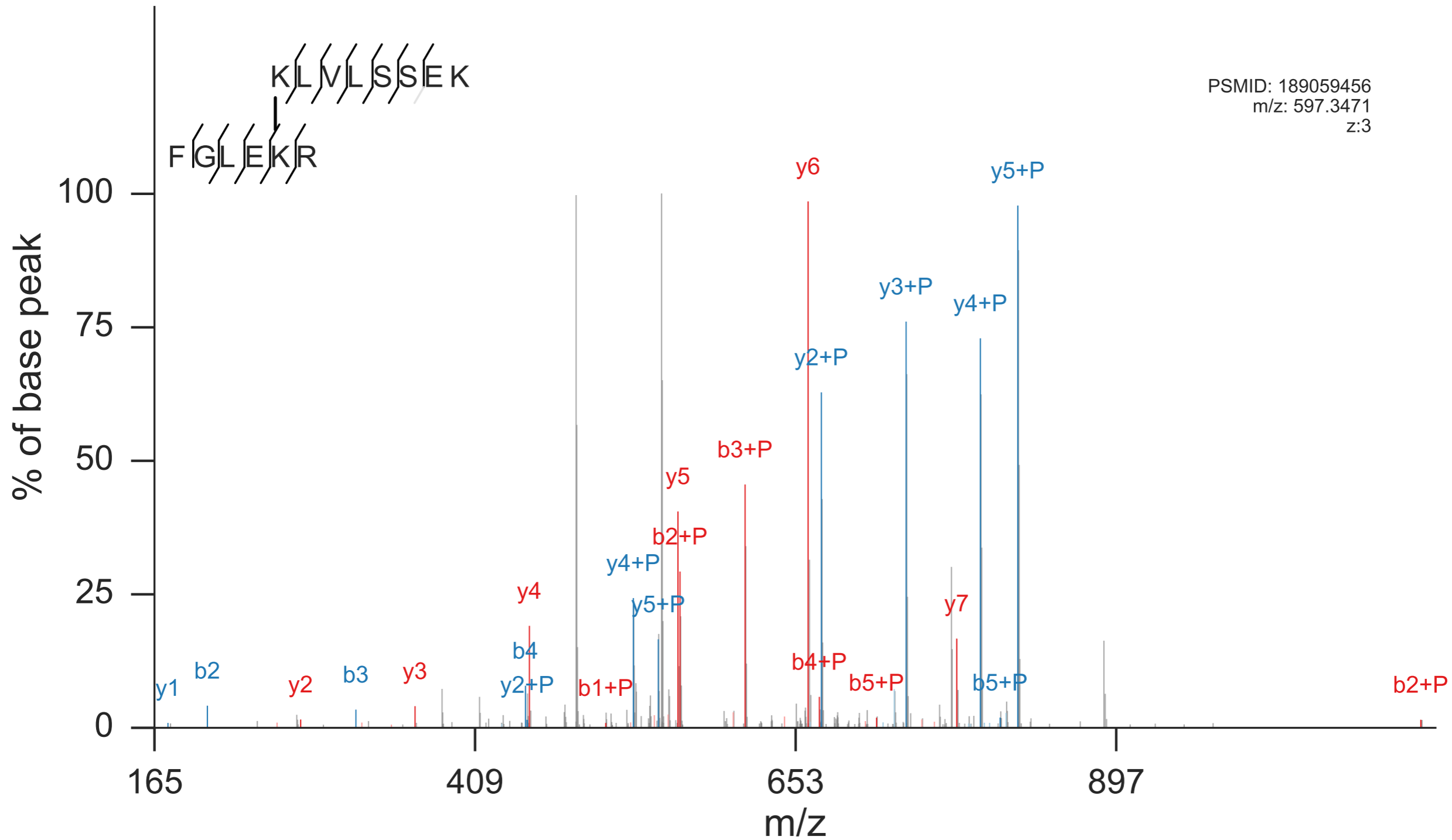


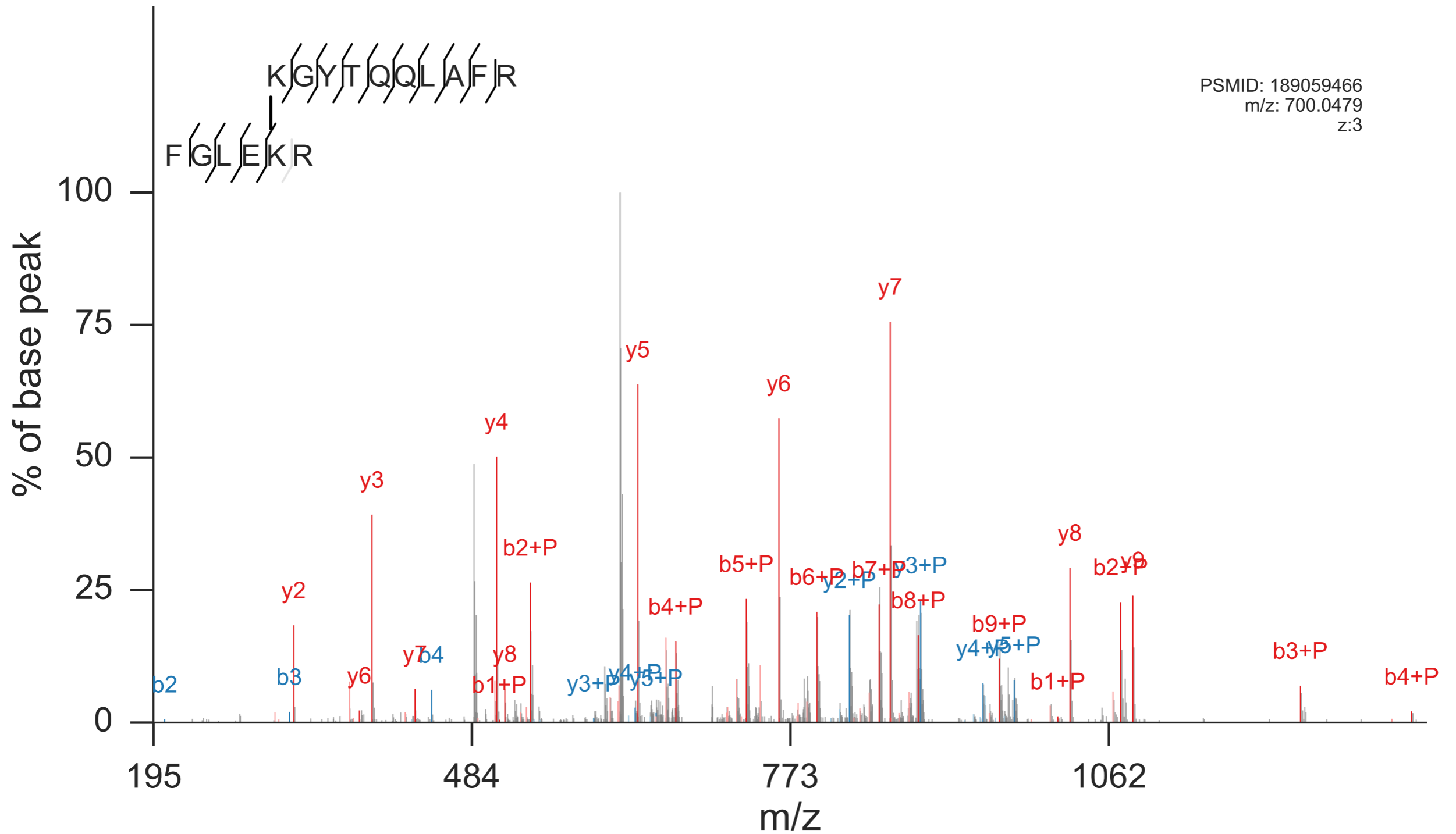


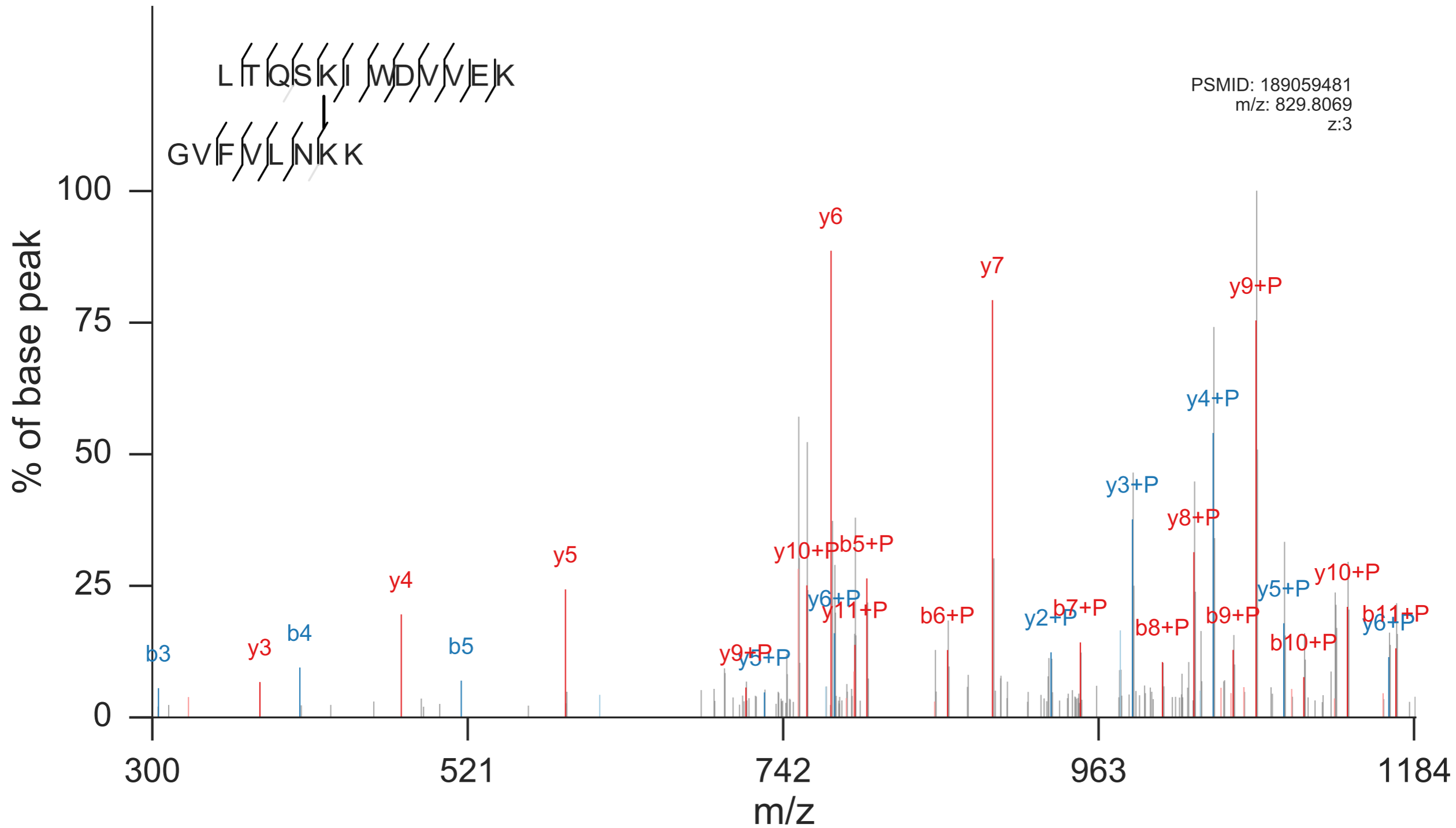


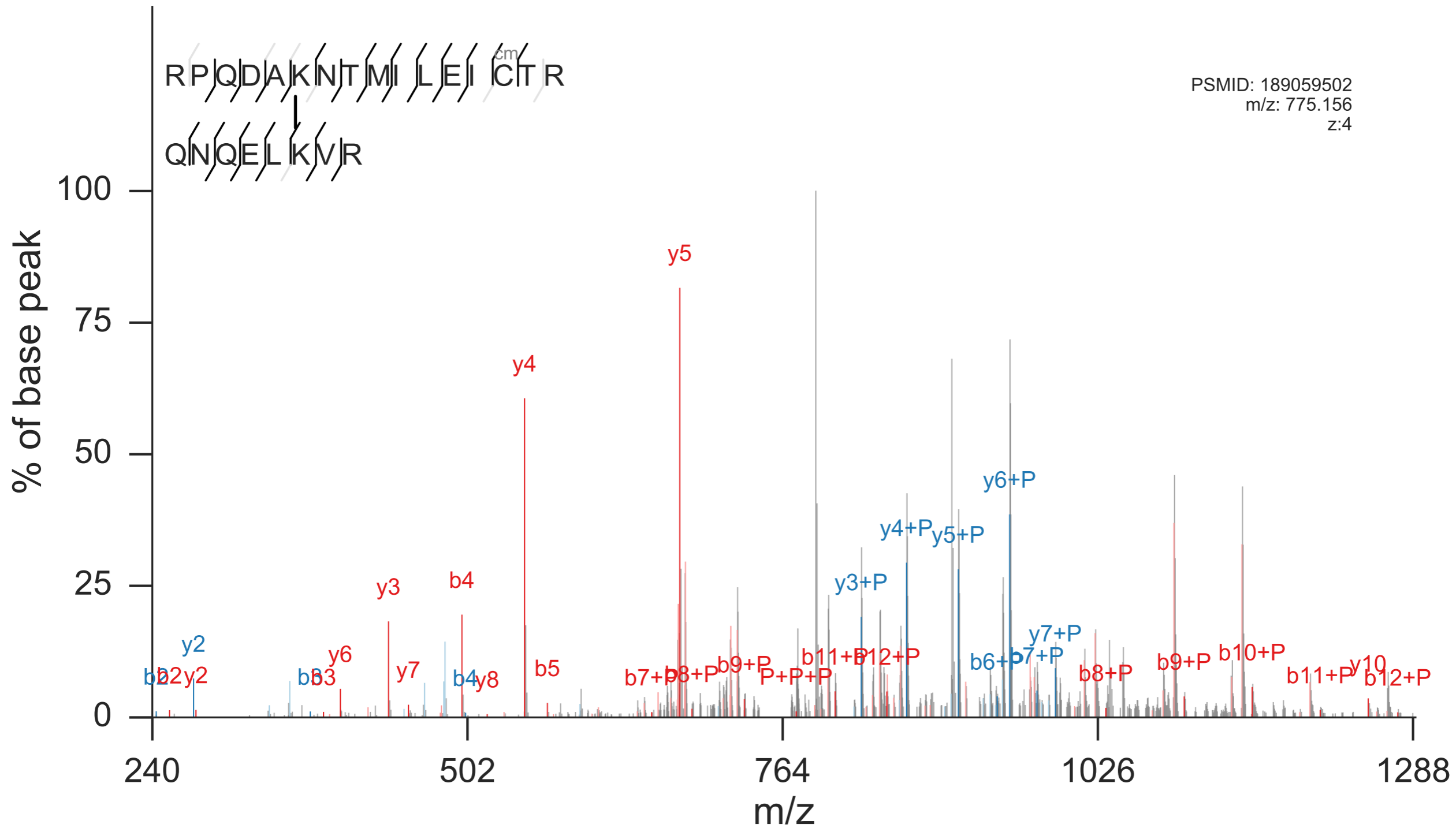


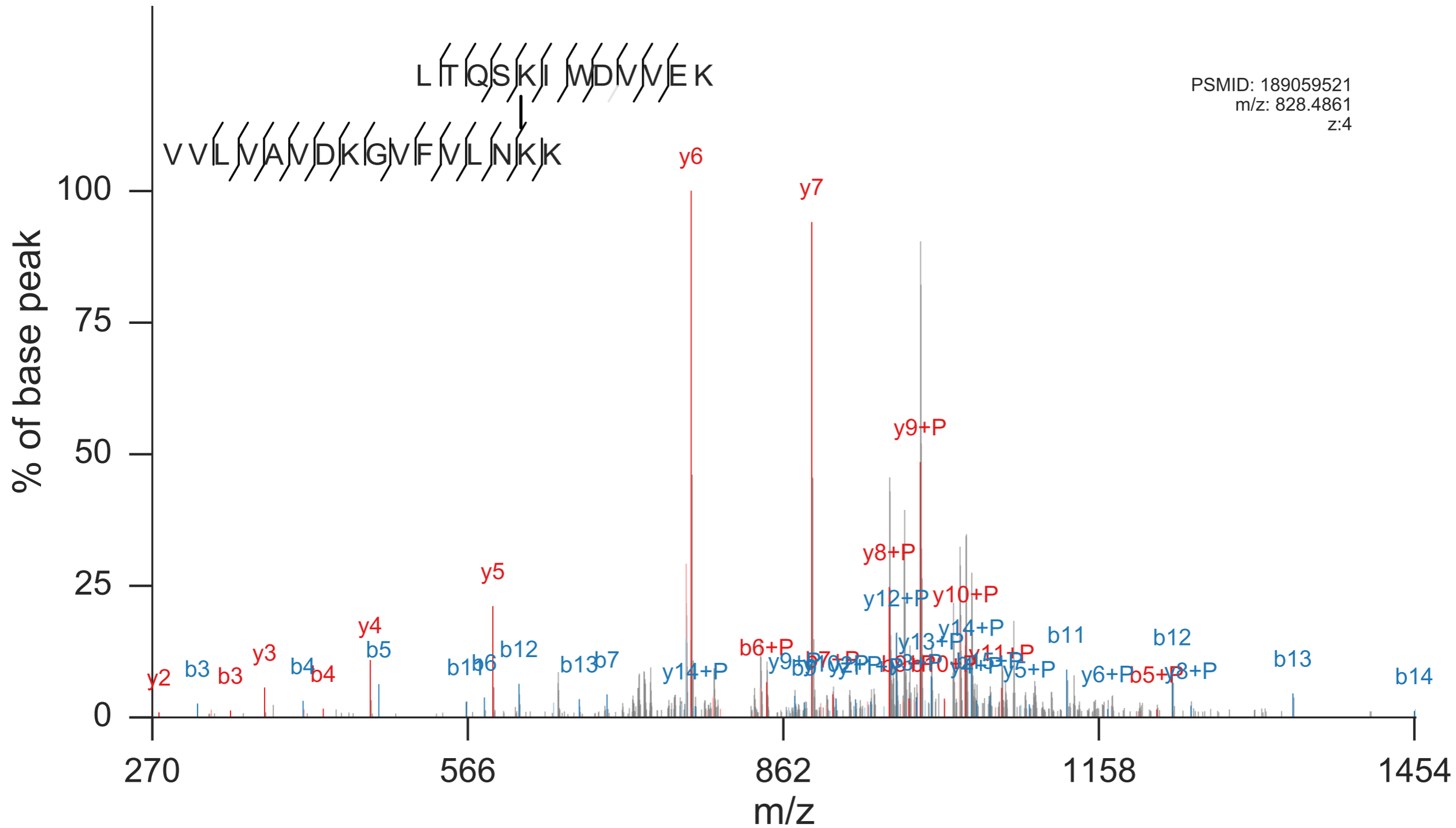


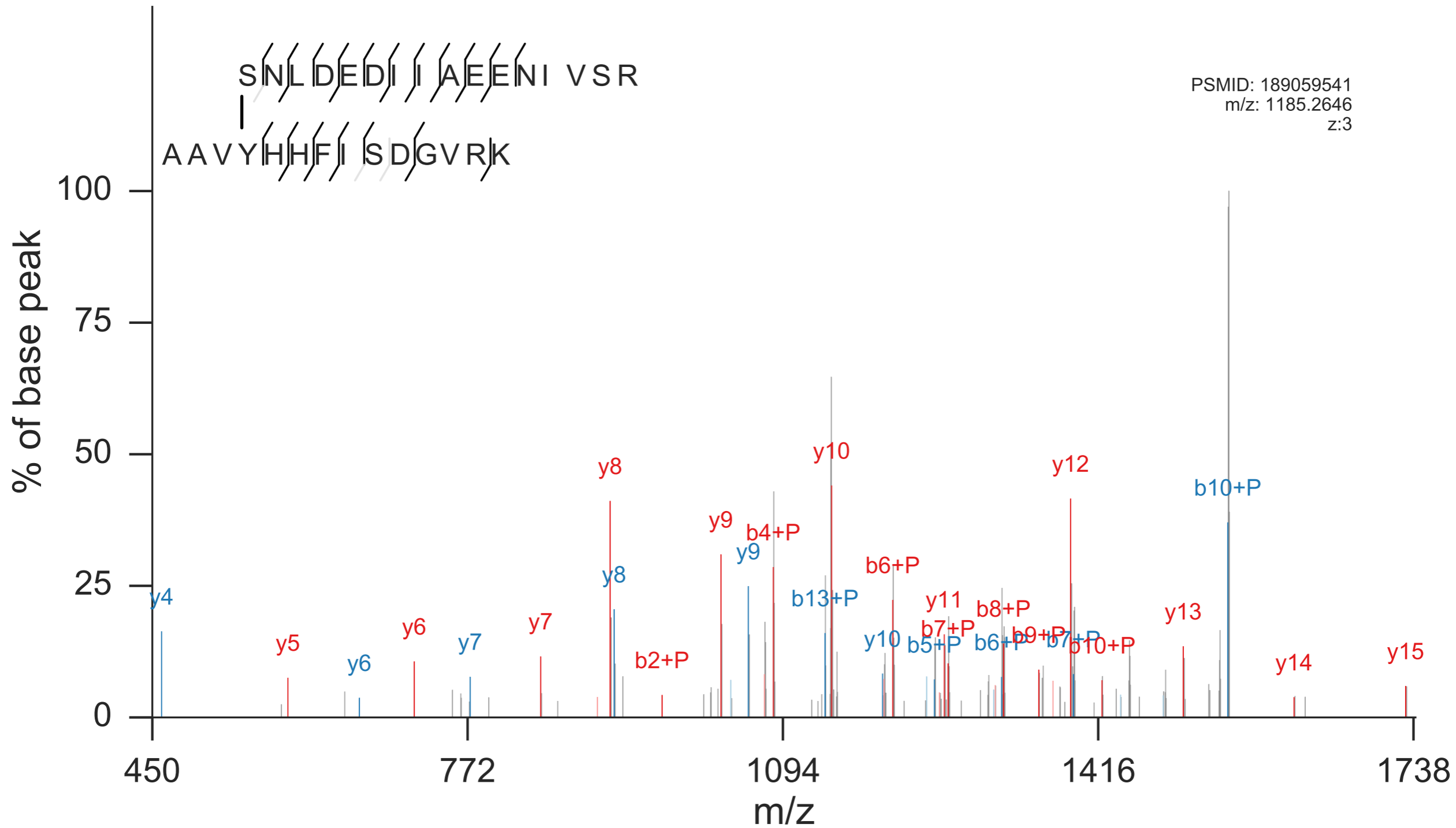


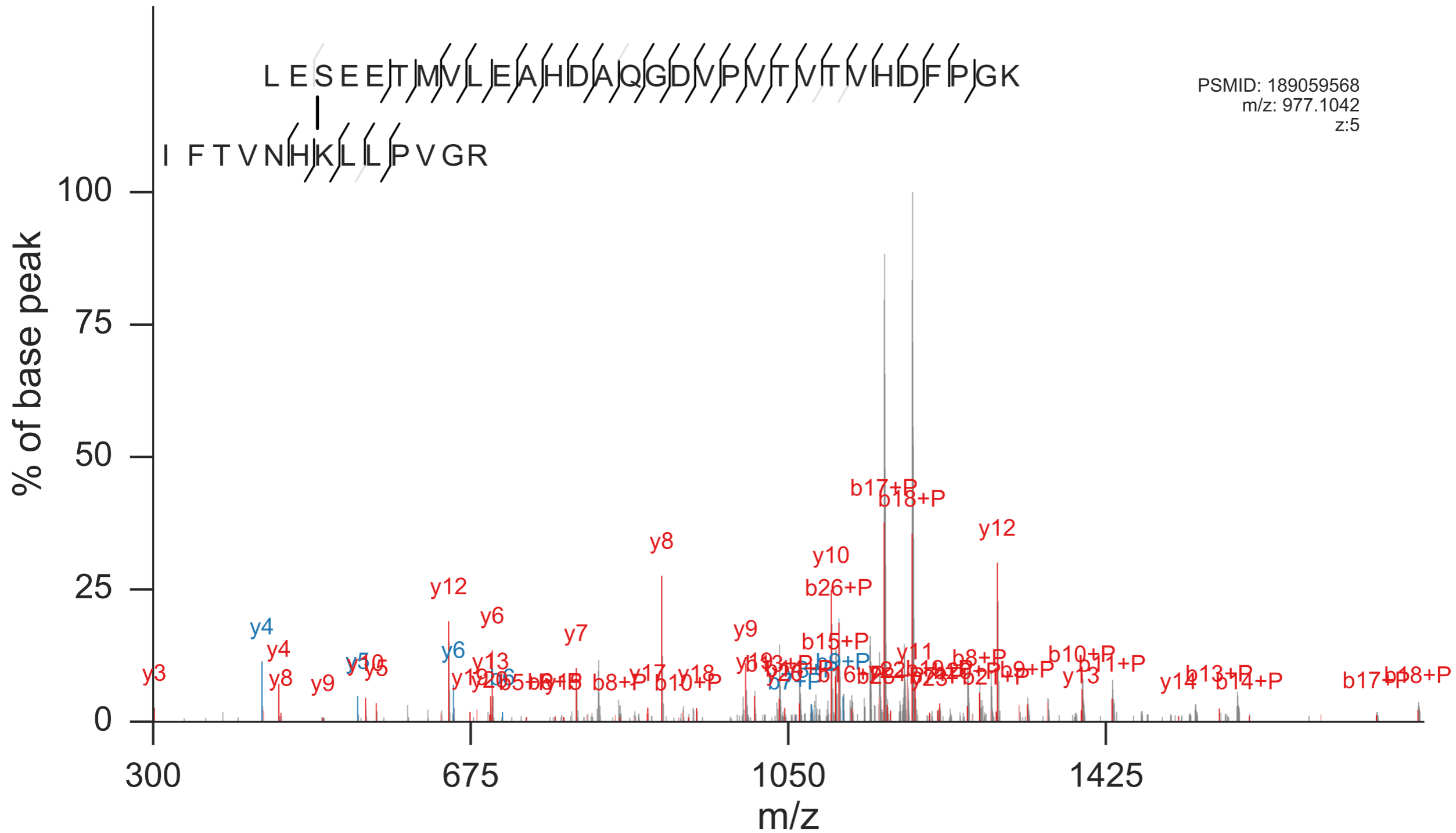


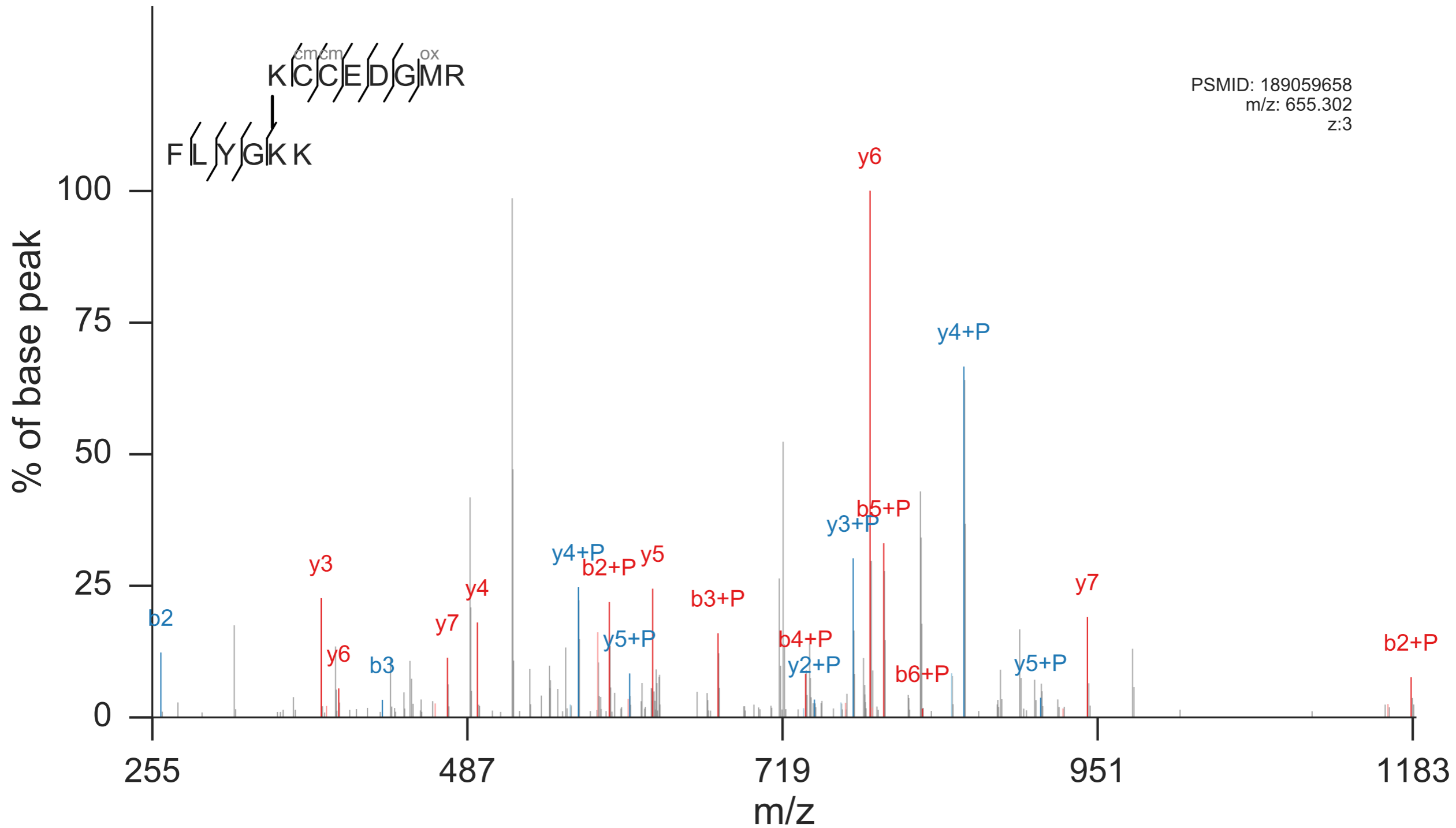


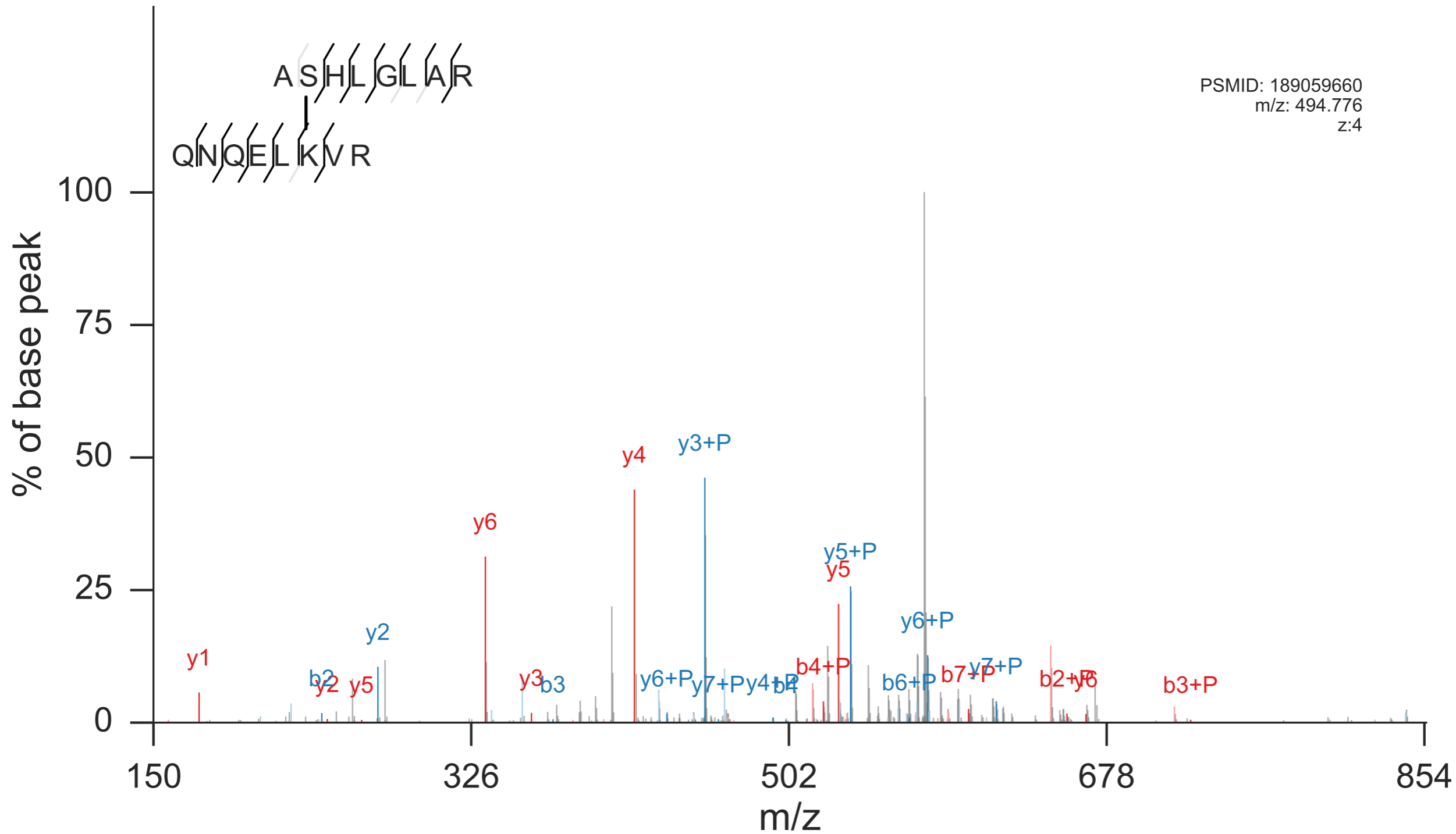


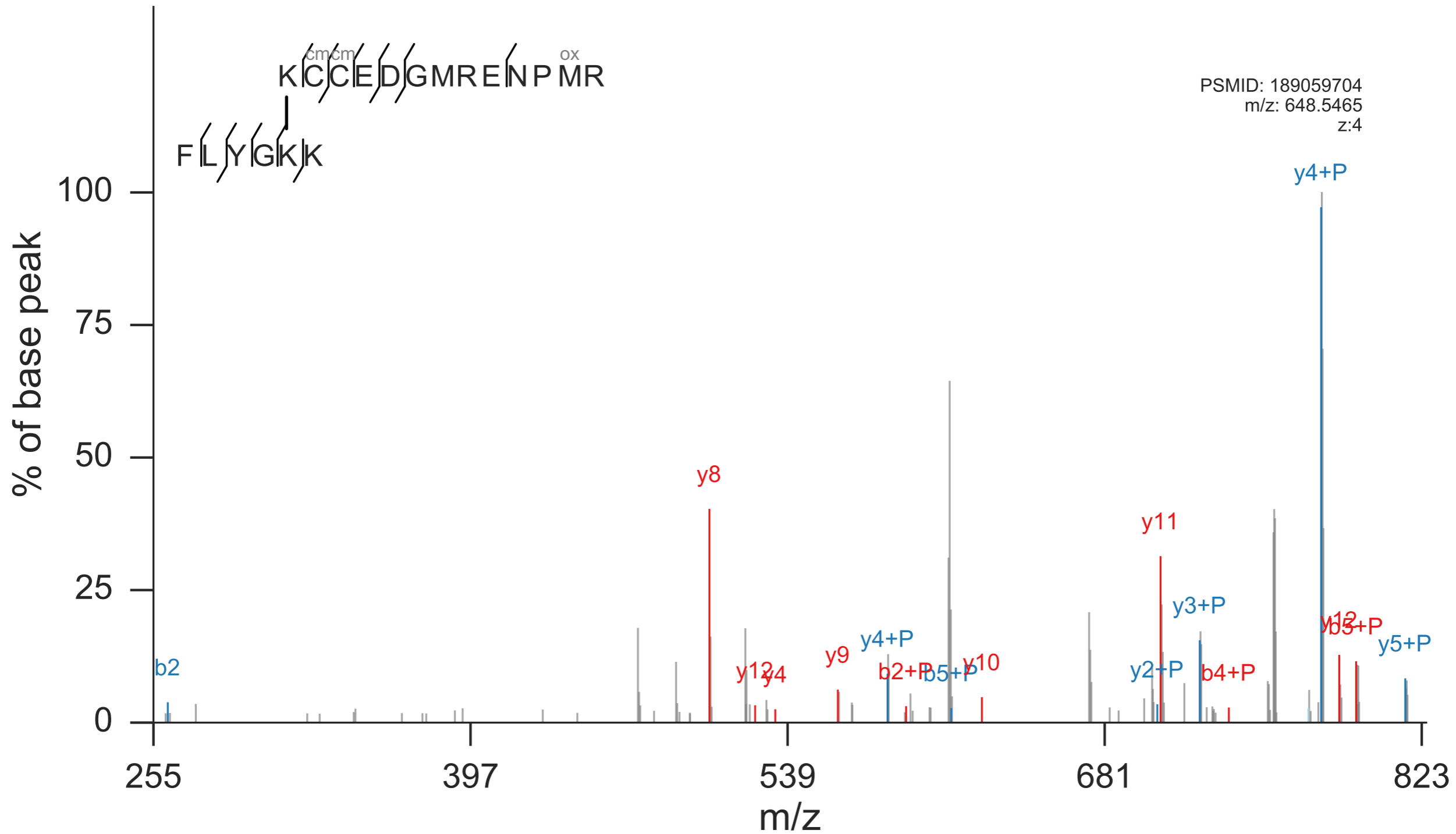


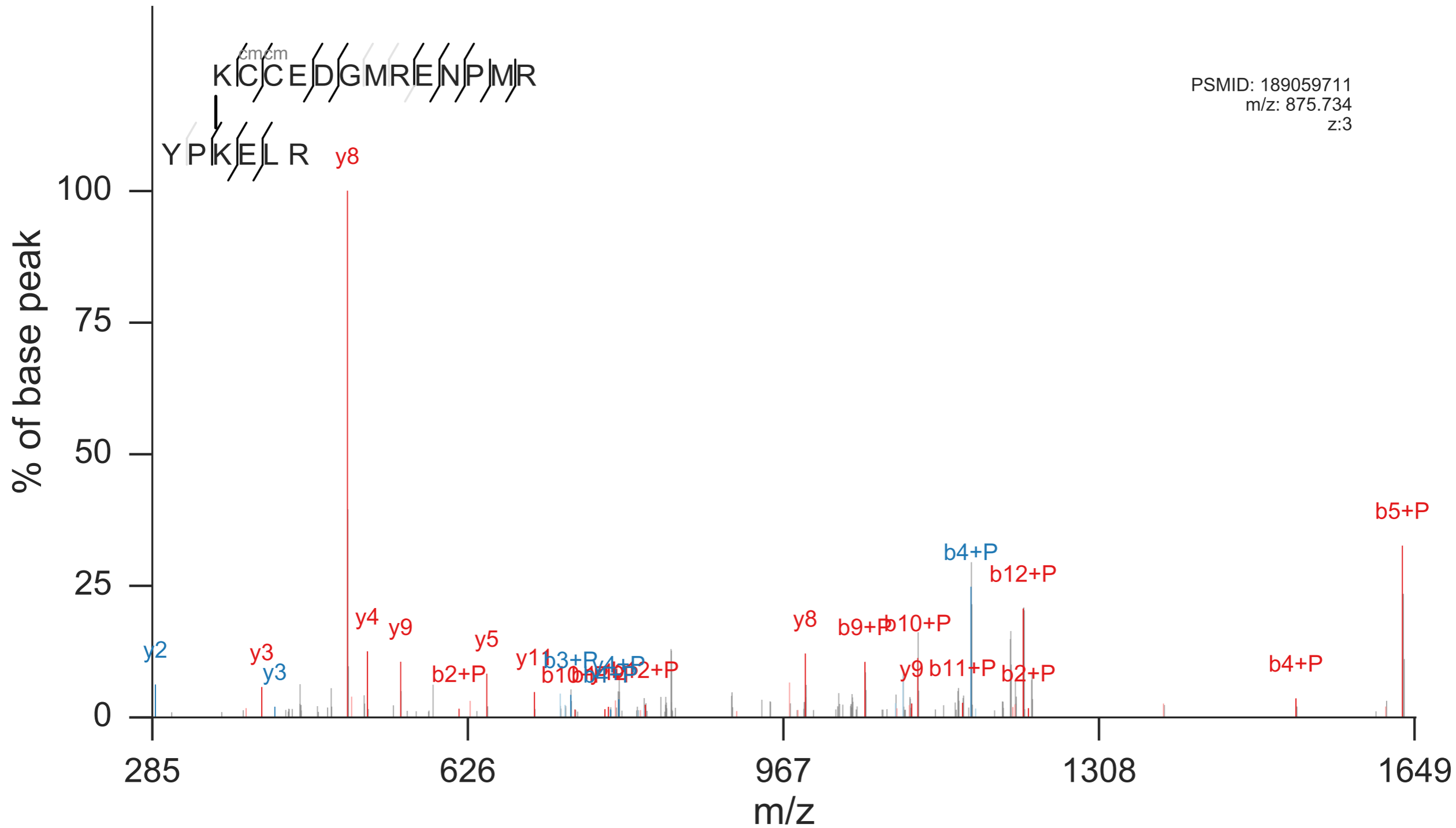


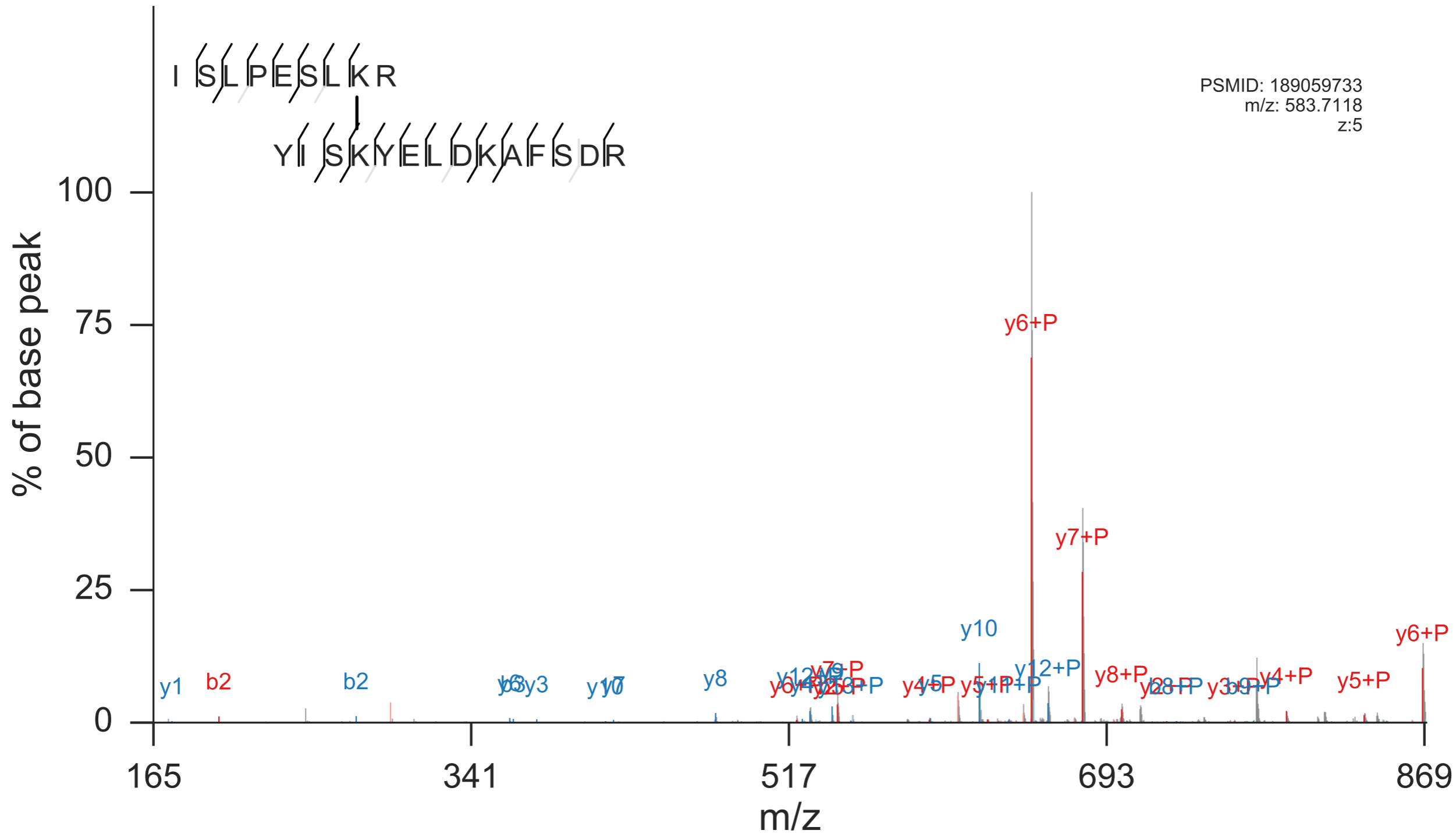


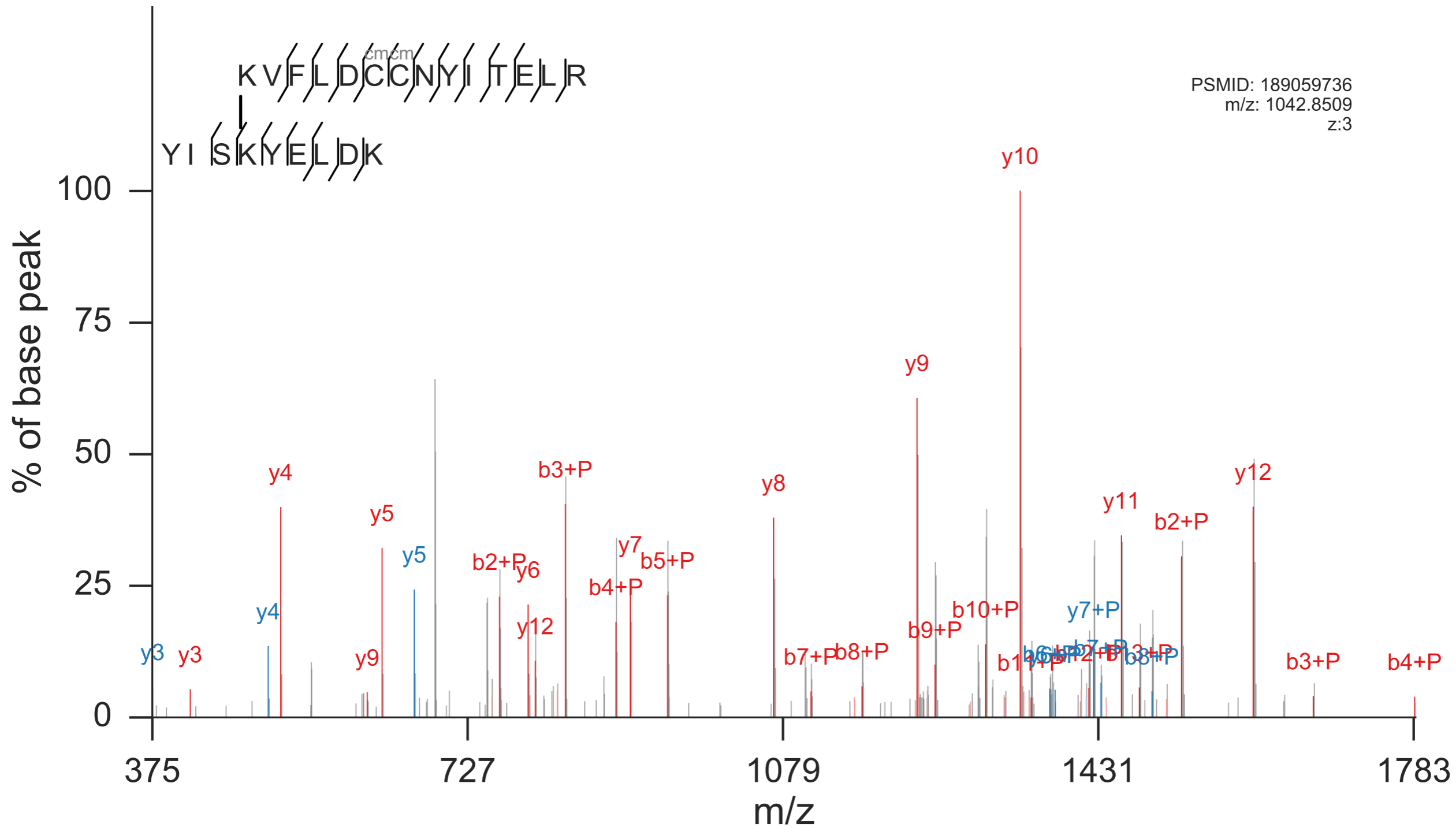


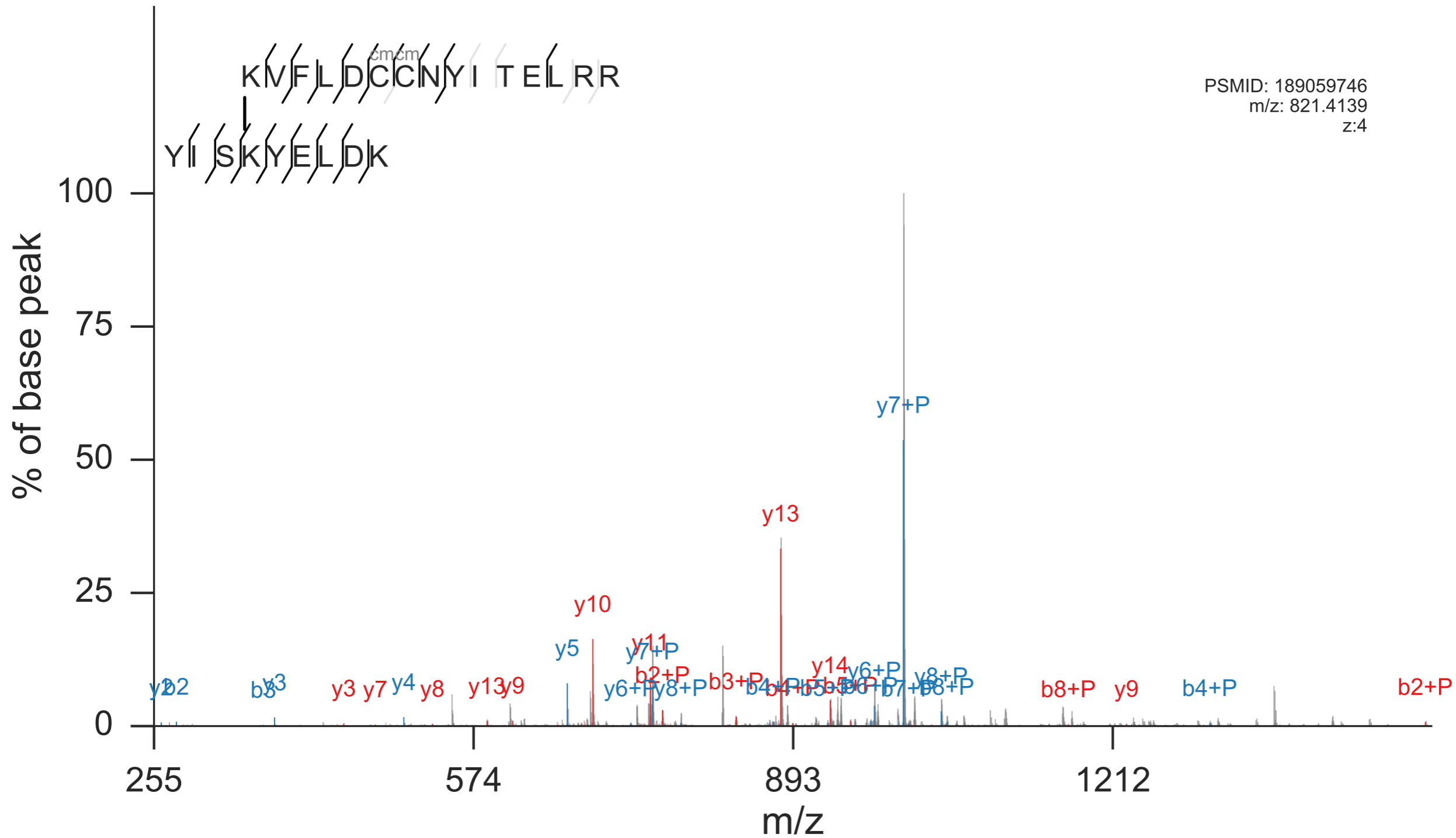


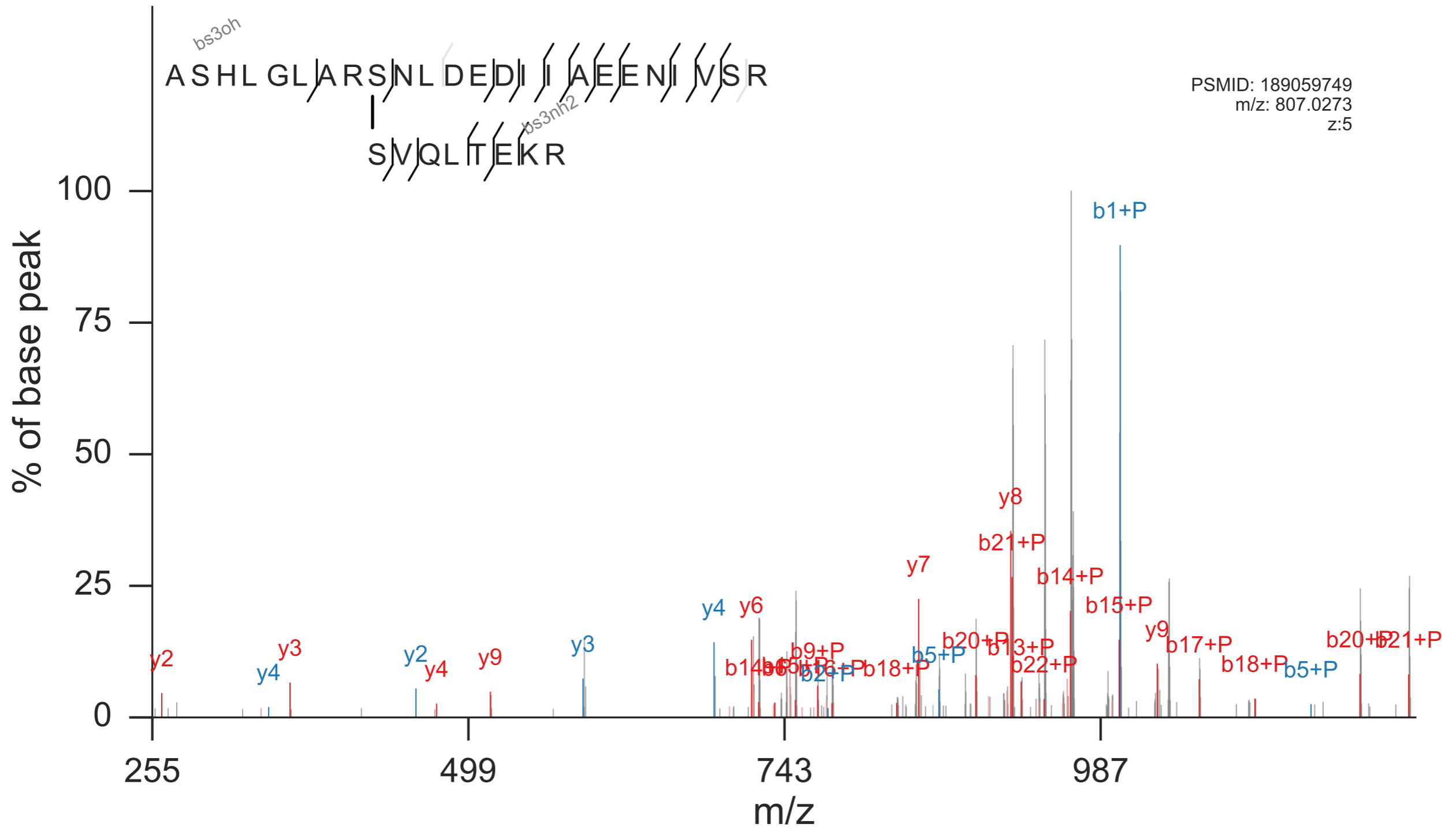


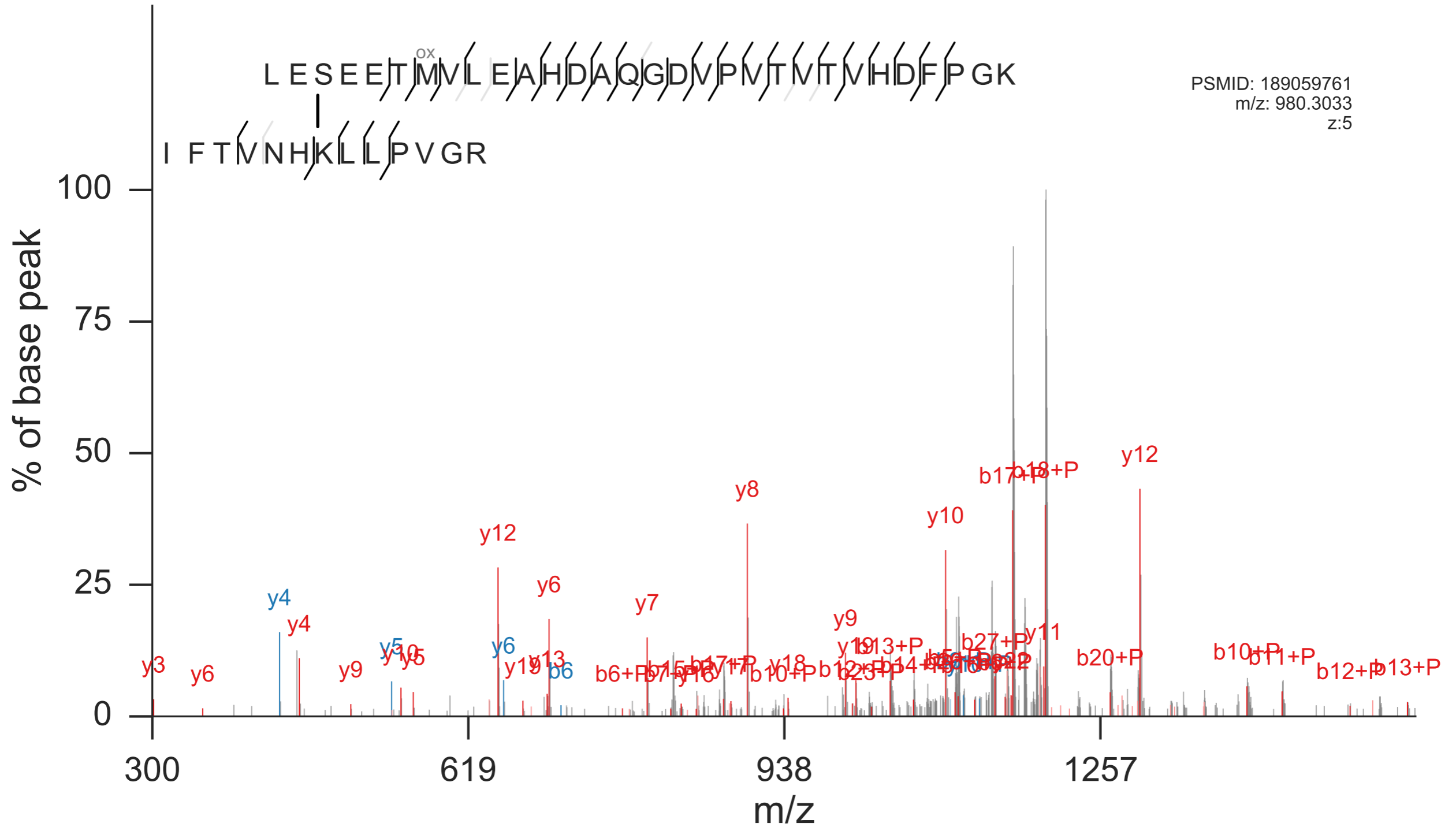


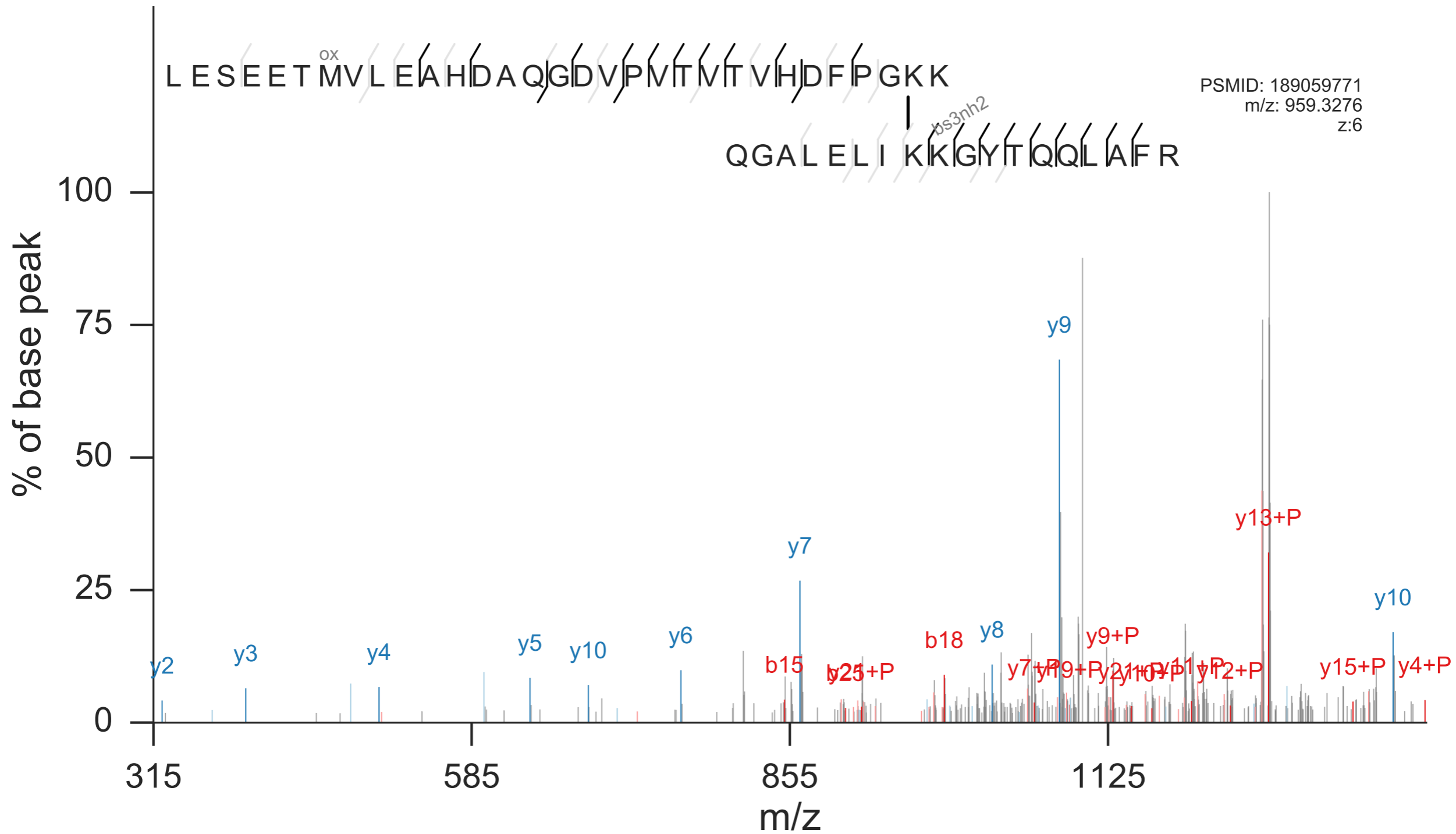


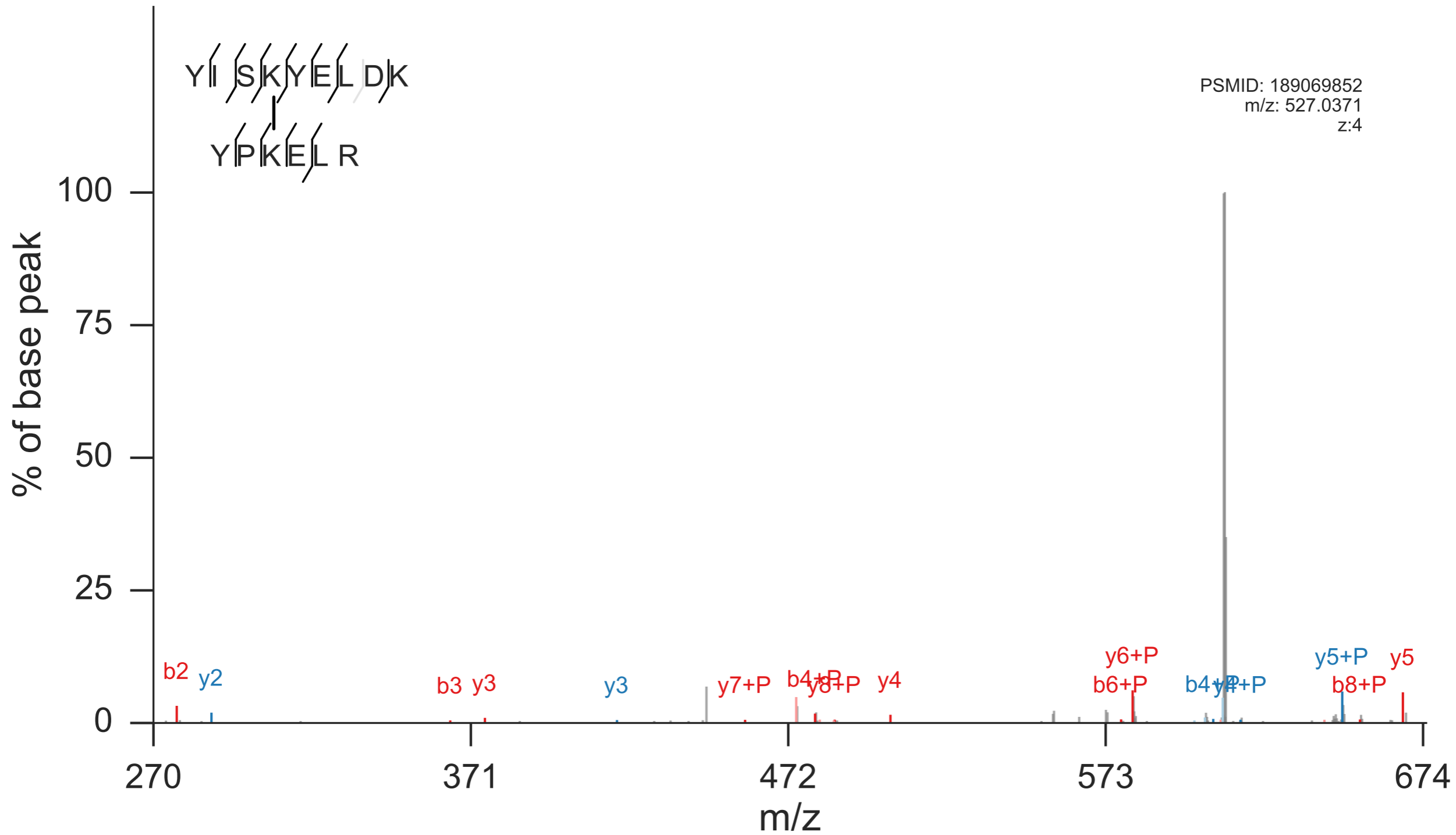


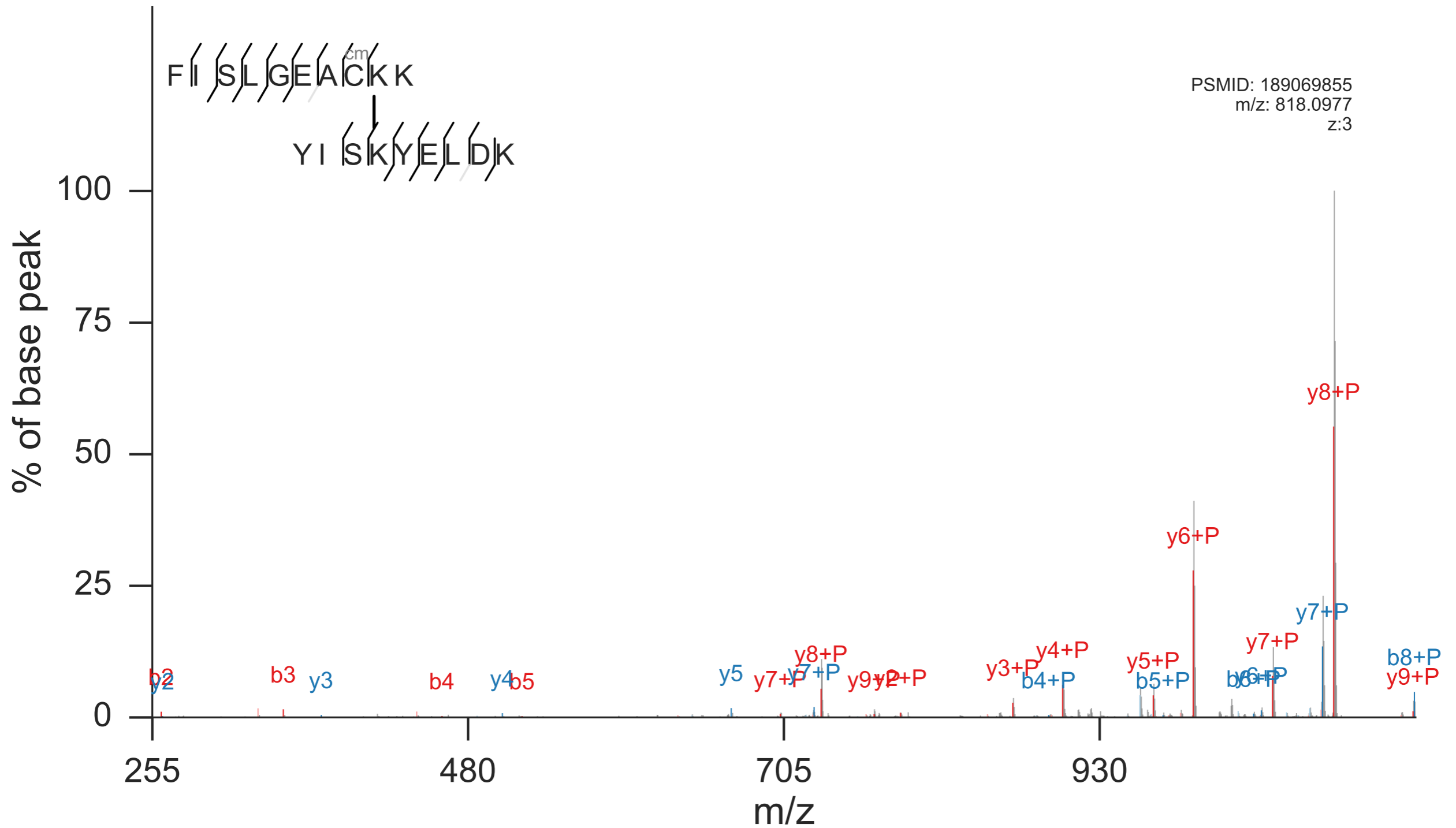


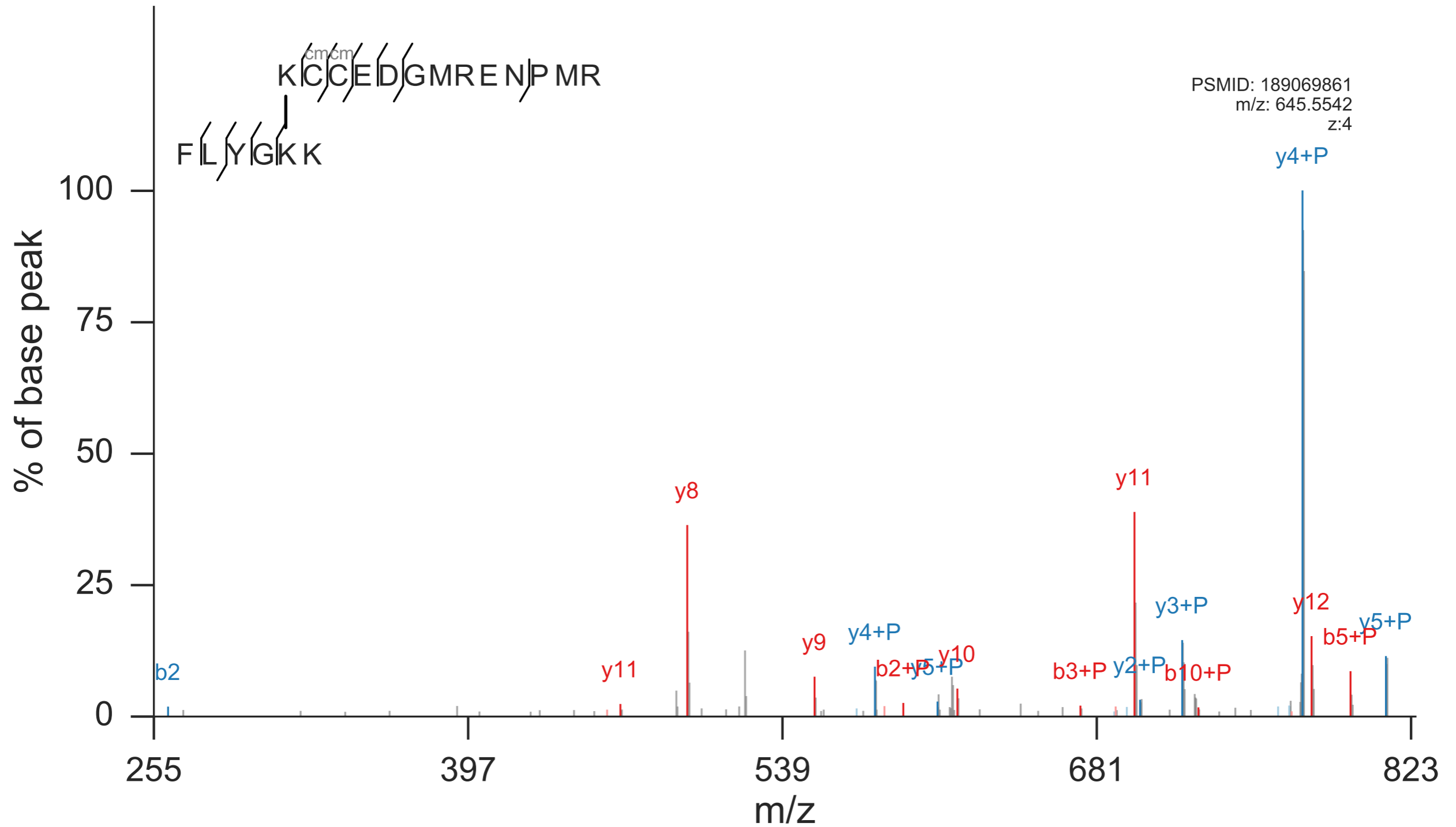


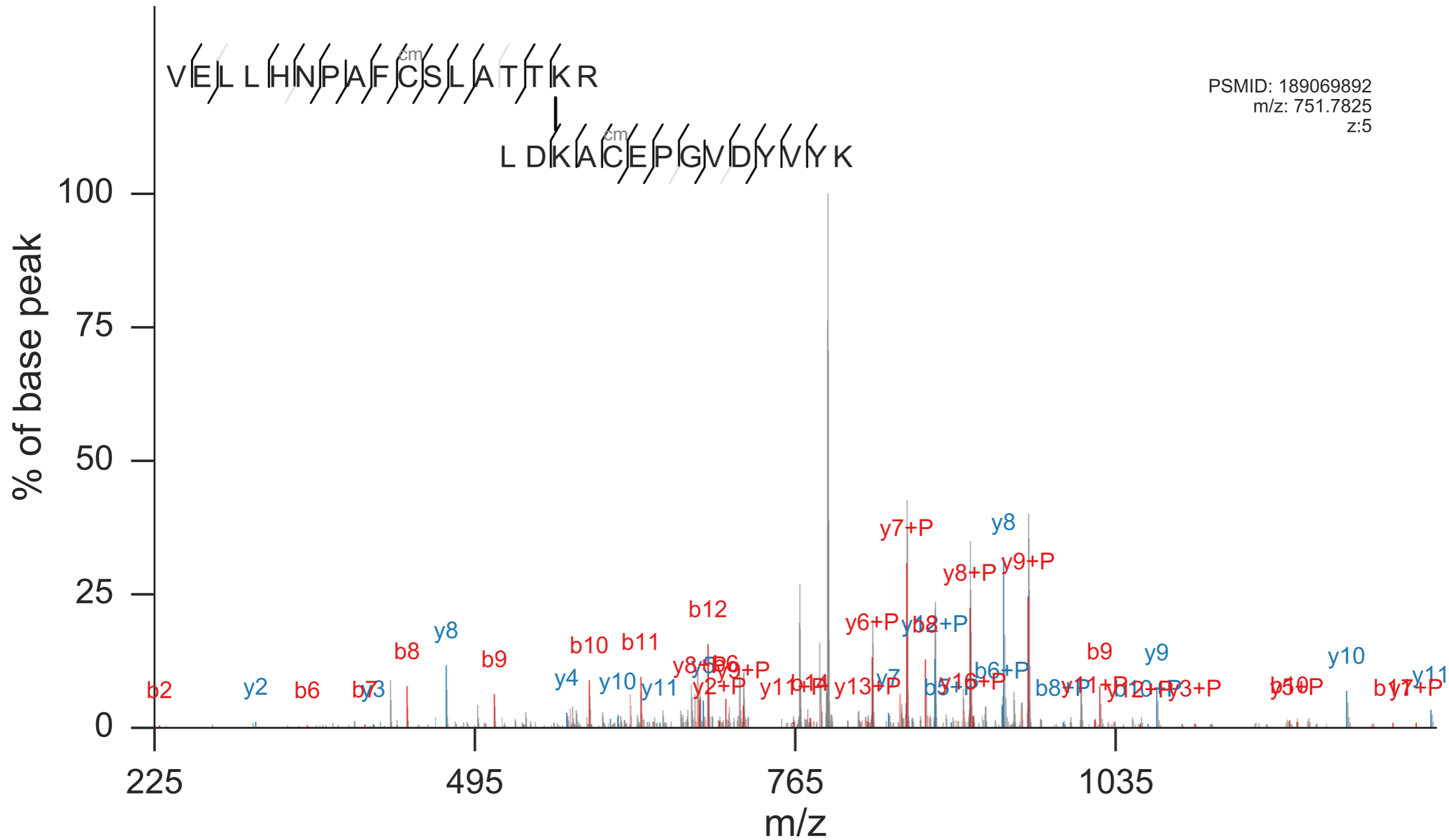


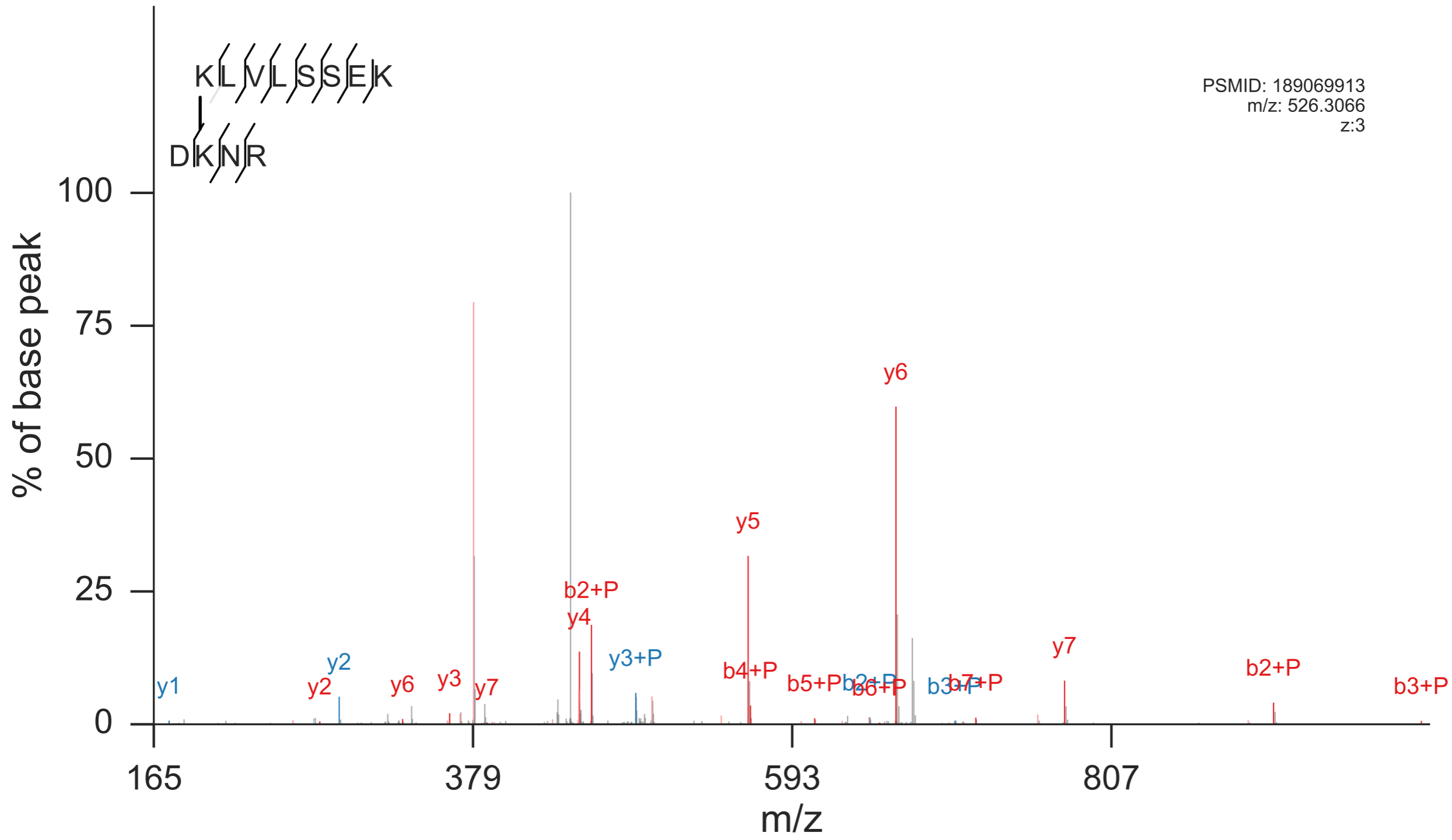


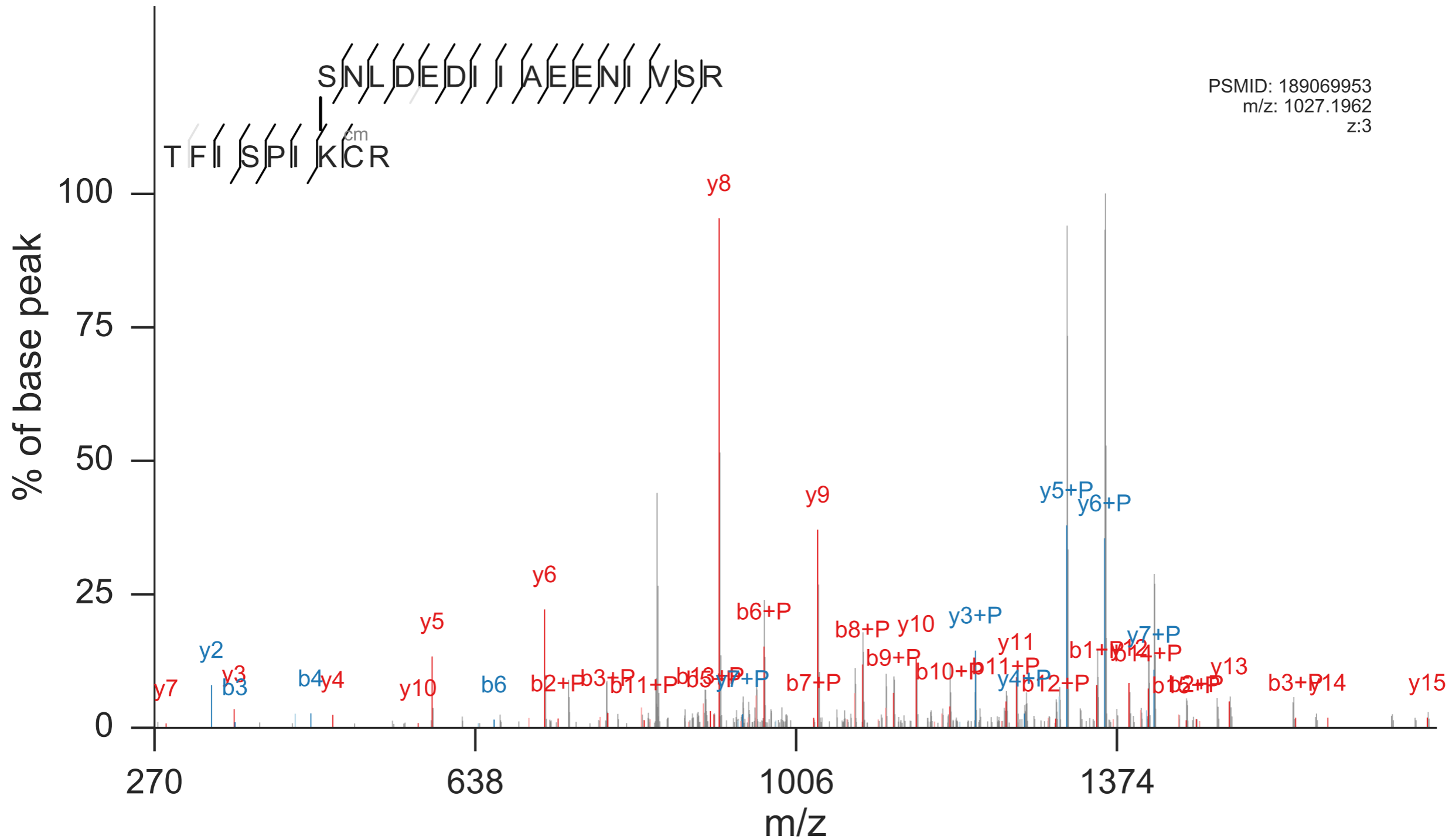


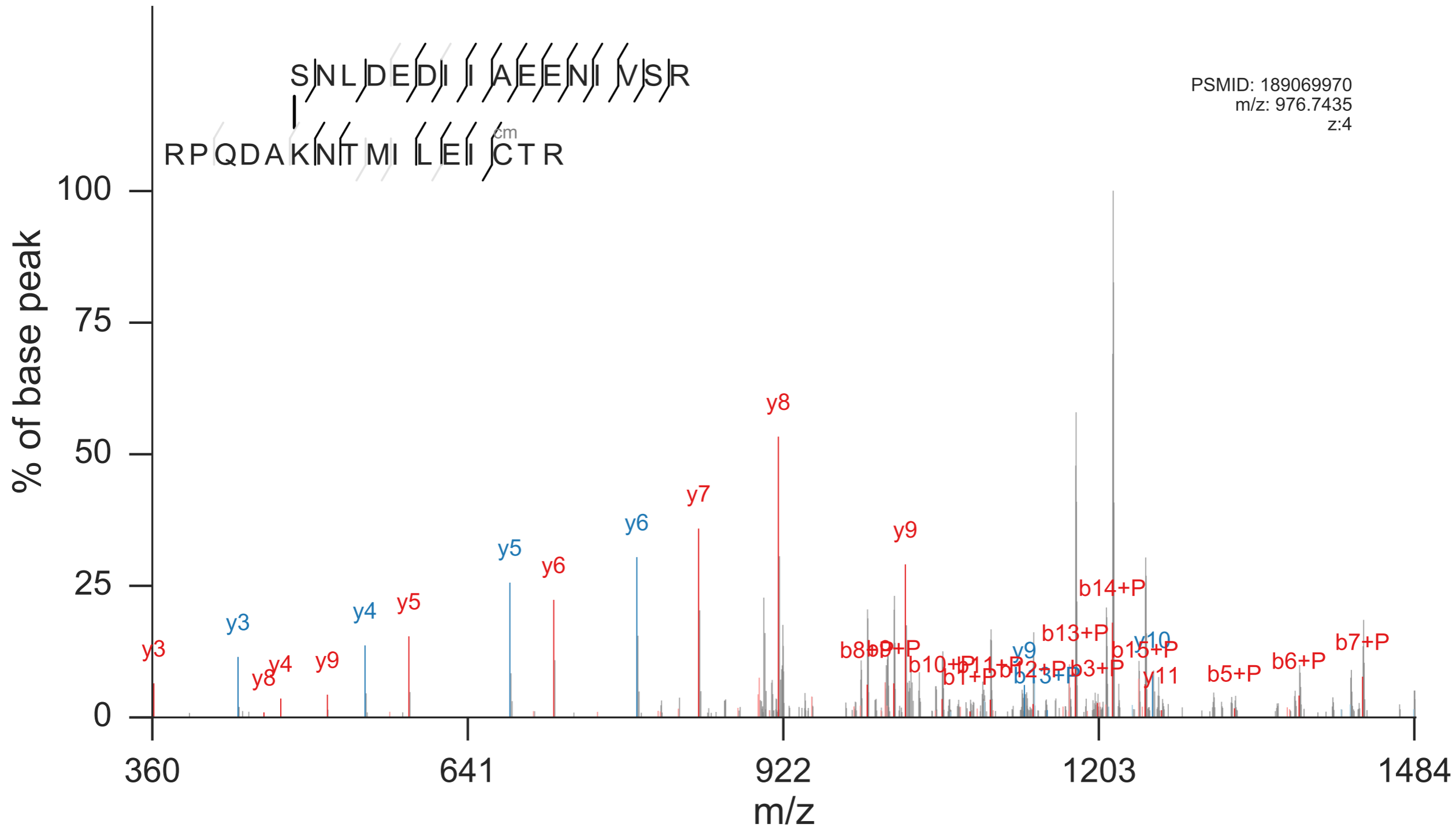


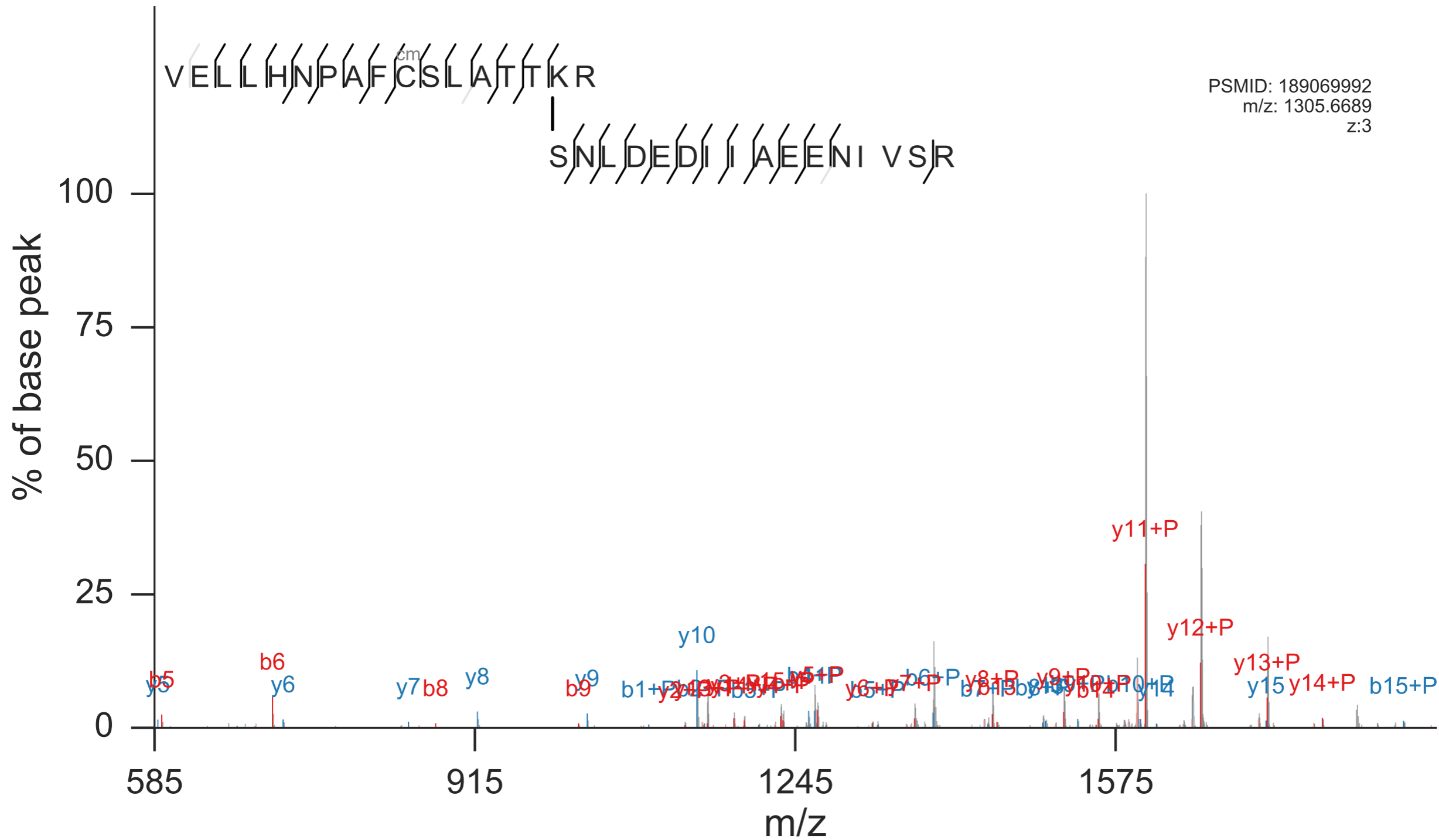


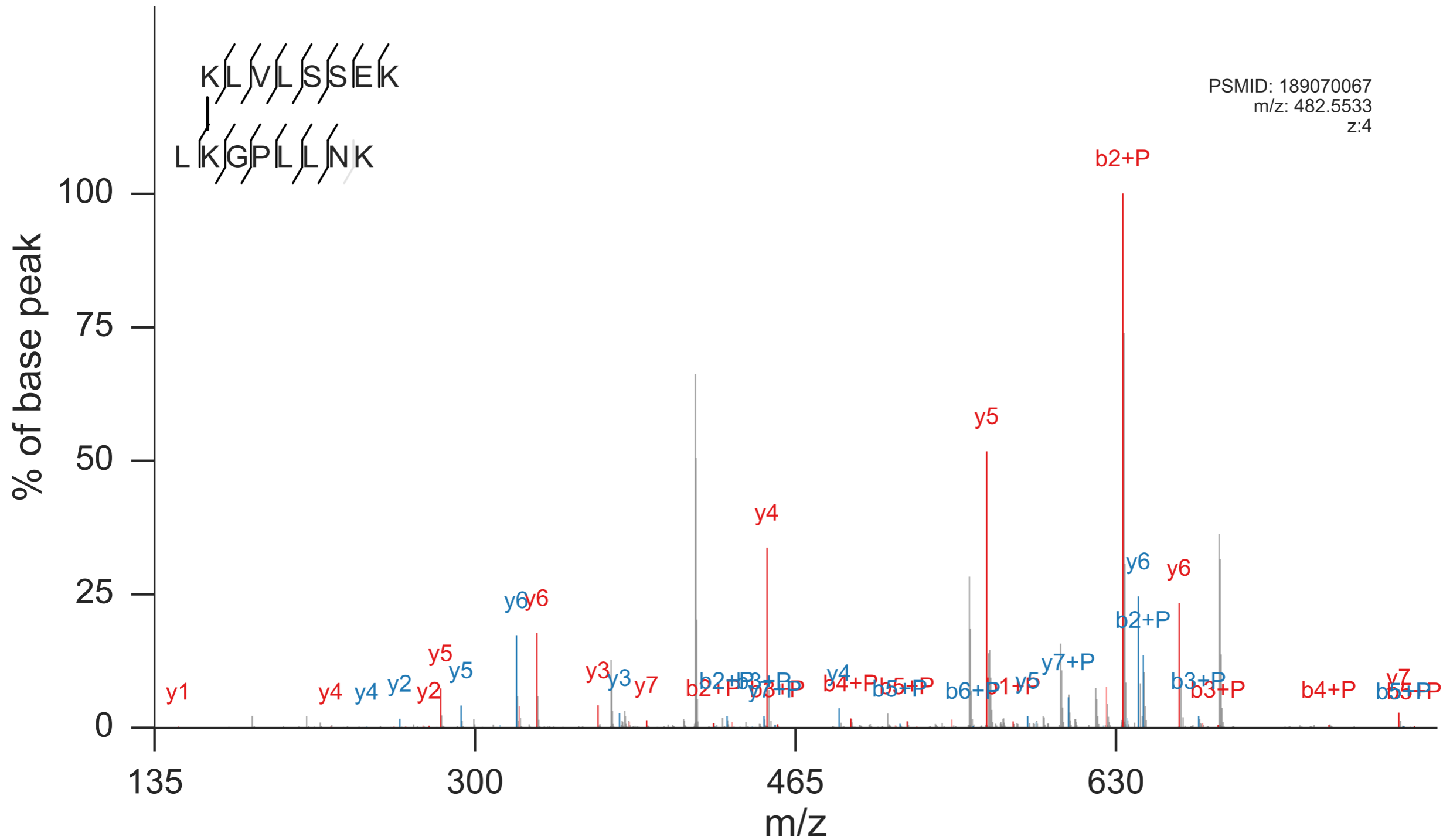


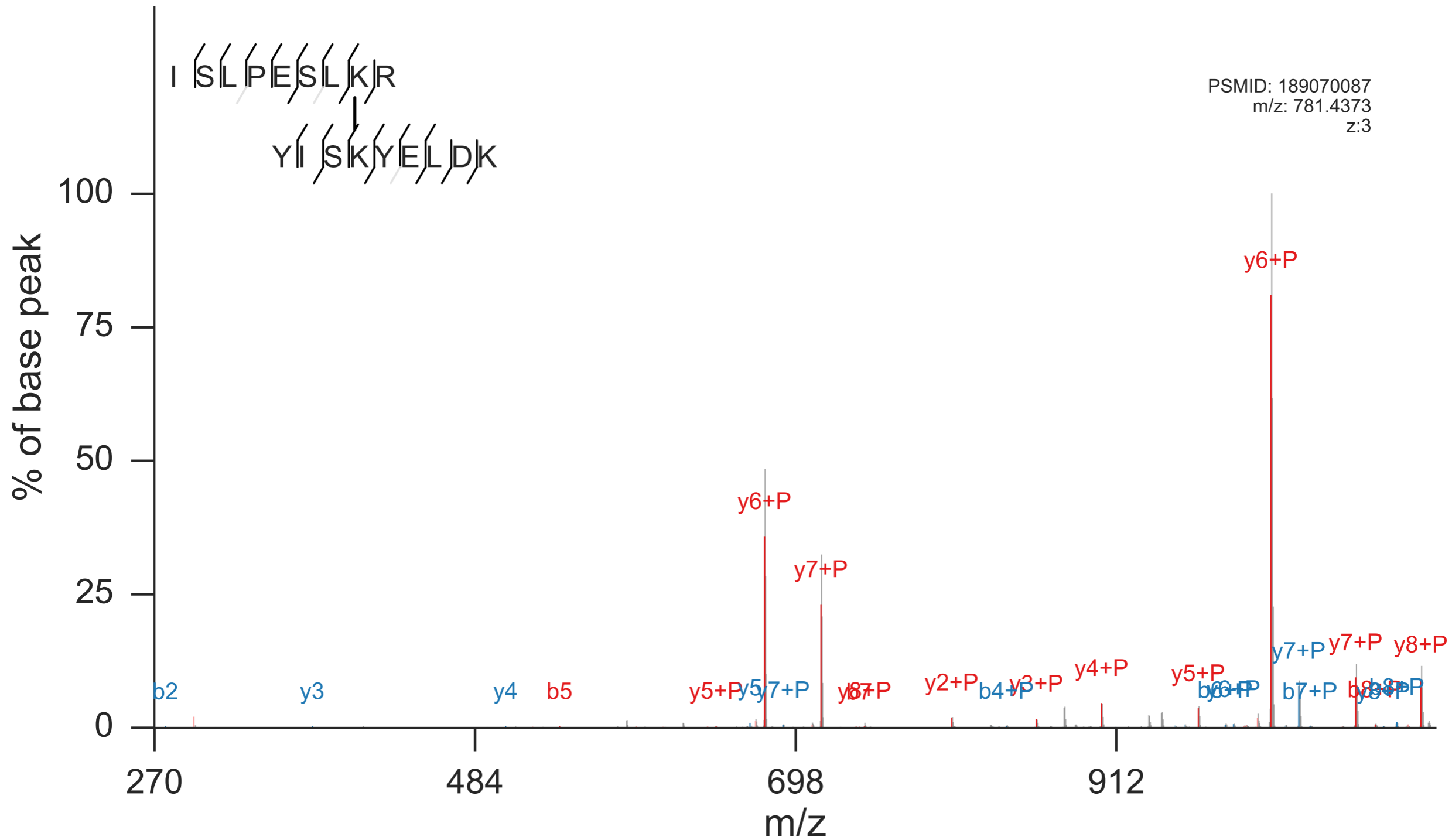


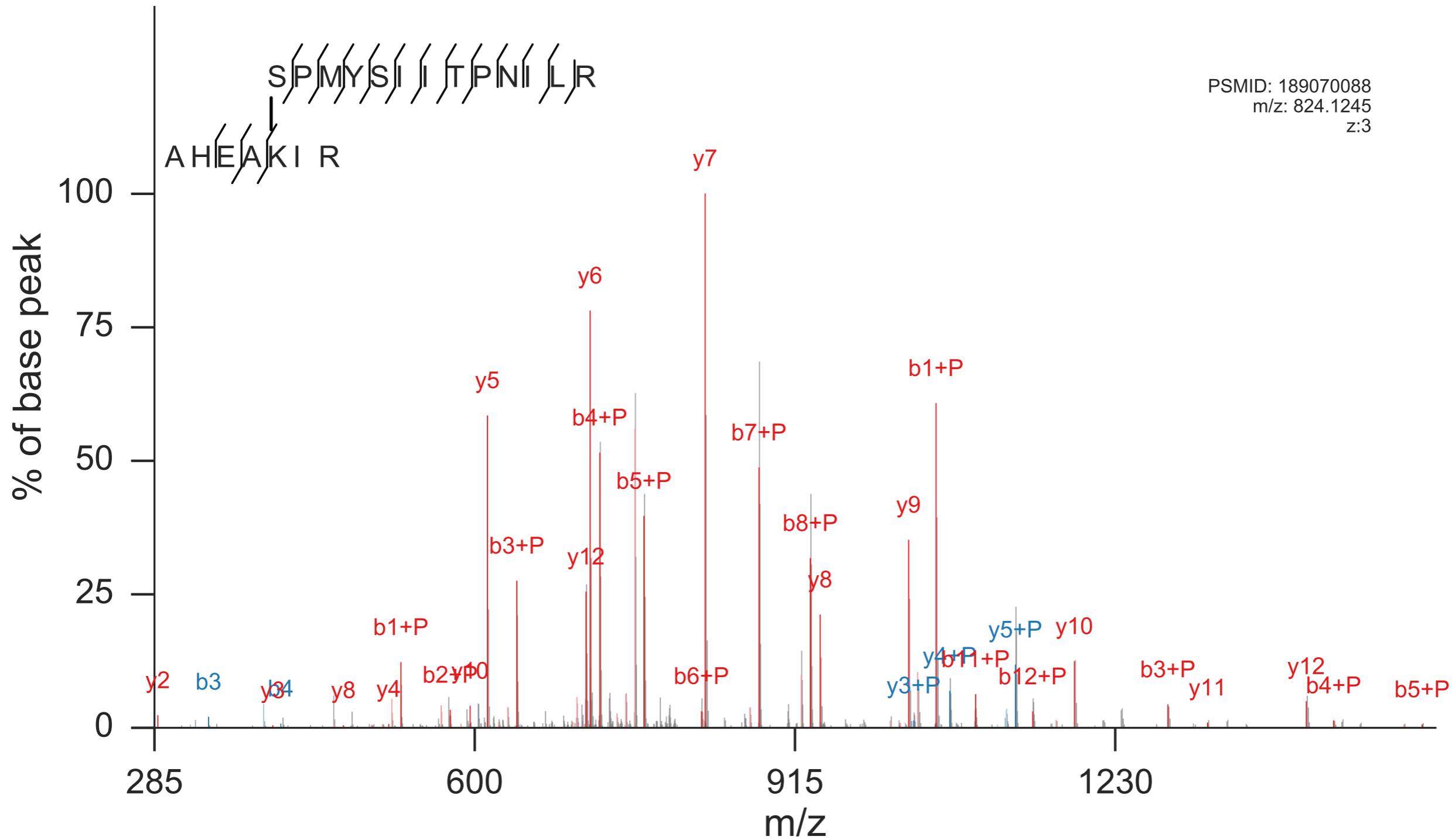


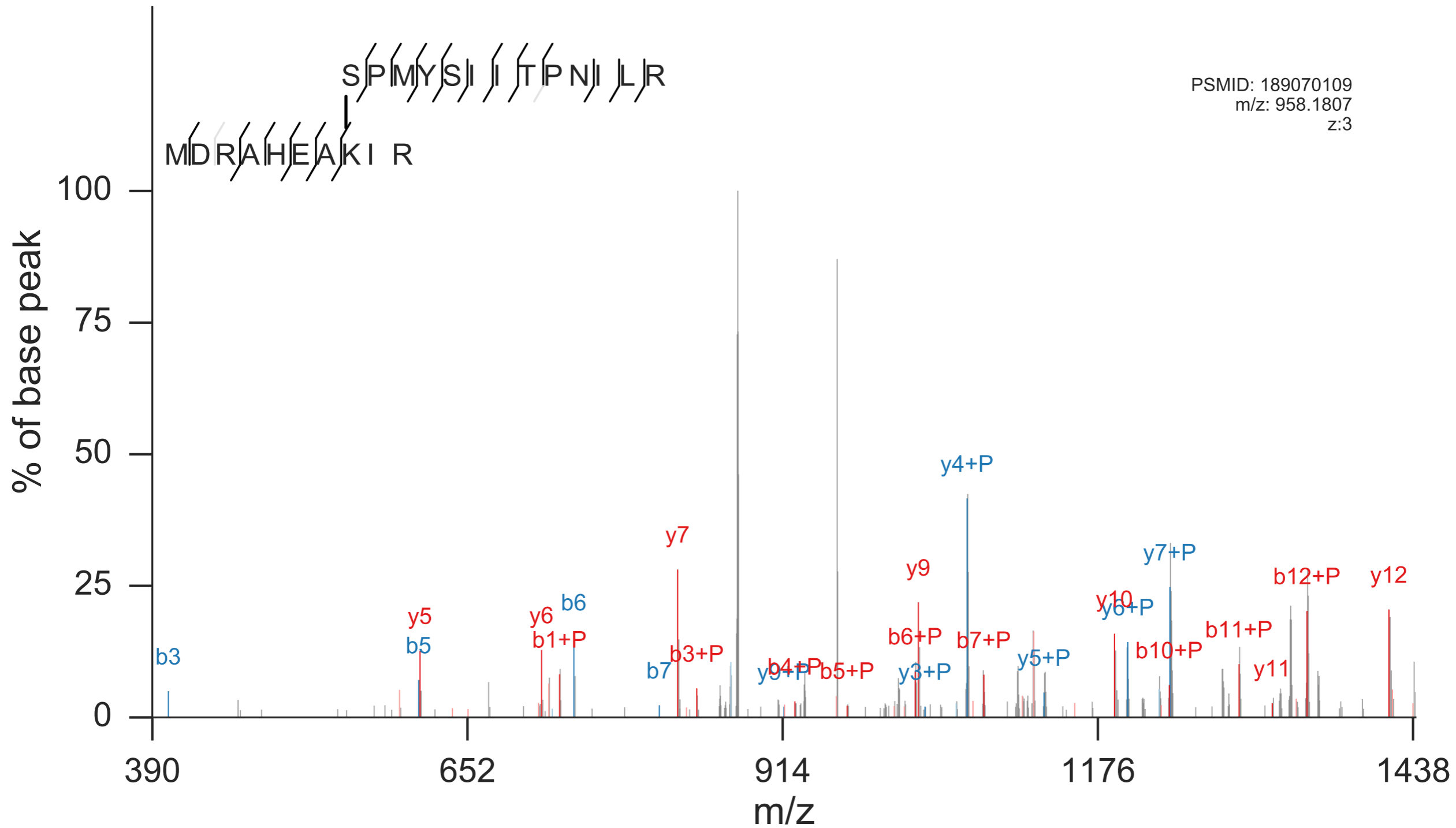


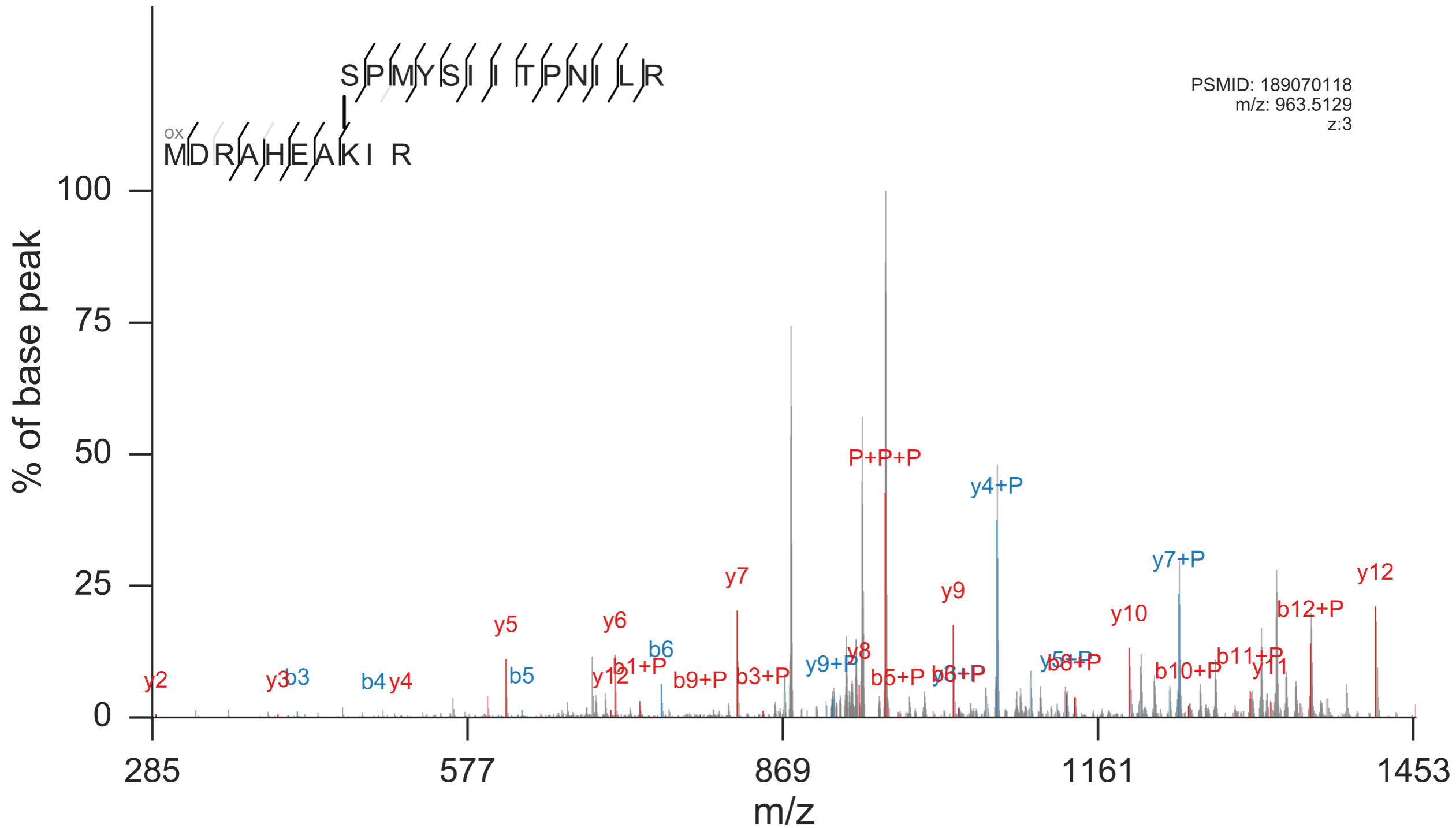


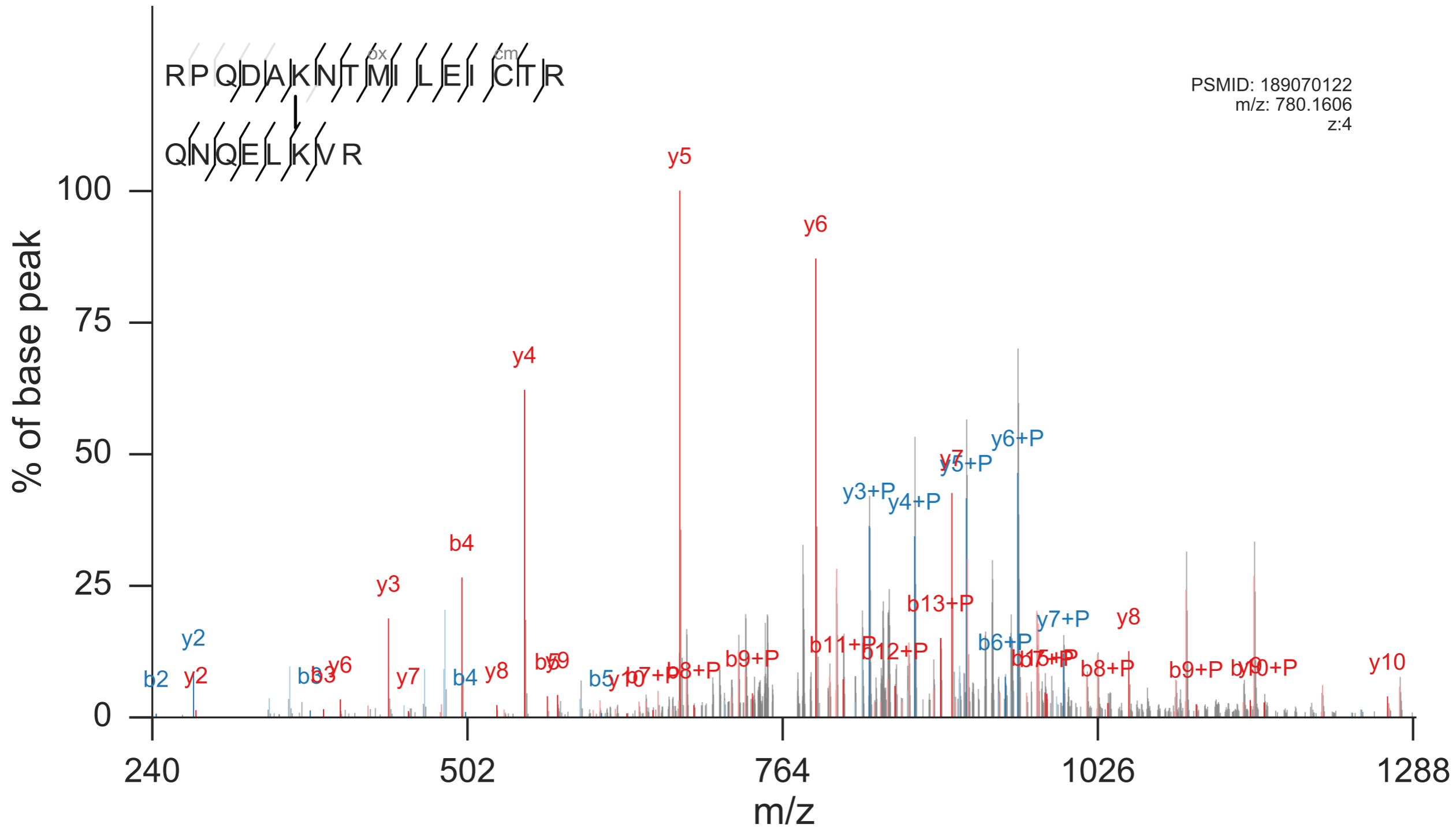


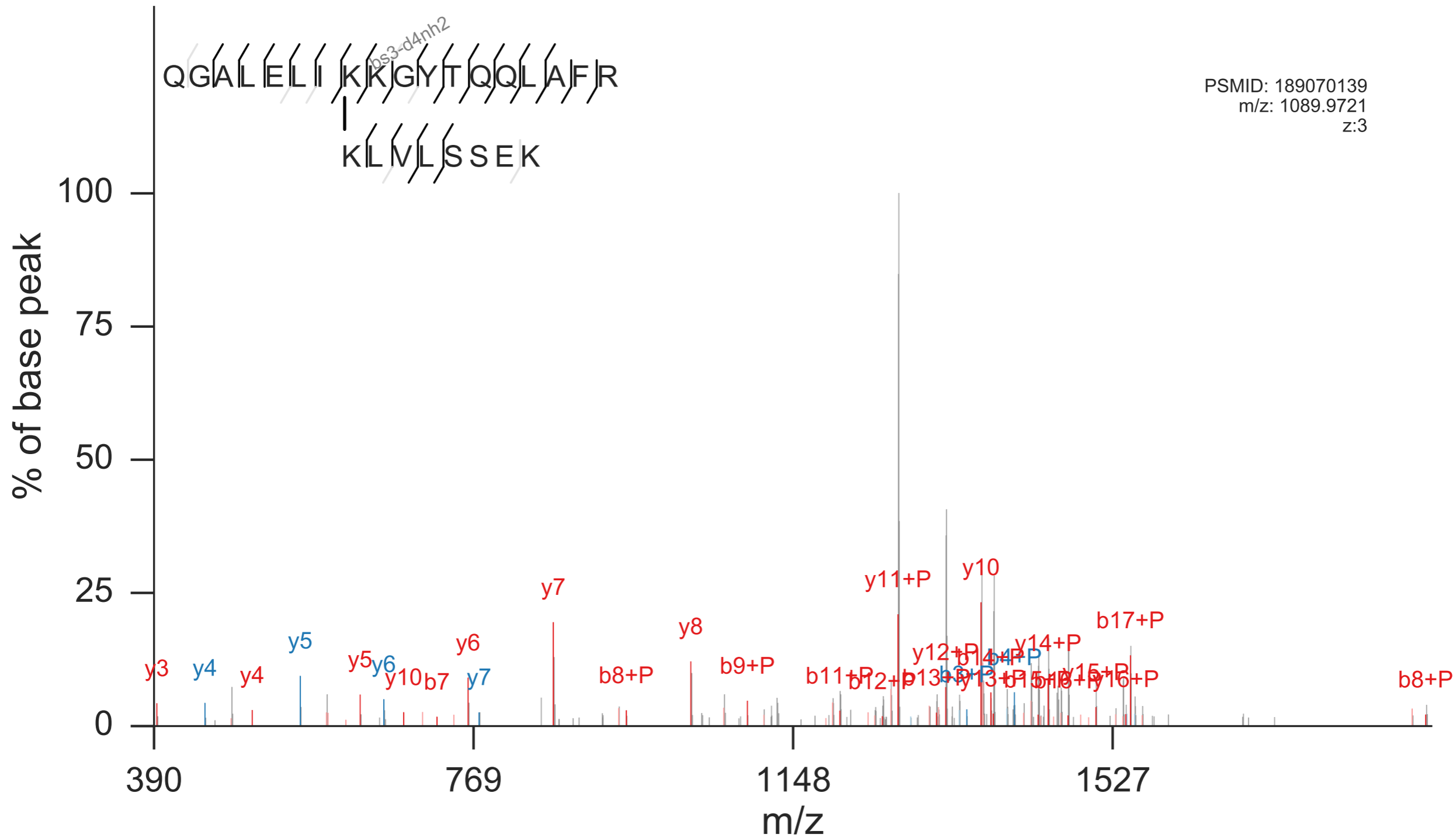


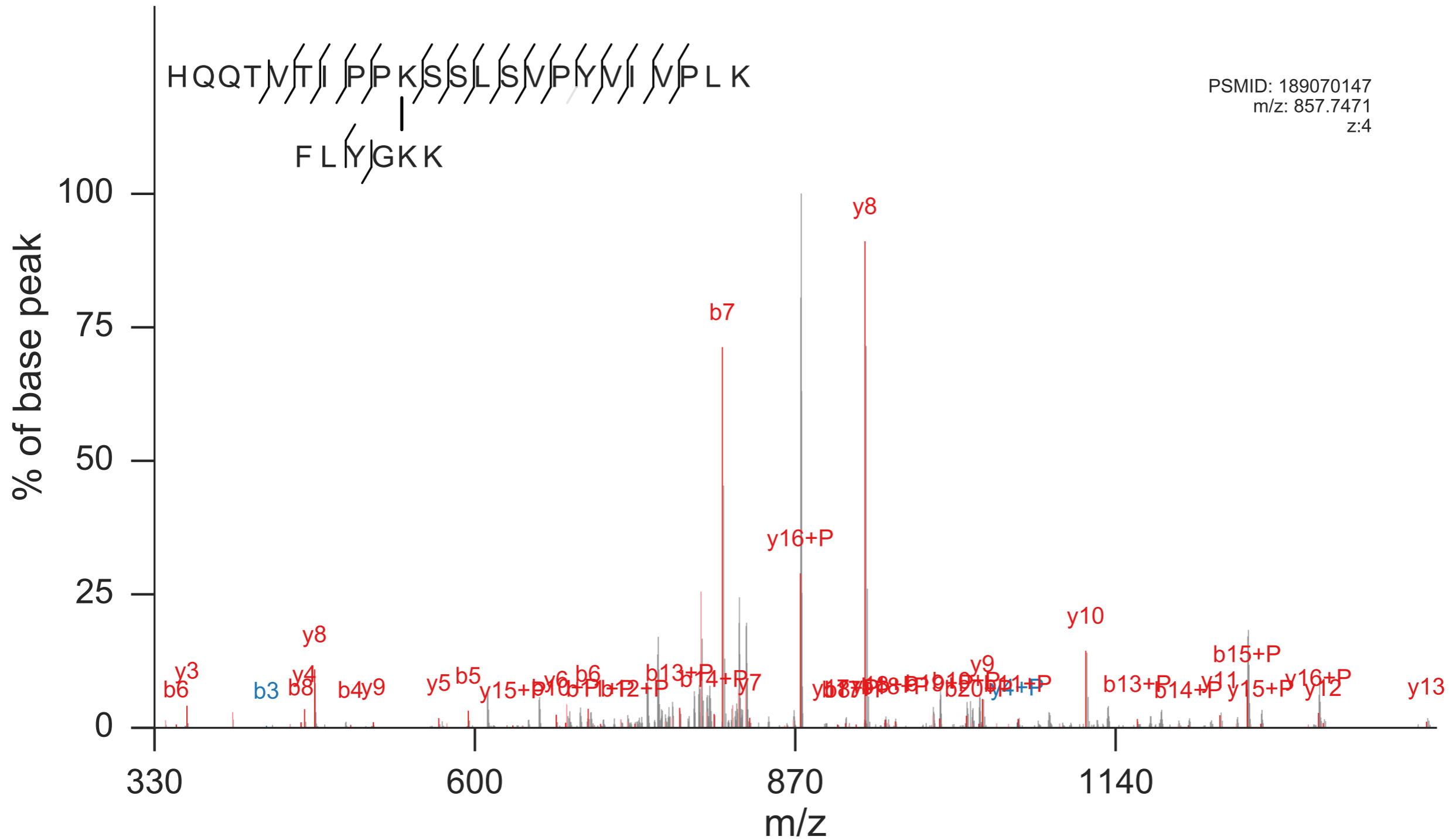


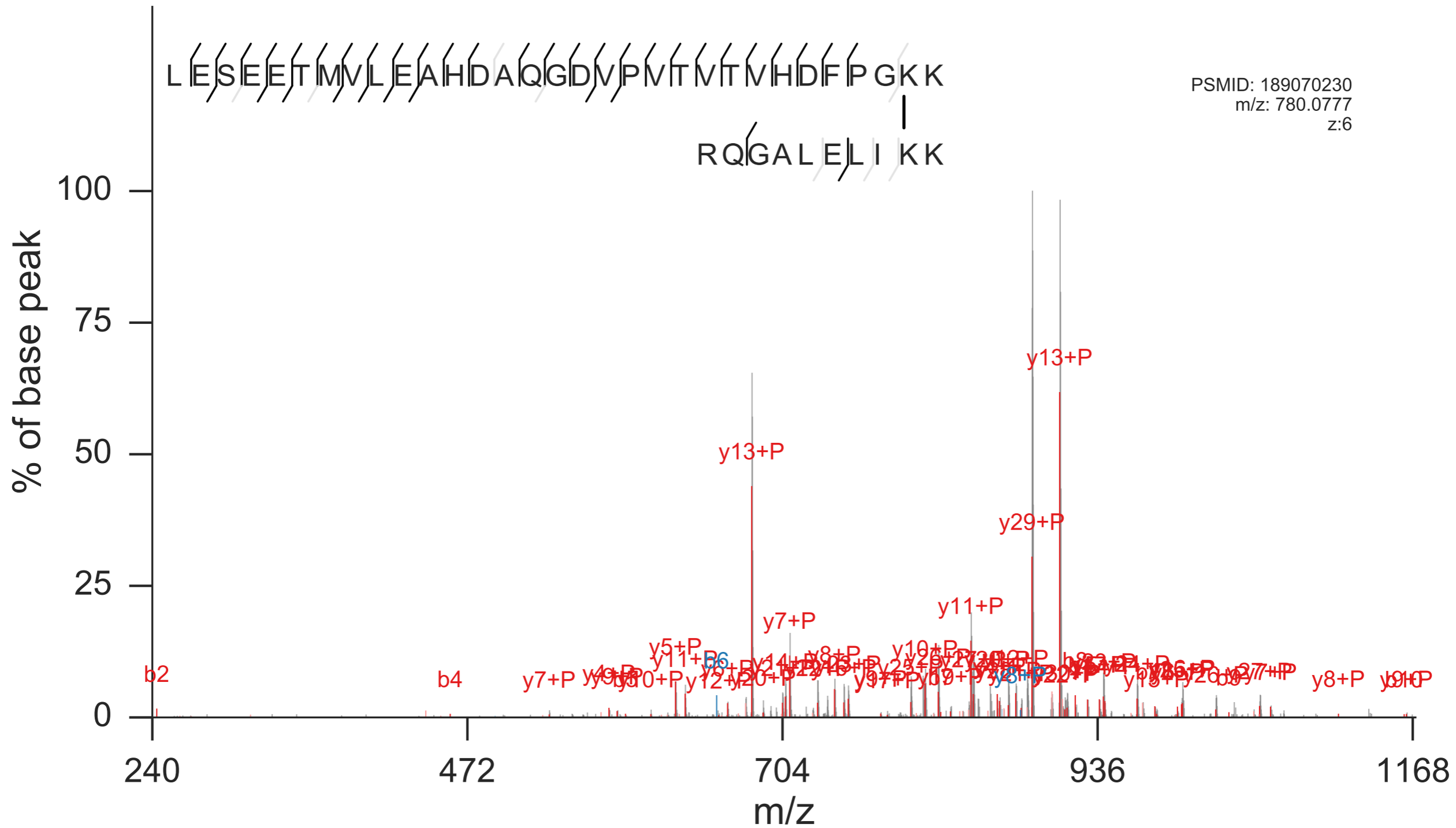


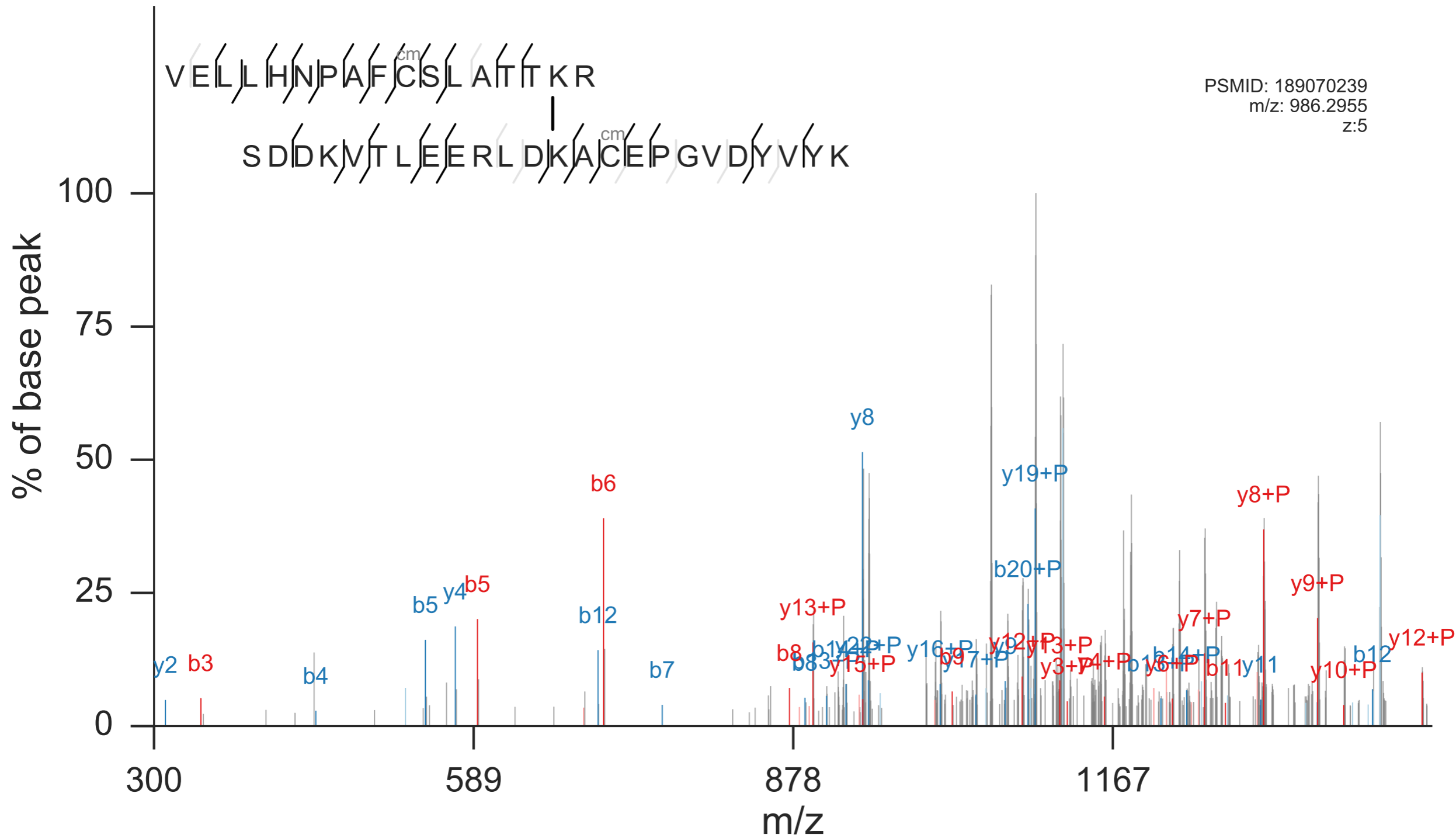


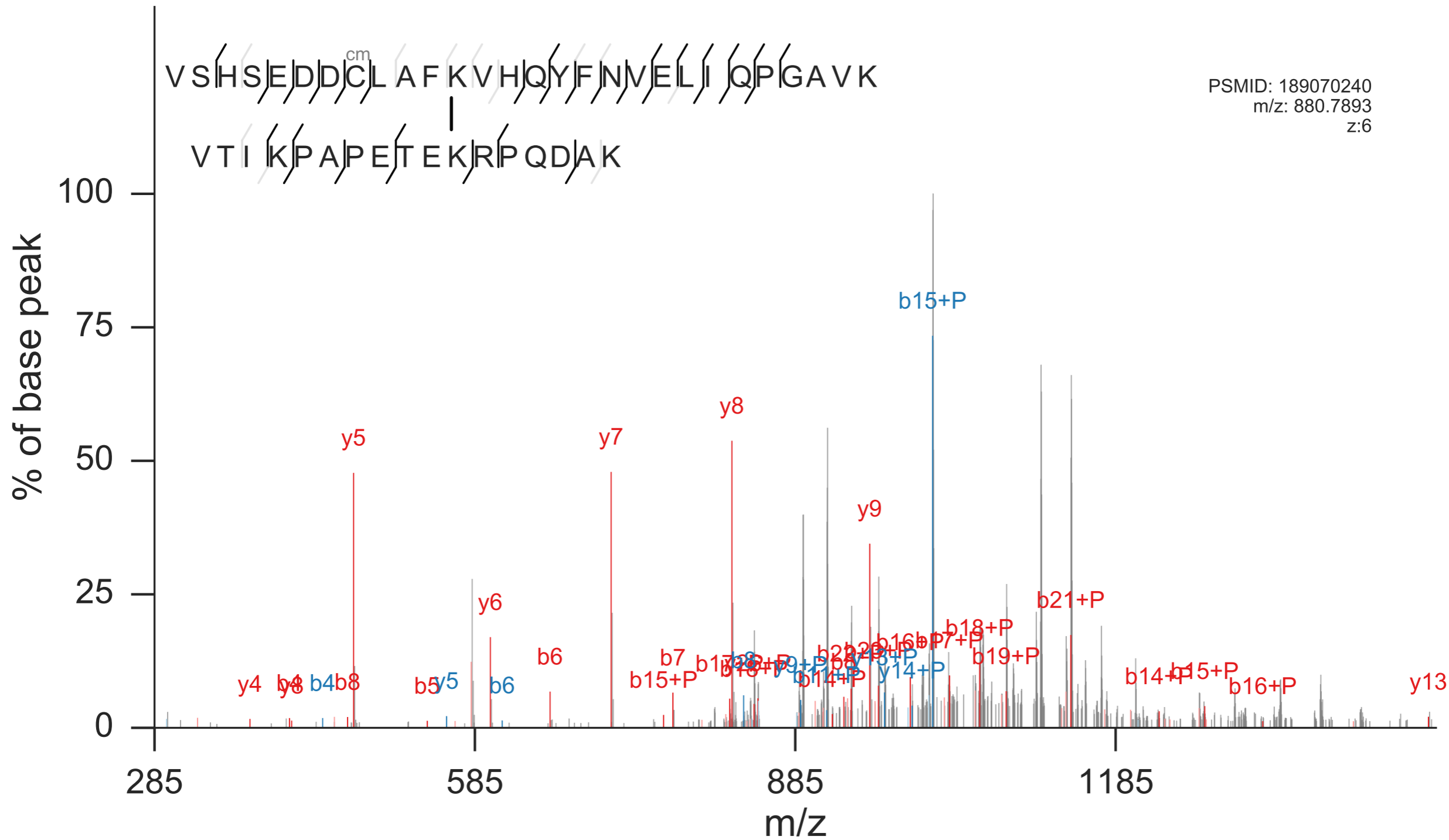


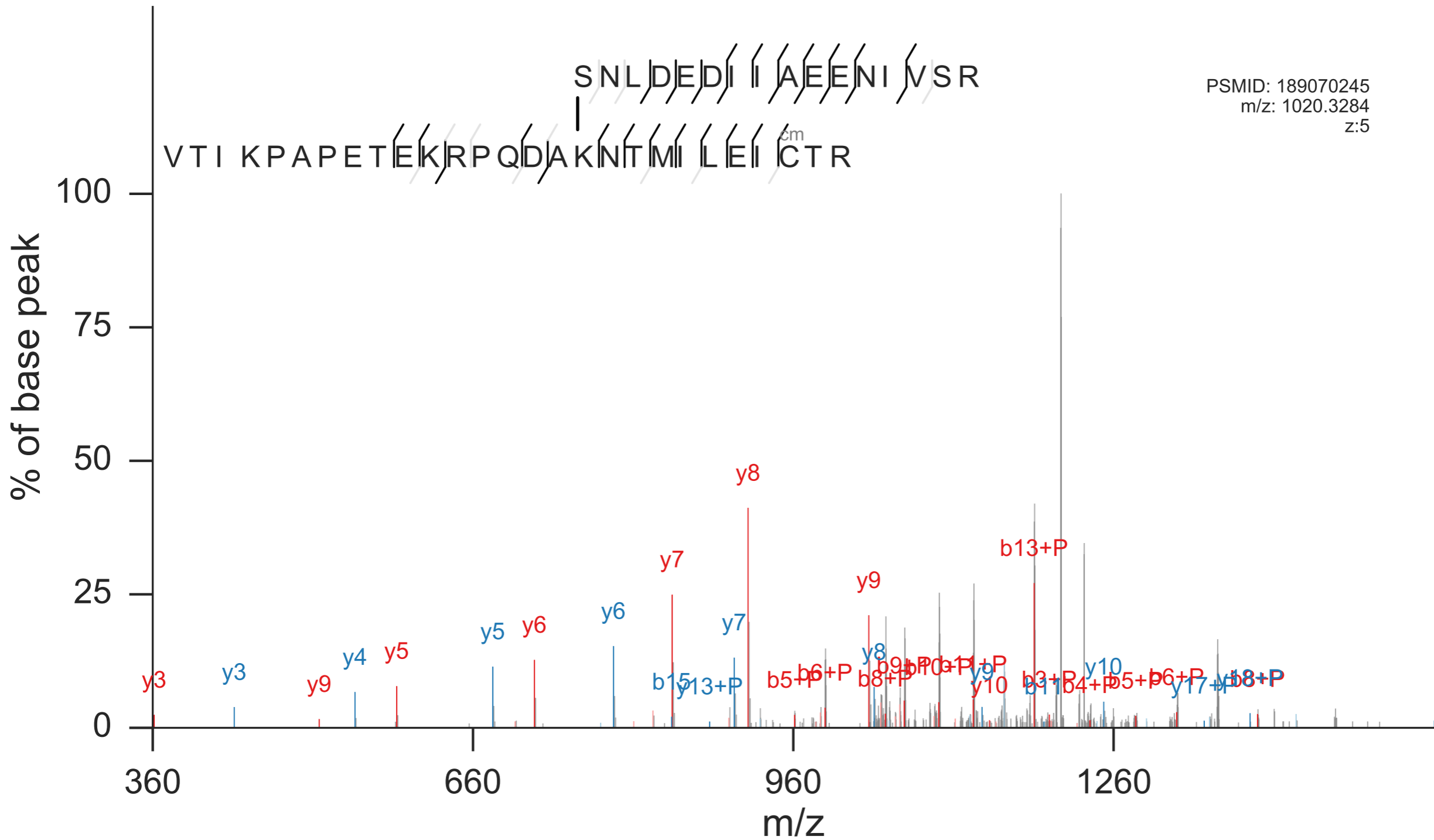


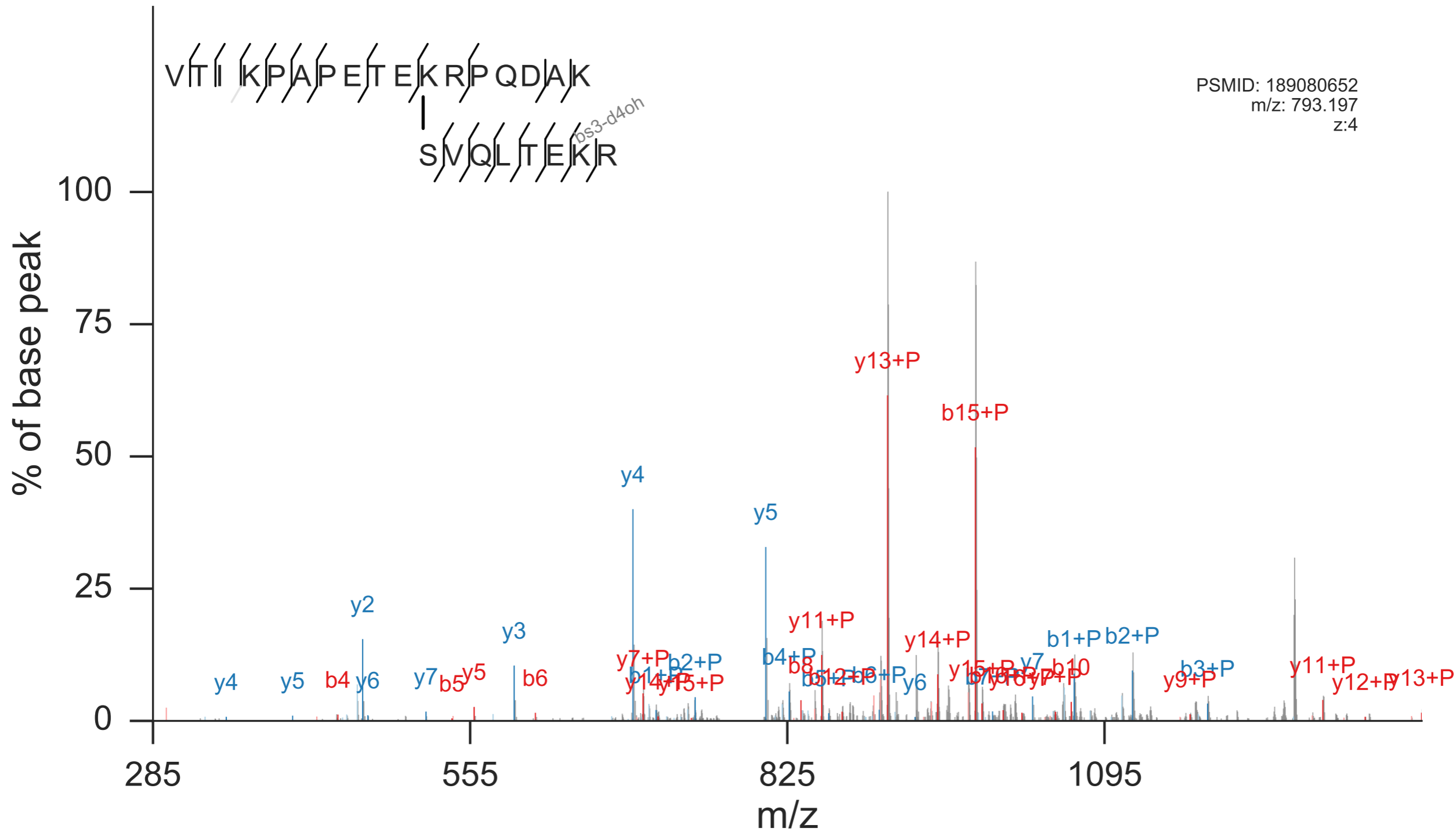


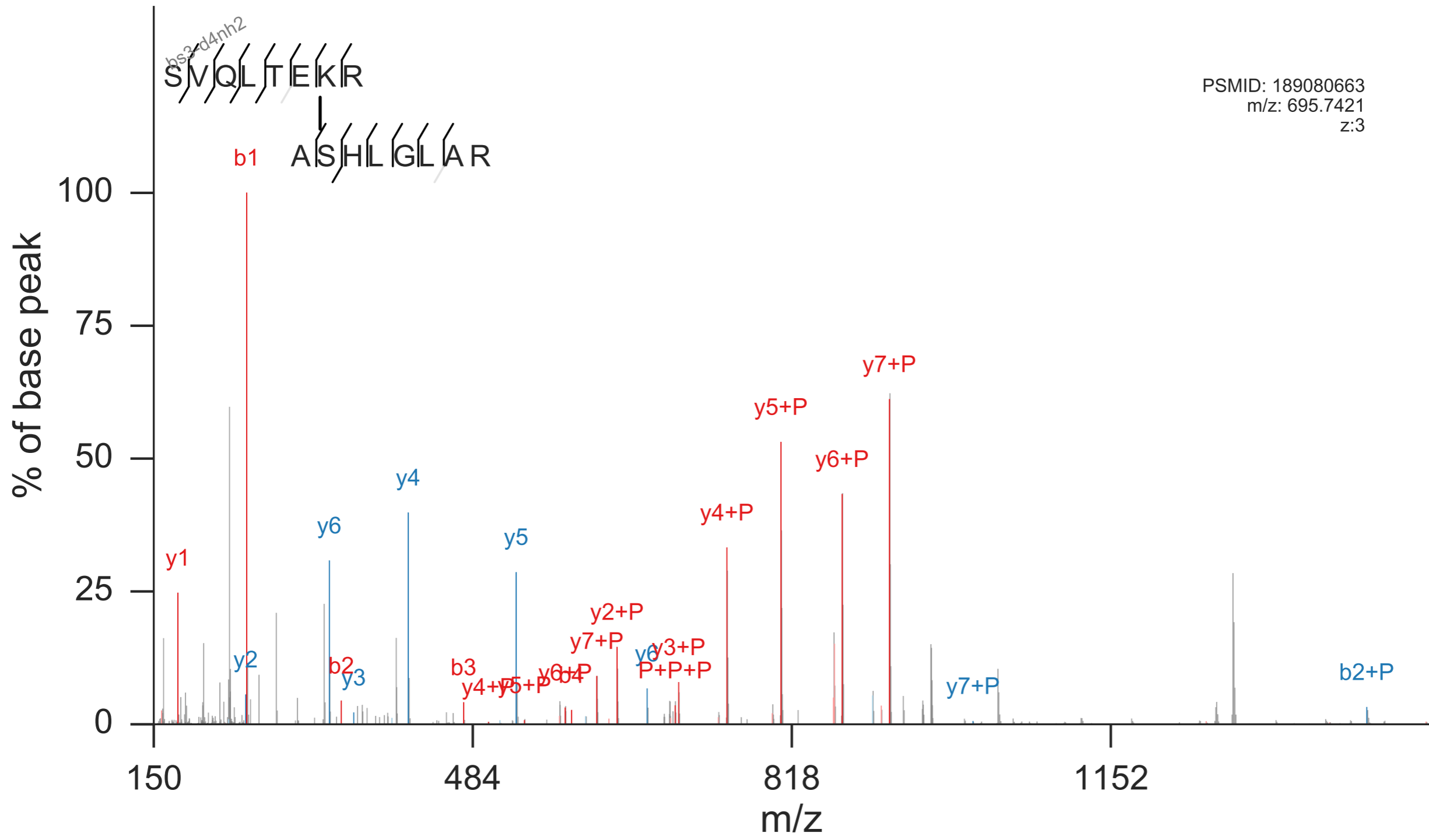


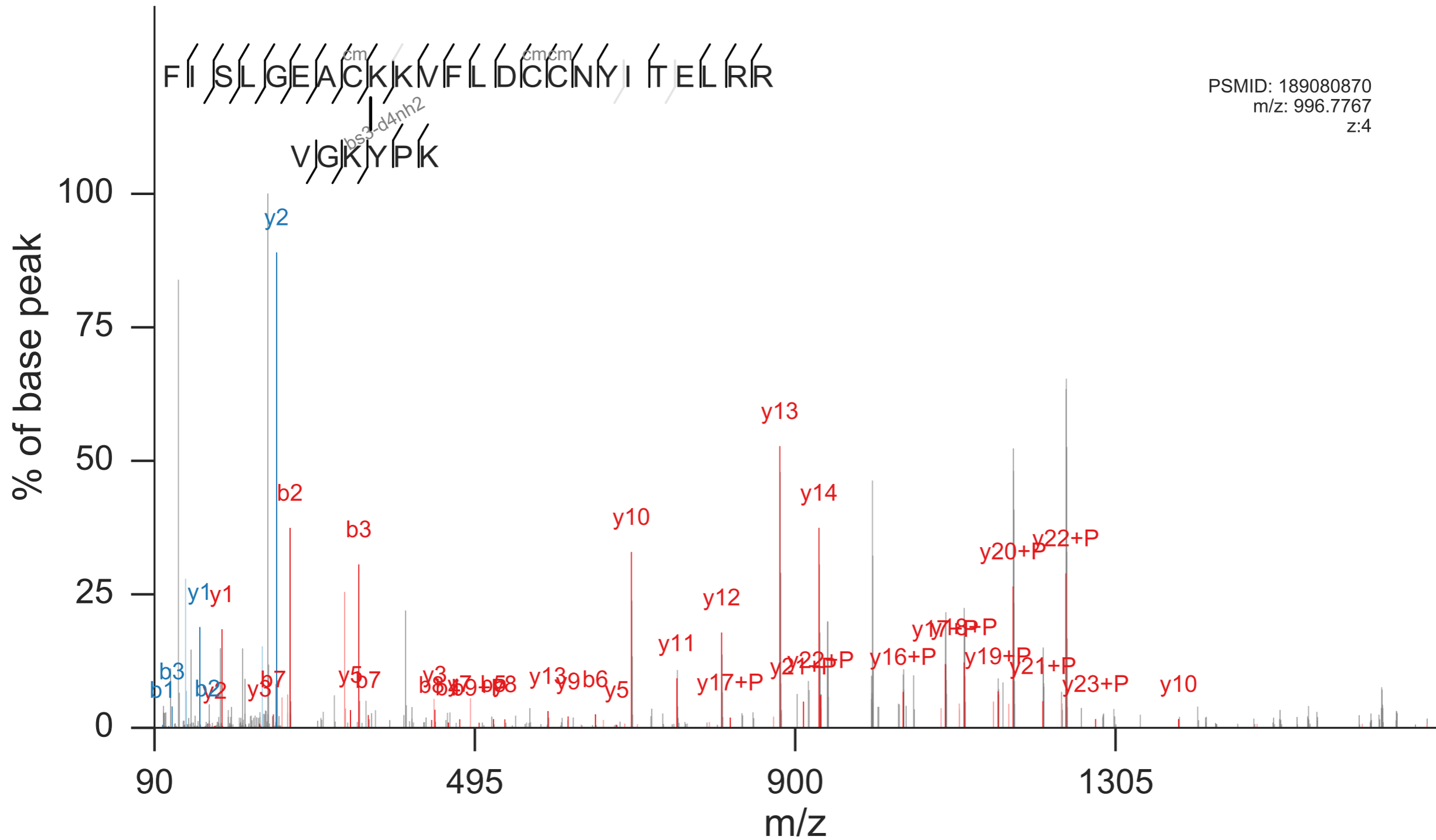












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V J G K Y P K

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bs3-d4nh2

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