Positive Probability Ltd

Note M1: Deisotoping – High Charge ESI Data

Introduction

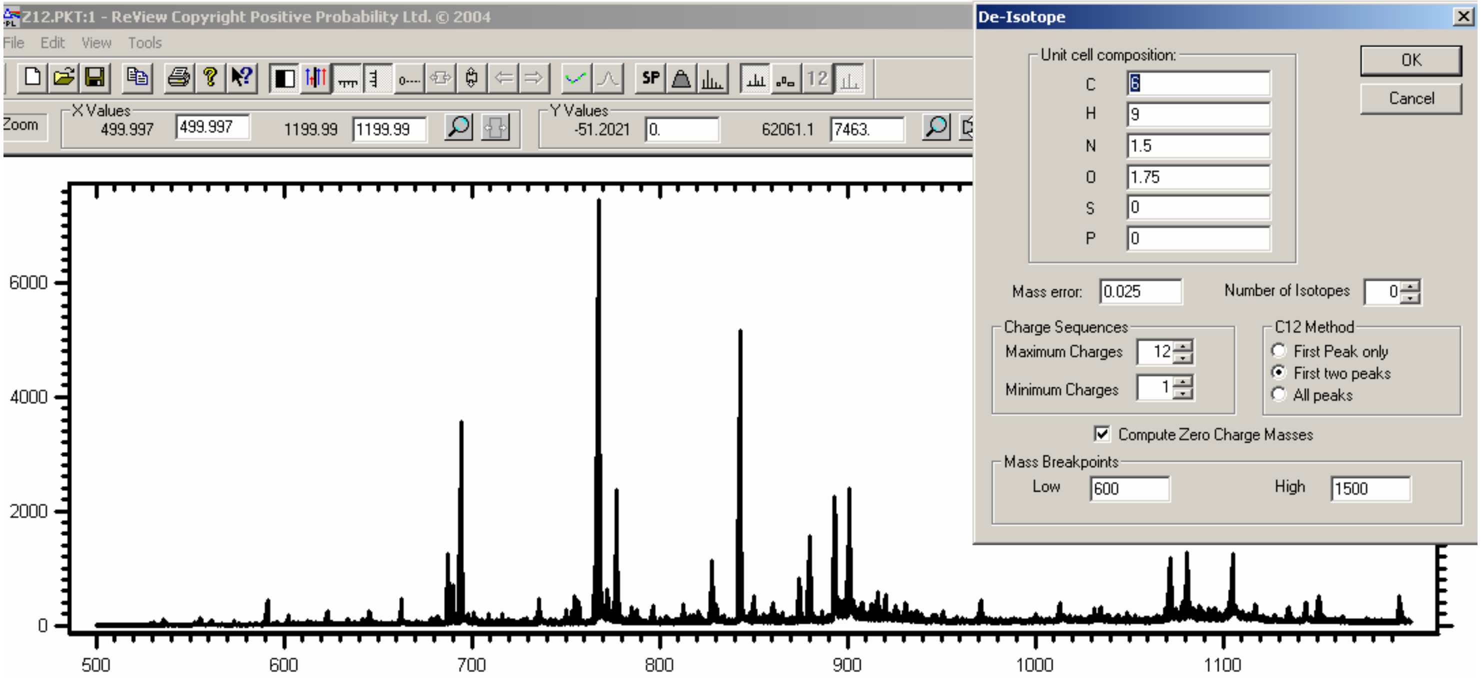
Depending on the experimental conditions, ESI spectra can contain high charge states for protein digests, particularly for higher molecular weight peptides. All generally available algebraic and data reconstruction deisotoping methods provided by manufacturers have the serious limitation that they are unable to deal with high charge states. Typically, data containing charges above 3 can create problems and it is rare that these programs will work reliably above Z5. This is unfortunate because higher mass peptides are much more diagnostic for protein identification than lower mass peptides. However, the ***ReSpect™*** algorithm and its ***Collapse™*** interface are able to reliably deisotope very high charge states.

In this example we show some protein digest data extracted from an LCMS run that contains charges from Z1 to Z12.

Data and Data Processing

The data described here concentrates on a peptide of mass 6611 Da to illustrate the ability of the ***ReSpect™*** algorithm to deisotope high charges. This intense peptide is “missed” by all other methods. Just like all methods, the program fits a model to the data. In this case the model is the expected isotope intensity profile for any m/z and charge state as determined from a user-defined empirical formula.

The data were first baseline corrected and then centroided to obtain a robust peak table with errors. The peak table was then filtered using 1 standard deviation and 68% confidence to remove all obvious noise before deisotoping to zero-charge masses. Input parameters are shown in the menu box on Figure 1.



X Values 499.997 499.997 1199.99 1199.99

Y Values -51.2021 0. 62061.1 7463.

Unit cell composition:
C 6
H 9
N 1.5
O 1.75
S 0
P 0

Mass error: 0.025

Number of Isotopes 0

Charge Sequences
Maximum Charges 12
Minimum Charges 1

C12 Method
○ First Peak only
● First two peaks
○ All peaks

☑ Compute Zero Charge Masses

Mass Breakpoints
Low 600
High 1500

Figure 1. Data and processing parameters.

Results and Discussion

The zero-charge result using the above parameters is shown in Figure 2 below.

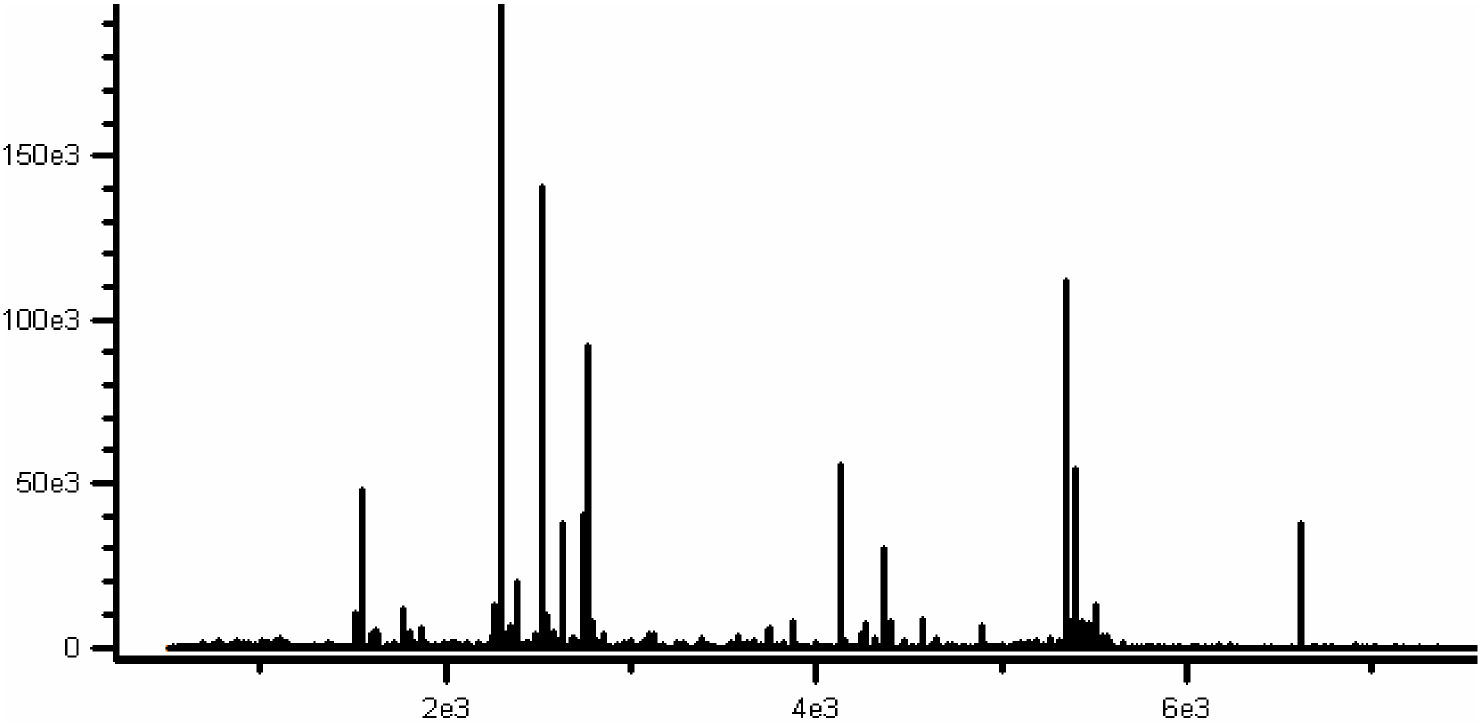


Figure 2. Zero-charge result.

The full output range goes to 15 kDa but there are no significant masses above 7 kDa. There is an intense mass at 6611.40 Da. The PPL implementation allow any mass to be highlighted and the evidence in the data viewed. The following figures show the evidence for this mass.

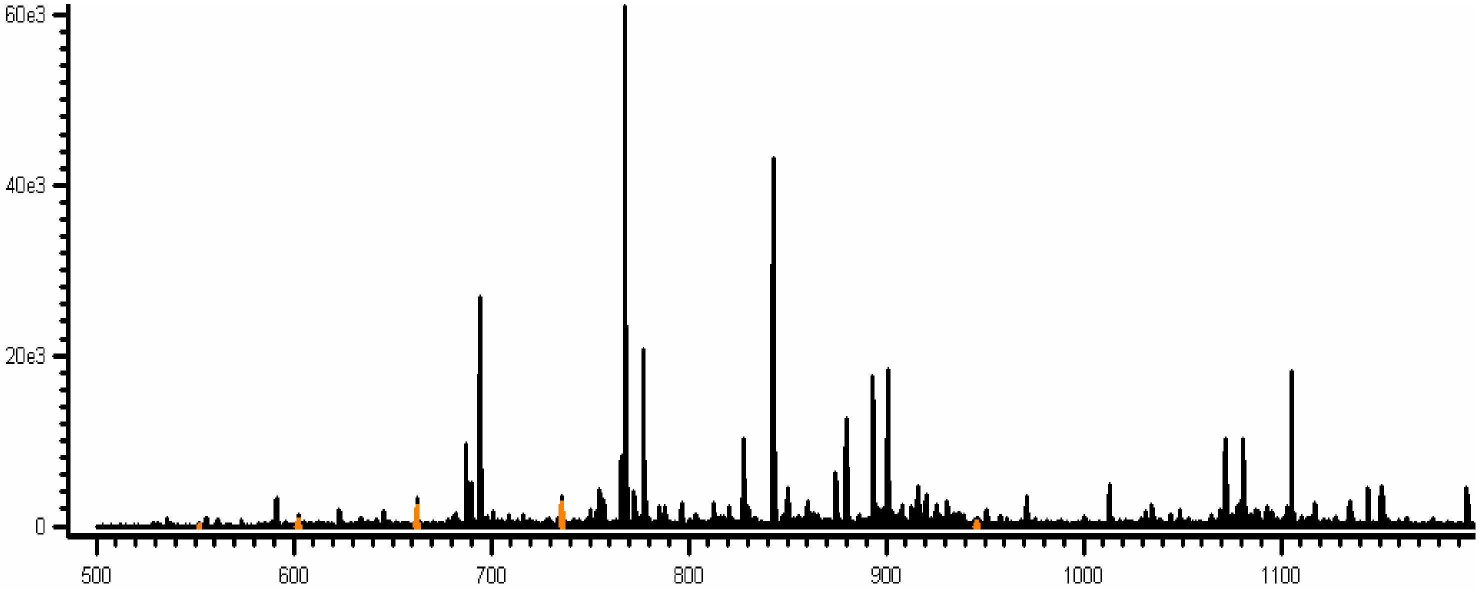


Figure 3. Data showing evidence for mass at 6611.40 Da (orange highlight).

A vertical expansion and annotation more clearly shows the highlighted charges, as illustrated in Figure 4 below.

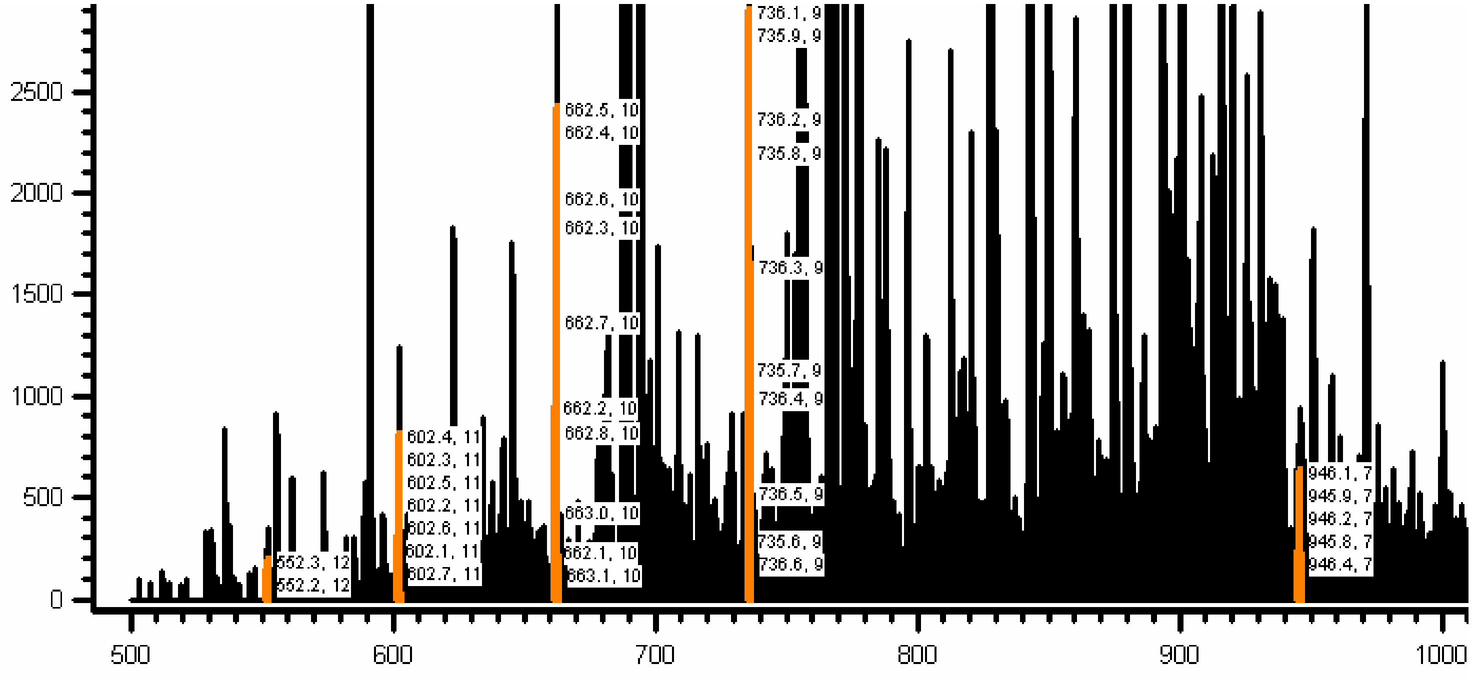
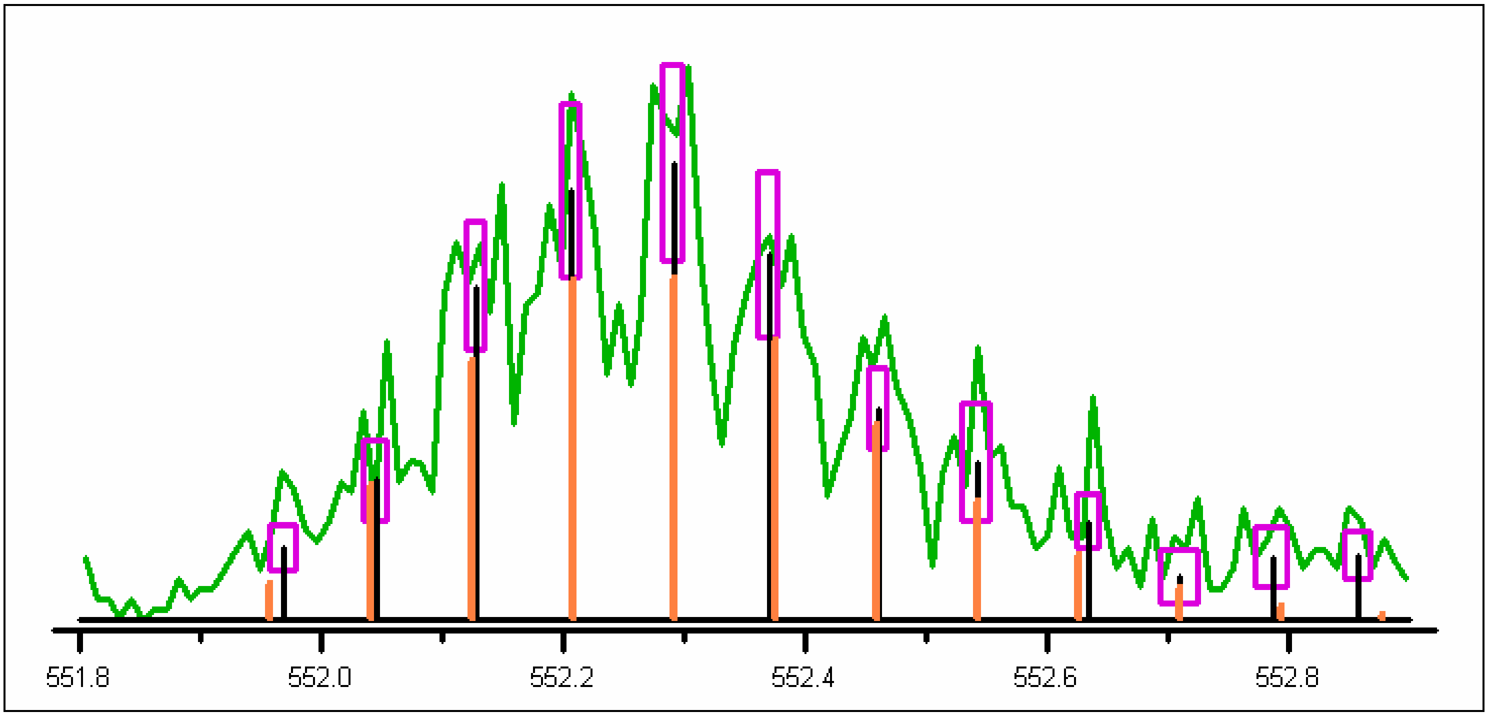


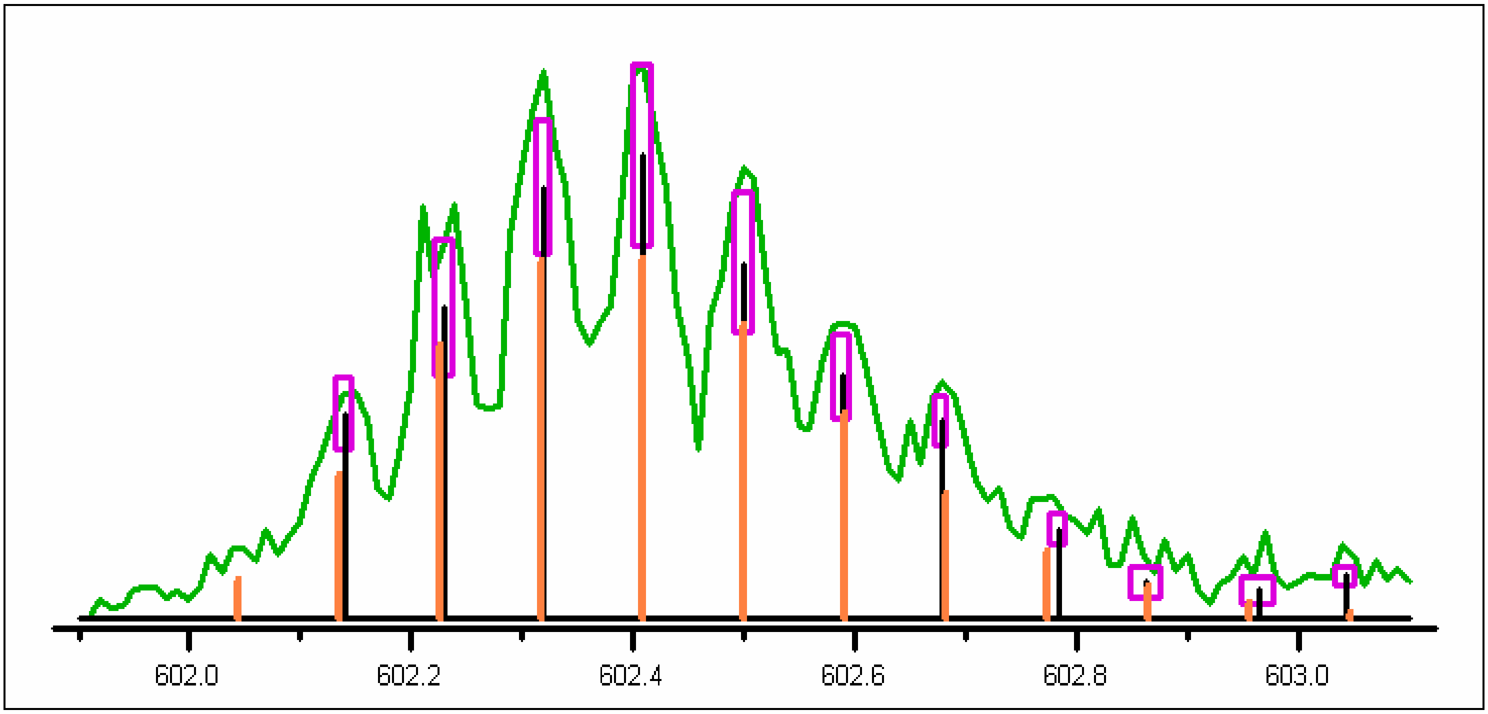
Figure 4. Reconstructed charges for mass = 6611.40 Da (orange highlight).

The following figures show each individual charge. In each figure the different colours show: green – data; black/magenta – spikes and 1 SD error bars; orange – reconstructed isotope pattern.

Evidence for Z12

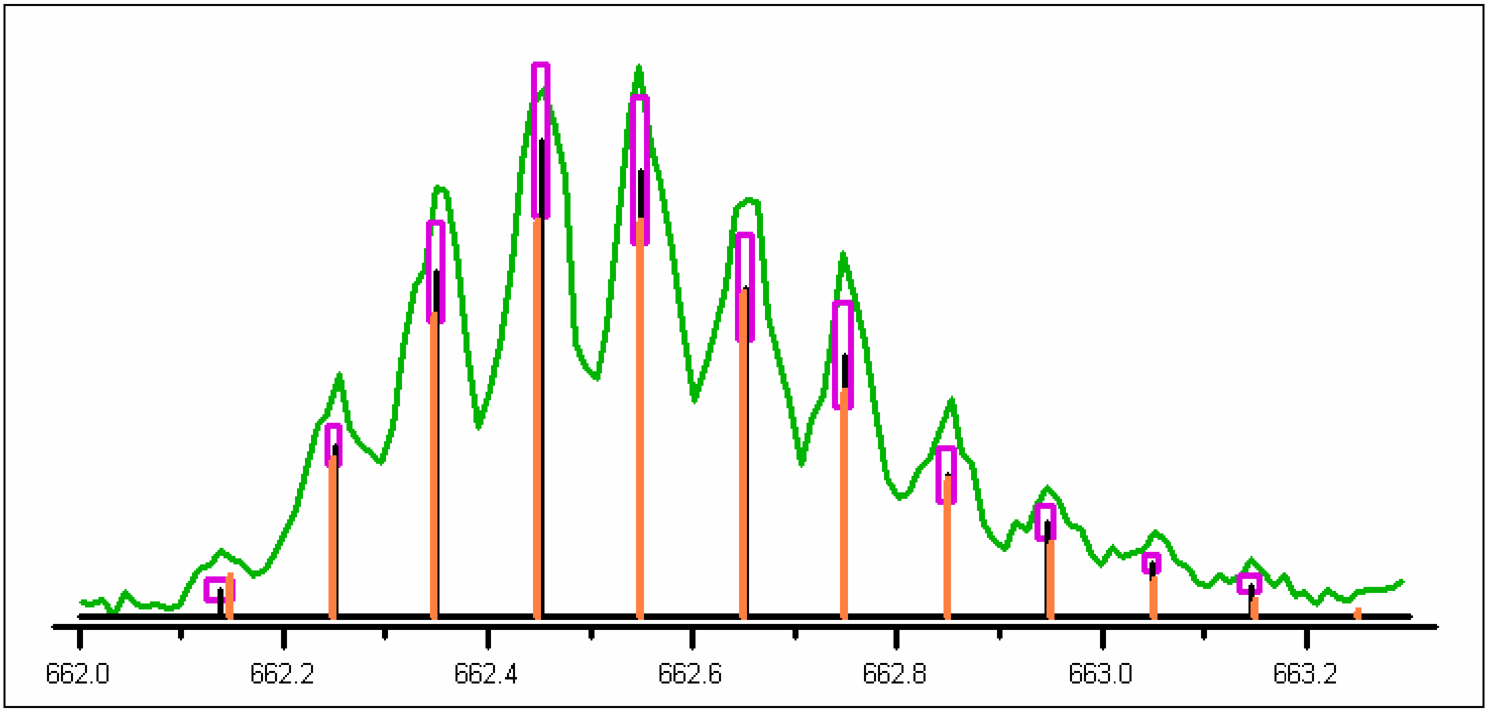


Evidence for Z11

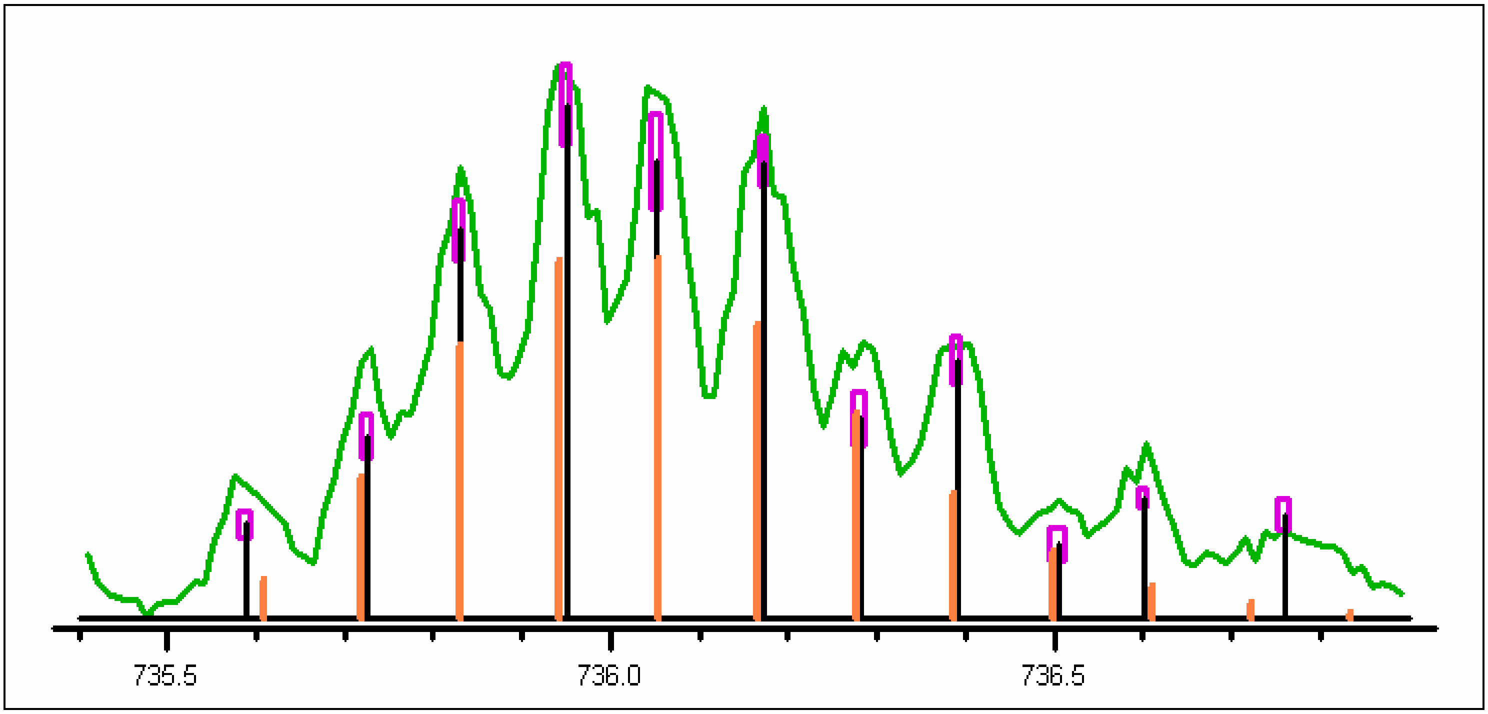


Note that the isotope peak at m/z ~602.04 has not been recovered by centroiding. This peak would have been recovered by deconvolving the data. Even so, ***ReSpect™*** correctly identifies where the C12 isotope should be. This is because ***ReSpect™*** fits ***all*** the data and the reconstructed intensity profile demands that the C12 is at m/z 602.04.

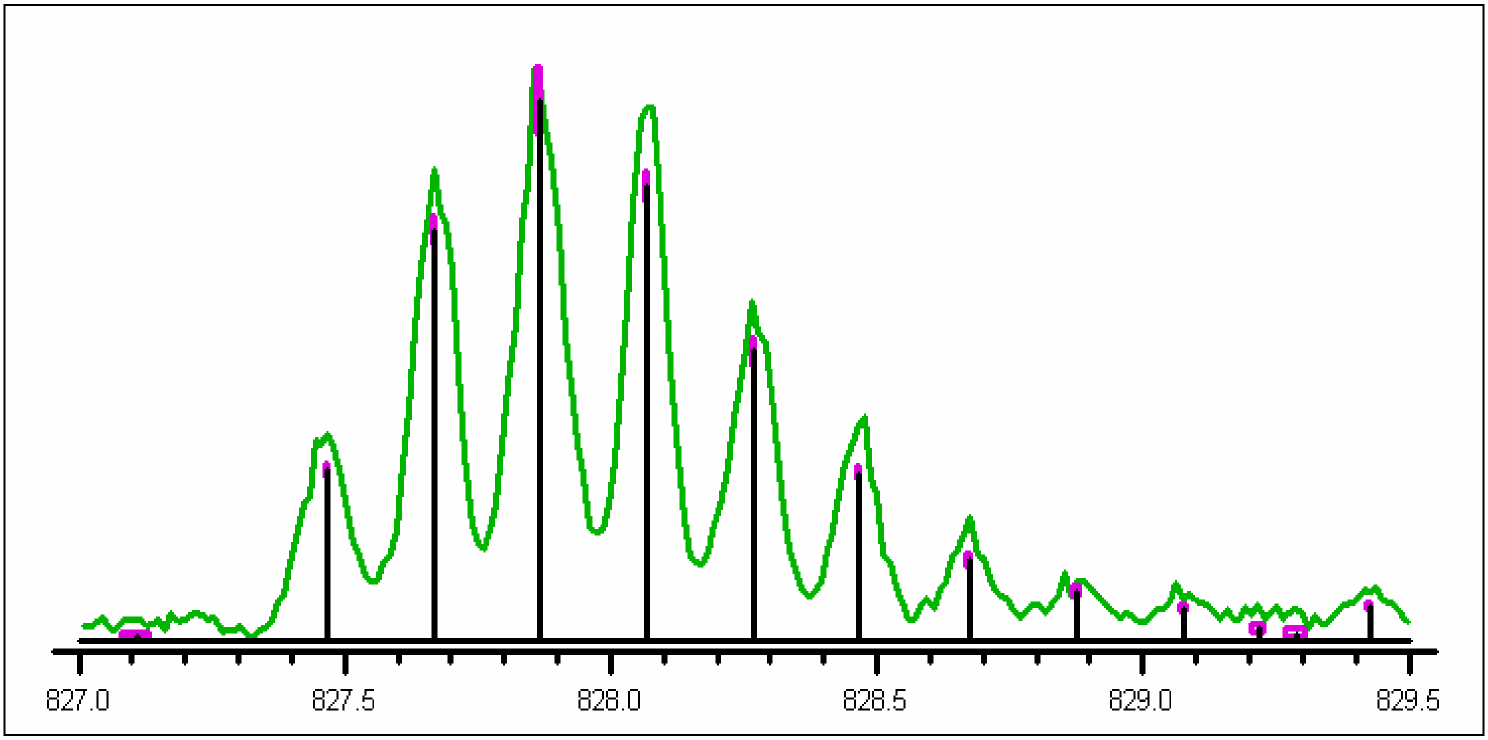
Evidence for Z10



Evidence for Z9

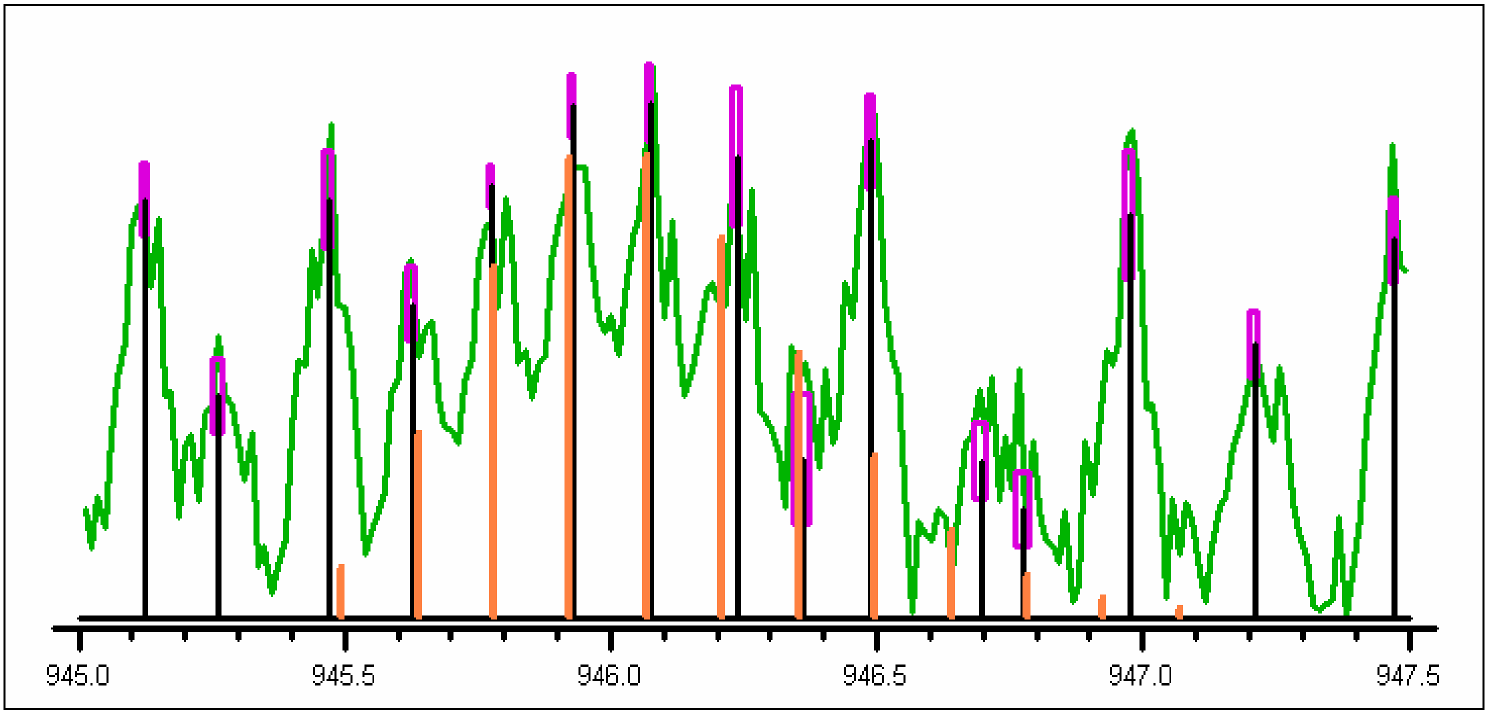


Z8 swamped by intense Z5



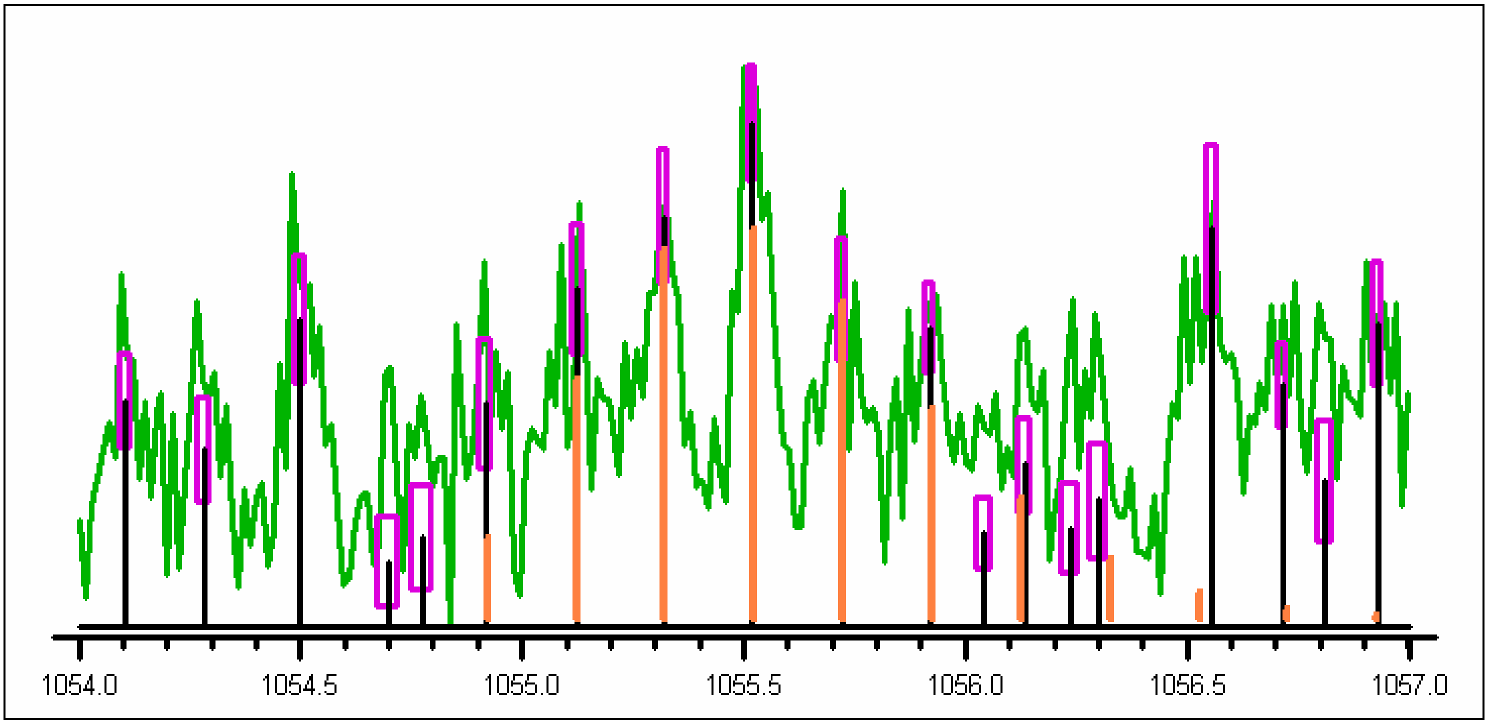
Note that the obvious Z5 ion is so intense that it has completely masked the expected Z8 ion for the peptide mass at 6611.40 Da.

Evidence for Z7



An important feature of the ***ReSpect™*** methodology is that there will always be positive evidence for any reconstructed mass. This is illustrated by selecting a weak mass and viewing the evidence for it in the data. A weak peptide at mass 5269.57 Da has an intensity <5% of the peptide at 6611.40 Da. Selecting this shows only a Z7 at m/z 1052.70. The evidence for this isotope cluster is shown below.

Evidence for Z7 of weak peptide of mass at 5269.57



Conclusions

The ***Collapse™*** deisotoping program will robustly deal with high charge data that other methods cannot handle. In addition, there is always evidence in the data for any reconstructed mass. The program does not produce artefacts.