Positive Probability Ltd

Note P7: Deisotoping Overlapping Charges

Introduction

Data reconstruction methods are particularly well-suited to complex problems such as deisotoping high resolution mess spectrometry data where different charge states may be overlapped. Because noise in the data and the peak position and intensity errors from a reconstruction method are taken into account, the deisotoped result is clean and free from artefacts other than those that may arise from using a compromise empirical formula.

Data and Data Processing

The data presented here are a narrow region from a tryptic digest (see Figure 1 below) and were acquired on and ESI-TOF instrument. This particular region shows a number of overlapping isotope clusters of different charge state. The data were first baseline corrected using the ***Nadir*™** program and then deconvolved with ***Sleuth*™** to provide a reliable peak table along with the m/z and intensity errors. The peak table was then analysed using the ***Collapse*™** interface to the ***ReSpect*™** algorithm.

Results and Discussion

The baseline corrected data and the deisotoped result are shown in the Figure 1 below.

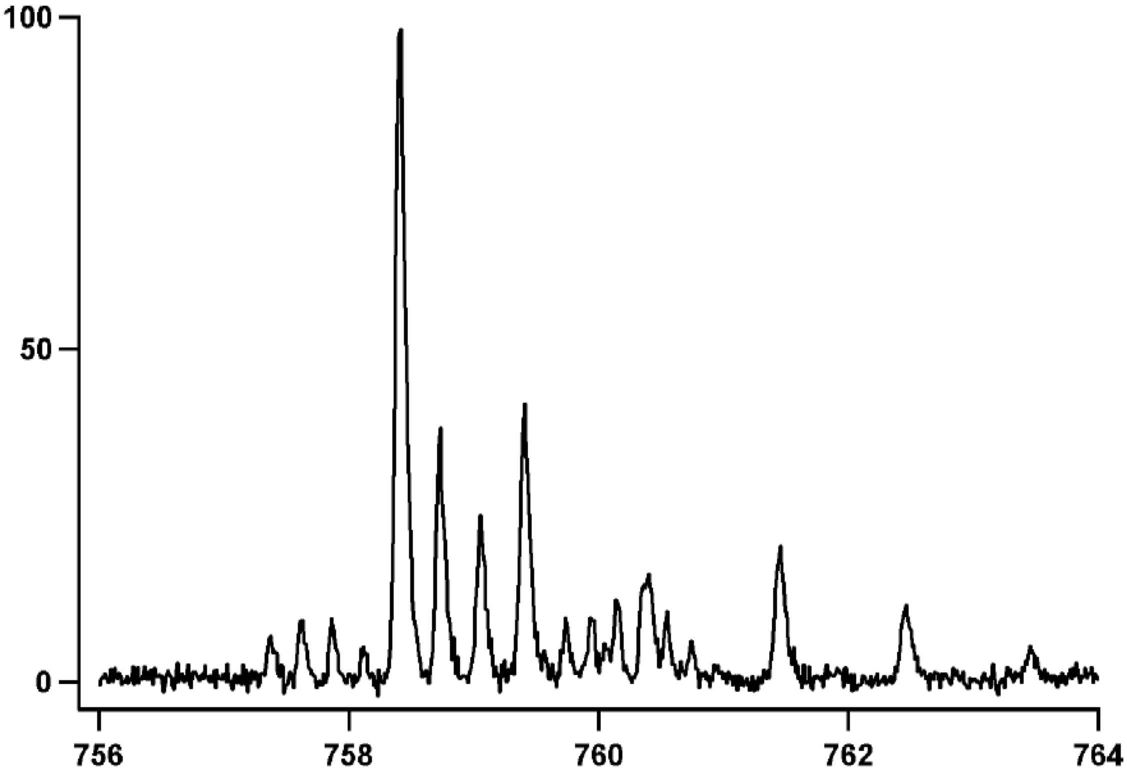

Figure 1. Part of a tryptic digest spectrum.

Figure 2 shows the deisotoped result.

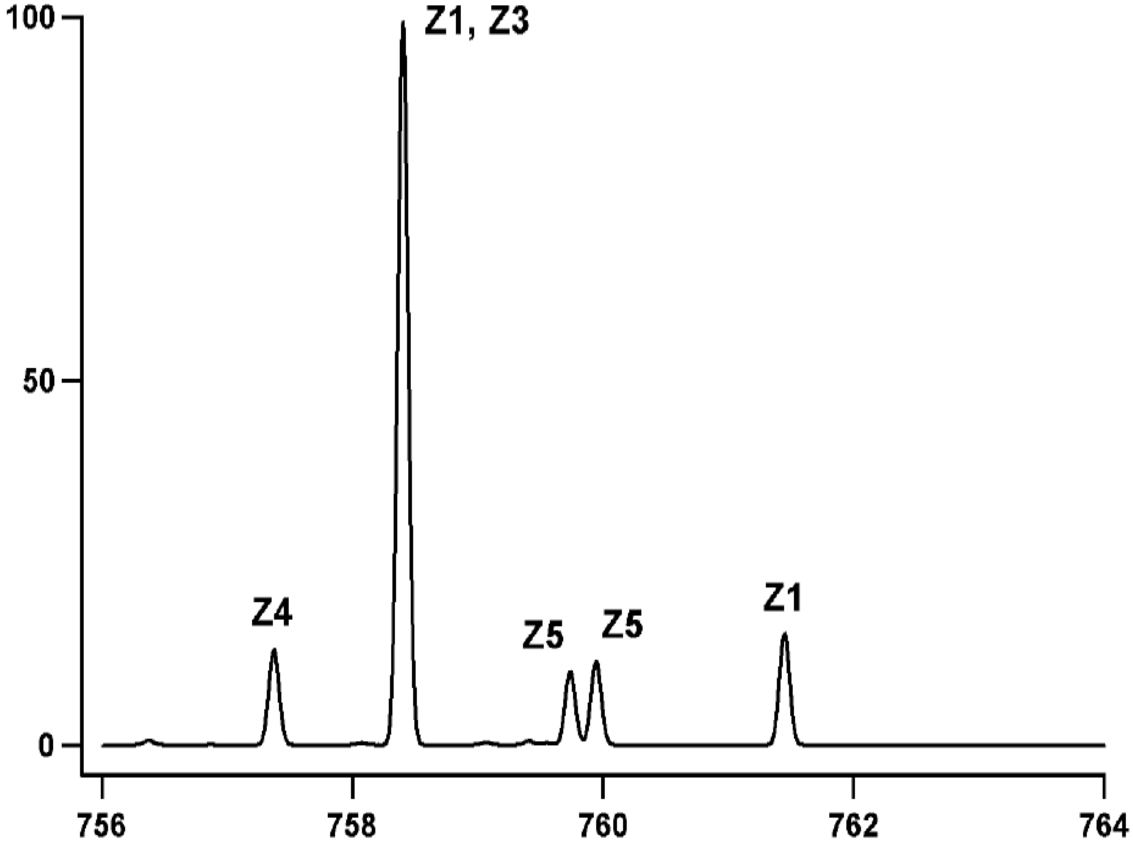

Figure 2. Deisotoped result.

Of course, the ***ReSpect™*** algorithm is able to directly provide and display zero-charge results. Plots may be in the form of a trace, as shown in Figure 2 where the width of the peaks is directly proportional to the size of the m/z errors (or mass errors for zero-charge results). Alternatively, a spike plot may be displayed where the height of the spikes is directly proportional to the true peak intensities.

The table below shows the reconstructed m/z and mass values along with their 1 standard deviation errors and intensities.

Reconstructed m/z Values and Masses

m/z	m/z error	Z	M	M error	Intensity
757.3699	0.0400	4	3025.4482	0.1602	408
758.4006	0.0404	3	2272.1782	0.1213	1620
758.4056	0.0401	1	757.3978	0.0401	1833
759.7392	0.0400	5	3793.6567	0.2000	360
759.9454	0.0402	5	3794.6880	0.2009	370
761.4519	0.0401	1	760.4441	0.0401	529

Note that although the Z3 and Z1 ions have very similar m/z values of 758.4006 and 758.4056 respectively, they are naturally not overlapped at zero-charge.

Entropy-based and other data reconstruction methods have a fundamental constraint that the result must have the same total intensity as that present in the data. It follows that the intensity of irrelevant peaks and noise must appear in the result as artefacts that fit the data in the best possible way. The ***ReSpect™*** algorithm has been specifically designed so that this unrealistic and unnecessary constraint does not apply. Consequently, the ***ReSpect™*** result may have a lower intensity than the data.

The table below shows the reconstructed intensities for each observed isotope peak present in the data. For the data processed here, the S/N is high and so most of the data are fitted (96.3%). Even so, other methods would be forced to include any left over intensity as artefacts.

Reconstructed Isotope Intensities

m/z	Int	Z4	Z1	Z3	Z5	Z5	Z1	Rec Int
757.3723	97	68						68
757.6193	130	116						116
757.8680	113	105						105
758.1176	60	66						66
758.4073	1636	33	1169	421				1623
758.7298	549	13		541				554
759.0550	377			374				374
759.4049	671		501	182				683
759.7390	118			70	38			108
759.9399	137				82	39		121
760.0593	48			23				23
760.1486	158				91	84		175
760.3660	227				71	94		165
760.4166	108		131	6				137
760.5477	115				43	73		116
760.7407	66			2	21	44		67
760.9547	24				9	22		31
761.4502	337		26				337	363
761.8392	15							
761.9275	17							
762.4592	198		4				145	149
763.4620	77		1				38	39
Totals	5279							5083
							% recovered intensity	96.3

Conclusions

The above example has served to show the effectiveness of data reconstruction methods to deisotoping overlapping isotope clusters. The result is free of artefacts and results may be presented as deisotoping to ions or to zero-charge masses.