Determine Monoisotopic and Average Mass for Intact Proteins Using a High Resolution Quadrupole Time-of-Flight Mass Spectrometry and Improved Deconvolution Algorithms

ASMS 2010

Ning Tang[1], Xiaoling Wu[1], Xiangdong Li[1], Joe Roark[1], Anthony Ferrige[2] and Keith Waddell[1], [1]Agilent Technologies, Santa Clara, CA, USA, [2]PPL, Isleham, UK

Determine Monoisotopic and Average Mass for Intact Proteins Using a High Resolution Quadrupole Time-of-Flight Mass Spectrometry and Improved Deconvolution Algorithms

Introduction

Analyzing intact proteins using quadrupole time-of-flight (Q-TOF) mass spectrometry has been the method of choice for the biopharma industry. Time-of –flight (TOF) technology offers excellent resolution and mass accuracy which are important to accurately measure large molecule molecular weights. Deconvolution algorithms have been used on protein electro-spray spectra to determine the average mass of large proteins. With the recent improvement of Q-TOF resolution from 20000 to 40000, modest size proteins (30 kDa) can be isotopically resolved. The increased resolving power allows the separation of small post-translational modifications (PTM) and adducts of large molecules. Although impressive, isotope separation has created a challenge but has also provided an opportunity to deconvolve spectra to obtain both monoisotopic and average mass of large molecules for improved characterization.

Experimental

Materials
Myoglobin was purchased from Sigma Aldrich (St. Louis, MO).

LC/MS analysis
All samples were analyzed using an Agilent 1290 HPLC coupled to Agilent 6530 or 6540 Q-TOF mass spectrometer. Agilent Poroshell 300SB-C8 Column 1x75mm was used.
Mobile phase: solvent A: 0.1% formic acid (FA) in water; solvent B: 0.1% FA in 90% acetonitrile (ACN) and 9.9% water.
Flow rate: 0.15ml/min
Gradient: 0-3min: 3% B
3-8min: 90% B
8-10min: 90%B
Q-TOF parameters:
Vcap: 4000V
Fragmentor: 175V
Drying gas: 10L/min; Drying gas temperature: 350 C
MS scan: 1 scan/second

Deconvolution algorithms
Three deconvolution algorithms were used including Maximum Entropy deconvolution and molecular feature extraction (MFE) in Agilent MassHunter Workstation and ReSpect from PPL.

Results

Comparison of myoglobin MS raw data on 6530 and 6540 Q-TOF

Figure 1 shows the raw data of the entire charge envelope and a zoom-in display of the +21 charge state of myoglobin. The isotopes were not resolved and a single peak was observed at a resolution of 20000, The isotopes were fully resolved (B) when it was acquired on 6540 Q-TOF with 40000 resolution.

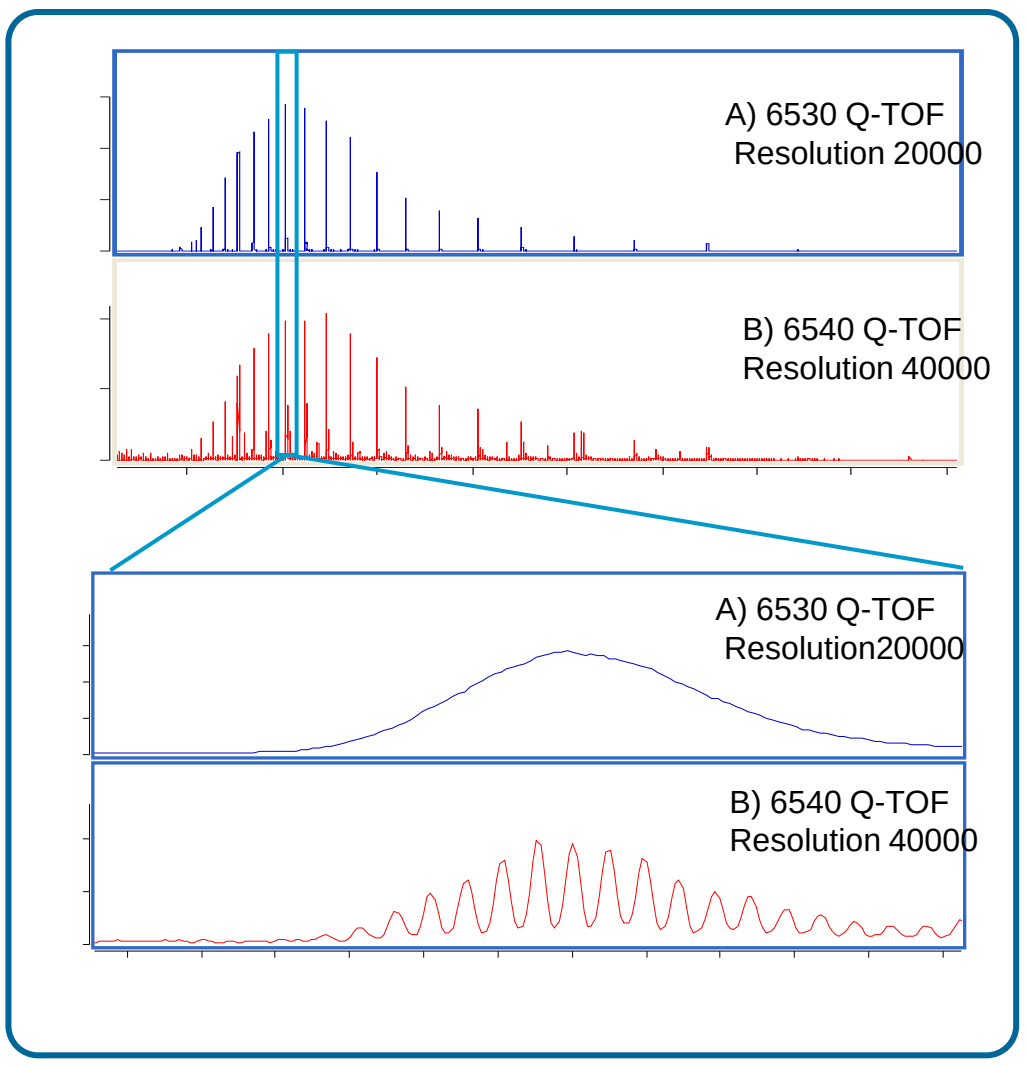


Figure 1. Q-TOF raw data of horse myoglobin A) acquired using 6530 Q-TOF in 4 GHz mode with resolution 20000. B) acquired using 6540 Q-TOF in 4 GHz mode with resolution 40000.

Maximum Entropy deconvolution algorithm

The maximum entropy technique has been well established in image processing for the last decade, Maximum Entropy deconvolution has become the most common algorithm for intact protein mass determination. This method transforms an m/z raw spectrum of one or more intact proteins, usually from averaging the mass spectra across the chromatographic elution time of the protein(s) of interest, into a zero-charge mass spectrum (in Dalton units). Maximum entropy deconvolution works very reliably for pure protein data or relative simple protein mixture.

Determine Monoisotopic and Average Mass for Intact Proteins Using a High Resolution Quadrupole Time-of-Flight Mass Spectrometry and Improved Deconvolution Algorithms

Results and Discussion

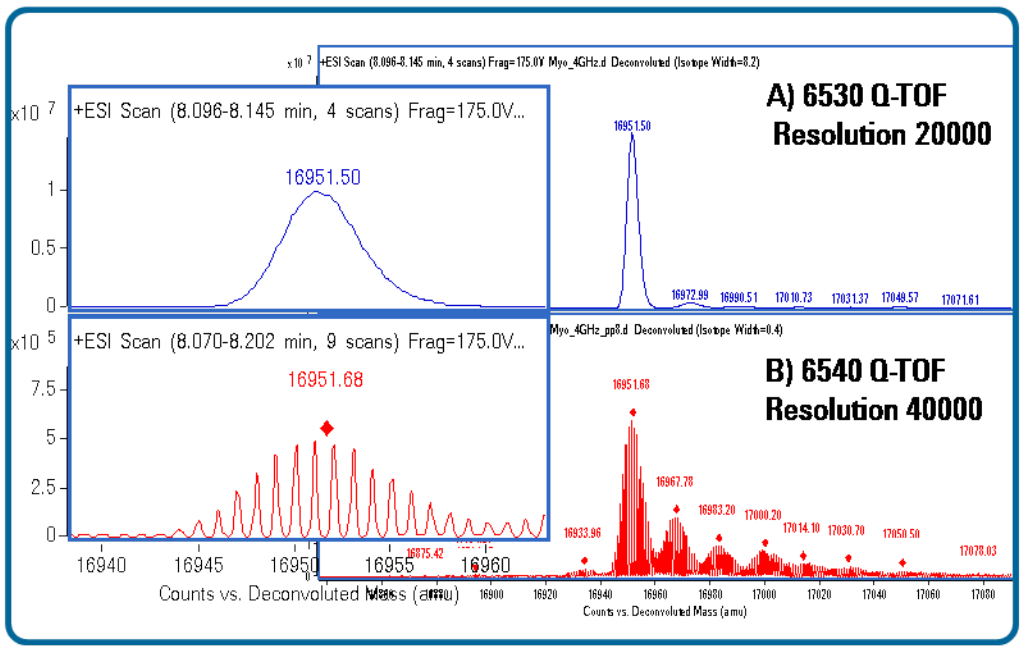


Figure 2. Maximum entropy deconvoluted results of horse myoglobin from 6530 and 6540 Q-TOF. A) acquired using 6530 Q-TOF in 4 GHz mode with resolution 20000. The deconvoluted result has a major peak at 16951.50 Da. B) acquired using 6540 Q-TOF in 4 GHz mode with resolution 40000. The deconvolution results from the 6540 Q-TOF had well-resolved isotopes with an average mass of 16951.68 Da.

Instrument	Deconvolved average mass	Theoretical mass	Mass error (ppm)	Modifications
6540 Q-TOF	16951.68	16951.60	4.4	Intact
	16967.78	16967.60	10.4	Intact + 1 Ox
	16983.20	16983.60	-24	Intact + 2 Ox
	17000.20	16999.60	35	Intact + 3 Ox

Table 1. Maximum Entropy deconvoluted results from high resolution (40000) 6540 Q-TOF.

Molecular Feature Extraction algorithm

Molecular feature extraction (MFE) algorithm works on resolved accurate mass TOF/Q-TOF LC/MS data. MFE maps MS signals in the 3-dimensional space of retention time and *m/z*. It then remove areas which only contain noise. It identifies covariant ions (isotope, adduct, dimmer, higher charge states, etc.) associated with a single compound across the entire LC/MS run and combine all the ion abundance to represent the compound. MFE determines the charge state based on isotope spacing and generates the neutral monoisotopic mass of the compound.

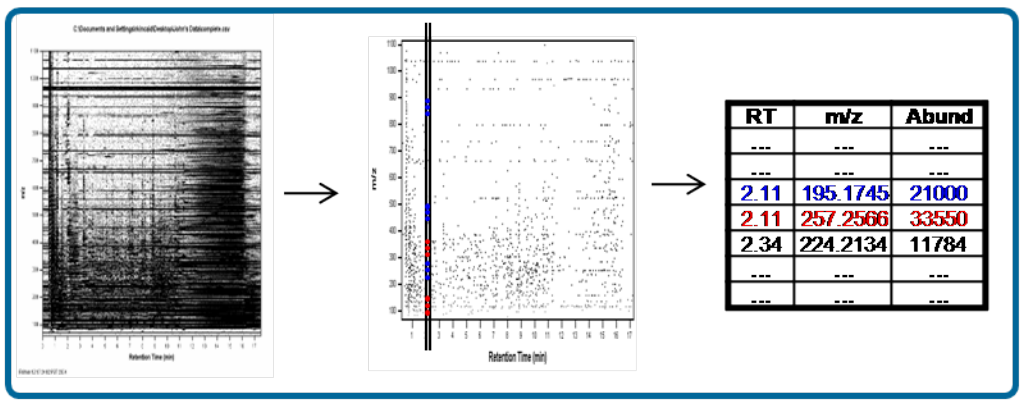


Figure 3. Illustration of MFE workflow. A) Map signals in the 3-dimensional space of retention time and *m/z*. B) Removal of noise. C) Identification of covariant ions and combination of all compound abundance.

Large proteins are not necessarily isotopically resolved. The **Large Molecular Feature Extraction (LMFE) algorithm** checks for signals that represent neighboring charge states in an envelope of multiply charged protein ions. The peaks within a given coelution group will contain the different charge states of the same protein, which are subsequently grouped together by algebraic charge state deconvolution. The average mass of the proteins are determined.

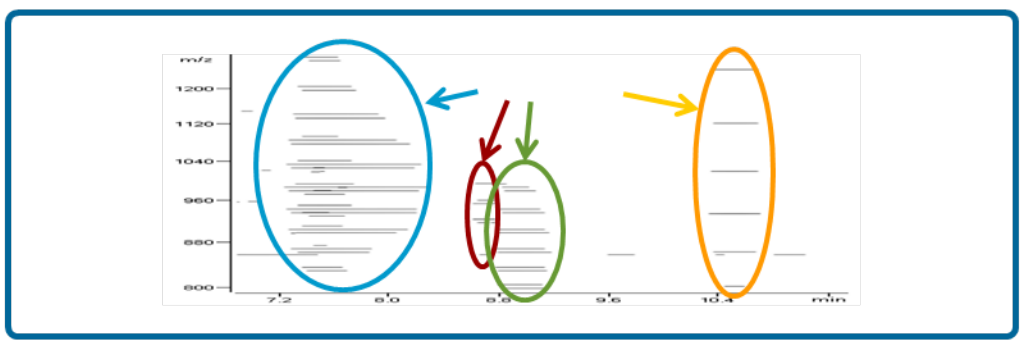


Figure 4. The peaks corresponding to different charge states of the same protein elute at the same time in the LC/MS run. Each oval corresponds to a unique coelution groups, each of which produces one or more protein compounds using algebraic deconvolution.

	Deconvolved mass	Theoretical mass	Mass error (ppm)	Modifications
Mono-isotopic mass (MFE)	16941.0235	16940.9651	3.45	Intact
	16957.0314	16956.9600	4.22	Intact + 1 Ox
	16973.0245	16972.9549	4.1	Intact + 2 Ox
	16988.9609	16988.948	0.7	Intact + 3 Ox
Average Mass (LMFE)	16951.5992	16951.60	-0.48	Intact
	16967.4661	16967.60	-8.28	Intact + 1 Ox
	16983.6669	16983.60	3.58	Intact + 2 Ox
	17000.1336	16999.60	31.24	Intact + 3 Ox

Table 2. MFE results on the high resolution (40000) Q-TOF.

Results and Discussion

Positive Probability Limited Algorithm

The algorithm at the core of the deisotoping and the charge deconvolution procedure used is called ReSpect. A noise estimate and a model are obtained from the data and fed into the data reconstruction program. The program generates a first estimate of the "sharp" information contained in the data. This is effectively a deconvolution and the result is then convolved with the model to obtain a reconstruction of the data. This is subtracted from the data to obtain a misfit, which is compared with the noise estimate. The sharp information is modified in an attempt to reduce the misfit and therefore provide a better fit of the data reconstruction . This cycle of events is repeated until the misfit is within the noise level. The process is faster than maximum entropy methods as it does not require a random number seed and gets to the "ideal" result faster.

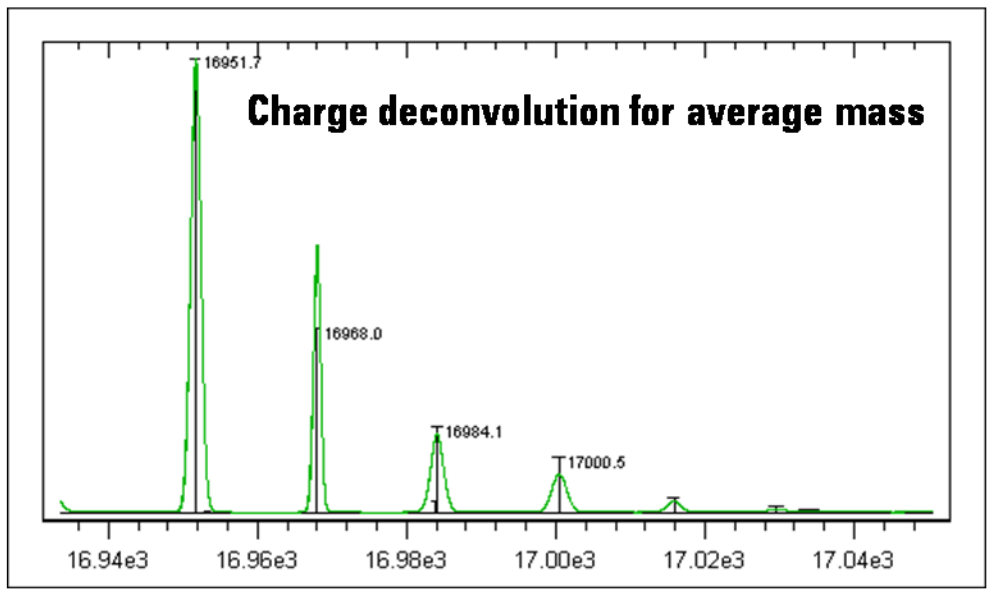
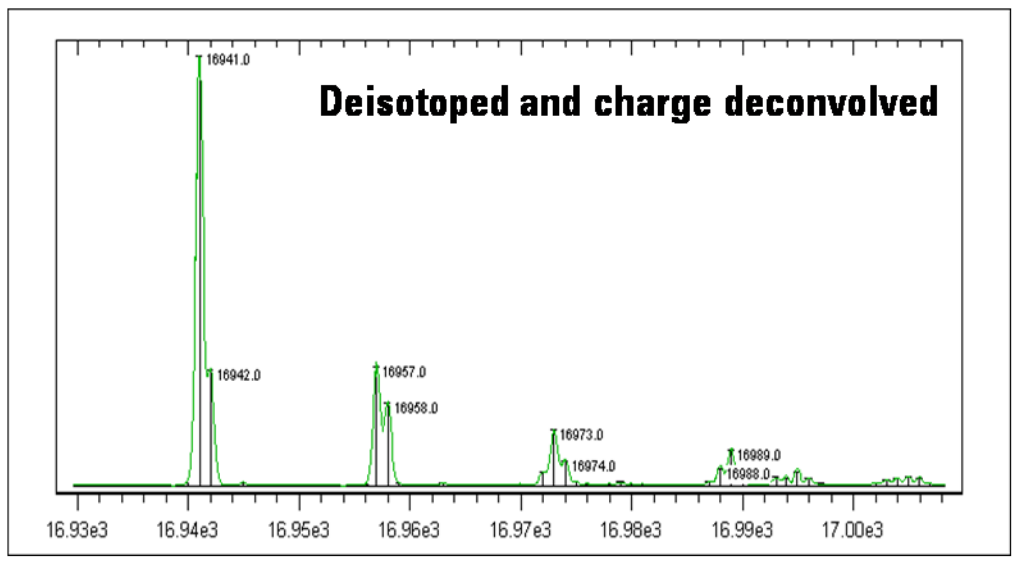


Figure 5. PPL deconvoluted average mass of horse myoglobin from 6540 Q-TOF. Peak modeled the entire isotope groups of a single charge state. The result reflects the average mass of myoglobin.



	Deconvolved mass	Theoretical mass	Mass error (ppm)	Modifications
Mono-isotopic mass	16940.9883	16940.9651	1.3	Intact
	16956.9785	16956.9600	1.0	Intact + 1 Ox
	16972.9785	16972.9549	1.4	Intact + 2 Ox
	16988.9609	16988.948	0.7	Intact + 3 Ox
Average mass	16951.7	16951.60	5.8	Intact
	16968.0	16967.60	23.5	Intact + 1 Ox
	16984.1	16983.60	29.4	Intact + 2 Ox
	17000.5	16999.60	53	Intact + 3 Ox

Table 3. PPL deconvolution results of the high resolution (40000) Q-TOF data.

Conclusions

- 6540 Q-TOF has improved resolution to greater than 40,000, which fully resolved the myoglobin isotope envelope.
- Different deconvolution algorithms have been applied to the high resolution data. Maximum Entropy, LMFE and PPL generated average mass. MFE and PPL generated monoisotopic mass.
- Deconvolved monoisotopic masses were more accurate (<5ppm) than the deconvolved average masses (50ppm).
- All deconvolution algorithms gave accurate deconvolution masses and the results were consistent between all three algorithms.

In Memory of Tony Ferrige

Sometimes the most significant advances in science are not necessarily due to the inventor of a new technique, they are due to those that laterally link a technology used in one science to that in another. This is how Tony came to introduce the technique of maximum entropy to electrospray mass spectrometry and successfully see the technique commercialized. Sadly, his contribution to science has come to an end and many of us will miss his ebullient approach to using the right mathematical algorithms : comment by Keith Waddell