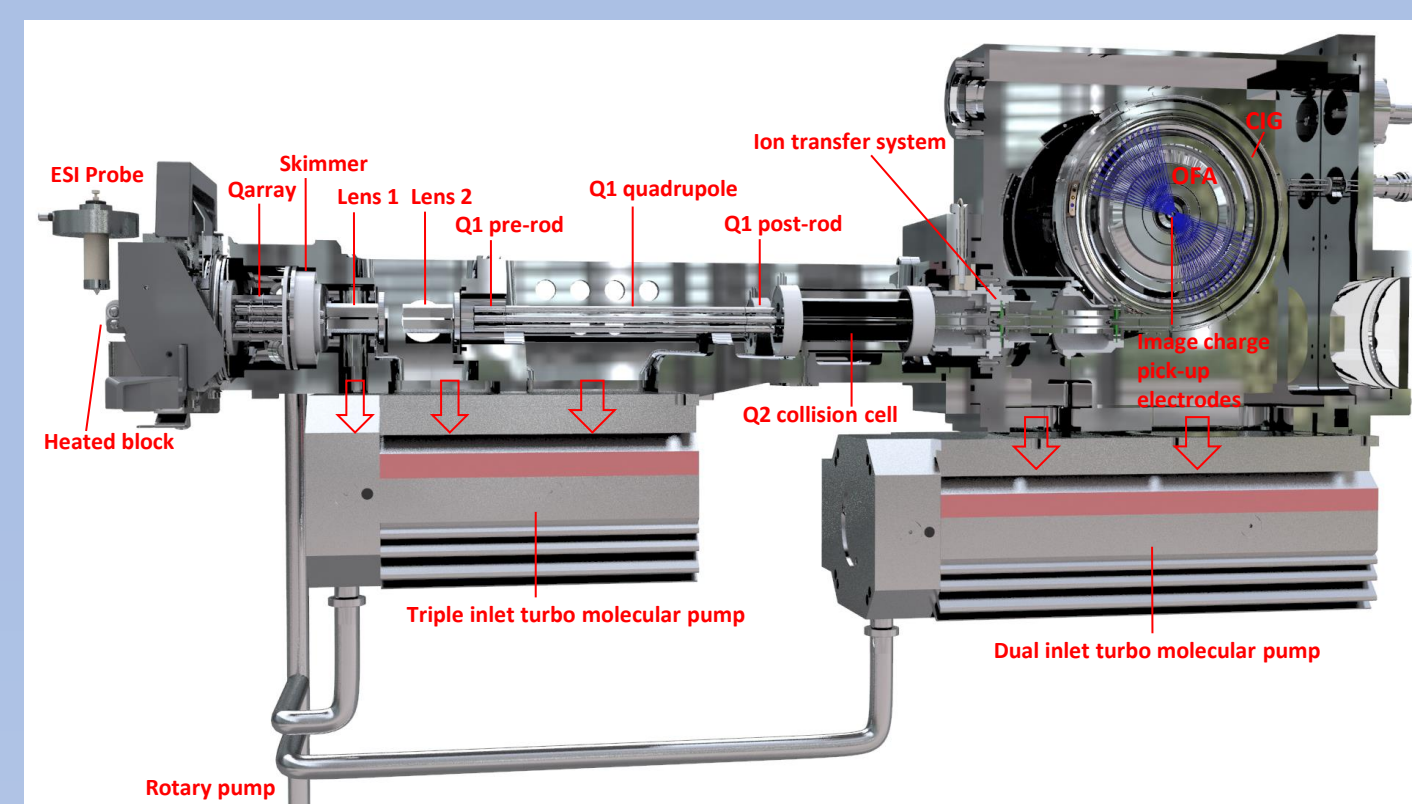
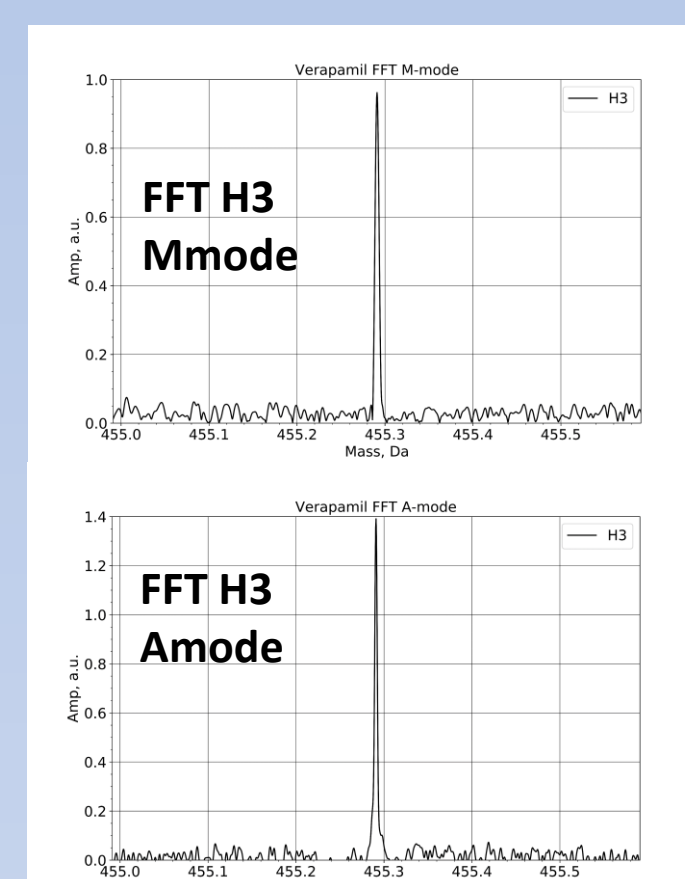
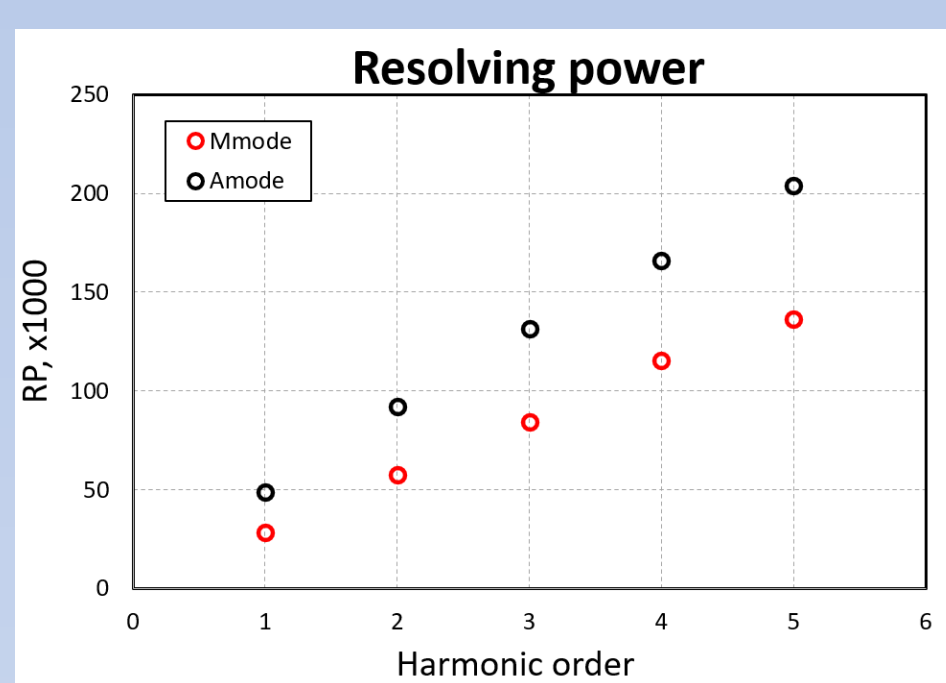


## CDMS OFA data processing

### 1. Orbital Frequency Analyzer (OFA)



- OFA is a High Resolution Mass Spectrometer working on Fourier Transform Mass Spectrometry (FTMS) principle
- Non-sinusoidal image charge signal delivers higher resolving power without acquisition time increase due to high order spectral harmonics
- Example of Verapamil MS (10nM syringe injection):



### 2. CDMS mode of OFA

- It is possible to measure individual ions signal in OFA to implement Charge Detection Mass Spectrometry (CDMS) mode of measurements
- In CDMS a single ion charge Q is measured which allows for true mass measurement:  $M=m/z \cdot Q$
- Mass-to-charge ratio measurement accuracy is high in OFA
- Q accuracy is a critical parameter. It is better with:
  - lower electronic noise level
  - longer measurement time
  - lower vacuum
- Data processing is necessary to measure Q properly:
  - Determine the frequency (or m/z)
  - Determine the lifetime (LT) of an ion
  - Calculate the average intensity of the ion signal over the determined LT and convert it into charge Q
  - Determine the mass by  $M = (m/z) \cdot Q$ , and plot all points as a mass histogram
- High multiplexing reduces overall experiment time and is a challenge for CDMS instruments

#### OFA in publications

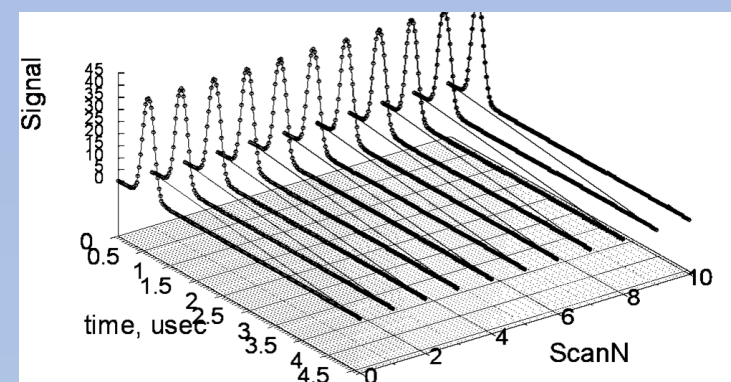
A simulation study of the planar electrostatic ion trap mass analyzer  
Li Ding, Ranjan Badheka, Zhengtao Ding, Hiroaki Nakanishi  
*J Am Soc Mass Spectrom.* 2013 Mar;24(3):356-64

High-Capacity Electrostatic Ion Trap with Mass Resolving Power Boosted by High-Order Harmonics  
Li Ding and Aleksandr Rusinov  
*Anal. Chem.* 2019, 91, 12, 7595-7602

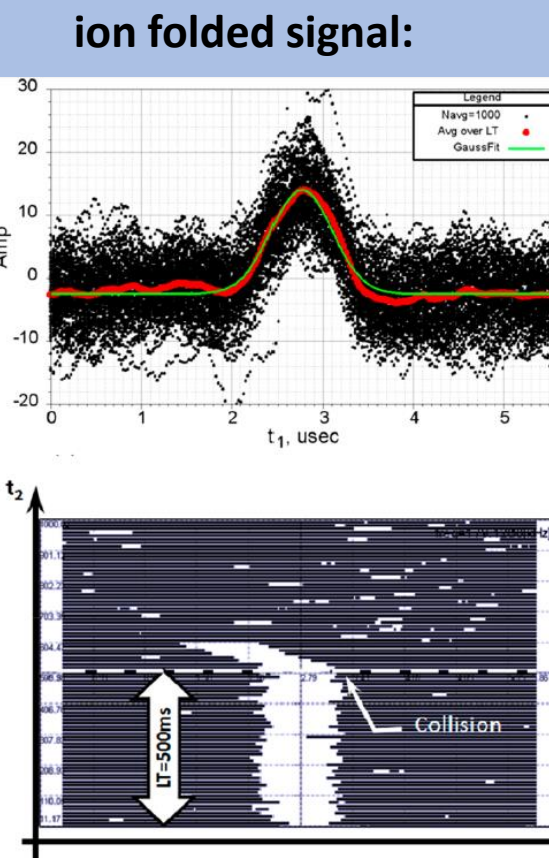
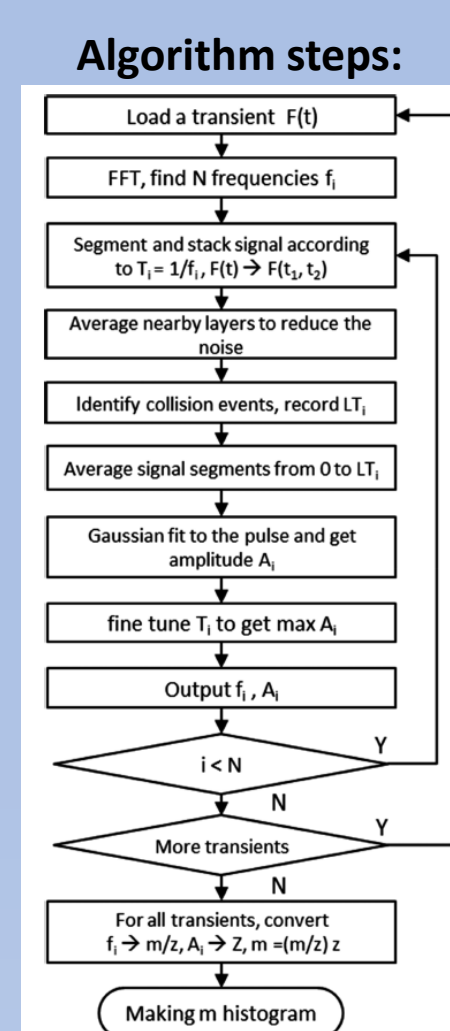
Protein Analysis by Electrospray-Orbital Frequency Analyzer with Charge Detection Mass Spectrometry Algorithm  
Aleksandr Rusinov, Li Ding, Sergey Smirnov, Patrick Knight, Roch Andrzejewski, and Hiroaki Waki  
*J. Am. Soc. Mass Spectrom.* 2021, 32, 5, 1145-1154

### 3. Folded time domain signal method

Ion frequency, LT and dynamics can be seen in folded time domain signal:

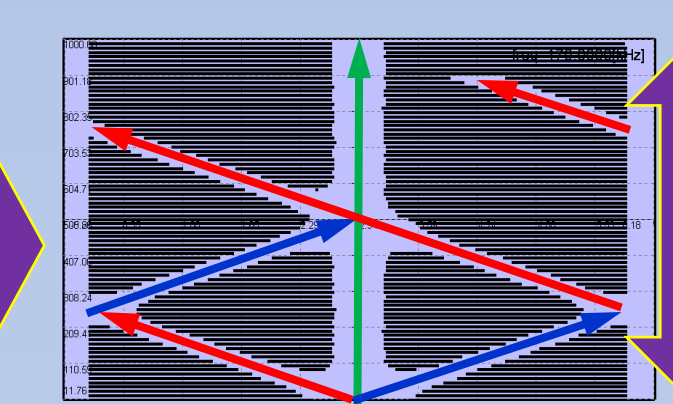


The method is very useful to watch for collisional events and ion frequency change.



Folded artificial signal of 3 ions:

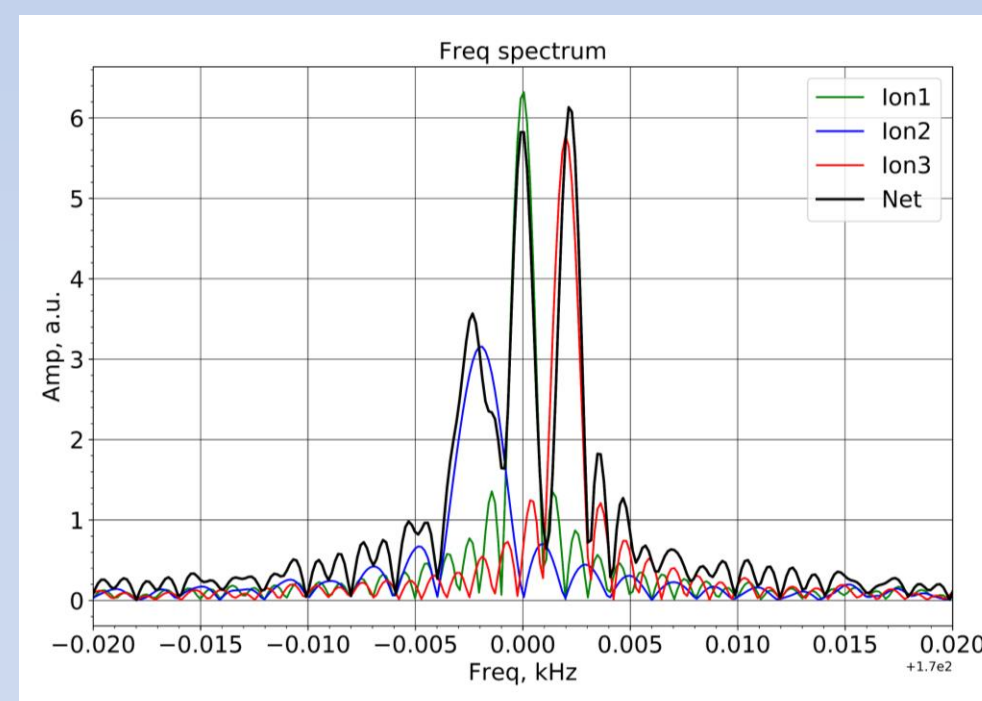
	M, Da	LT[ms]	Q
Ion1	40315.12	1000	50
Ion2	40316.07	500	50
Ion3	40314.17	900	50



### 4. Frequency domain fitting

Takes neighbour ions interference into account

Folded artificial signal of 3 ions:



- Distortion of the intensity (charge) of a target ion is diminished due to elimination of ions with nearby frequencies
- Elimination is performed with respective ions' LT
- Allows to process high multiplexity CDMS data

### 5. Scoring method

For each detected frequency (or  $(m/z)_0$ ) other probable frequencies (or m/z) are calculated:

$$(m/z) = \frac{(m/z)_0 Q_0^* + (n+k)m_1}{Q_0^* + k} \quad (m_1 = m_n = m_n \text{ is a nucleon mass})$$

Score is calculated for each guessed  $Q_0^*$  based on all actual frequencies presented in CDMS data. The best score determines actual (or improved) value of charge  $Q_0$

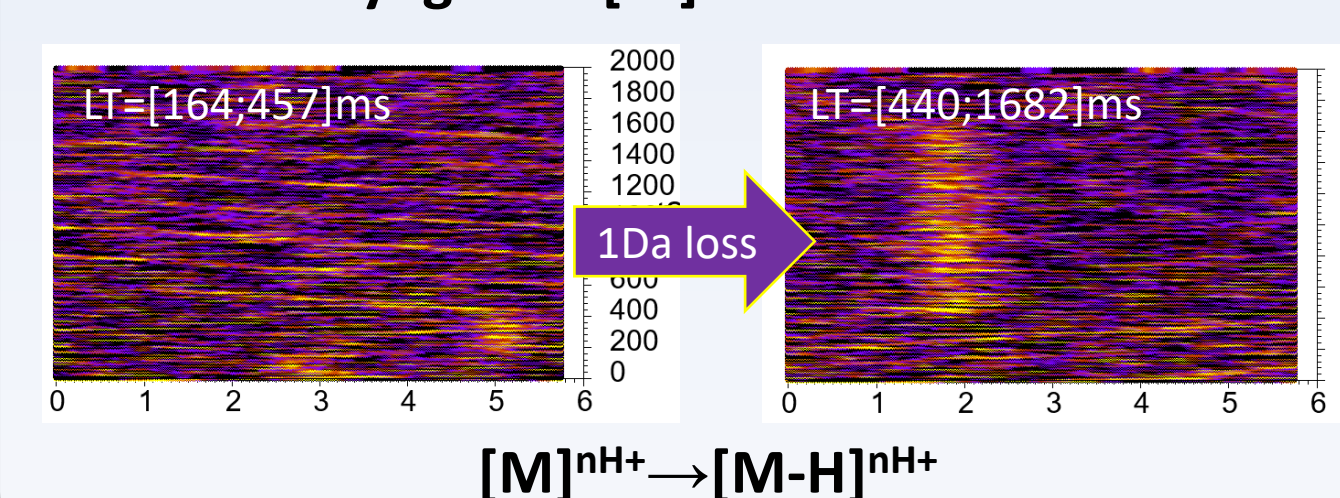
### 6. Mass Loss method (mICDMS)

Collisions of protein ions with gas cause neutral losses resulting in frequency change. Mass loss derived by means of frequency loss depends on charge of an ion → we can adjust charge Q:

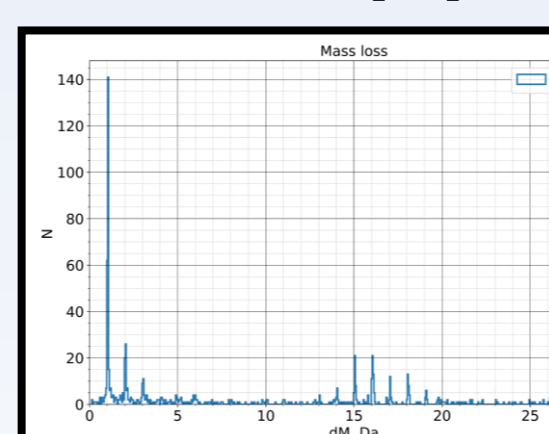
$$\Delta M = Q \left( \frac{m}{z}_1 - \frac{m}{z}_2 \right)$$

Proper charge Q – ideal MS!

Myoglobin [M]<sup>22H+</sup> loses 1Da:



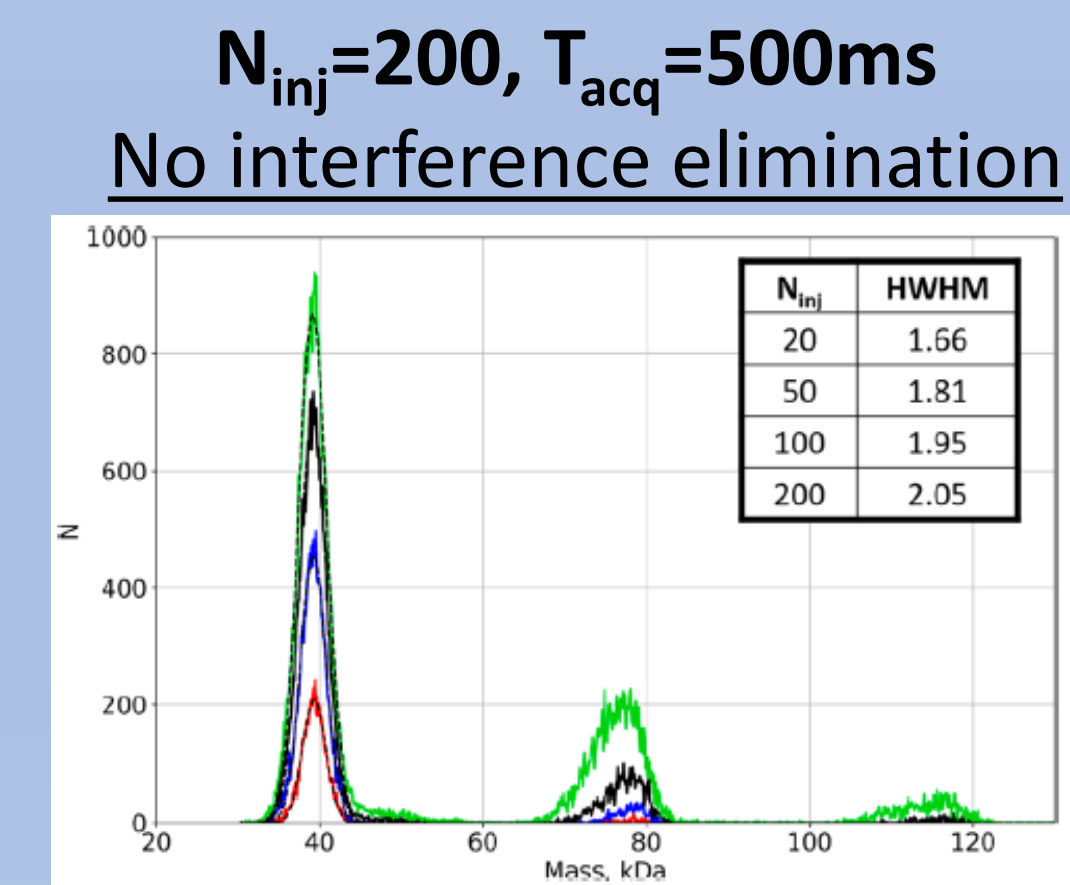
Observed mass losses for [M]<sup>22H+</sup>



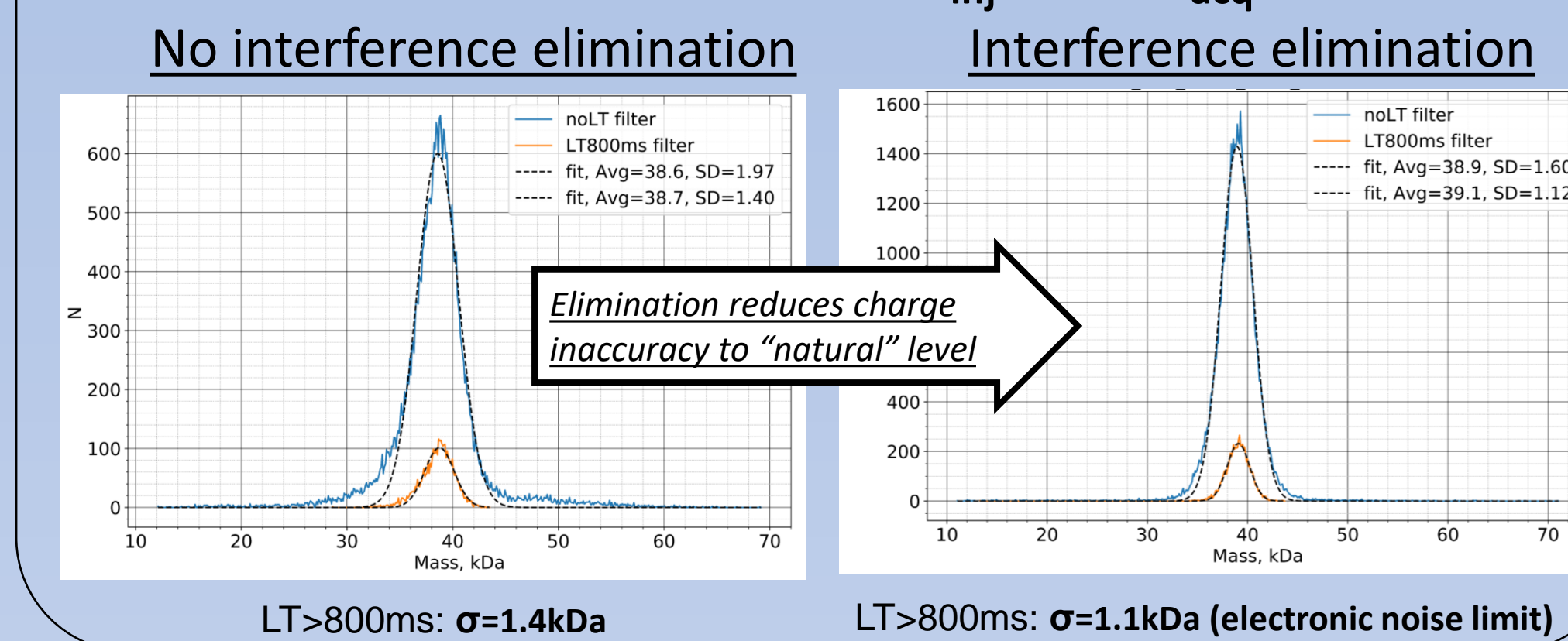
### 7. High multiplexing CDMS simulations

Folded time domain signal method:

Charge inaccuracy increases with number of ions per injection



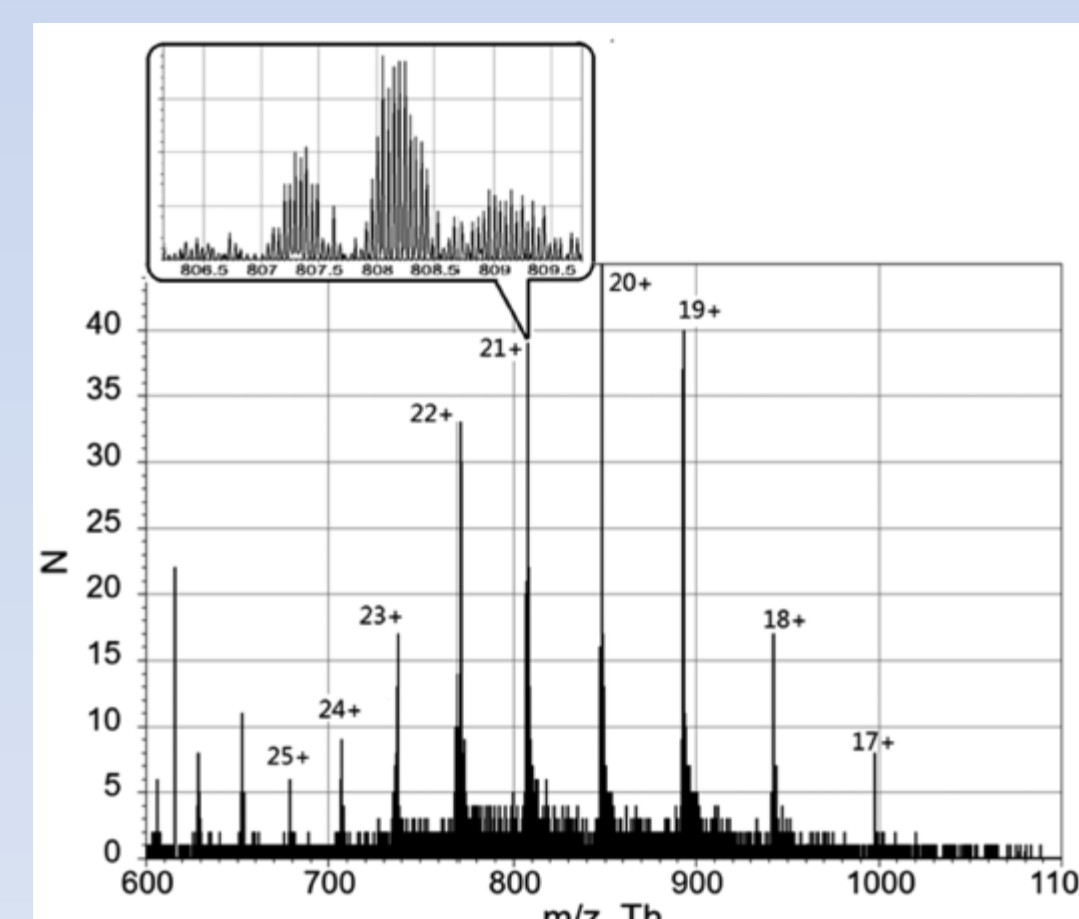
Frequency domain fitting,  $N_{inj}=200, T_{acq}=1000ms$



### 9. CDMS mode proteins measurements by OFA

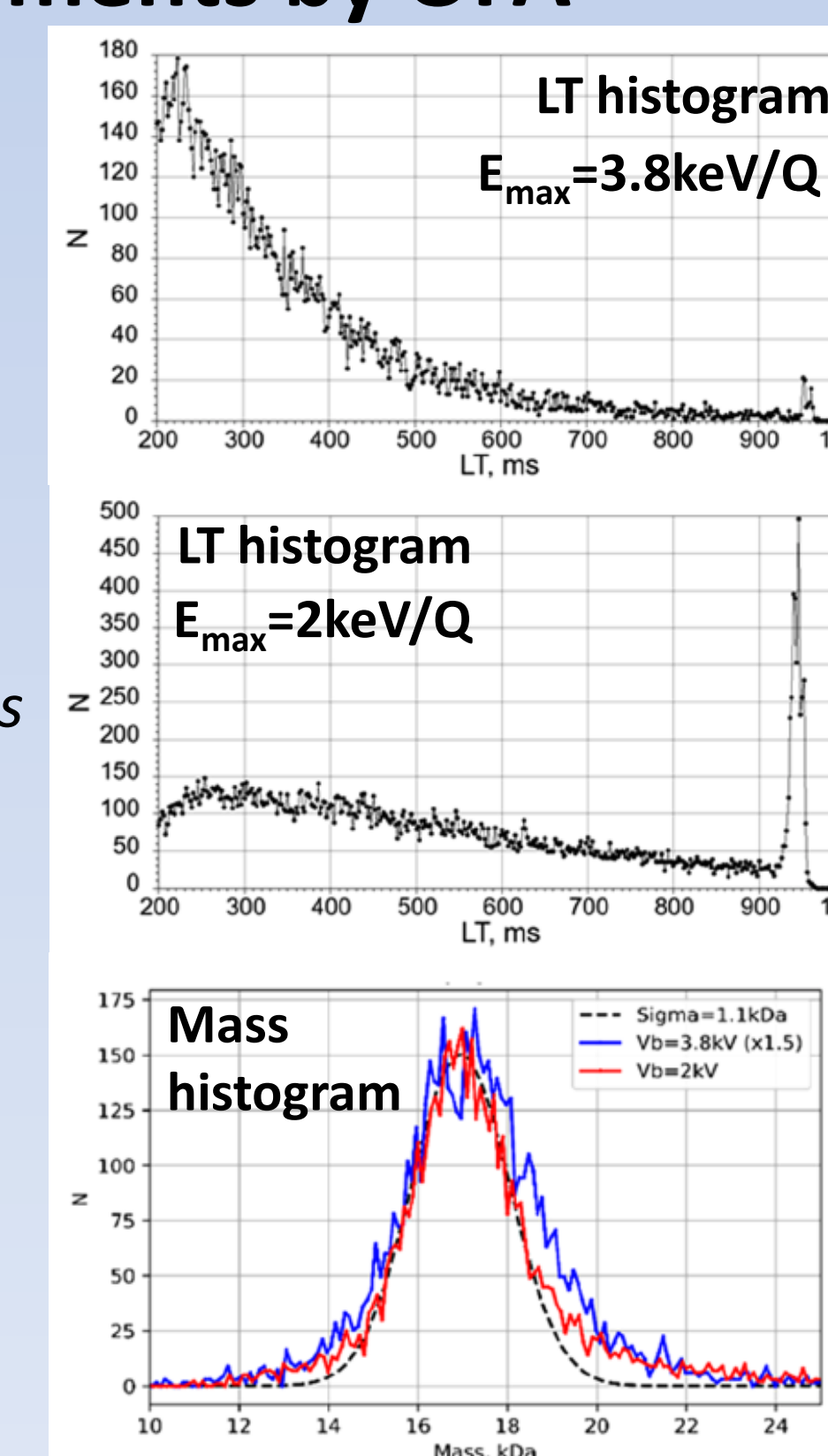
Myoglobin 40nM

Myoglobin isotopically resolved m/z histogram:



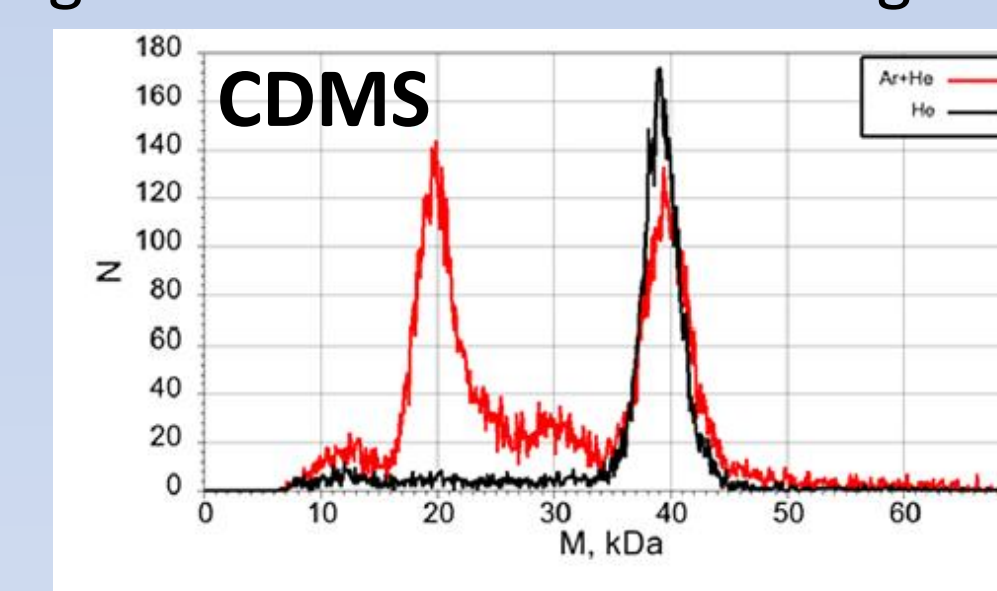
Ion energy reduction increases ions LT and improves Q accuracy

Much smaller concentrations are used to get reasonable m/z and mass spectra

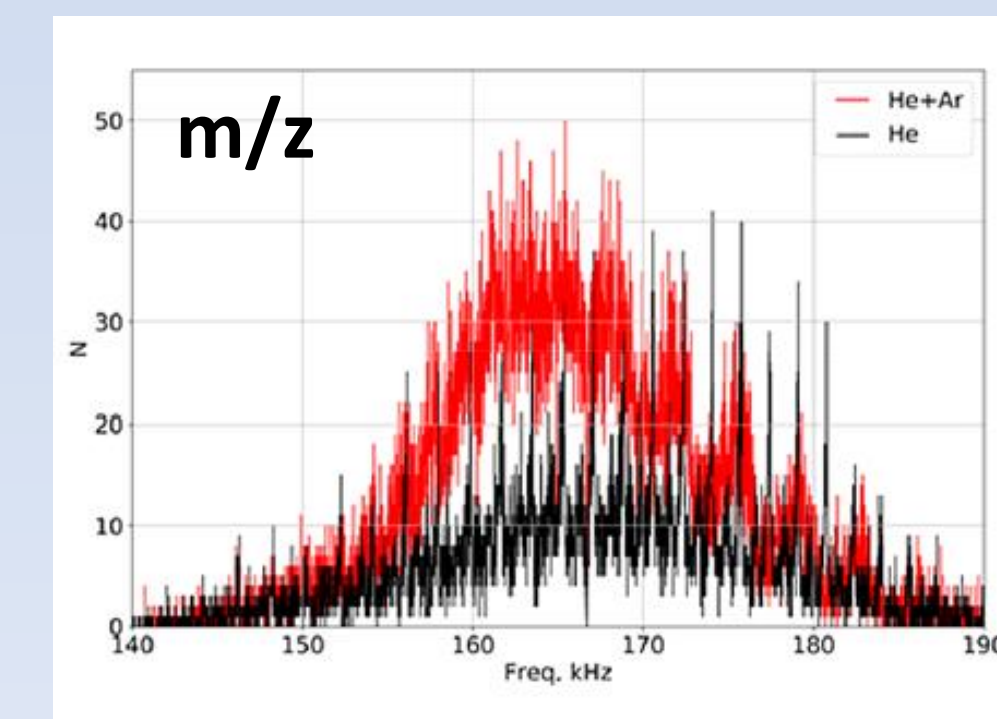


Aldolase 70nM

Fragmentation is seen with Ar gas:

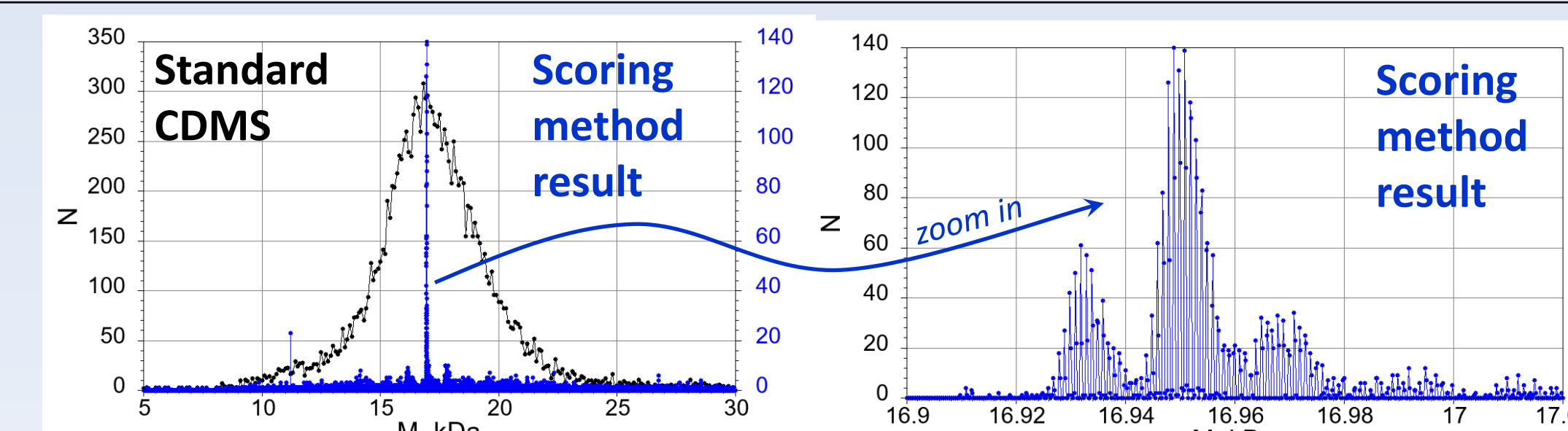


No fragmentation can be distinguished in m/z spectrum:



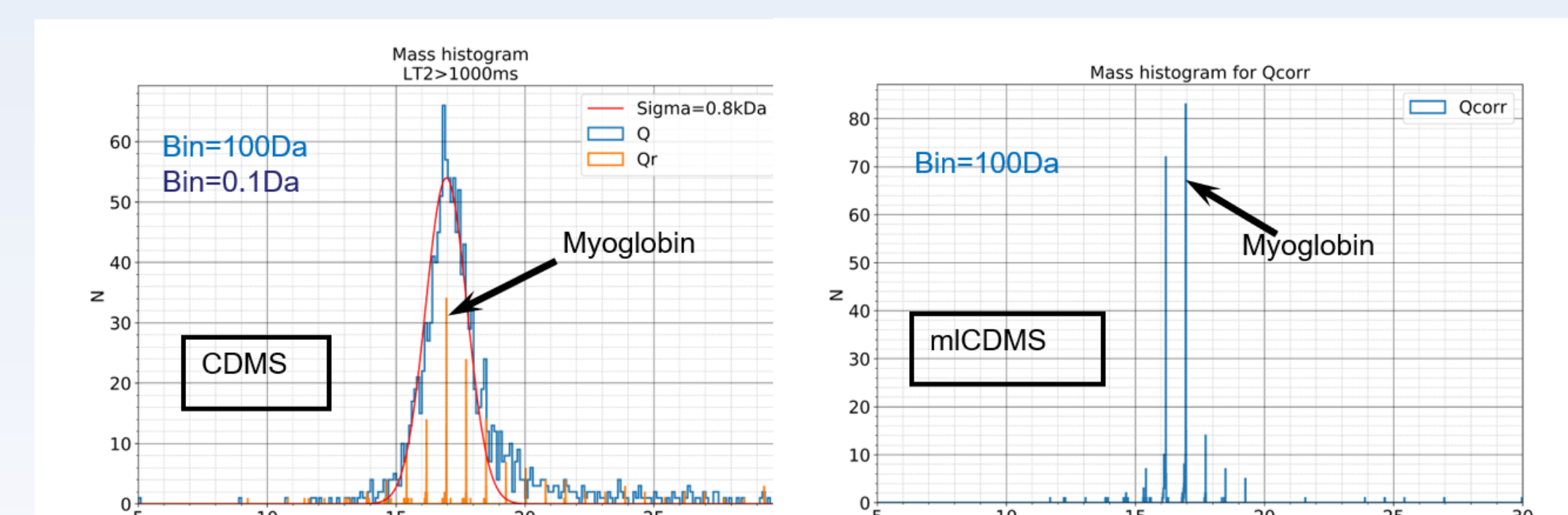
### 10. Example data processing

An example of Scoring method applied to Myoglobin data:



An example of Mass Loss CDMS method\* applied to Myoglobin data:

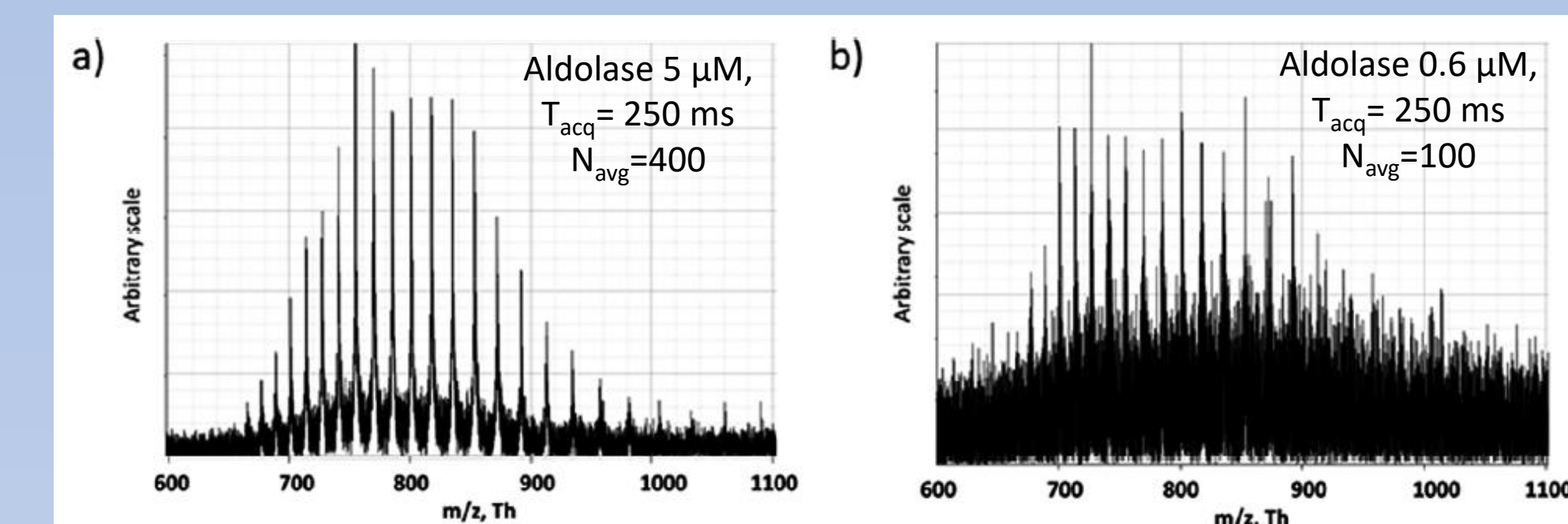
\*algorithm is being improved



### 8. Experimental conditions

- Proteins purchased from Sigma-Aldrich:
  - Myoglobin from equine heart
  - Aldolase from rabbit muscle
- Sample solutions were prepared using mixture of H<sub>2</sub>O and acetonitrile with ratio of 1:1 with 0.5% formic acid
- He/Ar gas for ions cooling
- Vacuum pressure (3-4)x10<sup>-10</sup> Torr

FTMS mode



High concentrations are required to get reasonable m/z spectrum

### 11. Conclusion

- In addition to a standard HR FTMS mode OFA can work in CDMS mode to measure high mass molecular ions
- Isotopic resolution can be achieved for proteins
- High multiplexing CDMS measurements can be utilized without measured charge accuracy deterioration
- Methods of charge accuracy improvement without electrical noise reduction have been developed and are being tested