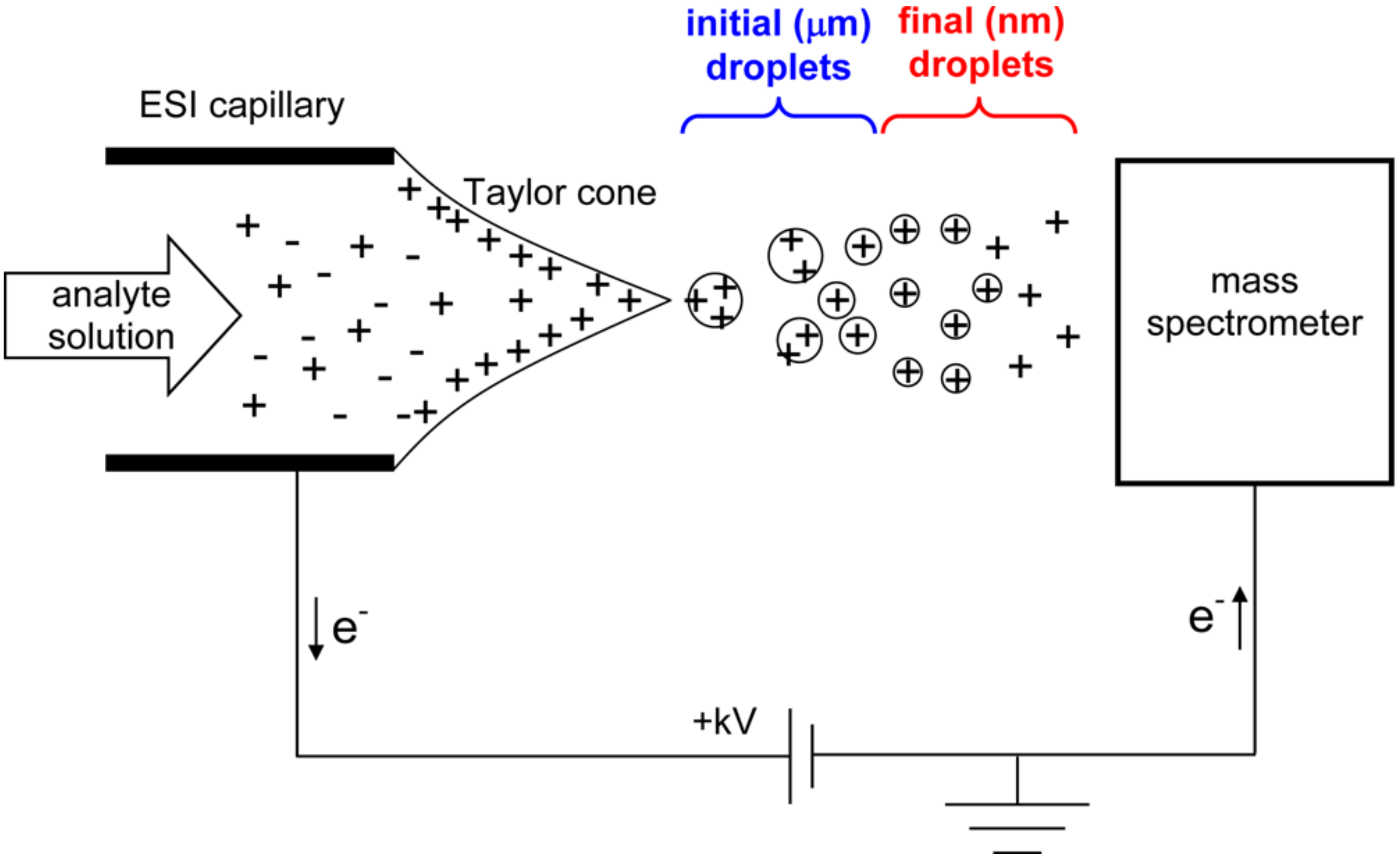
Electrospray ionization (ESI)



How can ions be generated from charged liquid droplets?

Ion evaporation model:

Electric field from a charged nanodroplet is sufficiently high to cause the ejection of small solvated ions from the droplet surface.

- Low molecular Wight species

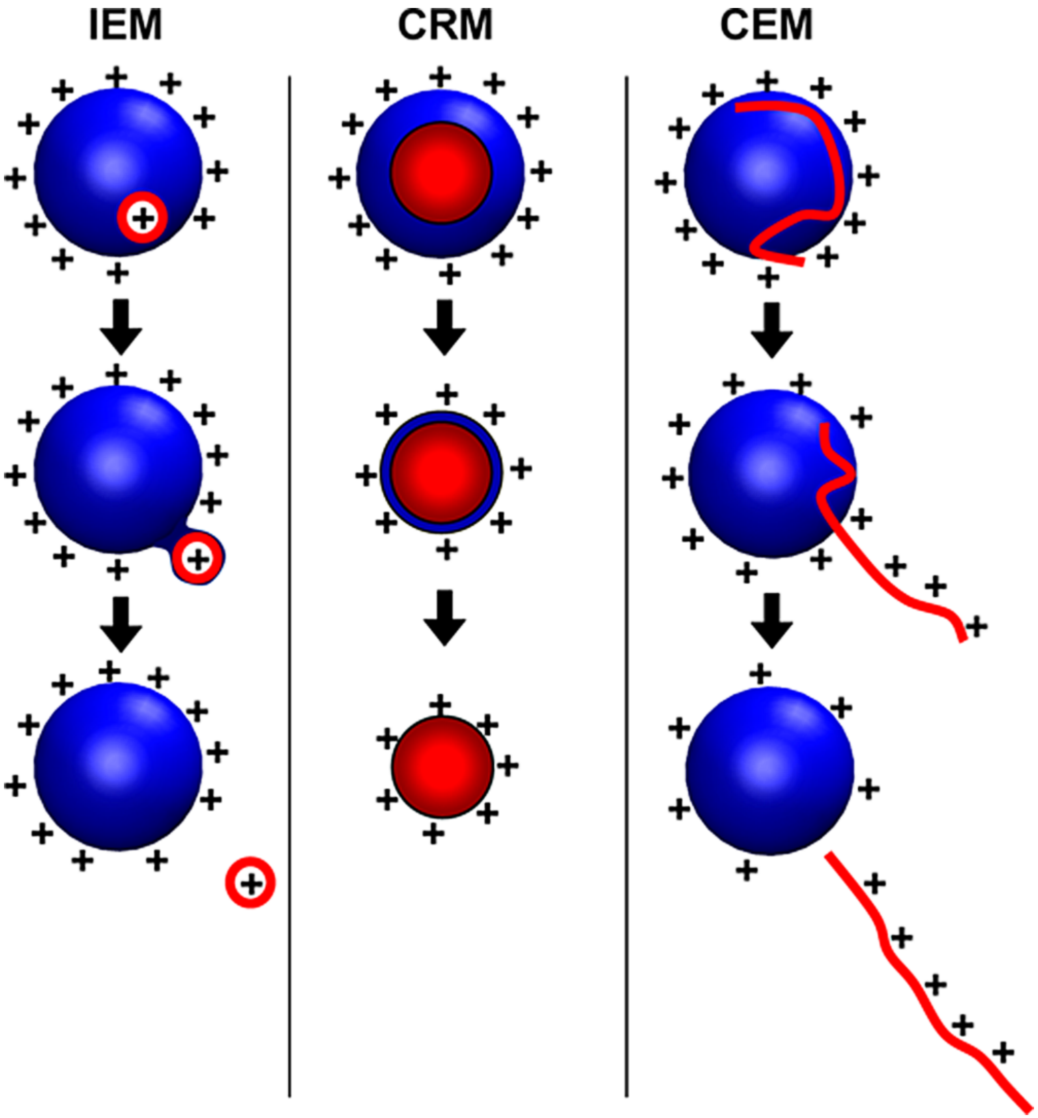
Charged residue model:

Nanodroplets that contain a single analyte evaporate to dryness. As the last solvent shell disappears, the charge of the vanishing droplet is transferred to the analyte.

- Large globular species

Chain ejection model:

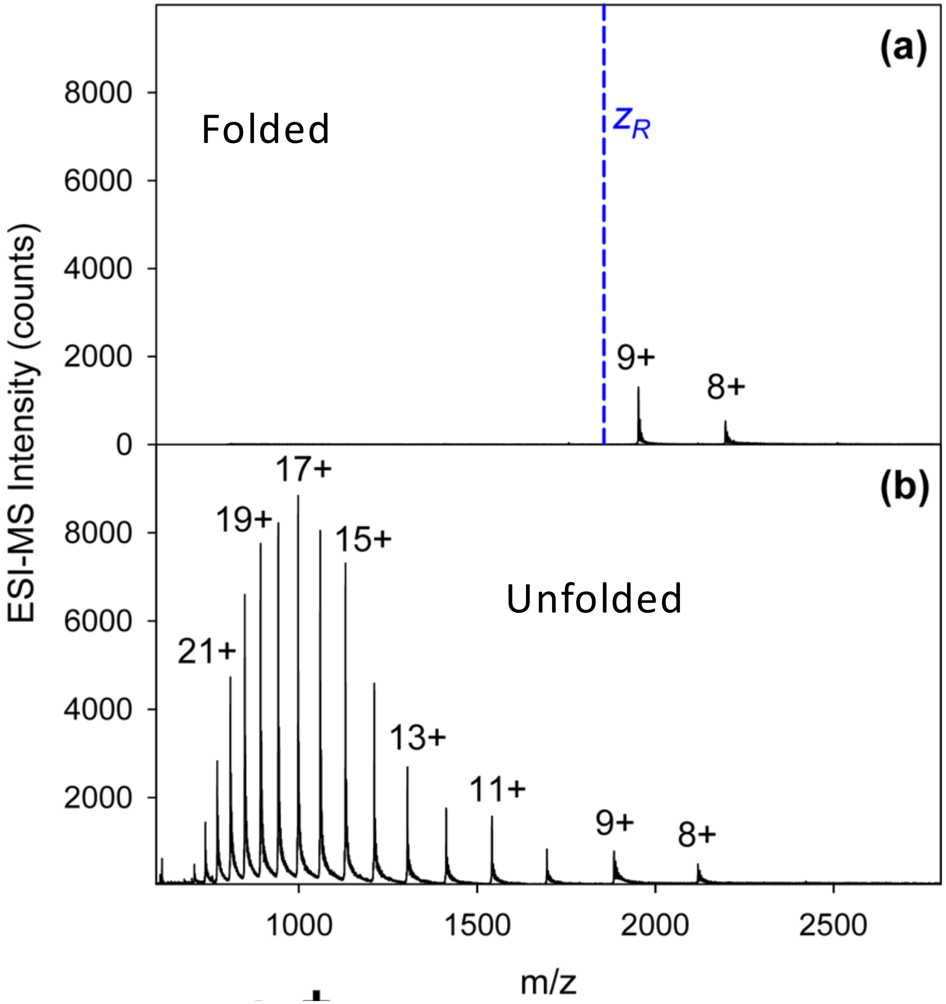
Unfolding switches the properties of the protein from compact/hydrophilic to extended/hydrophobic, which makes it unfavorable to reside in a droplet interior. They migrate to the droplet surface and get ejected.



Anal. Chem. 2013, 85, 2–9

Factors affecting ESI signal intensity

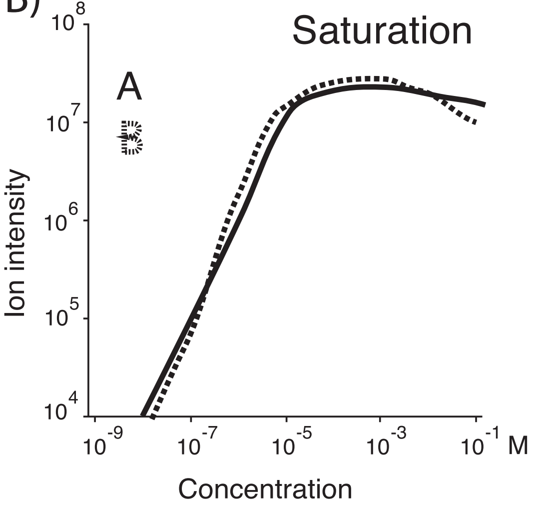
- Hydrophobic proteins are ionized more efficiently than hydrophilic ones
- Folded globular proteins tend to generate ESI mass spectra with relatively low intensities, whereas unfolded polypeptide chains provide more intense signals



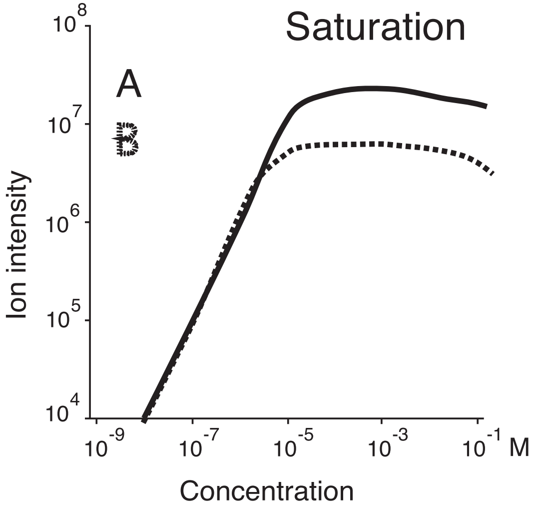
Factors affecting ESI signal intensity

Sample concentration

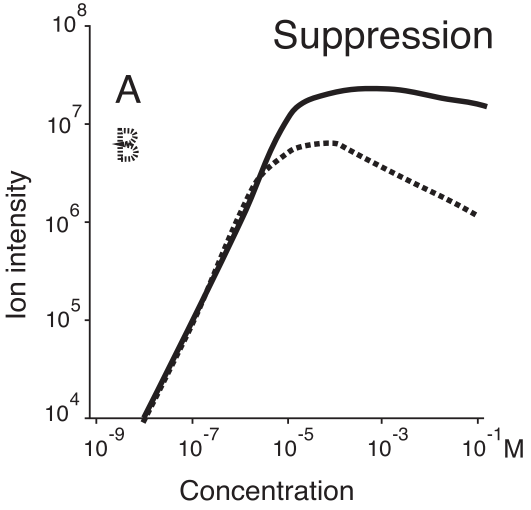
B)



Signal increases over 3 orders of magnitude and reaches saturation



2 samples may saturate at different intensities



More hydrophobic sample suppresses hydrophilic sample

Molecular & Cellular Proteomics 10: 10.1074/mcp.M111.009407, 1–8, 2011.

Calculating the mass of protein from and ESI spectrum

For two adjacent peaks (in a multiple-charge ion mass spectrum) m_1/z_1 and m_2/z_2 where $z_1 = z_2 + 1$

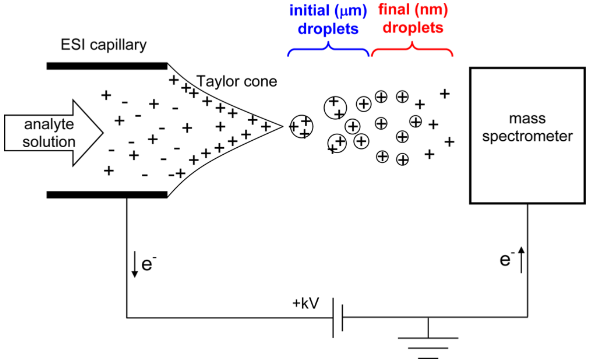
$z_2 = ((m_1/z_1) – 1) / ((m_2/z_2) – (m_1/z_1))$

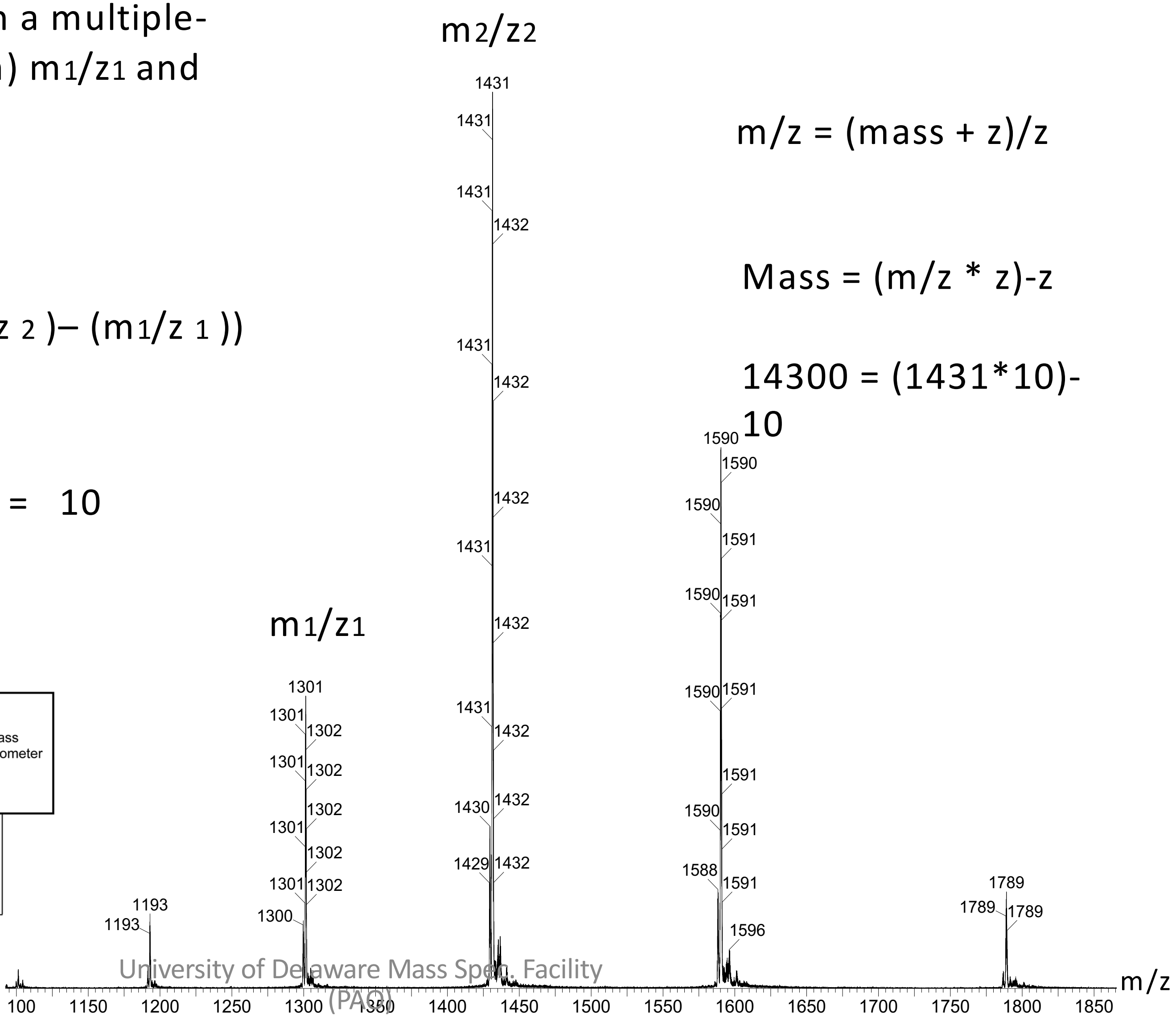
$Z_2 = 1301 - 1/ (1431-1301) = 10$

$m/z = (\text{mass} + z)/z$

$\text{Mass} = (m/z * z)-z$

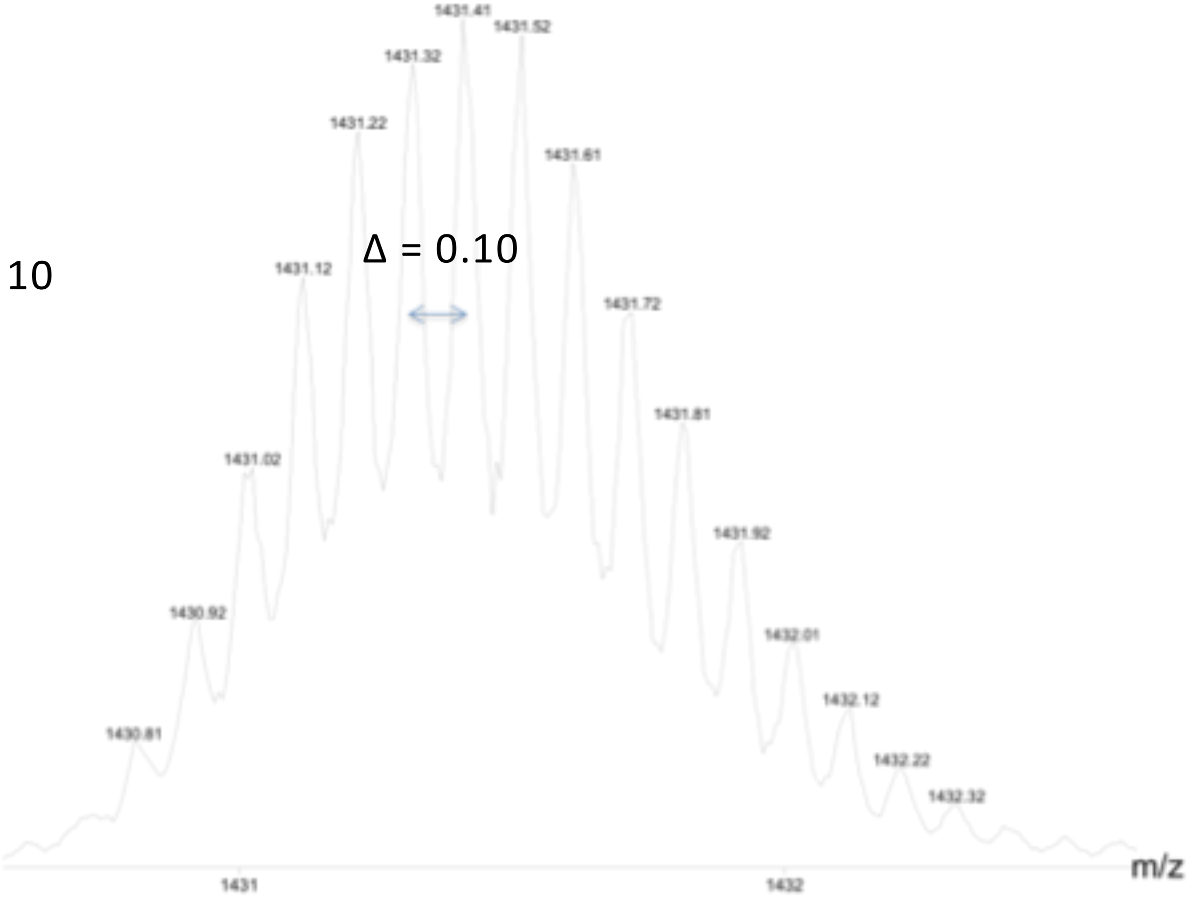
$14300 = (1431*10)-10$





Determining charge sate from isotopic distribution

Z = 1/ Δ = 10



Δ = 0.10

MaxEnt

- MaxEnt1 uses an entropy based deconvolution algorithm that utilizes probabilistic distance as a function of mass of parent ion.
- This requires translating mass spectra into vectors and probabilities
- A probability distribution is constructed from the mass spectrum and is compared to a model of multiply charged ion of the parent mass.
- A plot of this difference as a function of parent mass is the deconvoluted spectrum

J Am Soc Mass Spectrom 1992, 3, 207-215

MaxEnt1 deconvolution

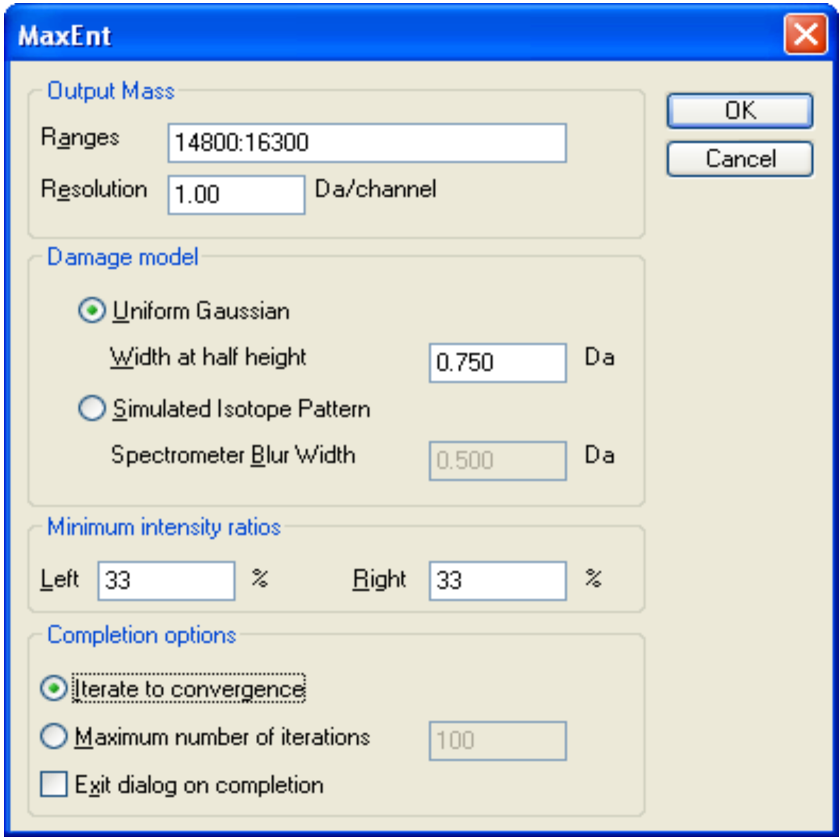
1. Ranges: This is the mass range which will be examined .

2. Resolution: Low numbers mean lots of resolution. ie The answer will be more precise but the processing will take longer. High resolution (long processing time) is approx 0.01. Low resolution (fast processing time) is approx 20.

3. Damage model: This is how Max Ent will evaluate the data. Uniform Gaussian is typically used.

Width at half height: Find a peak in the center of the charge state envelope and measure the width at half height. This is the value to be entered.

4. Minimum intensity ratios: This is the height of adjacent peaks relative to the most intense peak which will be considered as part of the charge state envelope. 33 – 33 usually works well.



MaxEnt

Output Mass

Ranges 14800:16300

Resolution 1.00 Da/channel

Damage model

● Uniform Gaussian

Width at half height 0.750 Da

○ Simulated Isotope Pattern

Spectrometer Blur Width 0.500 Da

Minimum intensity ratios

Left 33 % Right 33 %

Completion options

● Iterate to convergence

○ Maximum number of iterations 100

☐ Exit dialog on completion

MaxEnt result is quantitave: it reflects the peak intensities of species in the mass spectrum

Consideration for running modified protein samples

- Be aware of signal intensity saturation.
- Modification or breaking of disulfide bonds may cause protein to be extend → CEM, more efficiently ionized. Check charge state distribution.
- Some artifacts /peak harmonics may occur when using MaxEnt → check raw data to rule out artifacts.
- In a complex mixture, hydrophobic analytes can suppress the signal of hydrophilic analytes.