

What does a mass spectrometer do?

**1. It determines the mass of a compound better than any technique**

Sensitivity: peptides can be detected at attomol ( $10^{-18}$ ) levels (typically femtomols  $10^{-15}$ )

Accuracy: the difference which is observed between the theoretical mass and the measured mass of a compound.

$\Delta m$  accuracy =  $m_{(real)} - m_{(measured)}$

Expressed in parts per million (ppm)  $ppm = 10^6 \times \Delta m / m$  (measured)

less than 1ppm can be achieved (typically <10ppm)

Example: A 10 ppm error for a peptide of 1000Da =  $\pm 0.01$  Da (1000.01)

Resolution: The ability to separate two adjacent peaks masses

Resolution =  $mass / \Delta mass$

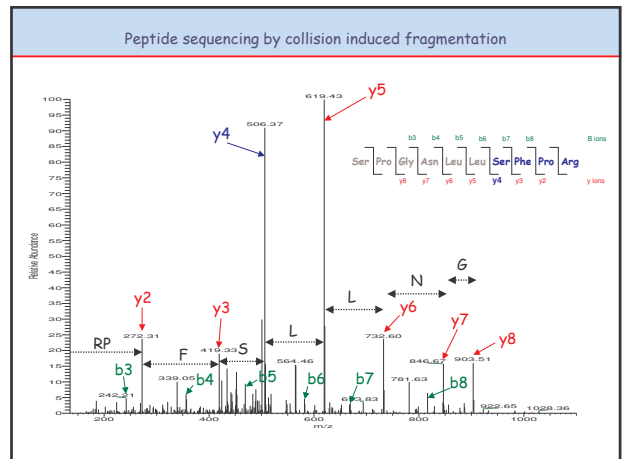
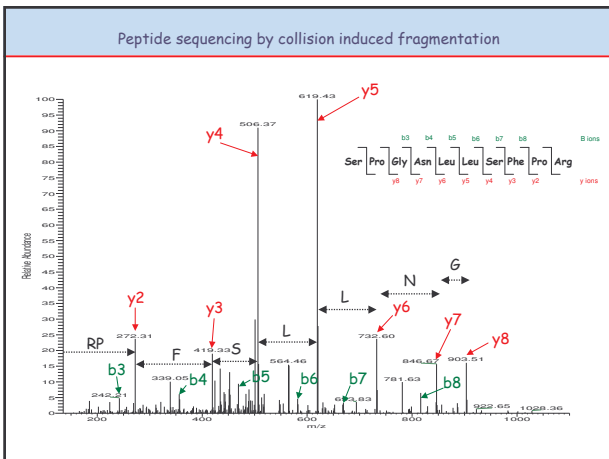
Trimethyllysine	42.0470 Da
Acetylysine	42.0106 Da
	00.0364 Da

Resolution of 500 000 @ 1000Da

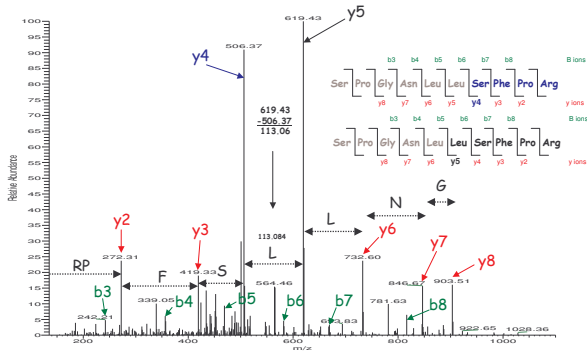
What does a mass spectrometer do?

**2. It can provide information about chemical structure**

Sequence information: Collision induced fragmentation



Peptide sequencing by collision induced fragmentation



What are mass spectrometers good for?

- Identify: peptide fingerprint mapping, de novo sequencing
- Verify structure: intact proteins, post translational modifications
- Quantify: labeling strategies, peak intensities

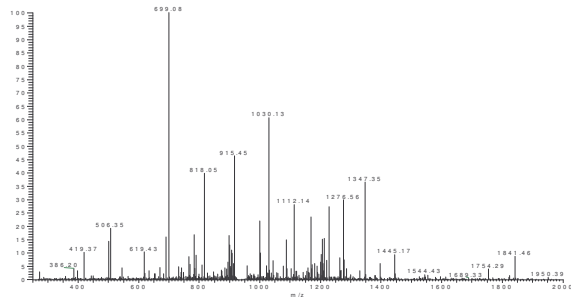
Applications:

- High throughput analysis of complex protein/peptide mixtures
- Targeted analysis of individual proteins

Phosphatidylinositol 5phosphate 4-kinase  $\beta$  (Type II PIPKinase)

MSMS fragmentation of peptide ion m/z 947.78 (Control)

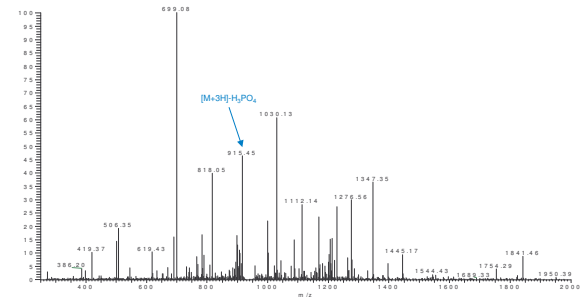
Parent mass  
947.78 3+ ion



MSMS fragmentation of peptide ion m/z 947.78 (Control)

Parent mass  
947.78 3+ ion

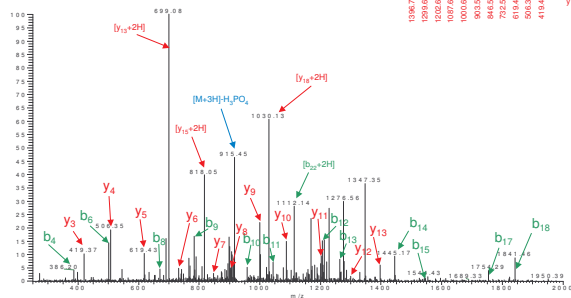
Phospho-N D G V G G L L C S Y G T P P D S P G N L L S F P R



MSMS fragmentation of peptide ion m/z 947.78 (Control)

Parent mass  
947.78 3+ ion

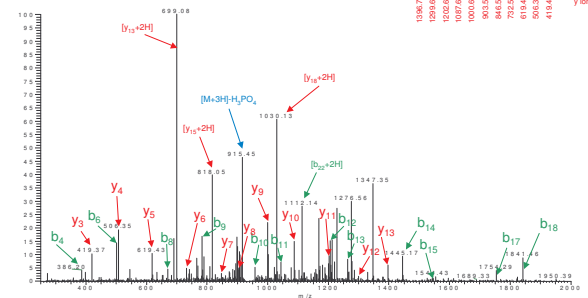
b ions  
Phospho-N D G V G G L L C S Y G T P P D S P G N L L S F P R  
y ions



MSMS fragmentation of peptide ion m/z 947.78 (Control)

Parent mass  
947.78 3+ ion

b ions  
Phospho-N D G V G G L L C S Y G T P P D S P G N L L S F P R  
y ions



Some interesting points

Intensities suggest  
a ratio of 1:1 (non-phospho:phospho)

Phosphopeptide elutes later  
than the non-phospho

