



Purdue-UAB Botanicals Center for Age-Related Disease

Tandem mass spectrometry of peptides

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Mass Spectrometry Methods in Botanicals Research**



Applications of tandem MS-MS to analysis of proteins

- **To confirm the identity of a protein from analysis of the sequence of its protease-cleaved peptides**
- **To identify the site of a posttranslational modification**

Instruments for tandem MS-MS

- **Although tandem mass spectrometry of peptides can be carried out on less sensitive instruments, the majority of peptide sequencing is performed on three major types:**
 - **A triple quadrupole instrument**
 - **An ion trap instrument**
 - **A hybrid quadrupole orthogonal-TOF (Q-tof or Qstar)**



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LC-MS-MS

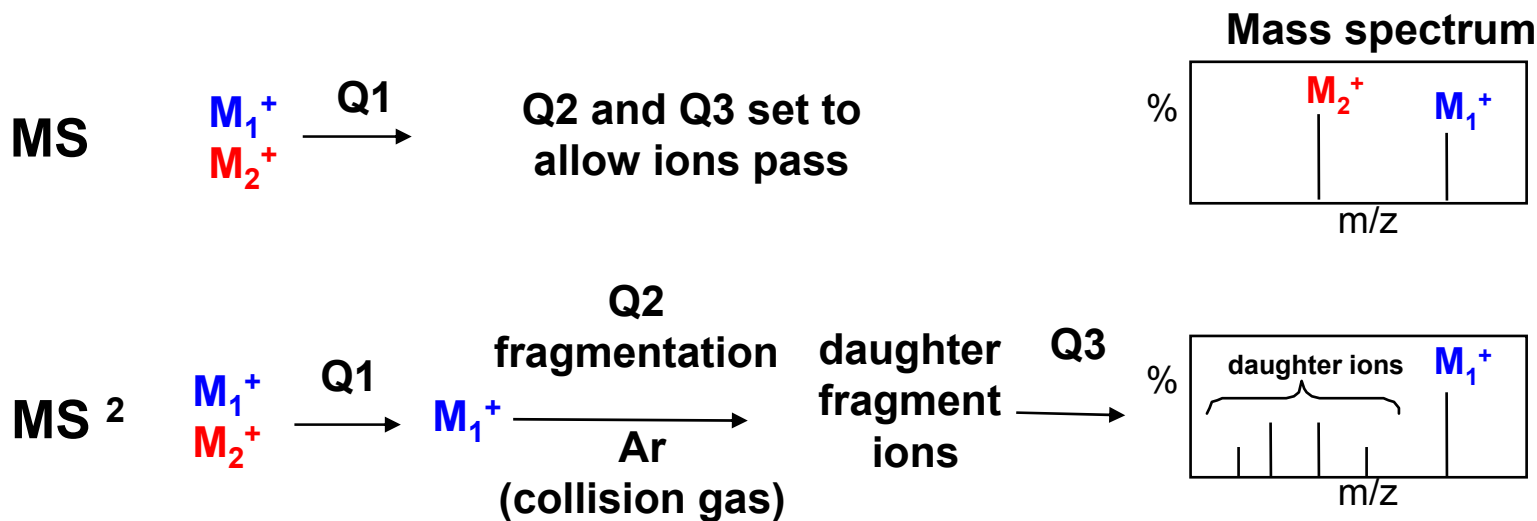
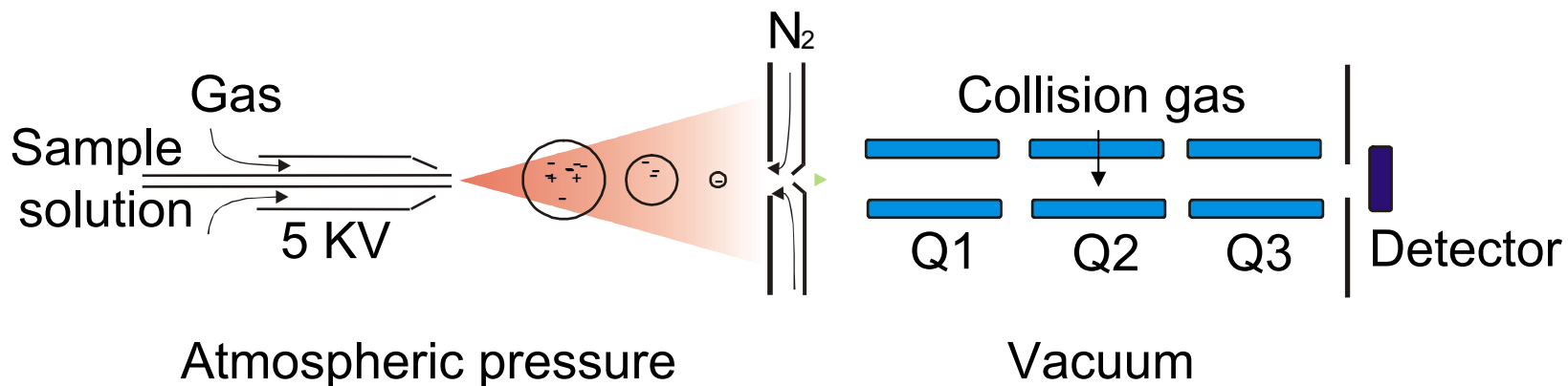
- **The complexity of the peptides in most samples requires some level of chromatographic separation - the search for modifications demands it**
- **Gradient LC analysis allows relatively large amounts of sample (2-6 μ l) to be loaded onto a reverse-phase column which is then eluted at 200-400 nl/min**

nanoLC-ESI-MSMS

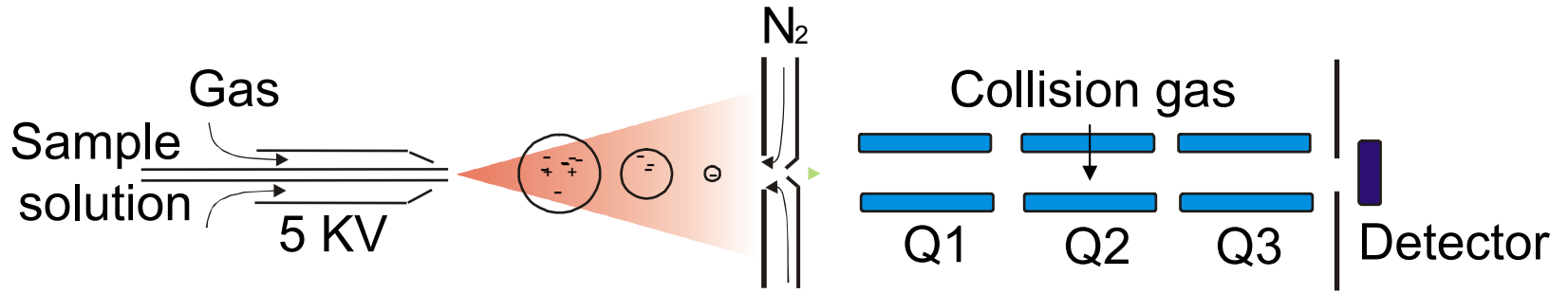
- For electrospray, smaller volumes are better. This has been taken to an extreme. Columns with diameters of $75\ \mu\text{m}$ or lower are routinely used



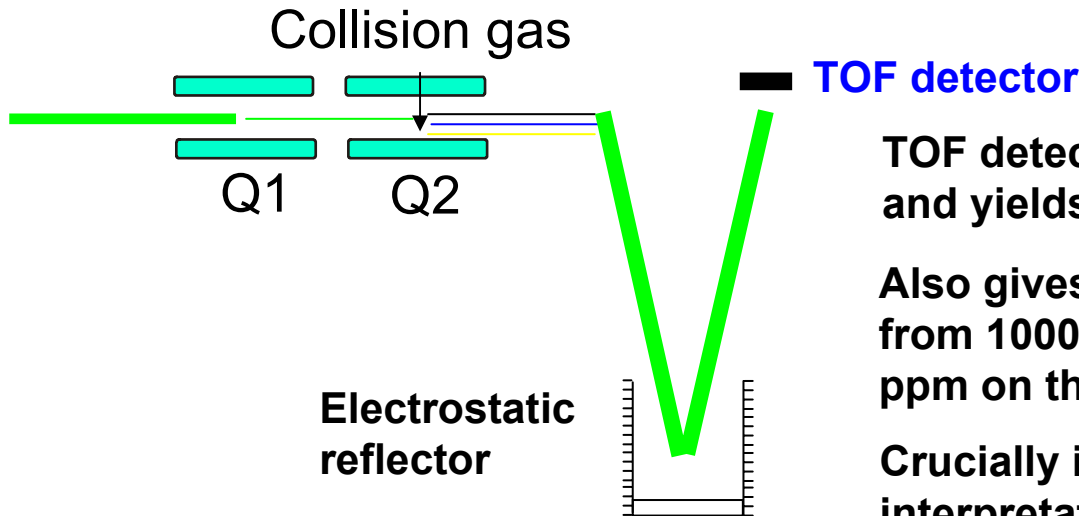
LC-ESI-MS Analysis



Triple quad versus Q-tof and sensitivity



The quadrupole analyzer (Q3) is slow and insensitive - it's a filter - thus throws away large amounts of data



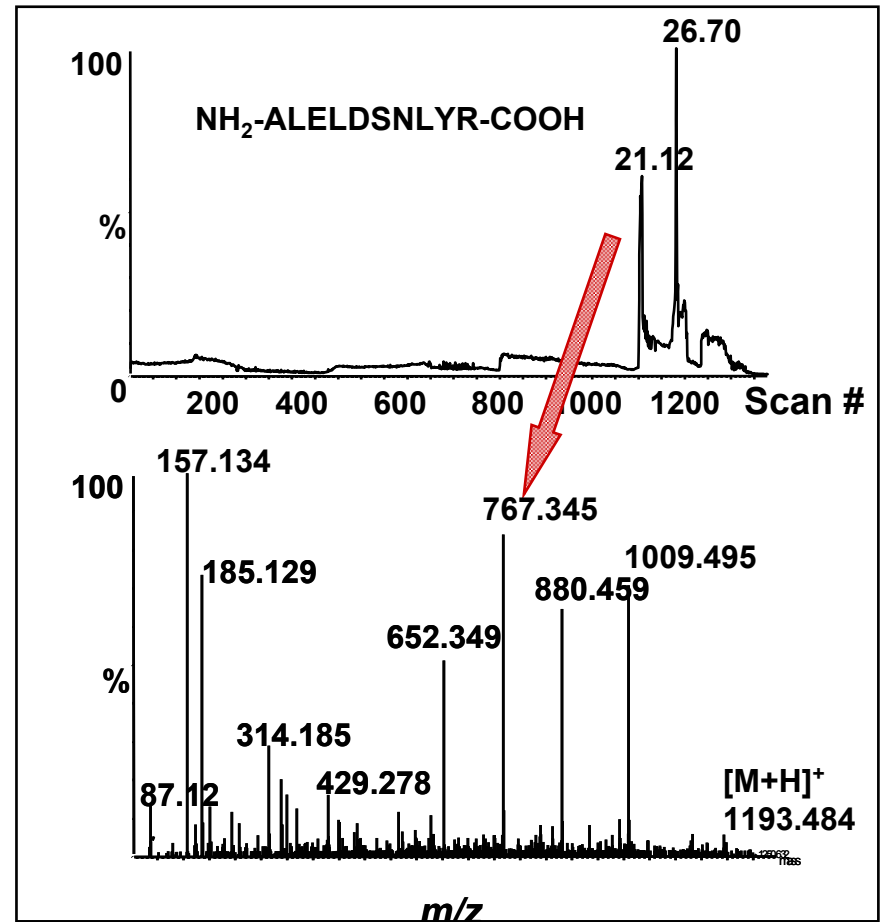
TOF detector collects all ions generated and yields fmol rather than pmol sensitivity

Also gives far greater mass accuracy - from 1000 ppm on the triple quad to 5-10 ppm on the Q-tof

Crucially important for automated interpretation of MS-MS spectra to yield amino acid sequence

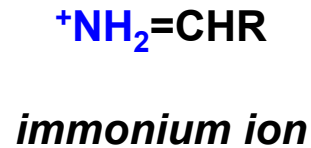
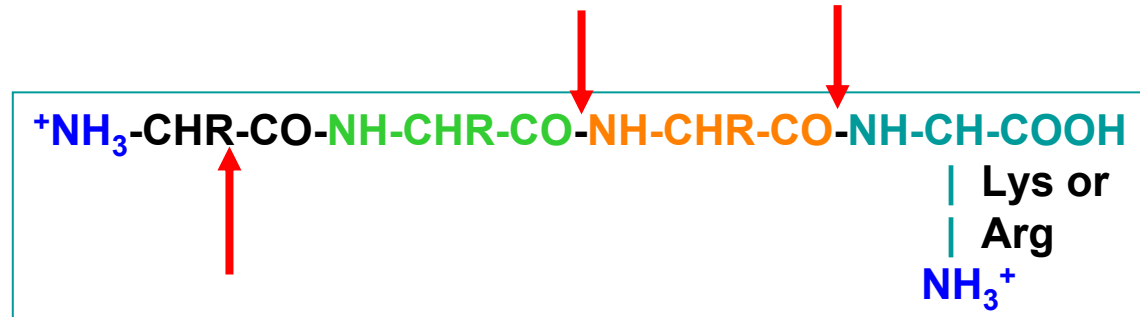
Reverse phase nanoLC-MSMS

- Peptides are separated on an acetonitrile gradient using columns with i.d.s of 0.05-0.30 mm. These operate at 200-2000 nL/min
- Peptides are introduced by electrospray and analyzed on a Qqtof. Ions are selected by a quadrupole filter, collision-dissociated and analyzed by time-of-flight (accuracy 5-10 ppm)

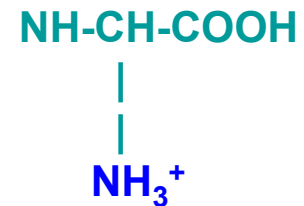


Fragmentation of peptide ions

Tryptic peptides are charged at each end



b2 ion



y1 ion

Residue masses of amino acids

Ala - A	71.0371
Arg - R	156.1011
Asn - N	114.0429
Asp - D	115.0269
Cys - C	103.0092
Glu - D	129.0426
Gln - Q	128.0586
Gly - G	57.0216
His - H	137.0589
Ile - I	113.0841
Leu - L	113.0841
Lys - K	128.0950
Met - M	131.0405
Phe - F	147.0684
Pro - P	97.0528

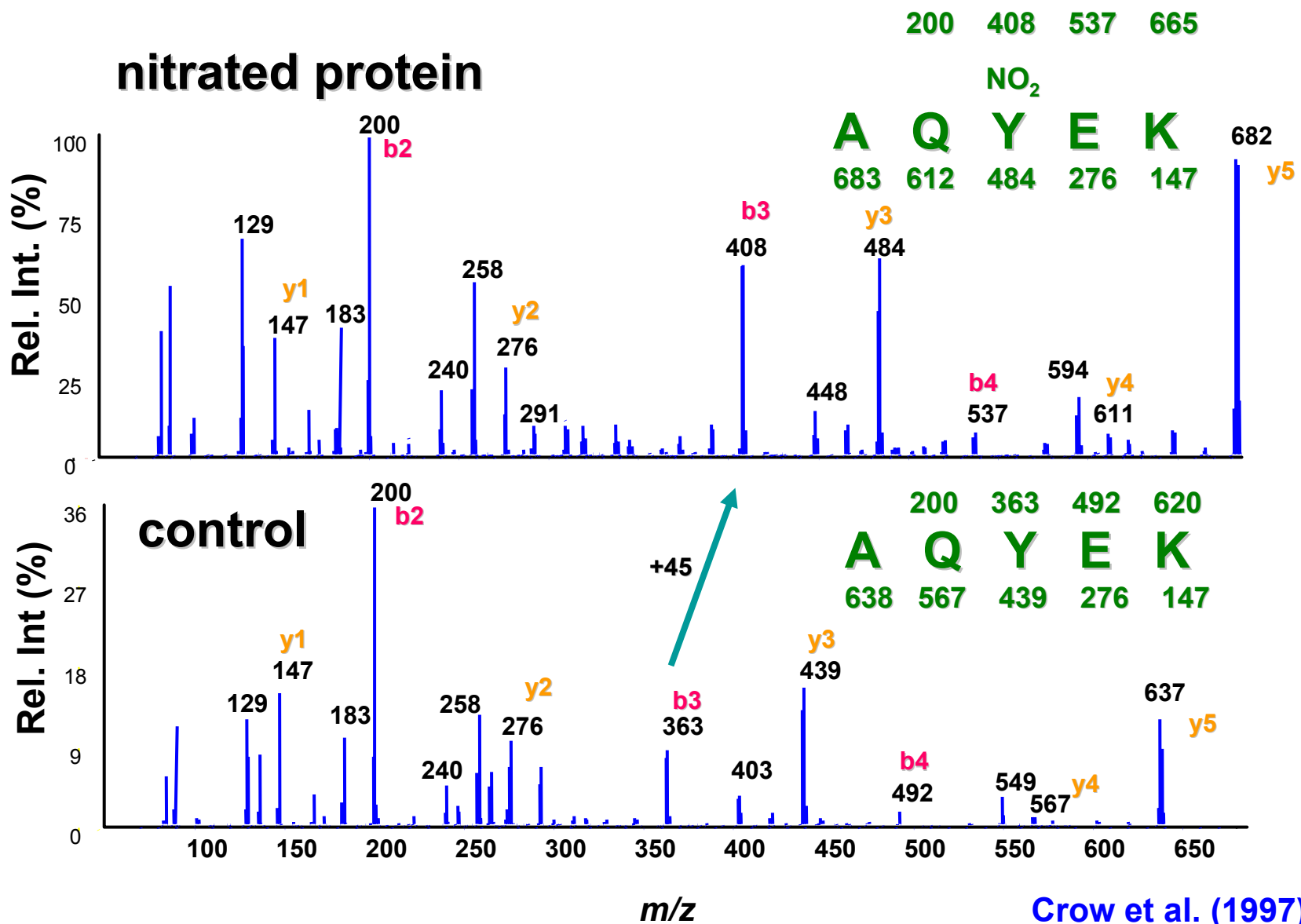
Ser - S	87.0320
Thr - T	101.0477
Trp - W	186.0793
Tyr - Y	163.0633
Val - V	99.0684

CMC 161.0147

Increase for CMC versus Cys

$$= (161.0147 - 103.0092)$$
$$= 58.0055$$

Site-specific nitration of a tyrosine-containing peptide using CID MS-MS spectra



Expected mass changes associated with modifications

<u>Biochemical</u>	<u>Δ</u>	<u>Chemical</u>	<u>Δ</u>
Disulfide bridge	-2	Oxidation S-OH	16
N-methylation	14	Oxidation S-O ₂ H	32
N-acetylation	42	Nitrosylation	29
Sulfation	80	Nitration	45
Phosphorylation	80	Carboxymethyl	58
O-glycosylation	204	4-hydroxynonenal	138
			156

<http://www.expasy.ch/tools/findmod/>

<http://www.abrf.org/index.cfm/dm.home>