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)**

LC-MS-MS

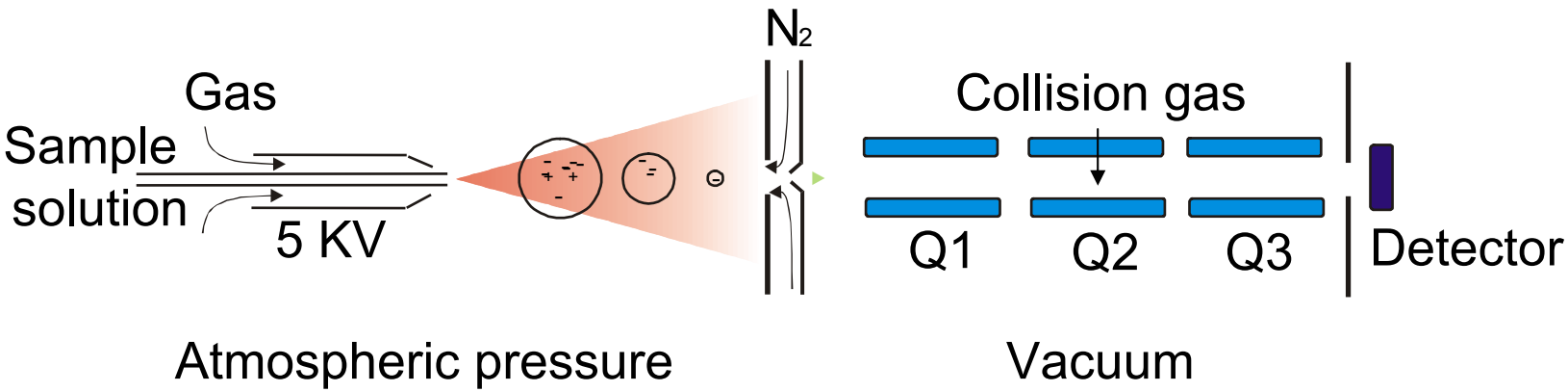
- The complexity of the peptides in most samples requires some level of chromatographic separation - the search for modifications demands it

- Gradient LC analysis allows relatively large amounts of sample (2-6 μl) to be loaded onto a reverse-phase column which is then eluted at 200-400 nl/min

nanoLC-ESI-MSMS

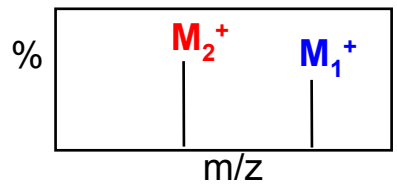
- **For electrospray, smaller volumes are better. This has been taken to an extreme. Columns with diameters of 75 μm or lower are routinely used**

LC-ESI-MS Analysis

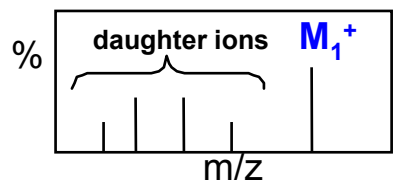


MS M_1^+ M_2^+ **Q1** → **Q2 and Q3 set to allow ions pass**

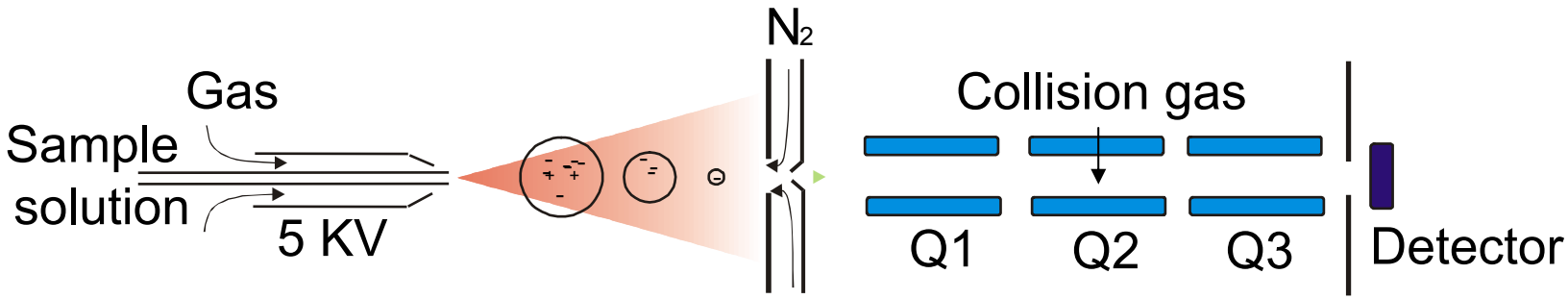
Mass spectrum



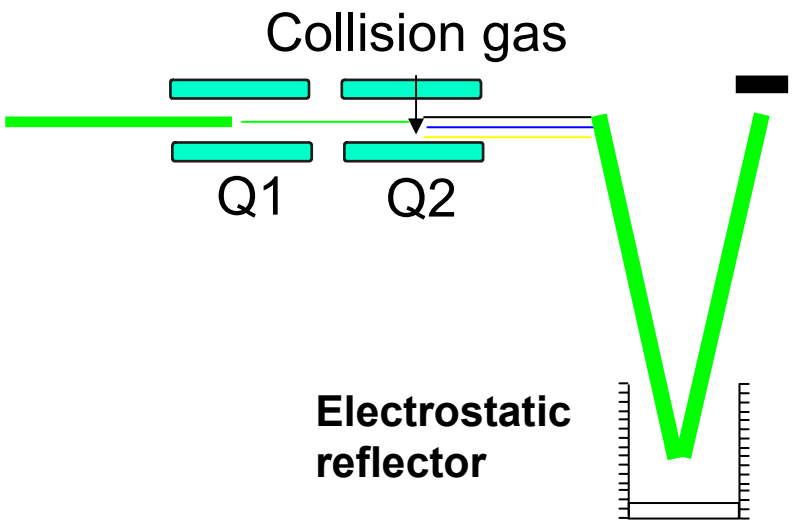
MS 2 M_1^+ M_2^+ **Q1** → M_1^+ **Q2 fragmentation** (**Ar** (collision gas)) **daughter fragment ions** **Q3** →



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

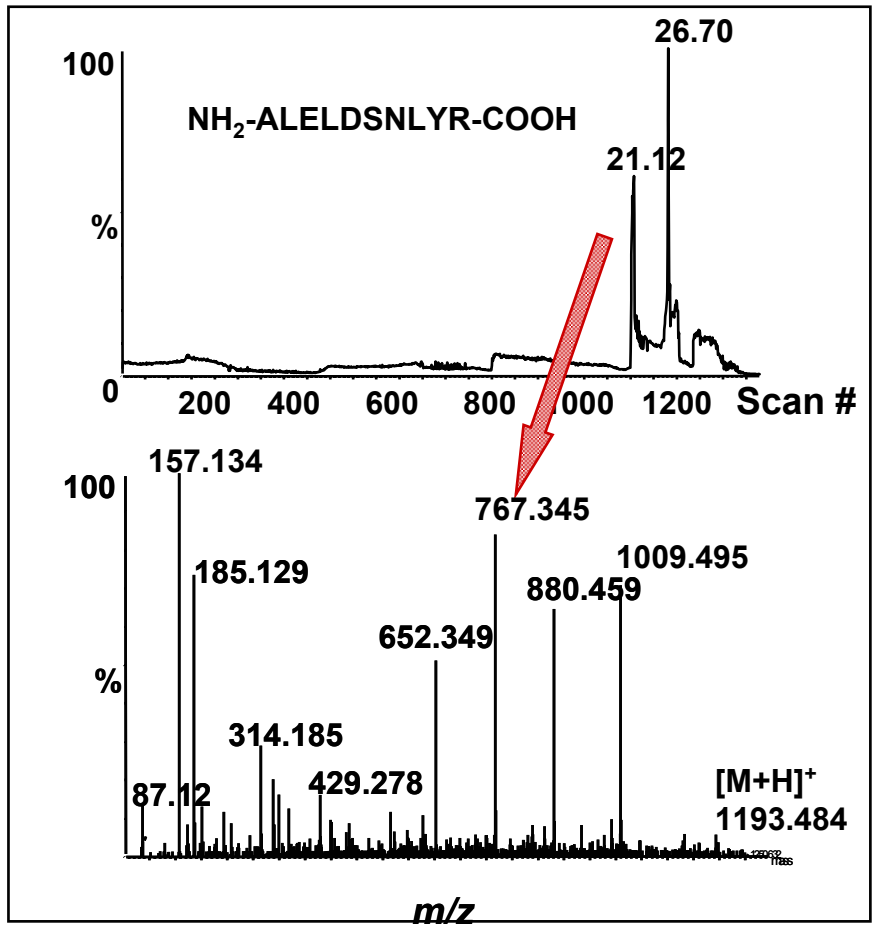
Also gives far greater mass accuracy - from 1000 ppm on the triple quad to 5-10 ppm on the Q-tof

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

Reverse phase nanoLC-MSMS

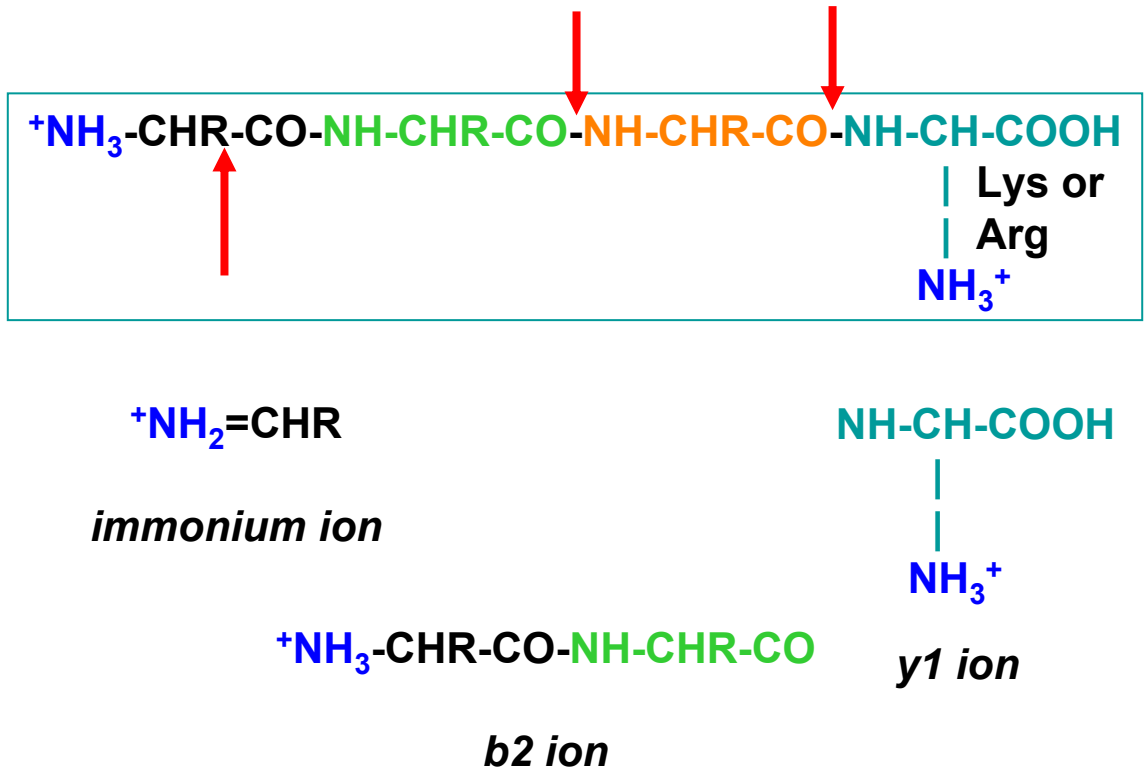
- Peptides are separated on an acetonitrile gradient using columns with i.d.s of 0.05-0.30 mm. These operate at 200-2000 nL/min

- 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



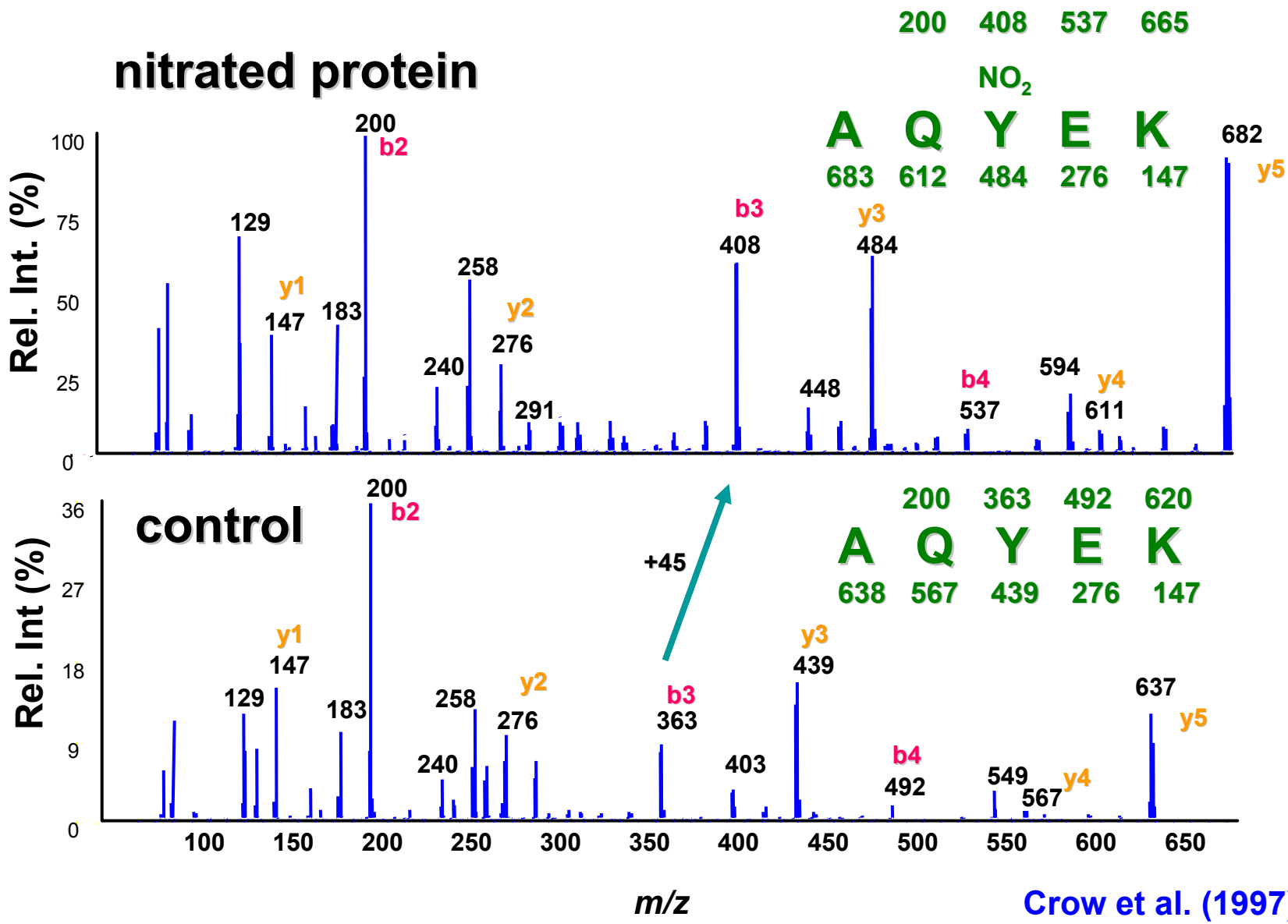
Residue masses of amino acids

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



Crow et al. (1997)

Expected mass changes associated with modifications

Biochemical	Δ
Disulfide bridge	-2
N-methylation	14
N-acetylation	42
Sulfation	80
Phosphorylation	80
O-glycosylation	204

Chemical	Δ
Oxidation S-OH	16
Oxidation S-O_2H	32
Nitrosylation	29
Nitration	45
Carboxymethyl	58
4-hydroxynonenal	138
	156

http://www.expasy.ch/tools/findmod/
http://www.abrf.org/index.cfm/dm.home