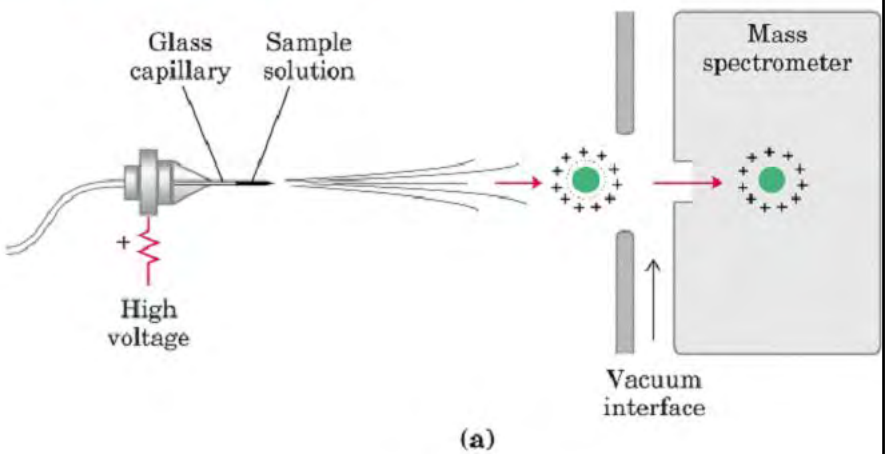
Mass Spectrometry

MS (mass spectrometry) is the production of ions, subsequently separated according to their mass-to-charge (m/z) ratio then detected.

The resulting mass spectrum is a plot of the (relative) abundance of the ions at each m/z ratio.

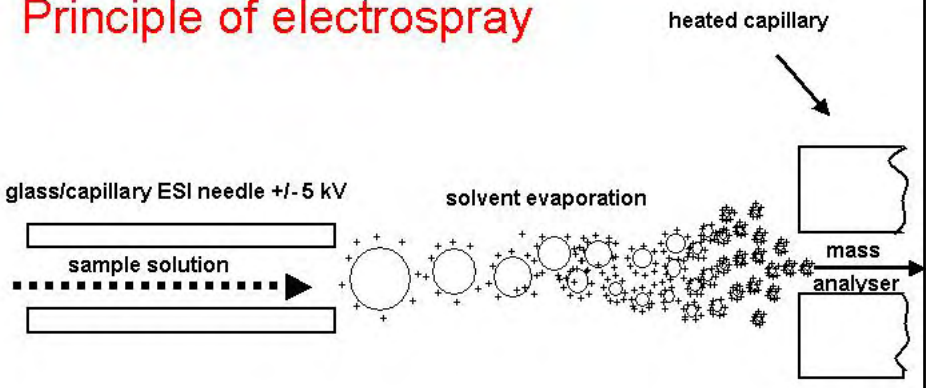
1

Quadrupole electrospray Mass Spectrometry

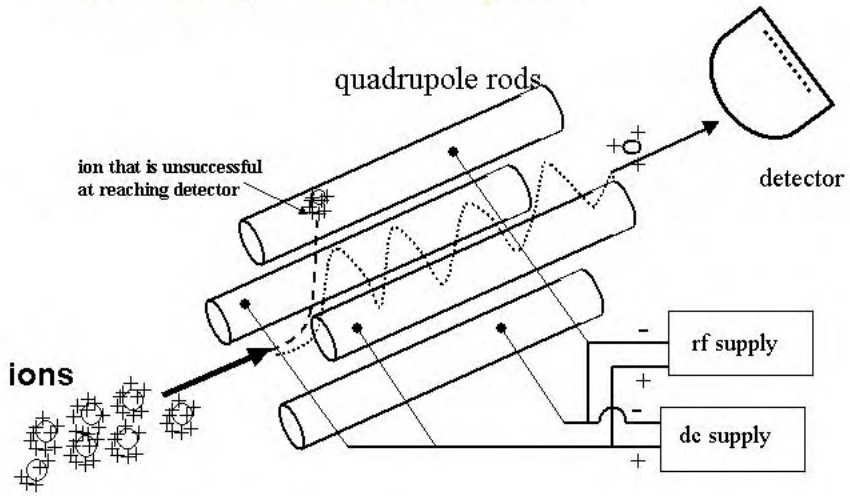


(a)

Principle of electrospray



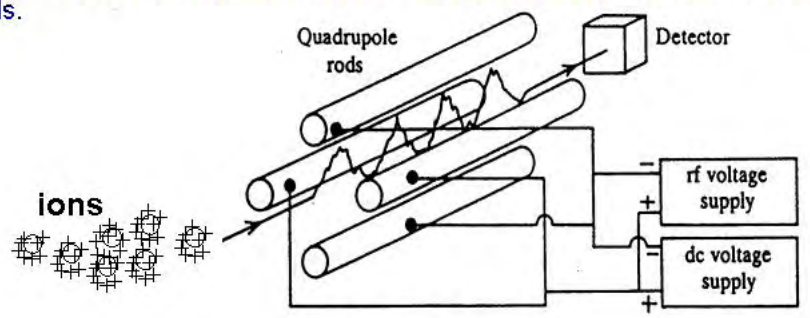
Quadrupole analyser



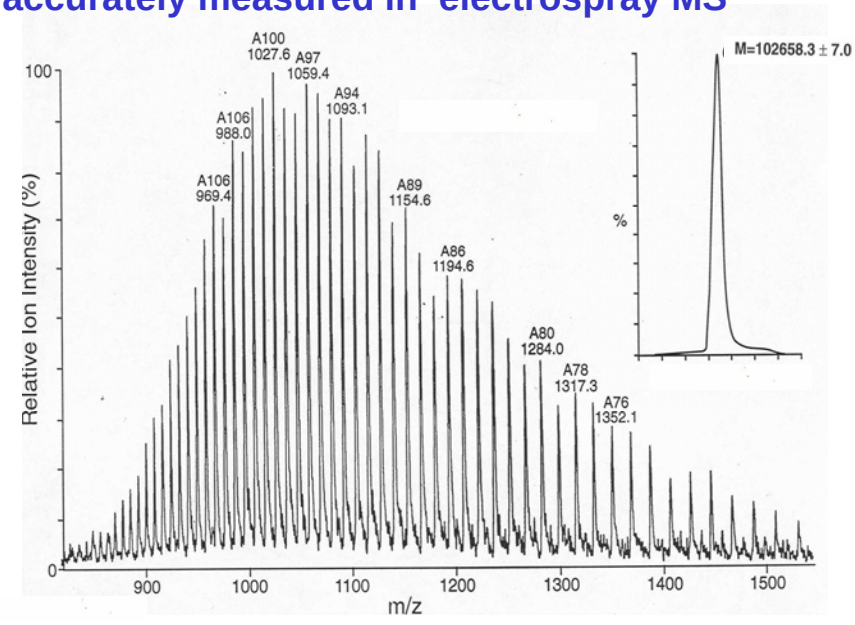
Quadrupole analyzer - how it works

The analyzer consists of four rods. DC and RF voltages are applied to each rod, creating a continuously varying electric field along the length of the analyzer. Once in this field, ions are accelerated down the analyzer towards the detector.

The varying electric field is precisely controlled so that, during each stage of a scan, ions of one particular mass-to-charge ratio pass down the length of the analyzer. Ions with any other mass-to-charge value impact on the quadrupole rods.



Large intact protein masses can also be accurately measured in electrospray MS



Mass Spectrometry sequencing

The most sensitive sequencing method is mass spectrometry

It uses protease cleavage (mostly by trypsin)* and identification of the fragments by subsequent mass charge ratio (m/z).

either as very accurate masses alone.

or by using a second fragmentation that gives ladders of fragments cleaved at the peptide bonds.

The protein is identified by searching databases of expected masses from all known peptides from every protein (or translations from DNA) and theoretical masses from fragmented peptides.

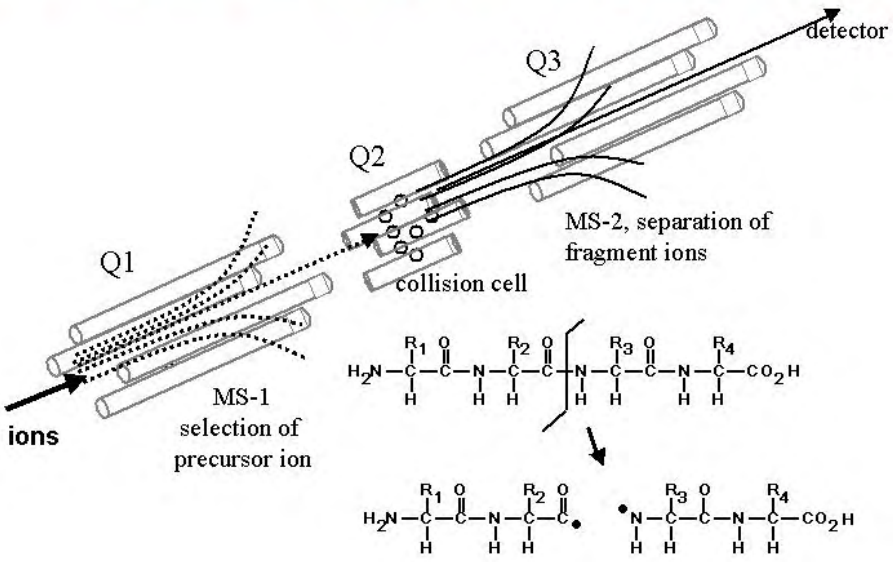
Method is used extensively on spots extracted from gels!

Sensitivity has been claimed down to zeptomole level

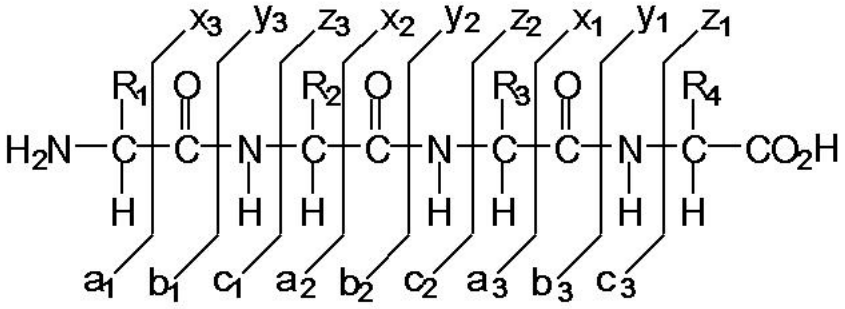
(N.B. nano,10^{-9} ; pico, 10^{-12} ; atto, 10^{-15} ; femto, 10^{-18} zepto, 10^{-21}).

* Tryptic peptides usually have basic groups at N- and C terminus which results in a high proportion of high energy doubly charged ions, that are more easily fragmented.

Quadrupole Mass Spectrometry Sequencing



Peptide Fragment ion nomenclature



The charge may be retained by either the N- or C- terminal fragment.

b and y series ions may predominate

Residue Masses of the protein amino acids

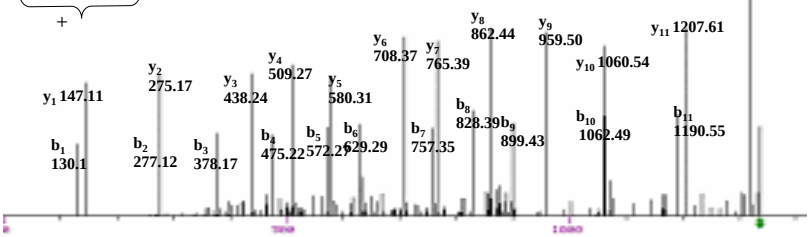
Name	Symbol	Residue Mass[1]	Side Chain
Alanine	A, Ala	71.079	CH_3-
Arginine	R, Arg	156.188	$HN=C(NH_2)-NH-(CH_2)_3$-
Asparagine	N, Asn	114.104	$H_2N-CO-CH_2$-
Aspartic acid	D, Asp	115.089	$HOOC-CH_2$-
Cysteine	C, Cys	103.145	$HS-CH_2$-
Glutamine	Q, Gln	**128.131**	$H_2N-CO-(CH_2)_2$-
Glutamic acid	E, Glu	129.116	$HOOC-(CH_2)_2$-
Glycine	G, Gly	57.052	H-
Histidine	H, His	137.141	imidazole-CH_2-
Isoleucine	I, Ile	**113.160**	$CH_3-CH_2-CH(CH_3)$-
Leucine	L, Leu	**113.160**	$(CH_3)_2-CH-CH_2$-
Lysine	K, Lys	**128.17**	$H_2N-(CH_2)_4$-
Methionine	M, Met	131.199	$CH_3-S-(CH_2)_2$-
Metsulphoxide	Met.SO	**147.199**	$CH_3-S(O)-(CH_2)_2$-
Phenylalanine	F, Phe	**147.177**	Phenyl-CH_2-
Proline	P, Pro	97.117	pyrrolidone-CH-
Serine	S, Ser	87.078	$HO-CH_2$-
Threonine	T, Thr	101.105	$CH_3-CH(OH)$-
Tryptophan	W, Trp	186.213	Indole-NH-CH=C-CH_2-
Tyrosine	Y, Tyr	163.176	4-OH-Phenyl-CH_2-
Valine	V, Val	99.133	$CH_3-CH(CH_2)$-

[1] NB Residue Mass is the mass in a peptide bond ie $-H_2O$.

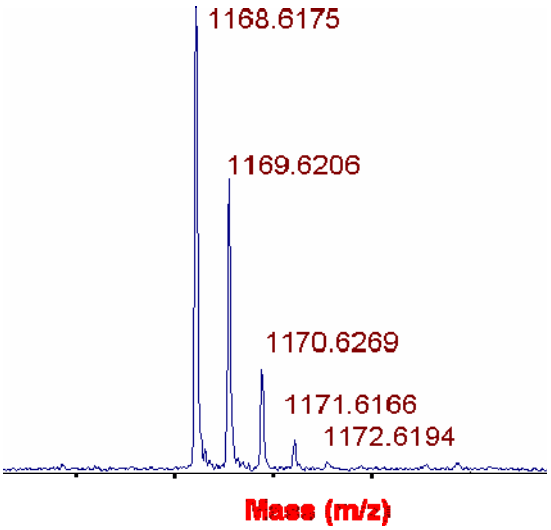
Tandem MSMS spectrum of a peptide

b_1 130.05	E FTPPGQAAYQK	y_{11} 1207.61
b_2 277.12	EF TPPGQAAYQK	y_{10} 1060.54
b_3 378.17	EFT PPGQAAYQK	y_9 959.50
b_4 475.22	EFTP PGQAAYQK	y_8 862.44
b_5 572.27	EFTPP GQAAYQK	y_7 765.39
b_6 629.29	EFTPPG QAAYQK	y_6 708.37
b_7 757.35	EFTPPGQ AAYQK	y_5 580.31
b_8 828.39	EFTPPGQA AYQK	y_4 509.27
b_9 899.43	EFTPPGQAA YQK	y_3 438.24
b_{10} 1062.49	EFTPPGQAAY QK	y_2 275.17
b_{11} 1190.55	EFTPPGQAAYQ K	y_1 147.11

Fragment ions in the spectrum represent cleavage of peptide bonds resulting in sequence information recorded from both the N- and C- termini of the peptide simultaneously. spectrum. Fragment mass information can be searched with "Sequest" program against protein databases (don't have to sit and work out the sequence).



High resolution of 13C isotopes



Finding the charge on a particular peptide using Zoom Scan.
This is useful for determining the high energy doubly charged tryptic peptides, for MS-MS for example.

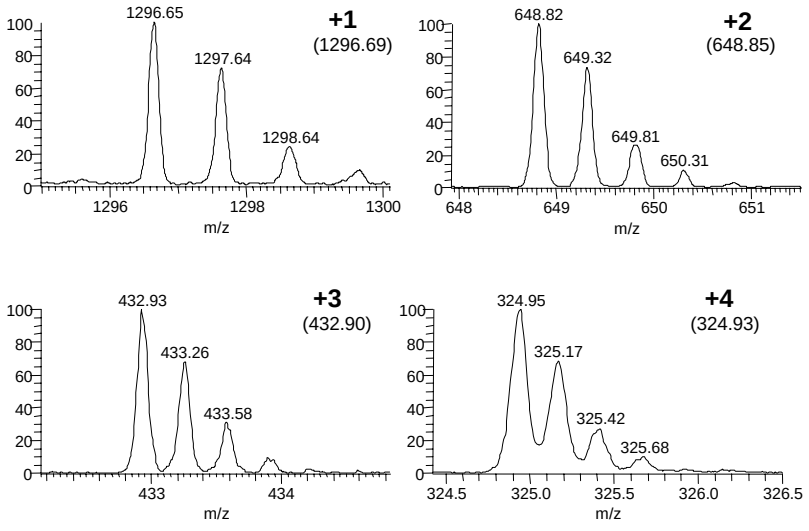
The mass detector operates on the basis of mass-to-charge ratio (m/z)

Mass Assignments are normally made assuming a single charge per ion (i.e. m/z = m - 1):

- Single charge - apparent mass = [(M + H) / 1] - 1
- Double charge - apparent mass = [(M + 2H) / 2] - 2
- Triple charge - apparent mass = [(M + 3H) / 3] - 3
- n charges - apparent mass = [(M + nH) / n] - n

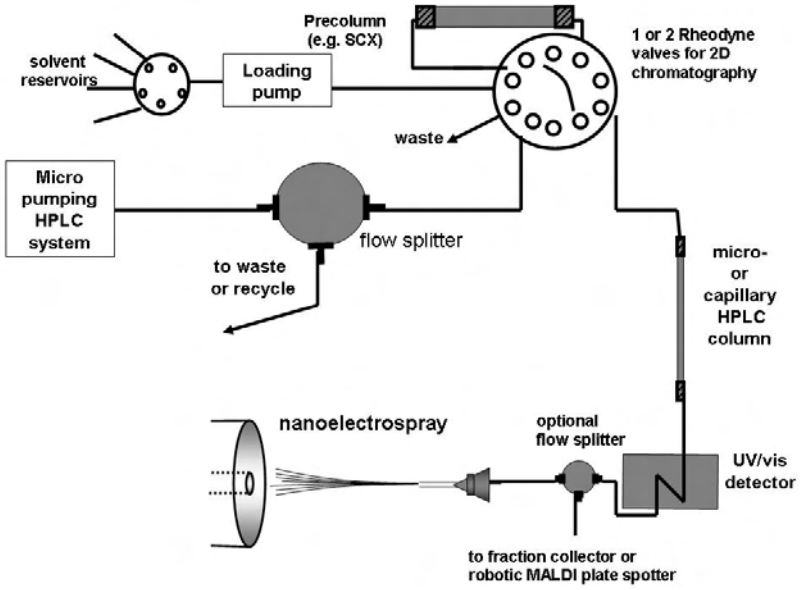
There is around 1.1% ^{13}C natural abundance, therefore with increasing size, peptides will have a greater chance of containing at least one ^{13}C and two ^{13}C etc.
A peptide of 20 residues has an approximately equal peak height of the all ^{12}C peptide and the peptide with one ^{13}C.

Zoom Scan *Angiotensin I (3 pmol/ml, 3 ml/min)*



Multi-dimensional LC Mass spectrometry

SCX = strong cation-exchange



Ion Trap Mass Spectrometry

The quadrupole ion trap mass analyser consists of three hyperbolic electrodes: the ring electrode, the entrance endcap electrode and the exit endcap electrode. These electrodes form a cavity in which it is possible to trap (store) and analyse ions. Both endcap electrodes have a small hole in their centers through which the ions can travel. The ring electrode is located halfway between the two endcap electrodes.

Ions produced from the source enter the trap through the quadrupole and the entrance endcap electrode. Various voltages are applied to the electrodes to trap and eject ions according to their mass-to-charge ratios. The ring electrode RF potential, an a.c. potential of constant frequency and variable amplitude, is applied to the ring electrode to produce a 3D quadrupolar potential field within the trapping cavity. This will trap ions in a stable oscillating trajectory confined within the trapping cell. The nature of the trajectory is dependent on the trapping potential and the mass-to-charge ratio of the ions. During detection, the electrode system potentials are altered to produce instabilities in the ion trajectories and thus eject the ions in the axial direction. The ions are ejected in order of increasing mass-to-charge ratio, focused by the exit lens and detected by the ion detector system.

LCQ DECA Ion Trap Mass Spectrometer

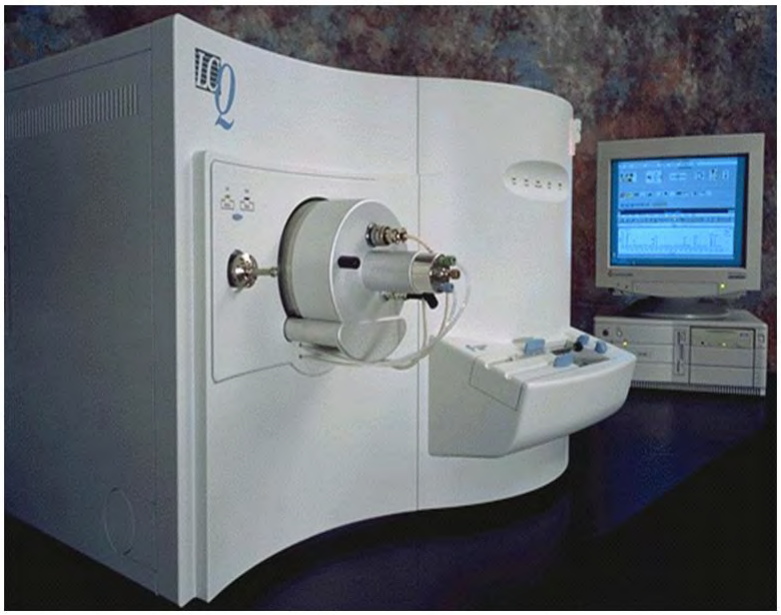
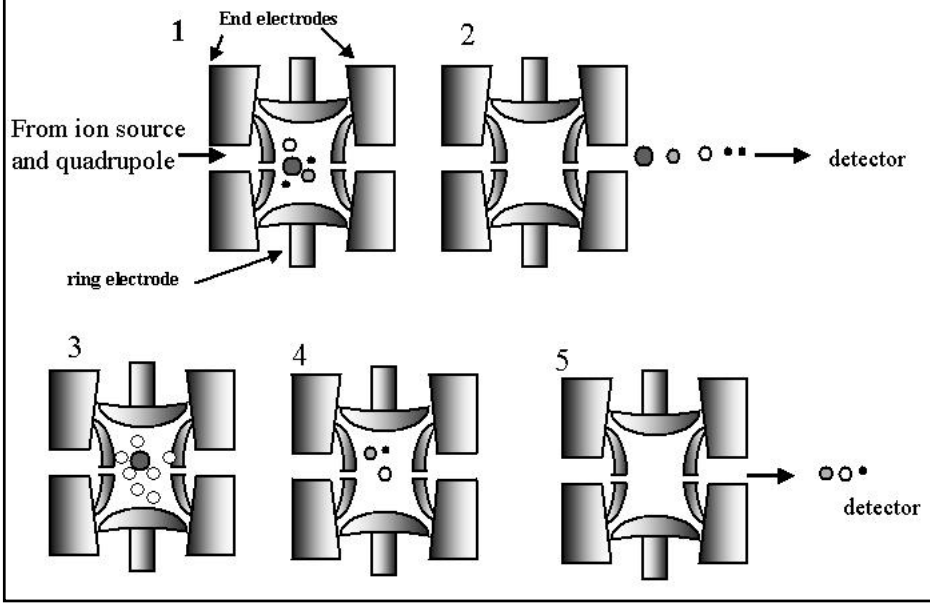
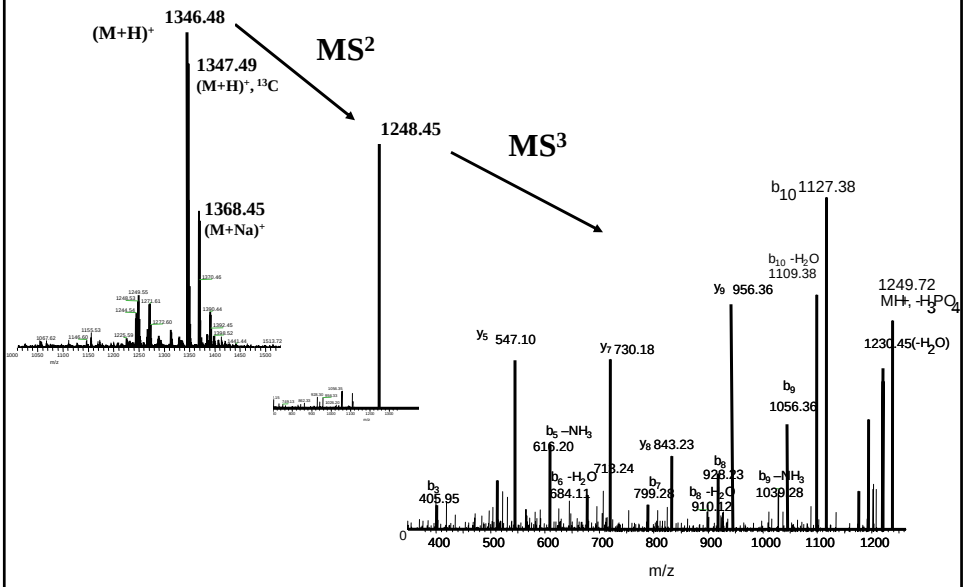


Diagram of an ion trap.



Identification of phosphopeptides in the ion-trap



In summary:

2 main types of mass spectrometer for proteomics.

A. Quadrupole MS (including triple quadrupole and ion-trap variants)

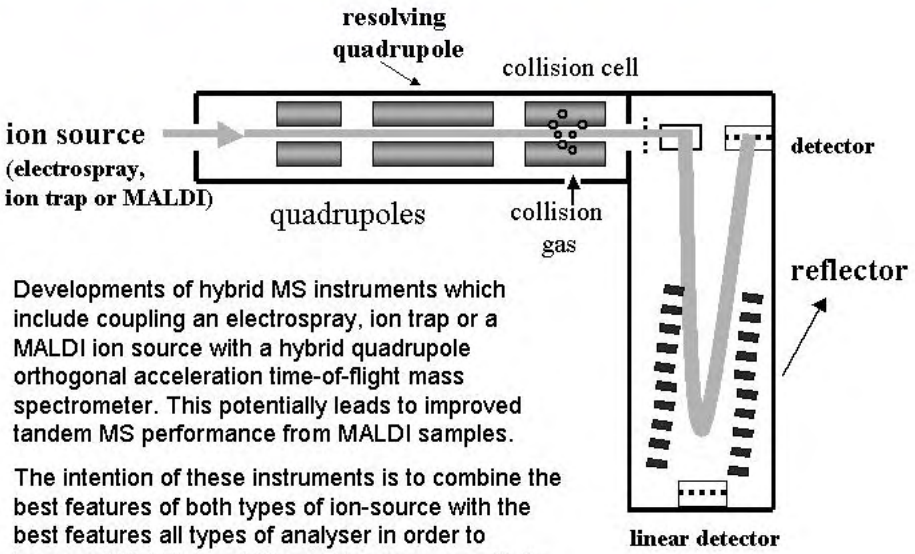
- More sequence information can be obtained.
- Can be coupled to HPLC and capillary LC for separation of complex mixtures.
- Better for analysis of post-translational modifications
- Tandem MS-MS used in quantitation (ICAT; SILAC; iTRAQ).

B. MALDI-tof MS.

- Can handle more complex mixtures since mainly singly charged species obtained.
- Mainly gives information for fingerprint analysis (sufficient if the genome of the species is sequenced. Almost certainly OK for any mammalian species).
- Very sensitive, rapid, lends itself to high throughput and automation.

[Mass spectrometry-based proteomics, review, R Aebersold, M Mann (2003) Nature, 422,198–207].

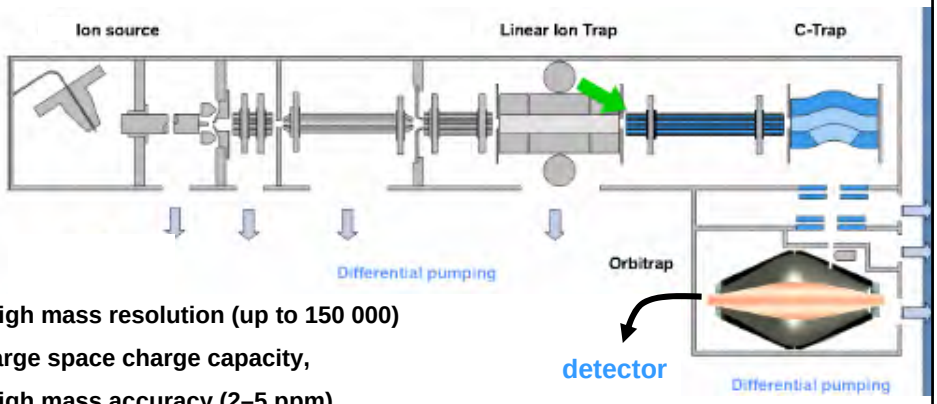
A hybrid quadrupole time of flight MS.



Developments of hybrid MS instruments which include coupling an electrospray, ion trap or a MALDI ion source with a hybrid quadrupole orthogonal acceleration time-of-flight mass spectrometer. This potentially leads to improved tandem MS performance from MALDI samples.

The intention of these instruments is to combine the best features of both types of ion-source with the best features all types of analyser in order to improve MS-MS capability and increase sensitivity.

Orbitrap mass spectrometer with electrospray ionization source (ESI)



high mass resolution (up to 150 000)

large space charge capacity,

high mass accuracy (2–5 ppm),

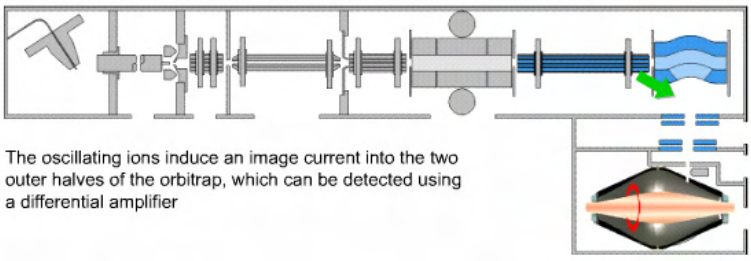
mass/charge range of at least 6000,

dynamic range greater than 10^3

Sensitivity: sub-femtomole

LTQ Orbitrap Operation Principle

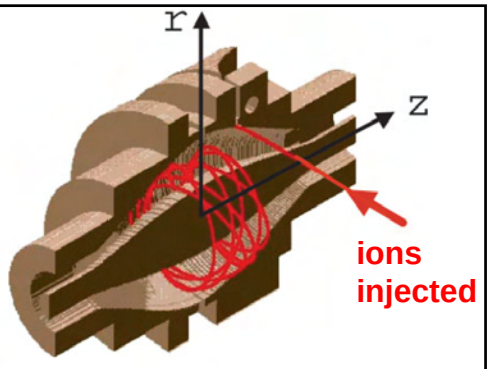
1. Ions are stored in the Linear Trap
2. …. are axially ejected
3. …. and trapped in the C-trap
4. …. they are squeezed into a small cloud and injected into the Orbitrap
5. …. where they are electrostatically trapped, while rotating around the central electrode and performing axial oscillation

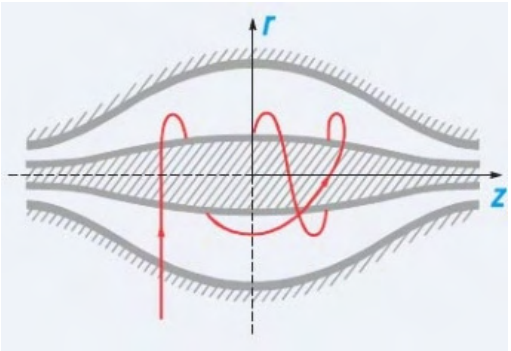


The oscillating ions induce an image current into the two outer halves of the orbitrap, which can be detected using a differential amplifier

Ions are transferred from the ESI source through three stages of differential pumping using RF guide quadrupoles. The third quadrupole, pressurized to less than 10^{-3} Torr with collision gas, acts as an ion accumulator; ion/neutral collisions slow the ions and cause them to pool in an axial potential well at the end of the quadrupole. Ion bunches are injected from this pool into the Orbitrap analyzer for mass analysis.

The Orbitrap operates by radially trapping ions about a central spindle electrode. An outer barrel-like electrode is coaxial with the inner spindlelike electrode and mass/charge values are measured from the frequency of harmonic ion oscillations, along the axis of the electric field, undergone by the orbitally trapped ions.
Ion frequencies are measured non-destructively, with subsequent fast Fourier transforms (FFTs) to obtain the mass spectra.





Surface Plasmon Resonance (SPR) technology,

Biacore enables the real-time detection and monitoring of biomolecular binding events

In addition to identifying binding partners to target molecules, Biacore provides quantitative information on:

- **Specificity** – how specific is the binding between two molecules?
- **Concentration** – how much of a given molecule is present and active?
- **Kinetics** – what is the rate of association / dissociation?
- **Affinity** – how strong is the binding?

Analysing Protein-protein interactions by Surface Plasmon Resonance (SPR) coupled to MALDI-TOF MS

Voyager-DE STR MALDI-TOF MS (Applied Biosystems)

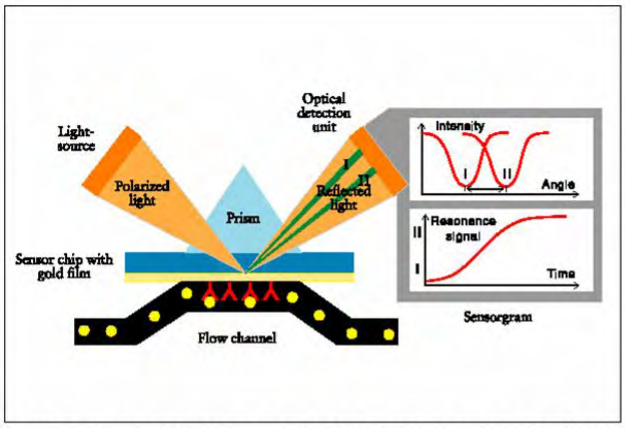
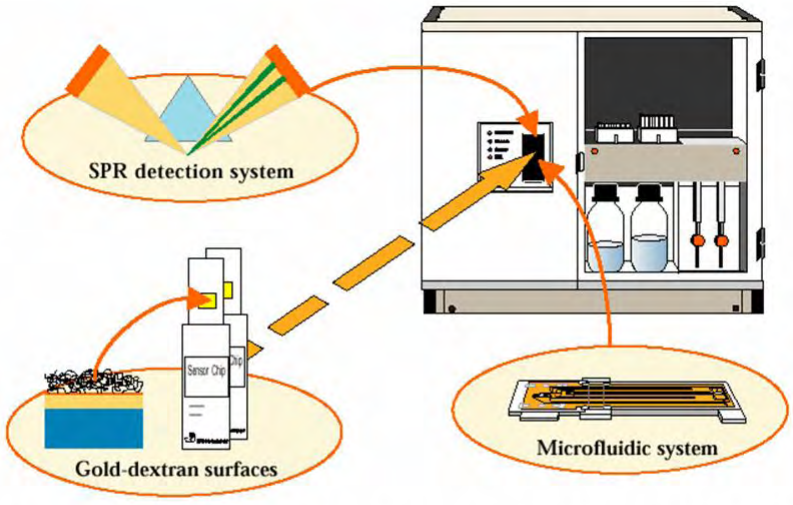


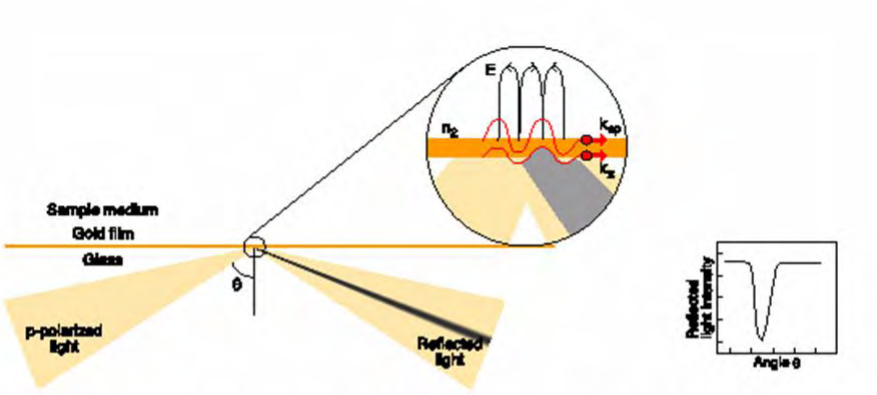
Figure 1. The SPR angle is sensitive to the mass concentration of molecules close to the sensor chip surface. As this concentration changes, the SPR angle shifts and produces a response.

In Biacore systems, the incident p-polarized light is focused into a wedge-shaped beam.

An increased sample concentration in the surface coating of the sensor chip causes a corresponding increase in refractive index which alters the angle of incidence required to create the SPR phenomenon (the SPR angle). This SPR angle is monitored as a change in the detector position for the reflected intensity dip (from I to II). By monitoring the SPR-angle as a function of time the kinetic events in the surface are displayed in a sensorgram.

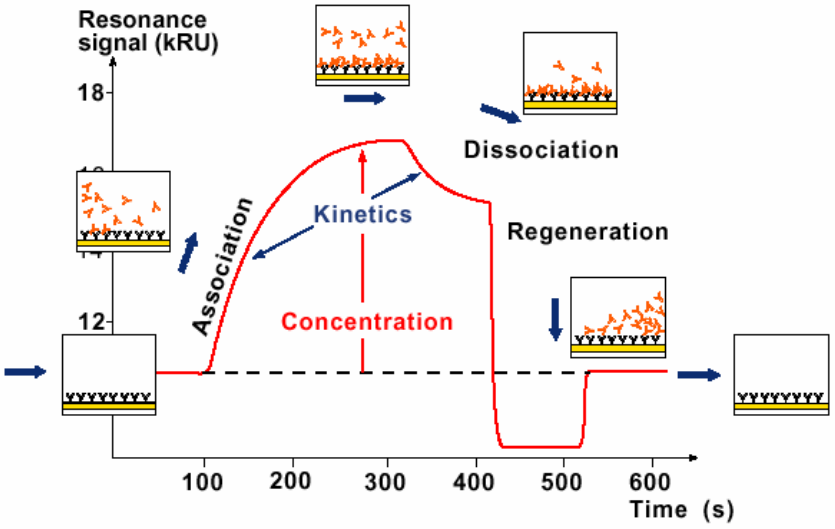


The sensor chip consists of a glass surface, coated with a thin layer of gold: the basis for a range of specialized surfaces to optimize the binding of a variety of molecules.

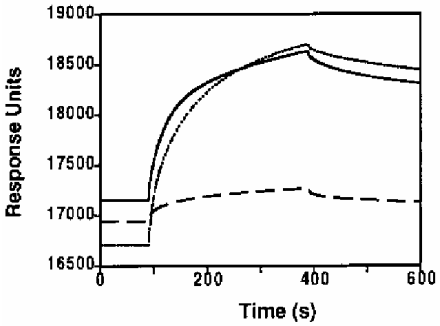


SPR is excited by polarized totally internally reflected light in a glass/metal film interface, the surface plasmon enhancing the evanescent field amplitude, E. In Biacore systems which use a sensor chip, this interface takes the form of an exchangeable gold coated glass slide. SPR is observed as a dip in the reflected light intensity at a specific angle of reflection.

The sensorgram



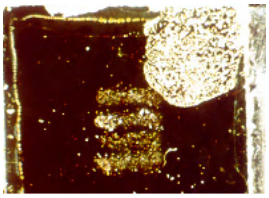
Binding of 14-3-3 protein to immobilized peptides

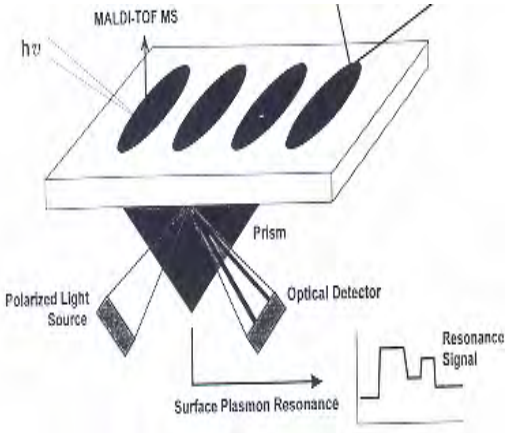


Sequence	K_{off}	K_{on}	K_D
	s^{-1} (x 10^{-3})	M^{-1} s^{-1} (x10^4)	nM
RL<u>S</u>H*p*SLP	2.05	3.68	55.7
RL<u>Y</u>H*p*SLP	1.08	2.89	37.4
RLSH*p*SL<u>G</u>	6.99	3.67	190.4

Peptide Binding to 14-3-3: Kinetic and Inhibition Analyses Using Surface Plasmon Resonance: 14-3-3 GST fusion protein (200 nM) was incubated with increasing amounts of the peptides, then infused over a sensor chip prederivatized with an excess of the peptides. The initial rate of binding, dR/dtinit, which indicates the amount of 14-3-3 not complexed to the inhibitor peptides, was measured and KIs corresponding to a 50% reduction in dR/dtinit determined.

Surface Plasmon Resonance Biomolecular Interaction Analysis Interfaced with Mass Spectrometry (BIA/MS)

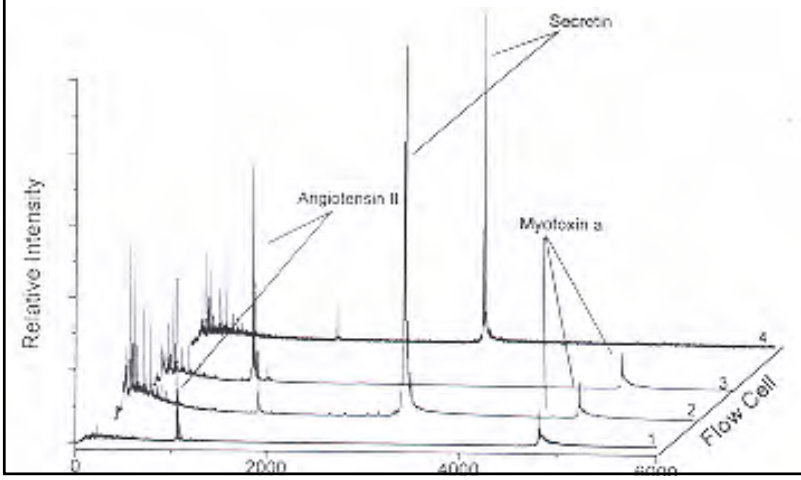




An SPR Chip Mounted on a Modified Voyager MALDI Plate. This magnified view clearly shows four flow cells after deposition of matrix. The upper right corner of the chip is a 1 µl spot of matrix added for comparison. This photo kindly provided by Dr. Alfred Yergey of the NIH.

MALDI TOF MS from Four Flow Cells on the Same SPR Chip.

Flow cells 1, 2, and 3, were modified with antimyotoxin α antibody; flow cell 4 was left blank. After exposure to purified myotoxin α and washing, MALDI matrix was added to each flow cell. Matrix containing angiotensin II was added to flow cells 1 and 3 while matrix containing secretin was added to flow cells 2 and 4. The samples were then analyzed by MALDI TOF

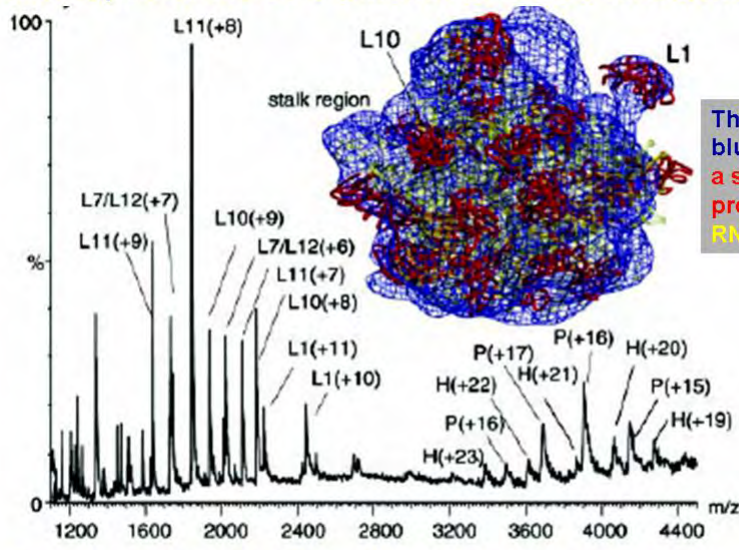


18

Analysis of Large Protein Complexes by Mass Spectrometry

Studying Protein Complexes by Mass Spectrometry. **Dynamic Protein Complexes: Insights from Mass Spectrometry**.Biol.Chem. 276, 46685–46688 (2001) Helena Hernandez and Carol V. Robinson

Mass spec of the 50 S subunit of *E. coli* ribosomes.



The ribosome is in blue mesh; a subset of the 55 proteins in red; RNA in yellow.

The peaks are labelled with the ribosomal protein and the charge state in parentheses. P and H represent pentameric and hexameric complexes. The inset structure is the intact *E. coli* ribosome oriented from the back of the 50 S subunit.

ES MS of 30S ribosomal subunits at pH 6.4

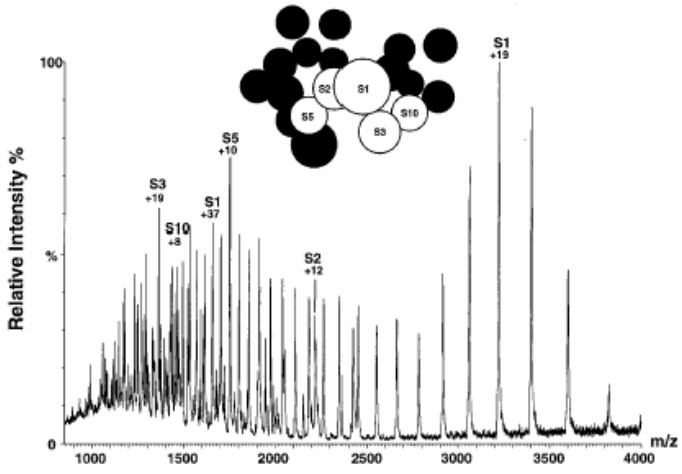


FIG. 2. Positive ion ESI mass spectra of aqueous solutions of 30S subunits at pH 6.4. Ninety-five percent of the peaks observed in this spectrum have been assigned to various charge states of the five 30S proteins indicated; only the most intense peak of the charge-state distribution for each protein is labeled (along with the total charge for that particular peak). (*Inset*) Representation of the topology of the 30S *E. coli* ribosomal subunit, derived from neutron scattering measurements of partially deuterated ribosomes (20). Proteins observed in the mass spectrometer are shown in labeled, white circles; unobserved proteins are represented as unlabeled, black circles.

ES MS of 70S ribosomes and 50Sribosomal subunits at pH 6.4

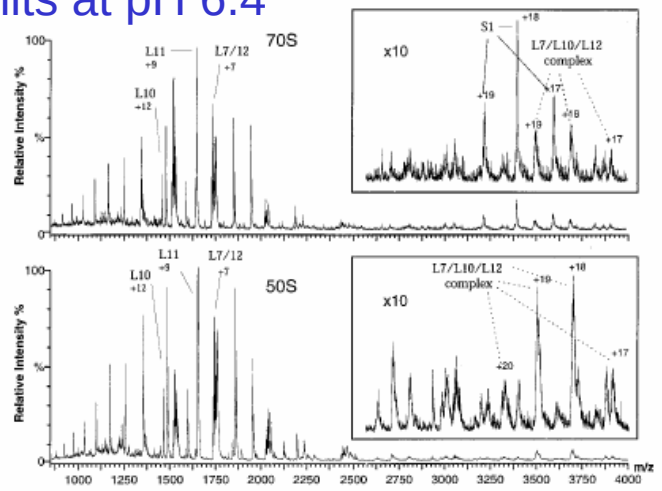
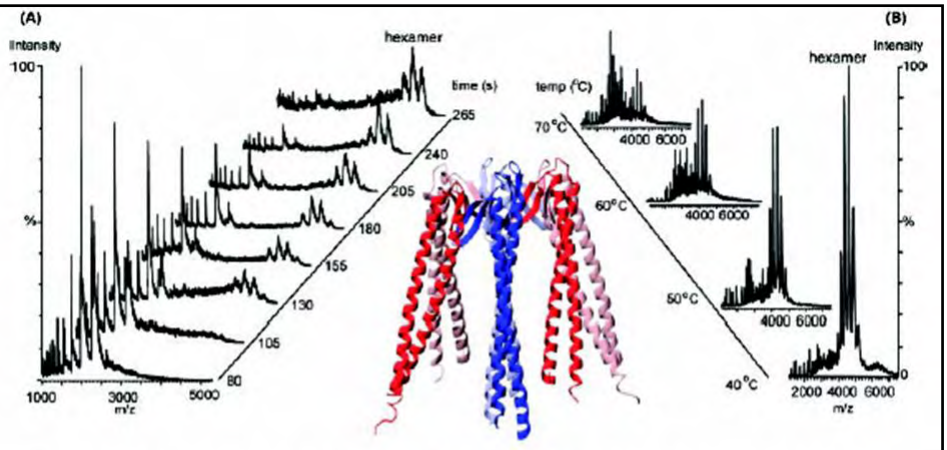
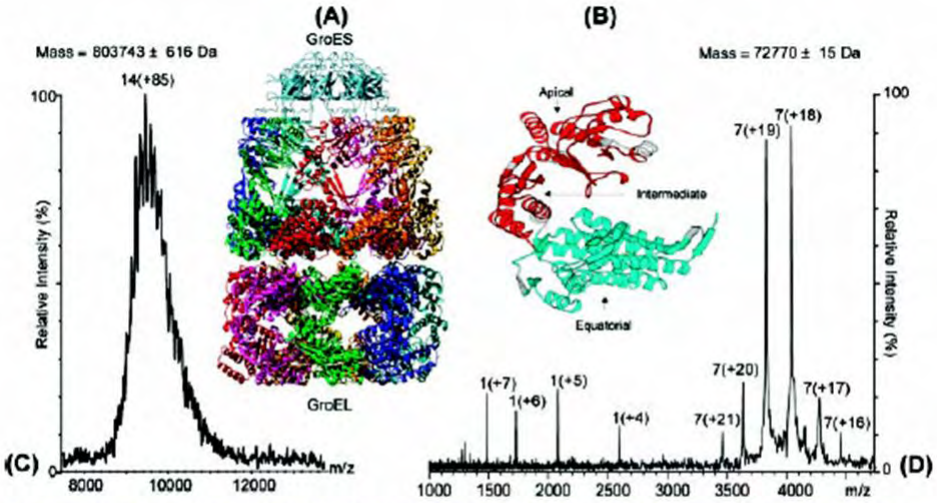


FIG. 1. Positive ion ESI mass spectra of aqueous solutions of intact 70S ribosomes and 50S subunits at pH 6.4. Only the most intense peak for each assigned protein is labeled (along with the total charge for that peak); each protein gives rise to a charge-state distribution of 4–10 identifiable peaks. (*Upper*) Intact 70S ribosomes. (*Lower*) 50S subunits. The spectra are highly reproducible; *ca*. 100 experiments with 16 separately prepared solutions of ribosomes yielded essentially identical spectra. Control spectra of acid denatured ribosomes show no peaks, confirming that only intact ribosomes are retained during the buffer exchange and that the proteins observed in the mass spectra dissociate from the ribosomes after introduction into the mass spectrometer.



The molecular chaperone MtGimC (related to eukaryotic chaperonin cofactor GimC/prefoldin) involved in the folding of actin and tubulin.
A. assembly of MtGimC from its component subunits monitored in real time by mass spectrometry.
B, the stability of the MtGimC hexamer investigated as a function of temperature.
Inset, x-ray structure of the MtGimC complex.



Structure of the GroELGroES-7ADP molecular chaperonin complex from *E. coli.*
B, regions of the GroEL monomer that undergo a 3-fold increase in H/D exchange kinetics (red) relative to equatorial domain (blue) when destabilized with 1.8 M GdnHCl. Q-ToF (**C**) and Fourier transform mass spectrometry (**D**) electrospray mass spectra recorded for the intact GroEL14-mer and GroES7-mer.
Peaks are labelled with the no. of subunits and charge state indicated in parentheses.