Mass Spectrometry  BT 301 Oct 24, 2023

# Biophysics

# Mass Spectrometry

# Matrix Assisted Laser Desorption Ionization (MALDI)

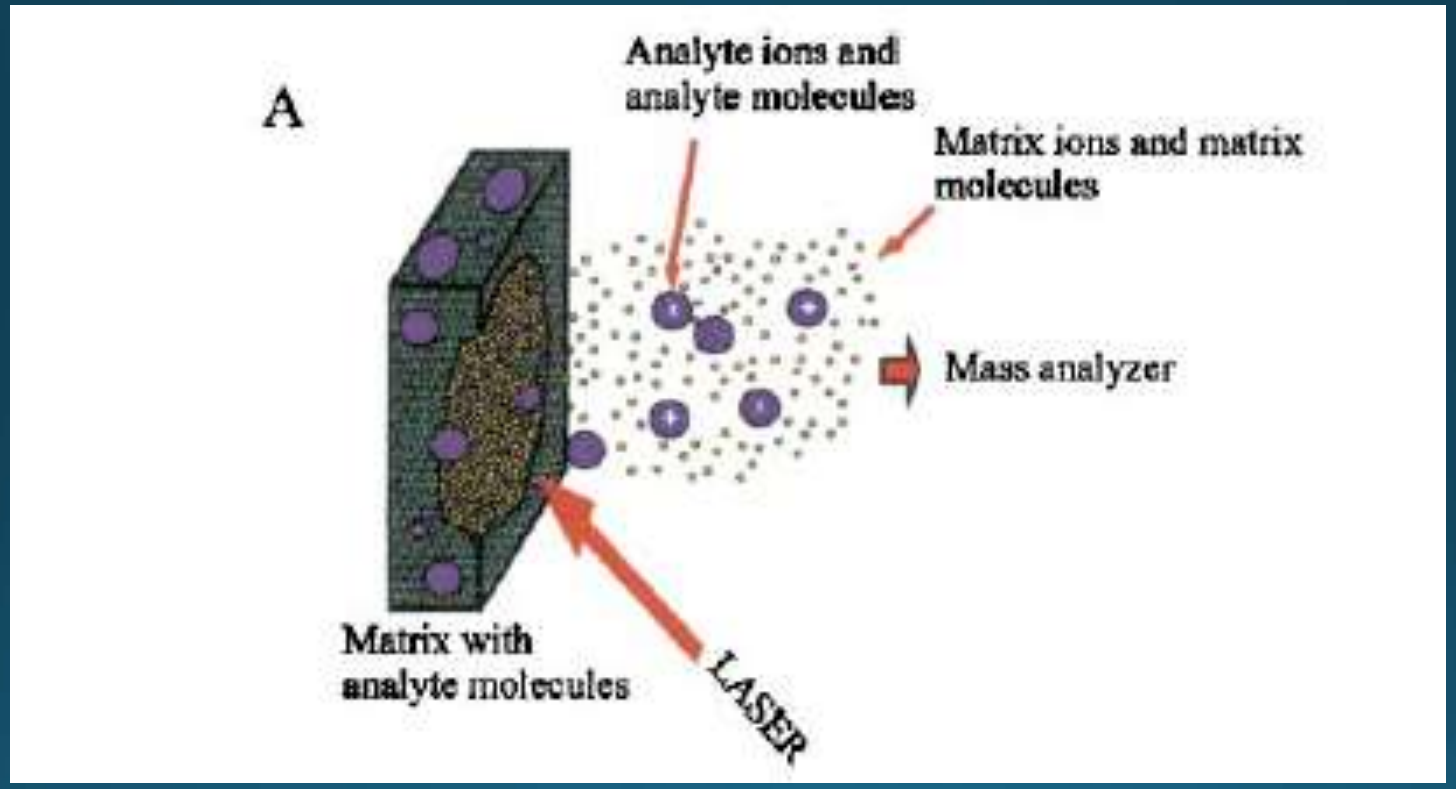



**Figure 1** Schematic of MALDI process and instrument. (*A*) A sample cocrystallized with the matrix is irradiated by a laser beam, leading to sublimation and ionization of peptides. (*B*)

# Electrospray Ionization (ESI)

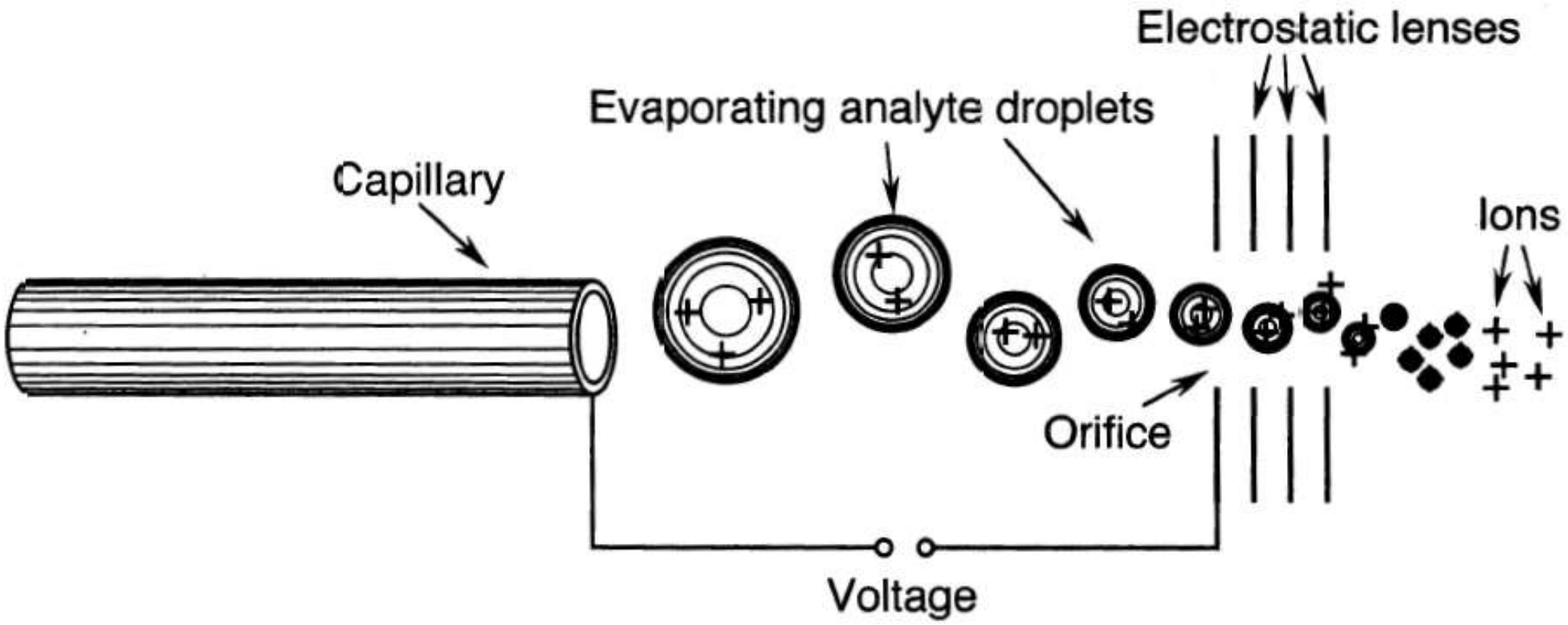



**Fig. 3.11** Electrospray ionization method. Analyte solutions delivered by liquid chromatography or a syringe pump are sprayed through the narrow, heated capillary leading into the mass spectrometer. A voltage of typically 200 V – 5 kV is applied between capillary and orifice in front of the electrostatic lenses. Ions form in vacuum by evaporation of the analyte solution of charged droplets

# MALDI-Time of Flight Spectrometer (TOF)

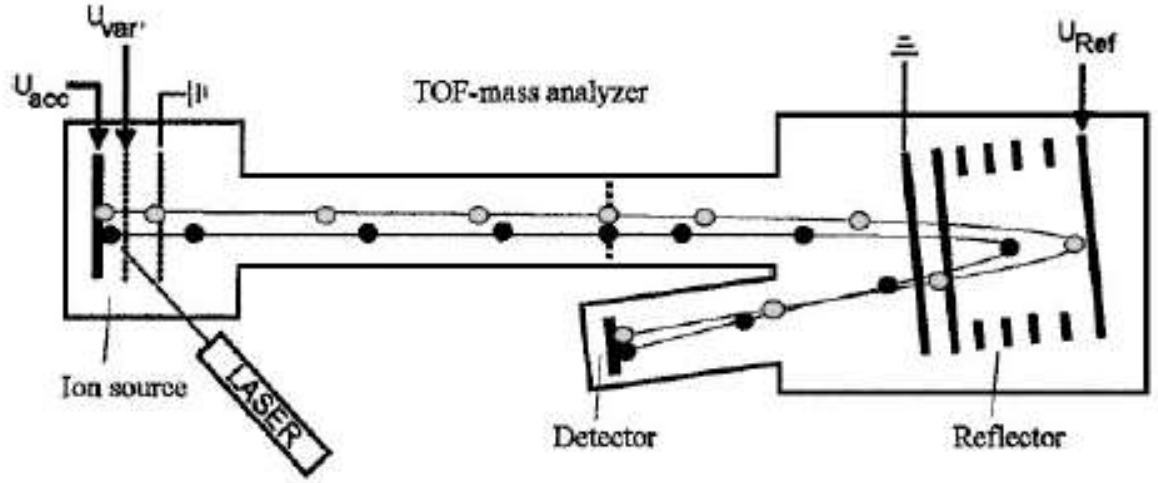



**Figure 1** Schematic of MALDI process and instrument. (*A*) A sample cocrystallized with the matrix is irradiated by a laser beam, leading to sublimation and ionization of peptides. (*B*) About 100–500 ns after the laser pulse, a strong acceleration field is switched on (delayed extraction), which imparts a fixed kinetic energy to the ions produced by the MALDI process. These ions travel down a flight tube and are turned around in an ion mirror, or reflector, to correct for initial energy differences. The mass-to-charge ratio is related to the time it takes an ion to reach the detector; the lighter ions arrive first. The ions are detected by a channeltron electron multiplier.

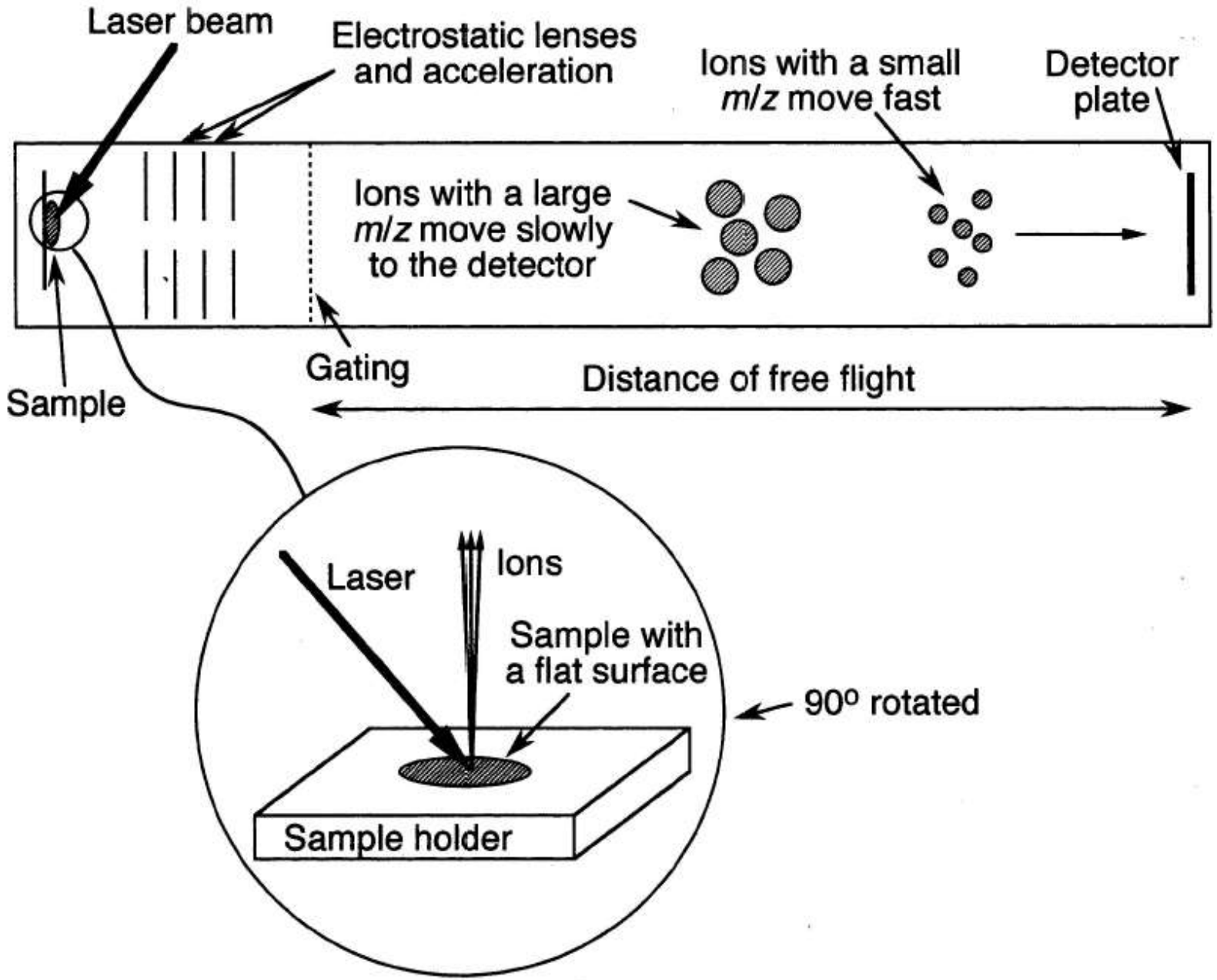



**Fig. 3.6** Linear time-of-flight mass spectrometer (TOF) with matrix-assisted laser desorp-

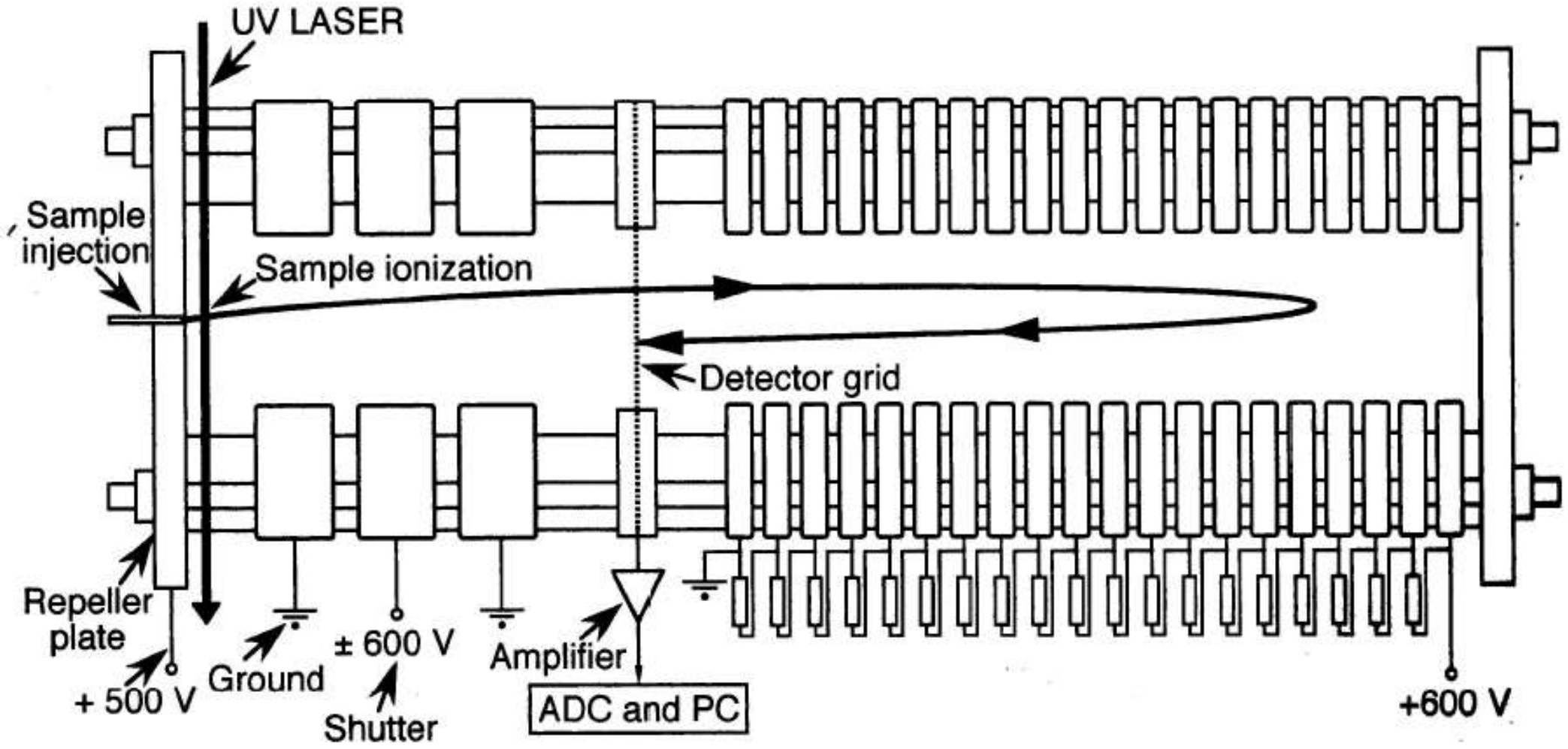



**Fig. 3.7** A simple reflectron time-of-flight mass spectrometer (e.g., Bryden, 1995). The reflector enhances mass-spectrometric resolution: it increases the time of flight and can focus ions. Here a voltage pulse at the shutter electrode causes a uniform starting time of the ions

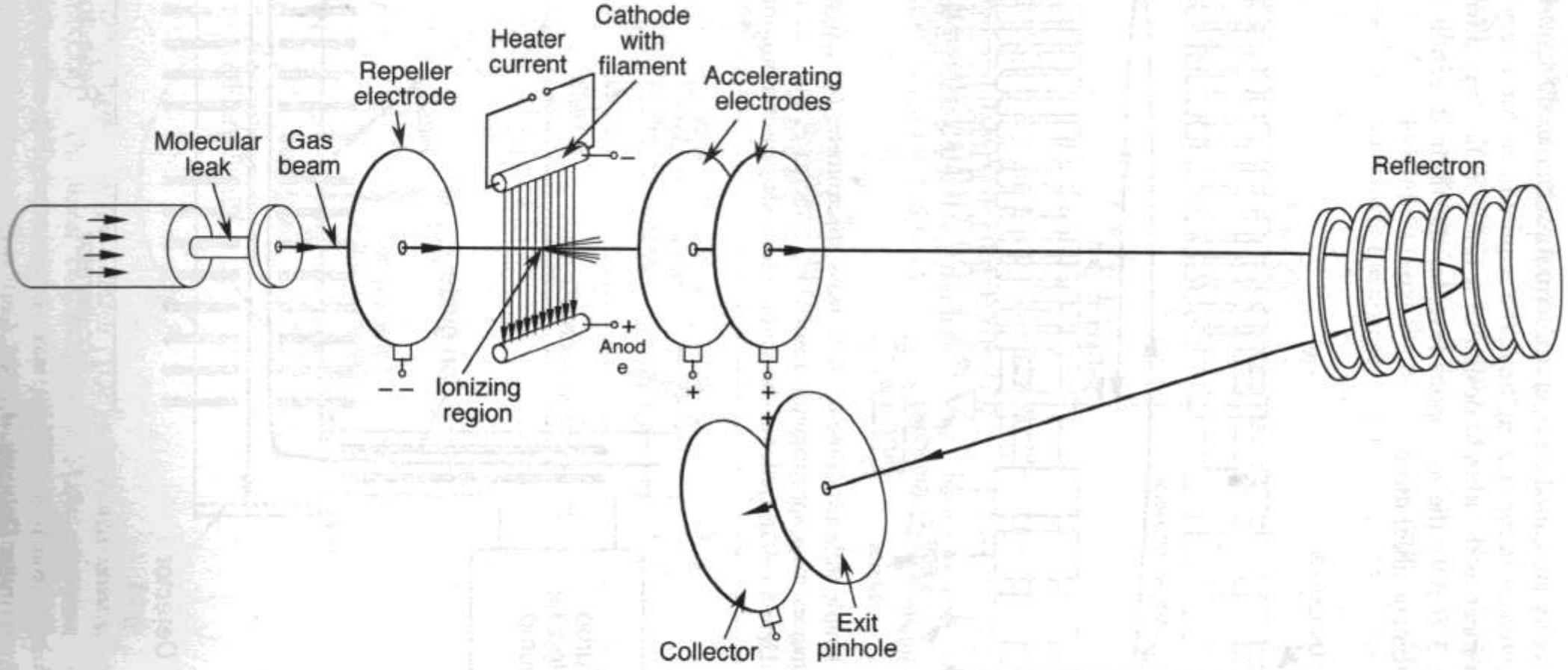



**Fig. 3.9** A high-resolution reflectron time-of-flight mass spectrometer. For further details see, e.g., IonSpec Corp., Irvine, CA; JEOL USA, Inc., Peabody, MA; Micromass and Waters Corporation, Milford, MA; Thermo Finnigan, San Jose, CA; Varian Instruments, Walnut Creek, CA

### 3.1.2 Quadrupole mass spectrometer

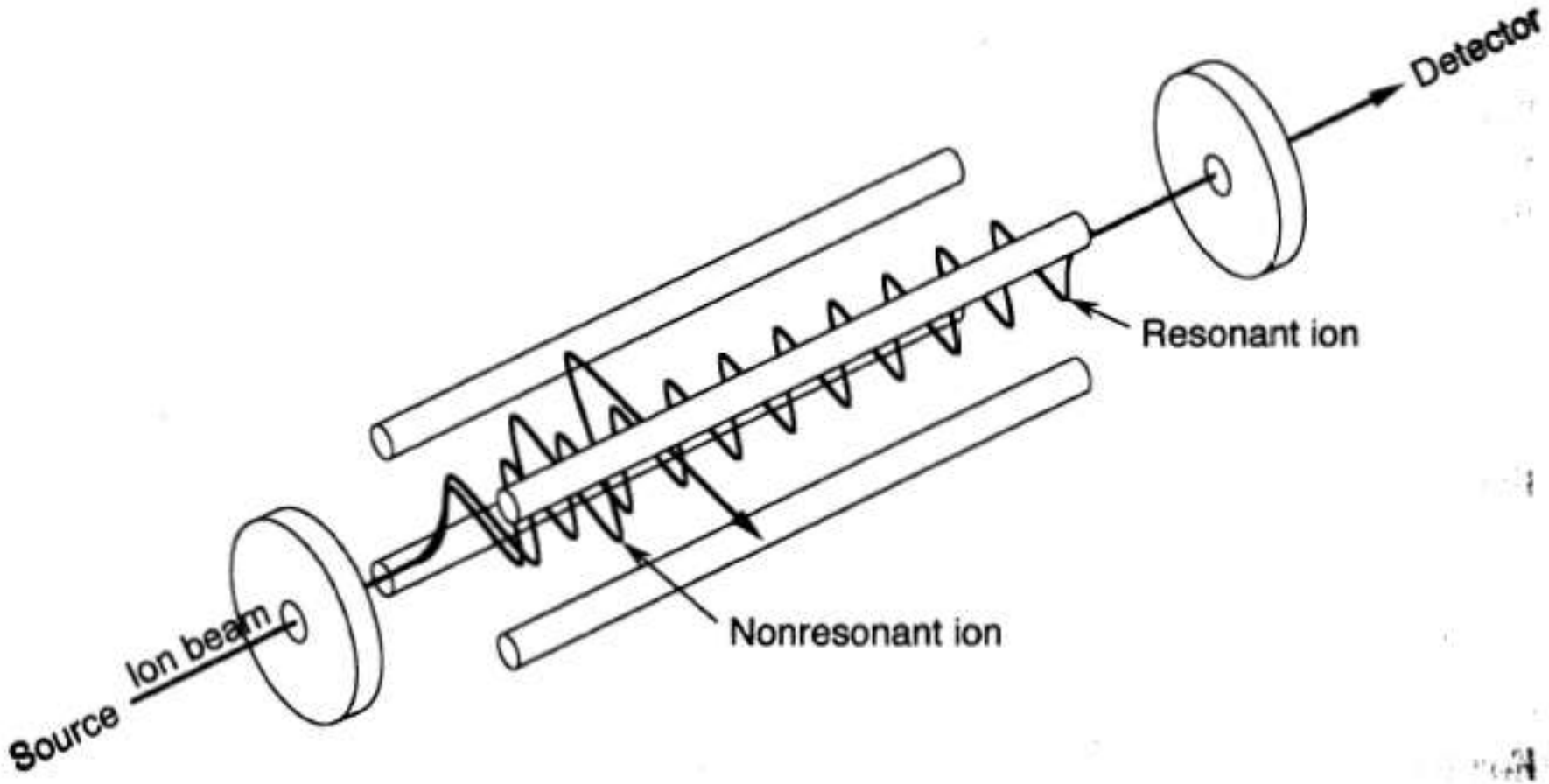



**Fig. 3.4** Quadrupole mass spectrometer. The ion beam is accelerated to a high velocity by an electric field and passed through the quadrupole mass analyzer comprising four metal rods. DC and AC potentials are applied to the quadrupole rods in such a way that only ions with one mass-to-charge ratio ($m/z$) can pass though the analyzer at a time. To scan different $m/z$, DC and AC potentials are varied

### 3.1.3 Ion trap mass spectrometer

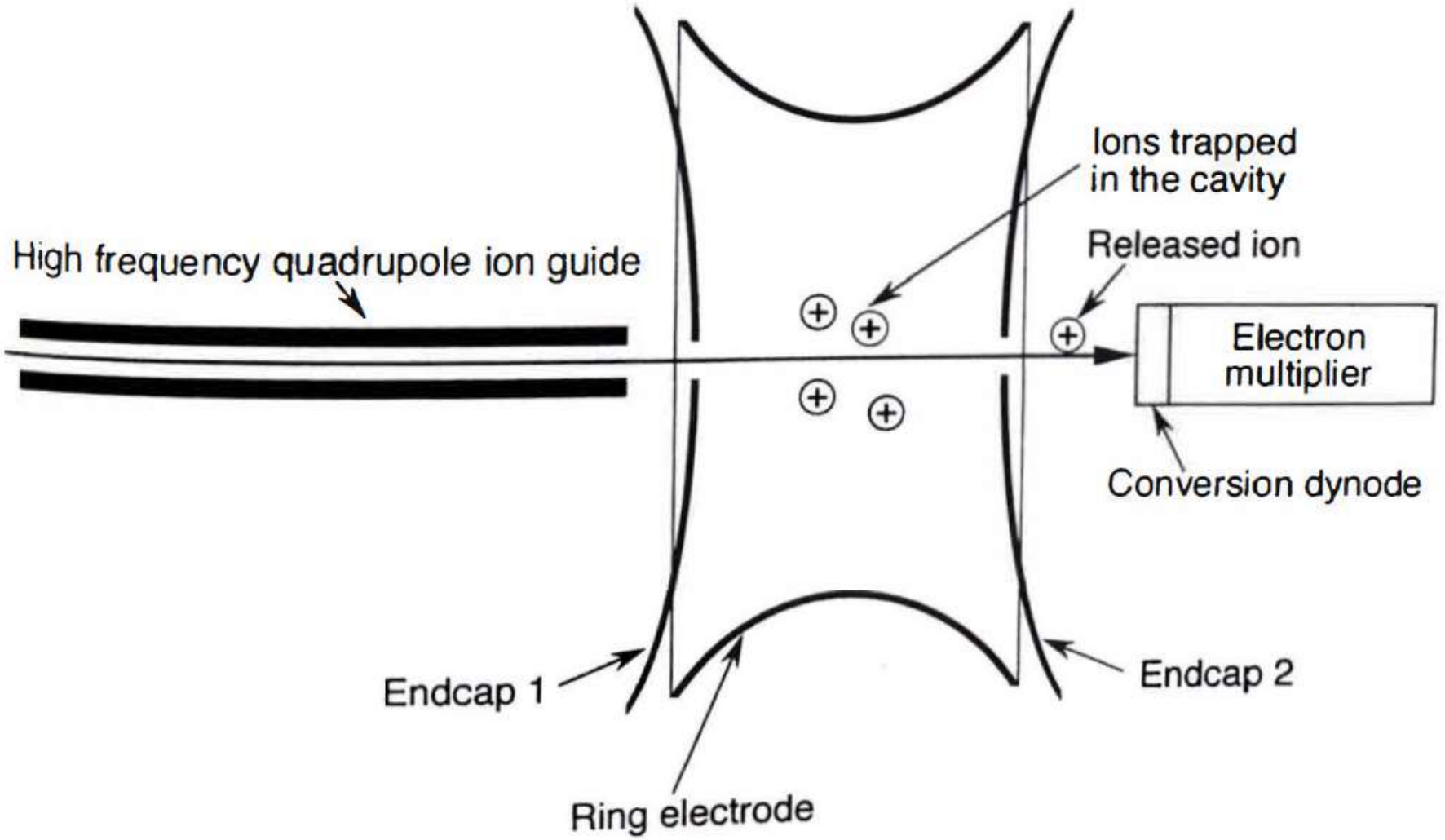



**Fig. 3.5** Ion trap mass analyzer. With the help of different radio frequency signals applied to the ring electrode and the endcaps, all ions are trapped in the cavity and then sequentially ejected according to their $m/z$

### 3.1.5 Fourier transform mass spectrometer

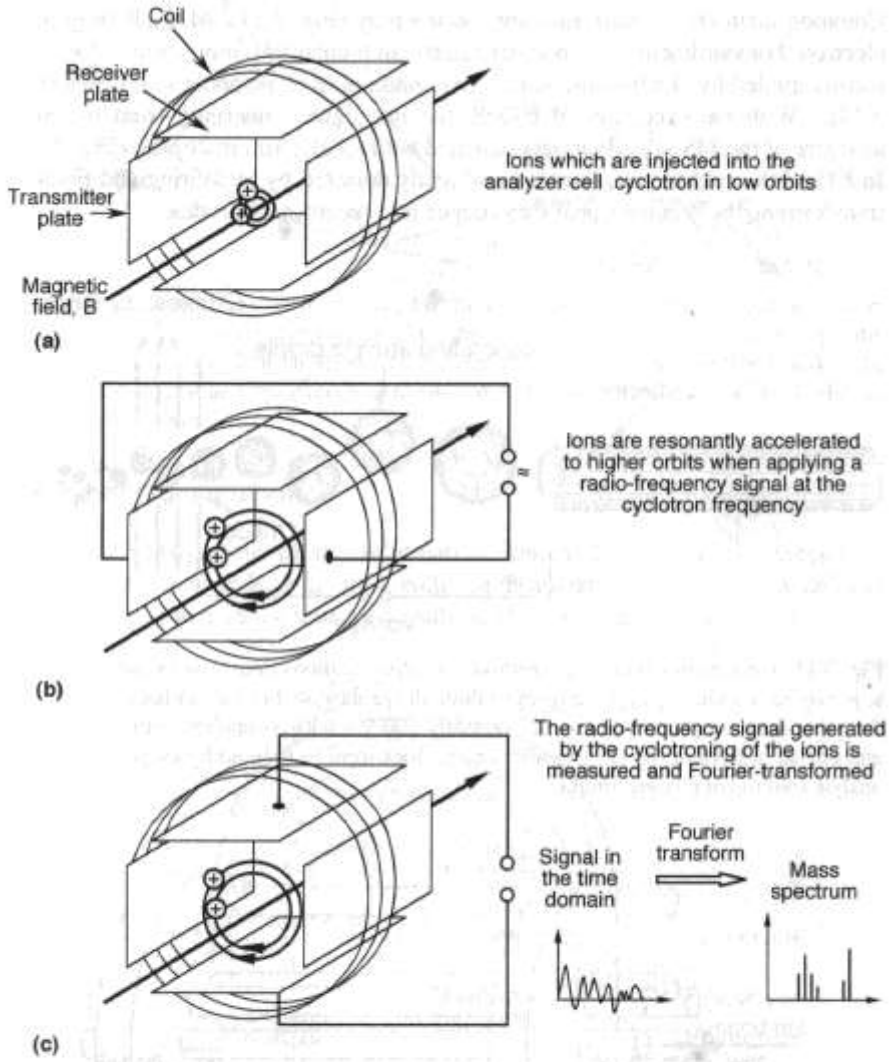



**Fig. 3.10** Principle of operation of a Fourier transform mass spectrometer (FTMS), also called "ion cyclotron resonance mass spectrometer" (see, e.g., IonSpec Corp., Lake Forest, CA; Bruker Daltonik, Bremen, Germany). **(a)** Ions are injected into the analyzer cell of the spectrometer. The magnetic field forces the thermal ions on orbits with small radii that depend on their mass-to-charge ratio. **(b)** An applied radio frequency pulse resonantly moves the ions to higher orbits. **(c)** The radio-frequency signal generated by the cyclotroning of the ions is measured and Fourier-transformed. For the method of Fourier transform see also Sect. 4.1.1. The striking characteristic of FTMS is the high resolution, $R$, typically in excess of 100,000

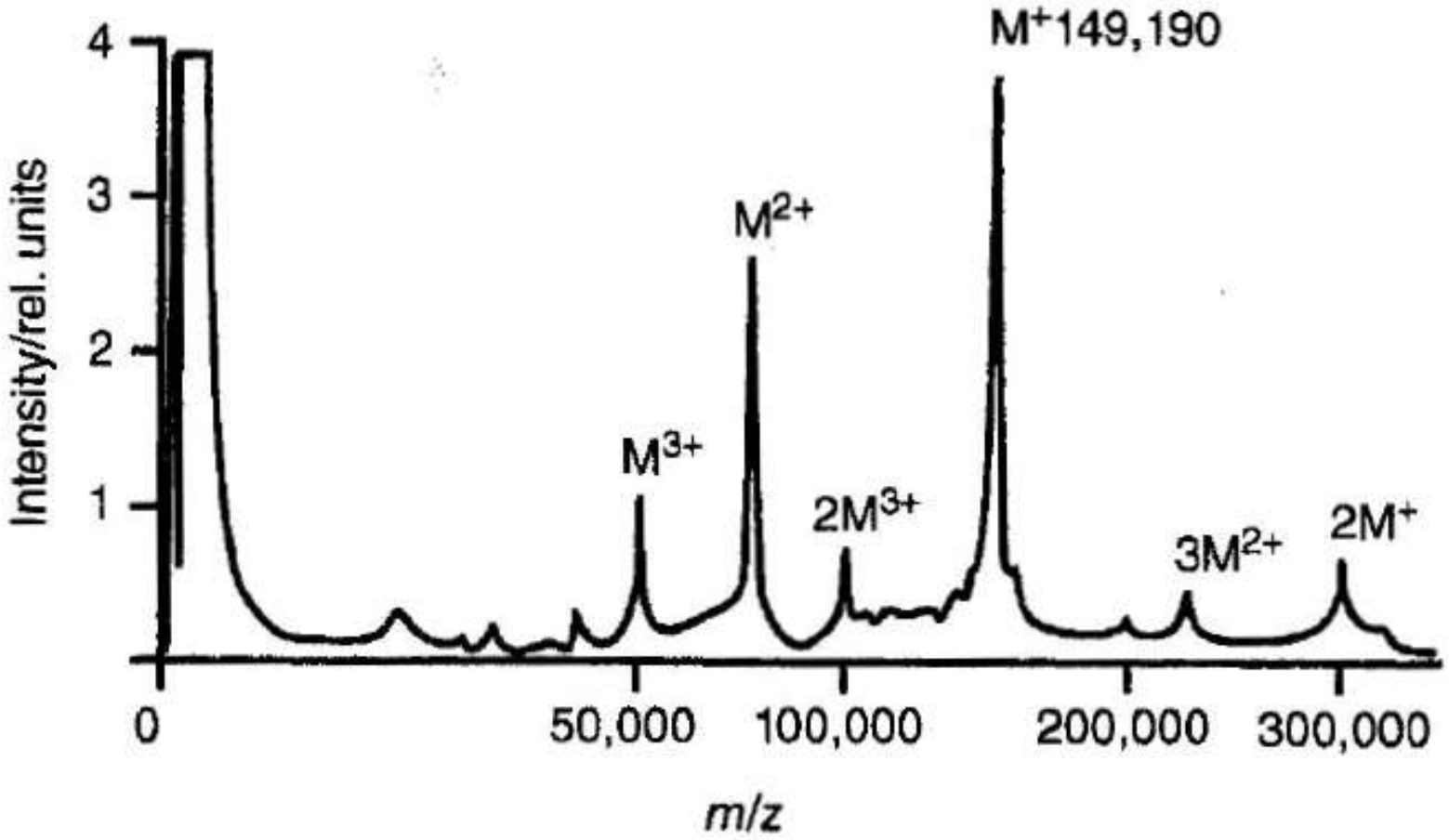



**FIGURE 19-4.** The MALDI mass spectrum of a monoclonal antibody. Reprinted from F. Hillenkamp and M. Karas, Mass spectrometry of peptides and proteins by matrix assisted ultraviolet laser desorption/ionization, *Methods Enzymol.* **193**, 280 (1990). © 1990, with permission from Elsevier.

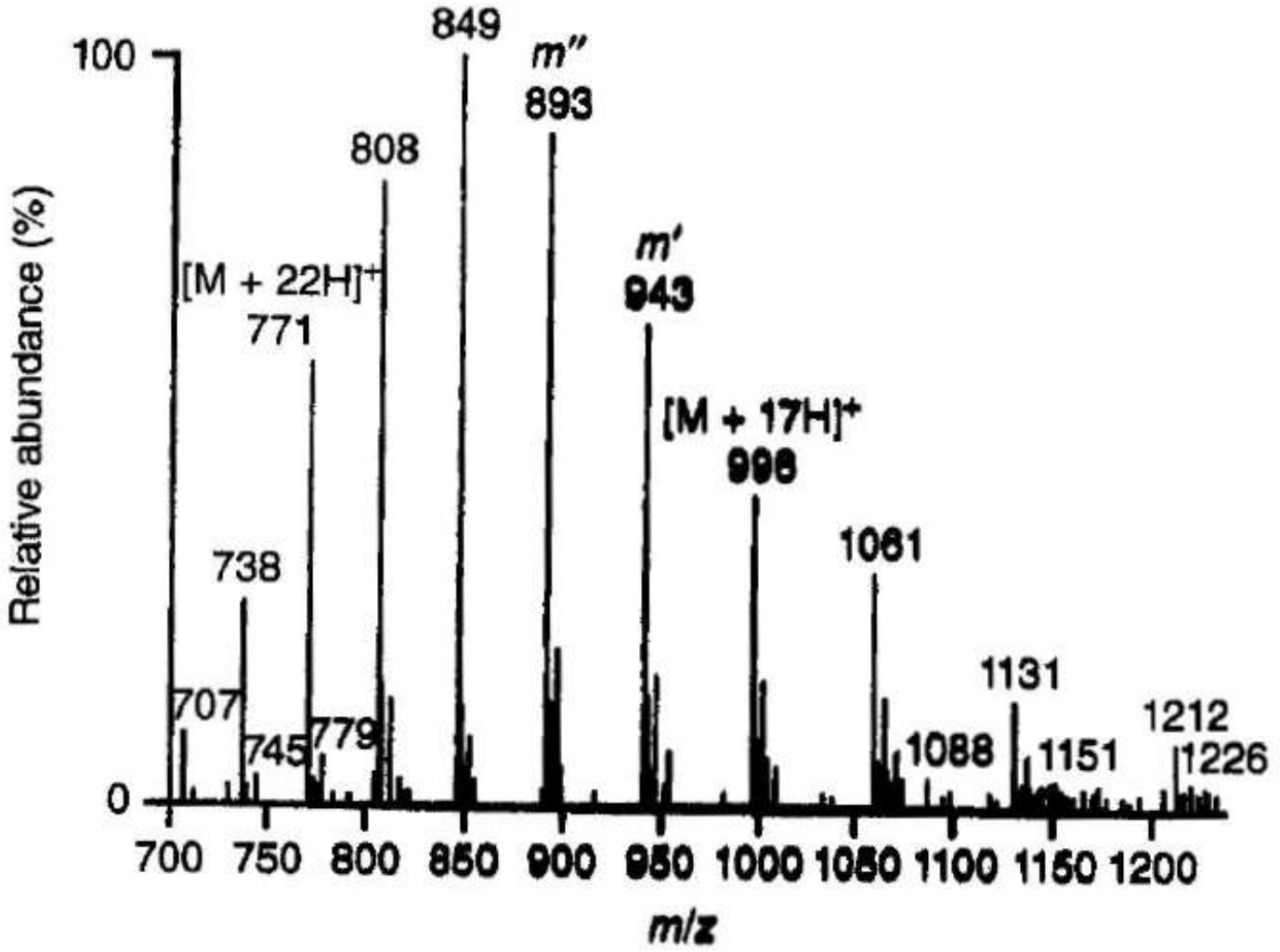



**FIGURE 19-5.** An electrospray ionization (ESI) mass spectrum of horse myoglobin. Each peak is characteristic of myoglobin with a different charge and number of protons. From C. Dass, *Principles and Practice of Biological Mass Spectrometry*, Wiley, New York, 2001, p. 43. Reprinted with permission of Wiley © 2001.

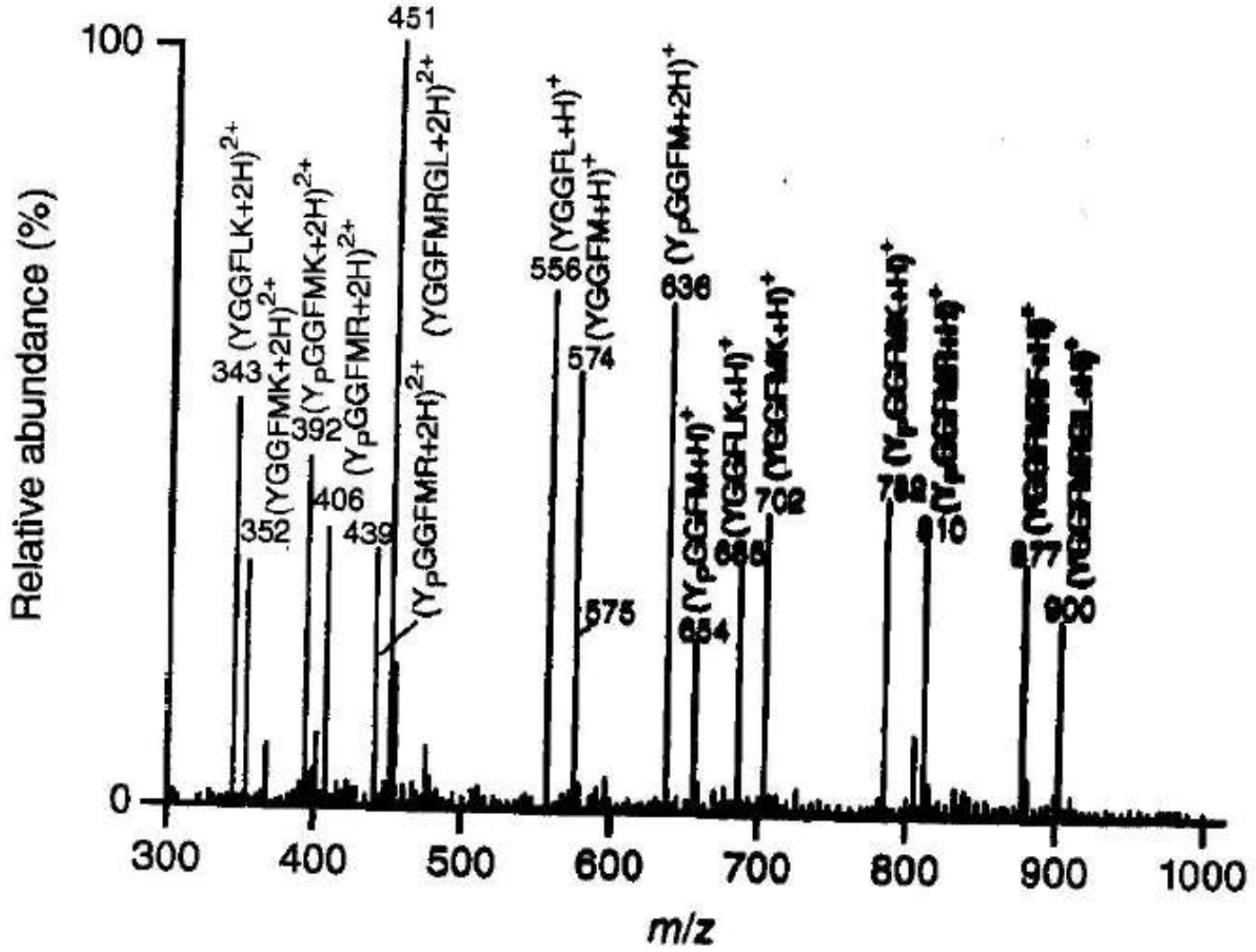



**FIGURE 19-6.** The positive ion ESI mass spectrum of a mixture of 10 peptides. The masses and amino acid sequences of the major peaks are indicated. Reproduced with permission from C. Dass in B. S. Larsen and C. N. McEwen (eds), *Mass Spectrometry of Biological Materials*, Marcel Dekker, New York, 1998, pp. 247–280.

### 3.1.7 Ion fragmentation

Significant enlargement of the information content of spectra is achieved by fragmenting the sample, e.g., in a collision chamber (Fig. 3.14) or a helium-containing cavity of an ion trap mass analyzer (Fig. 3.5; see also Sect. 3.2).

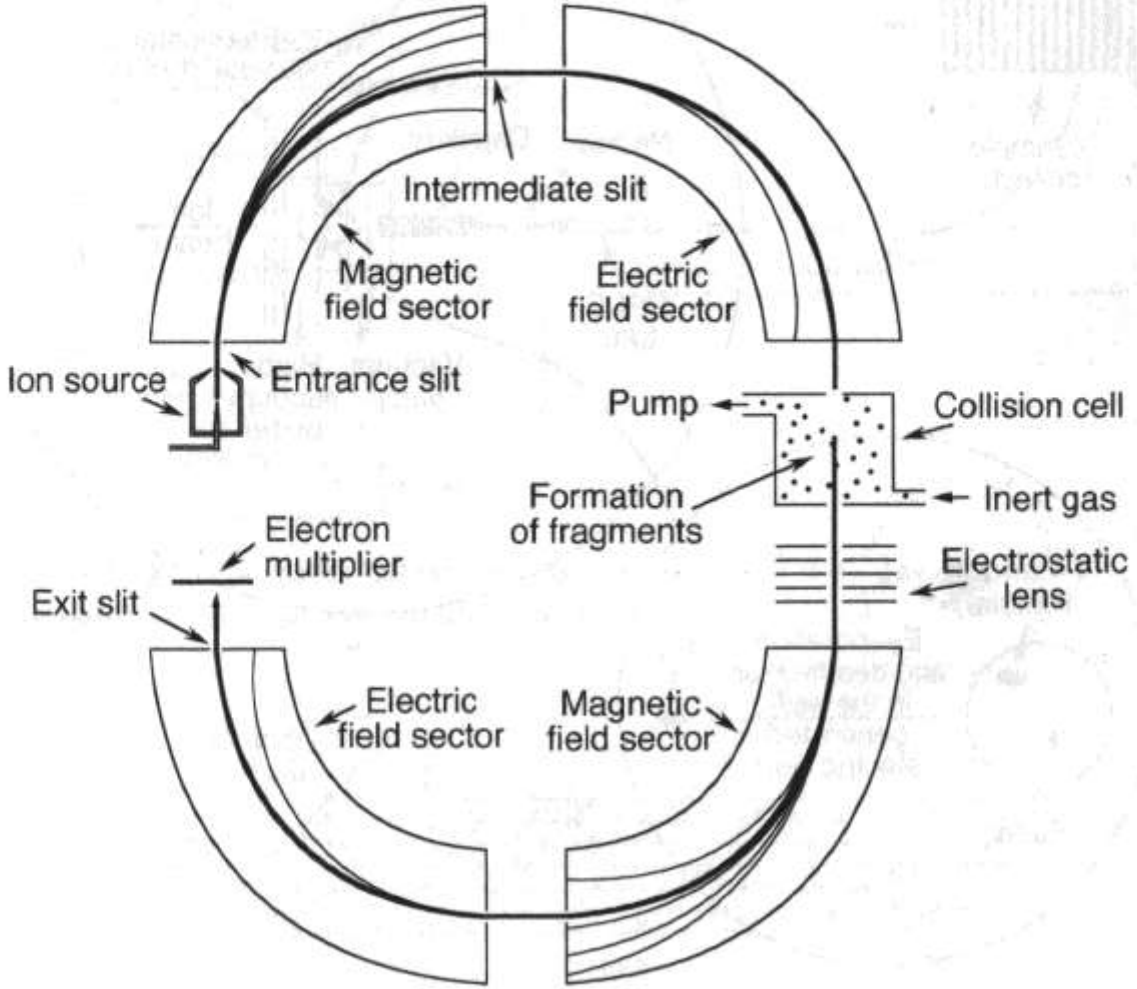



**Fig. 3.14** High resolution sector MS with a collision chamber

# Combining Chromatographic separation with Mass Spectrometry

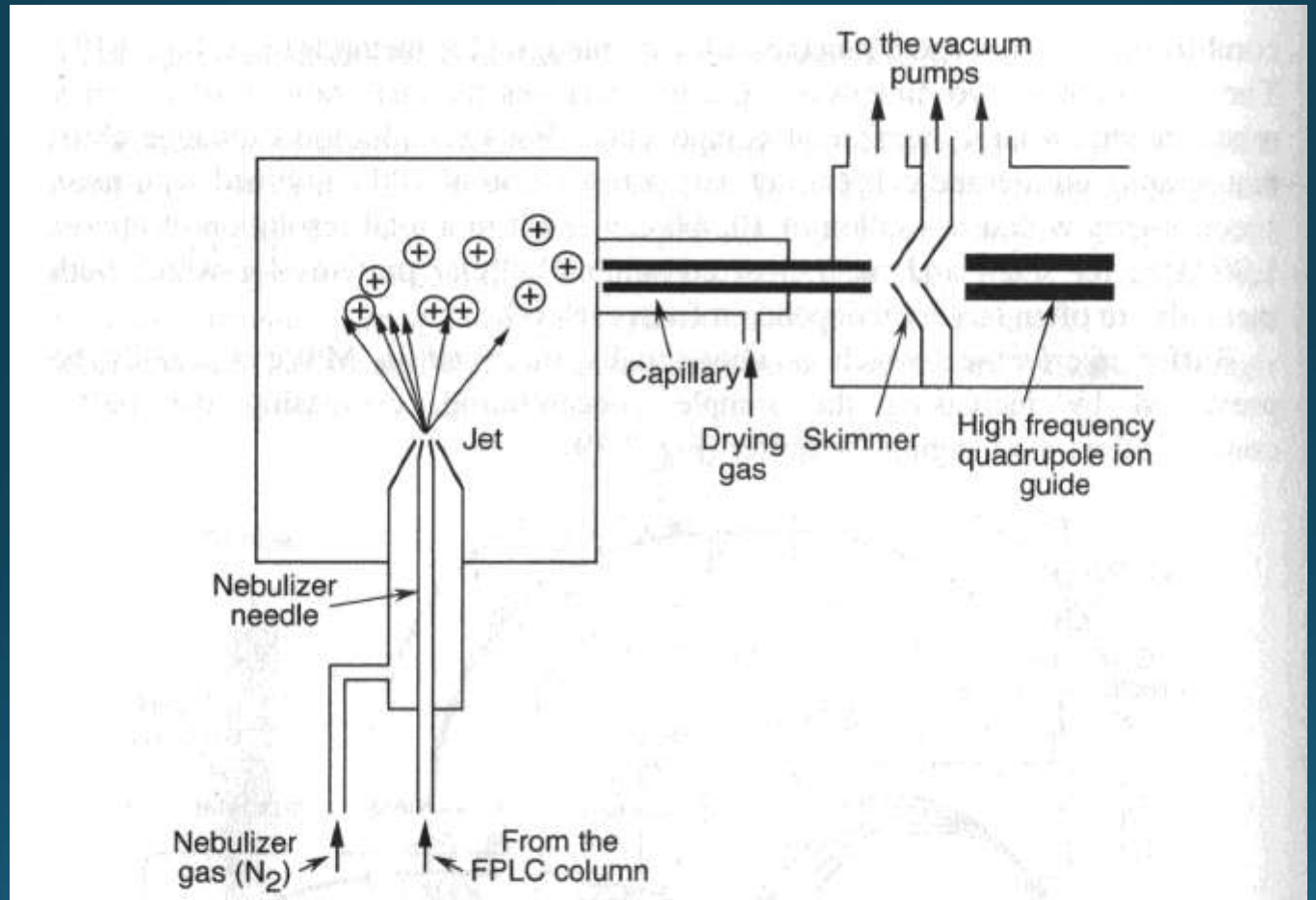



**Fig. 3.18** FPLC/MS connector. In several stages the solvent is removed from the analyte solution by application of dry nitrogen and vacuum. The quadrupole ion guide leads the ions to the mass analyzer of the mass spectrometer

# Combining Chromatographic separation with Mass Spectrometry

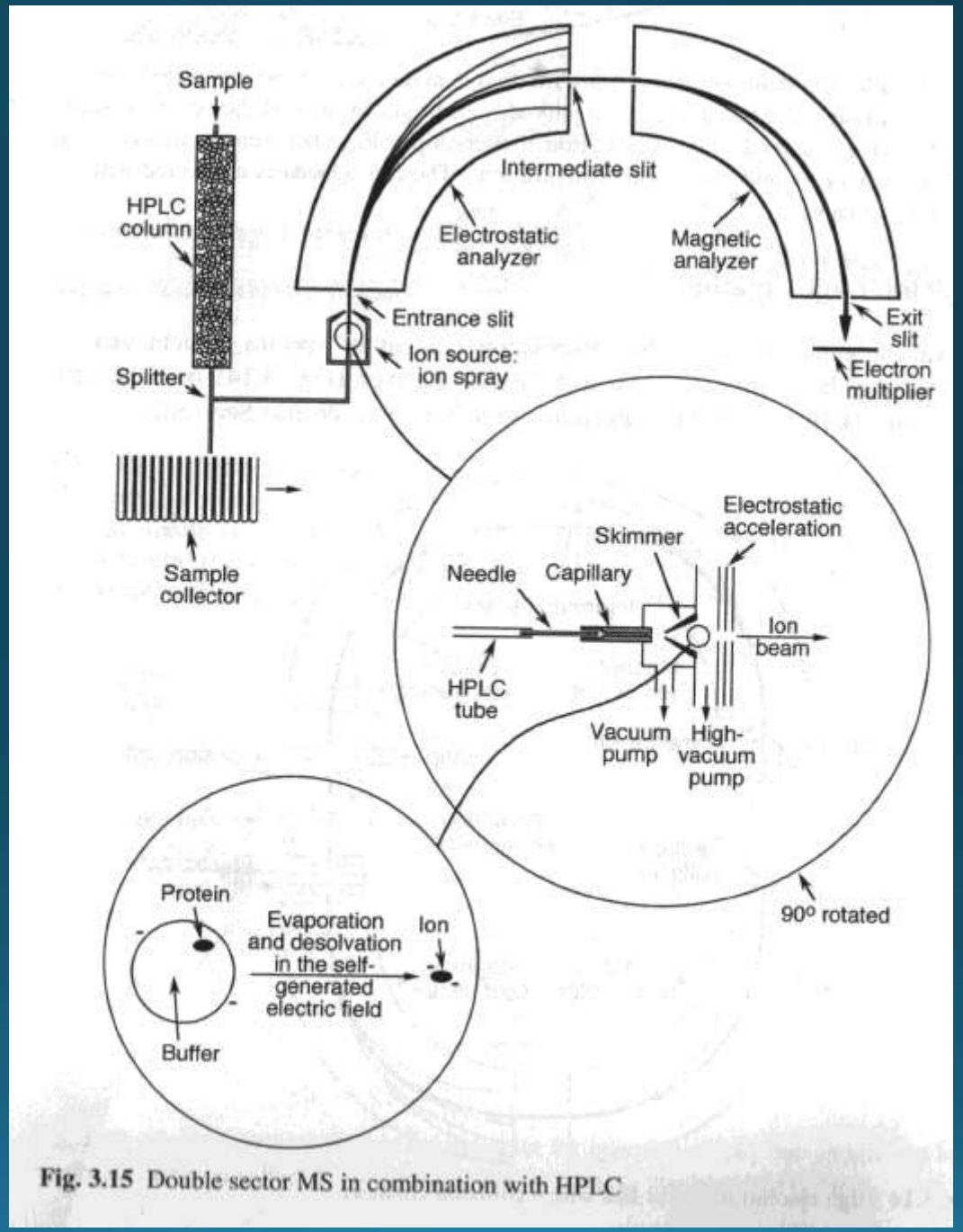
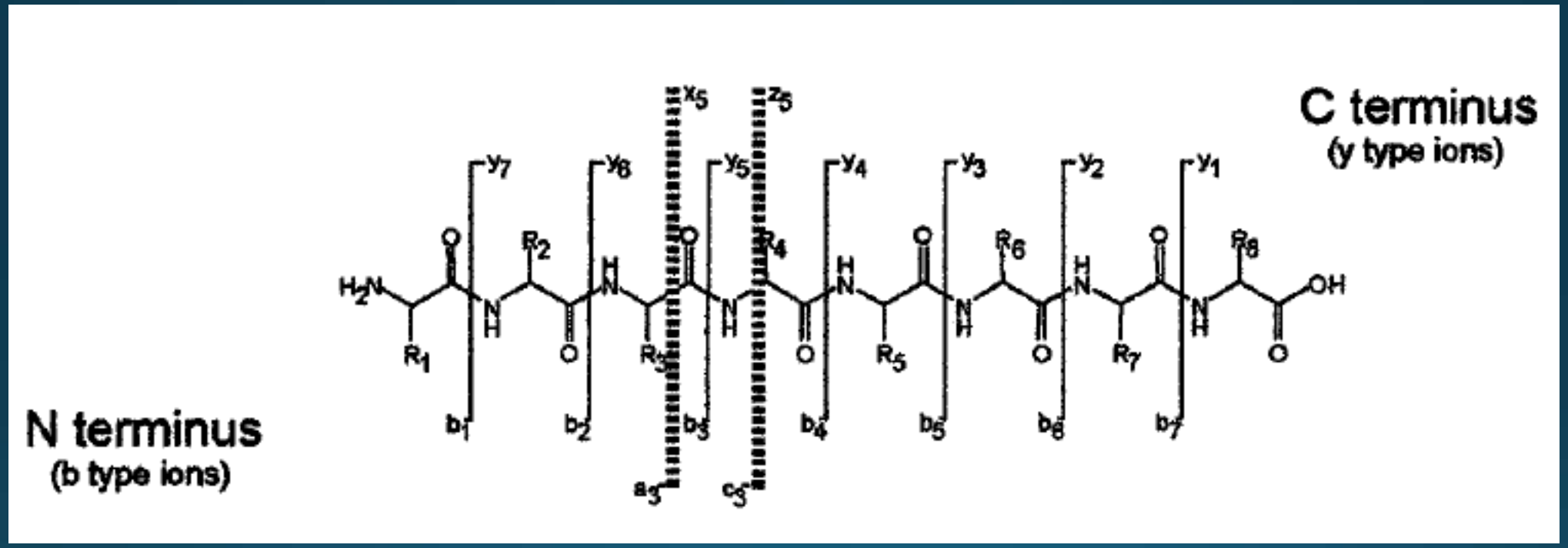
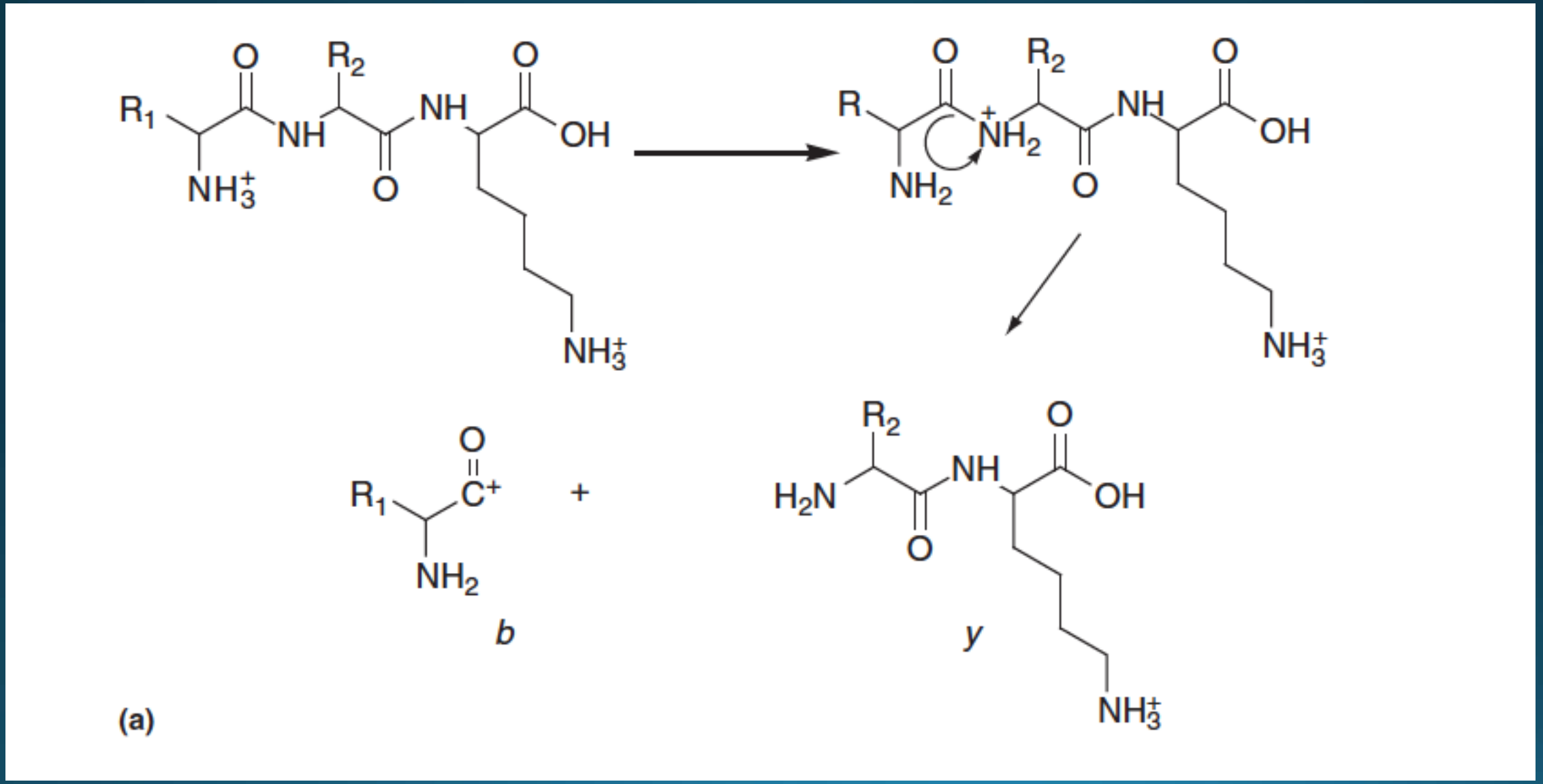



Fig. 3.15 Double sector MS in combination with HPLC

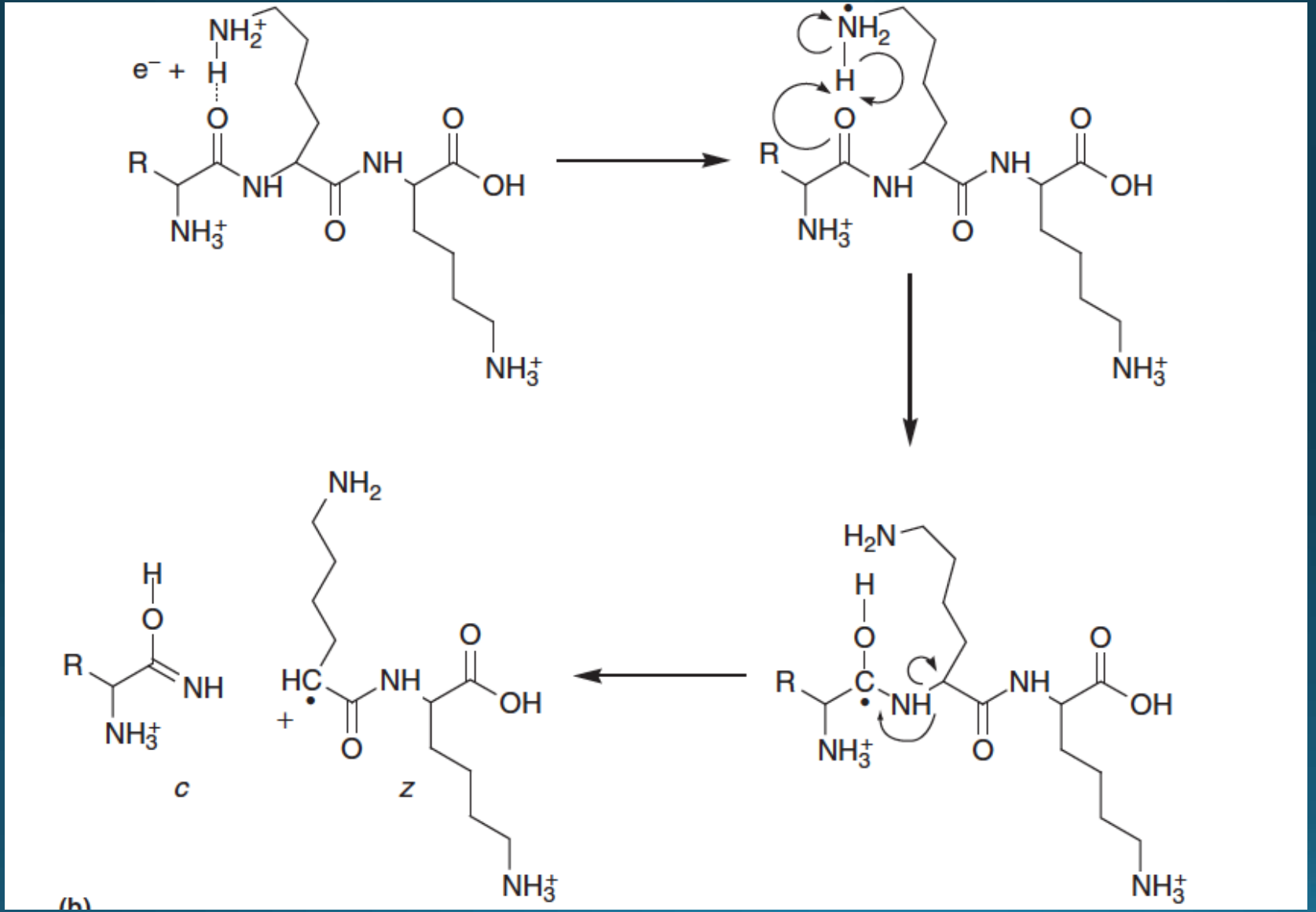
# Collision Induced Dissociation



# Collision Induced Dissociation



# Electron Transfer Dissociation



# Hydrogen-Deuterium Exchange

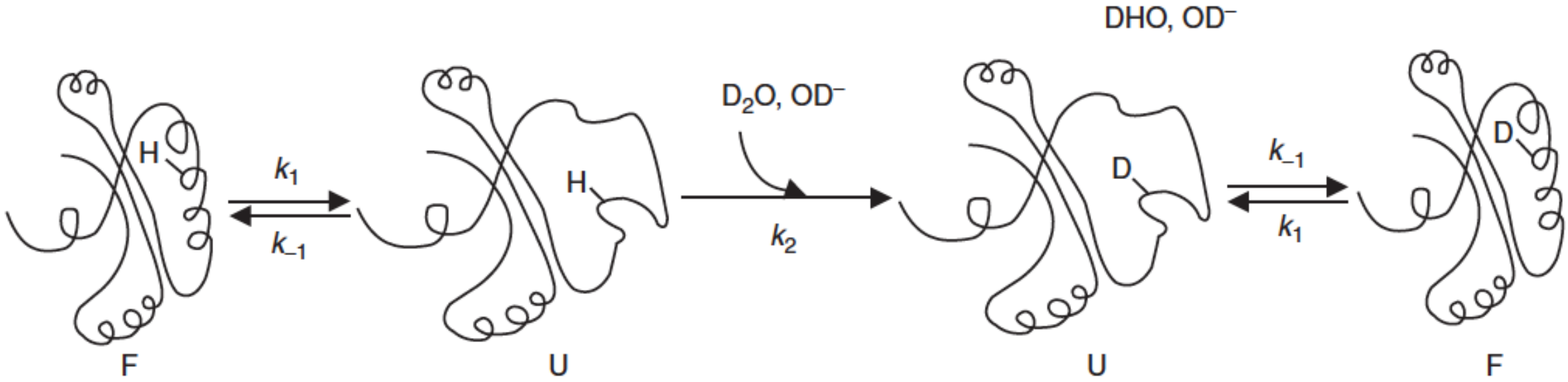
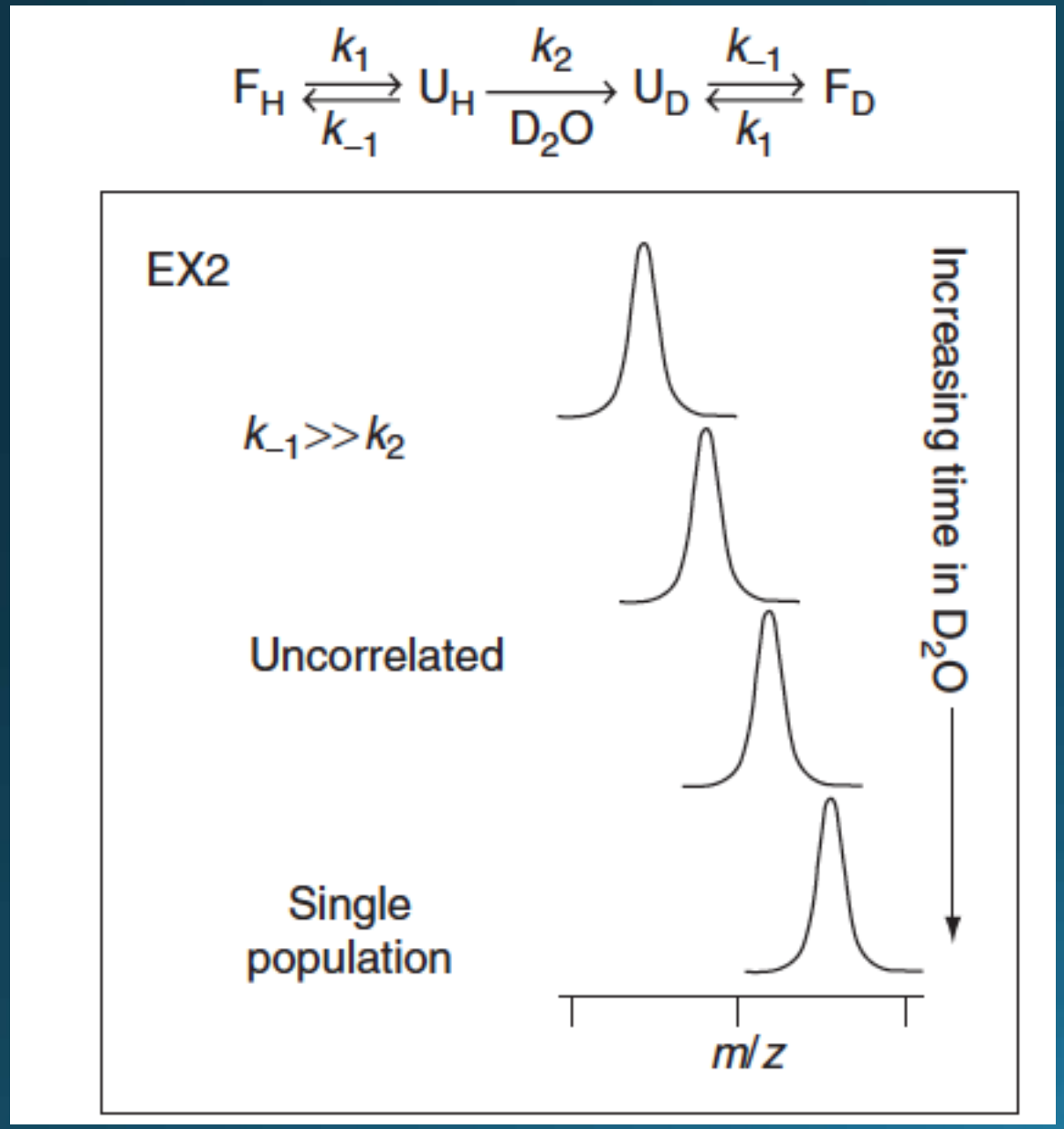



**Figure 6** Pictorial representation of a folded (F) protein unfolding in a small region to facilitate partial or complete isotope exchange. The kinetics of the unfolding and refolding processes are described by rate constants $k_1$ and $k_{-1}$, respectively, and exchange from the unfolded polypeptide is described by $k_2$. Reprinted with permission from Deng, Y.; Zhang, Z.; Smith, D. L. Comparison of continuous and pulsed labeling amide hydrogen exchange/mass spectrometry for studies of protein dynamics. *J. Am. Soc. Mass Spectrom.* **1999**, *10*(8), 675–684.

# Hydrogen-Deuterium Exchange



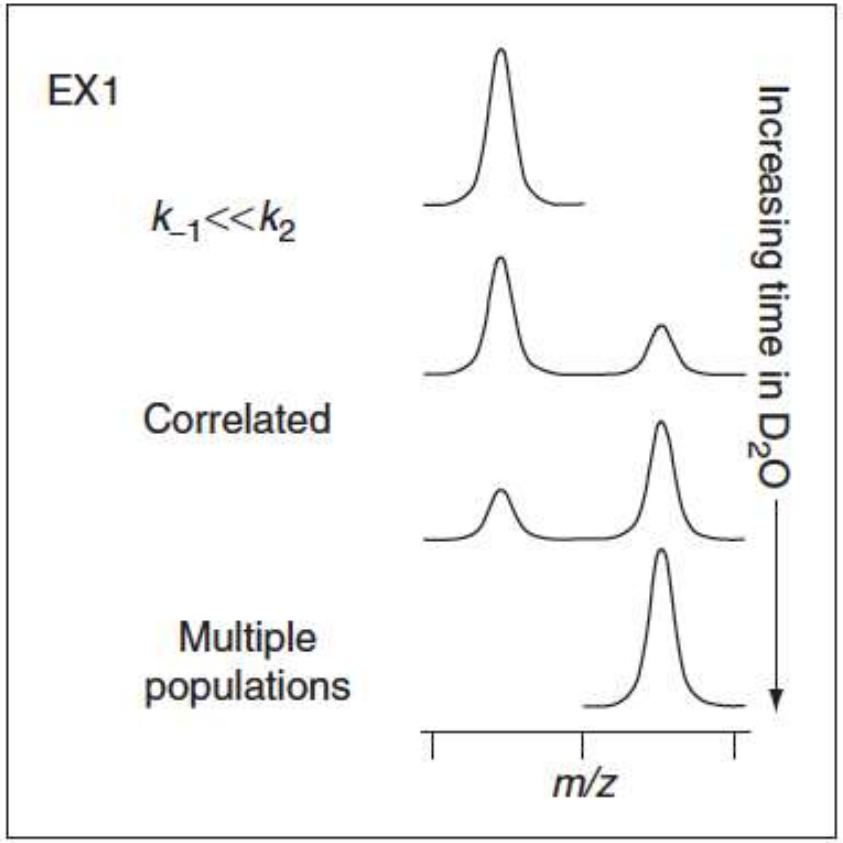




**Figure 7** Protein unfolding and deuterium labeling in hydrogen exchange mass spectrometry, where the two kinetic extremes, EX1 and EX2, are depicted. Reprinted with permission from Weis, D. D.; Wales, T. E.; Engen, J. R.; Hotchko, M.; Ten Eyck, L. F. Identification and characterization of EX1 kinetics in H/D exchange mass spectrometry by peak width analysis. *J. Am. Soc. Mass Spectrom.* **2006**, *17*(11), 1498–1509.

# NASA Mars Mobile with TOF MS

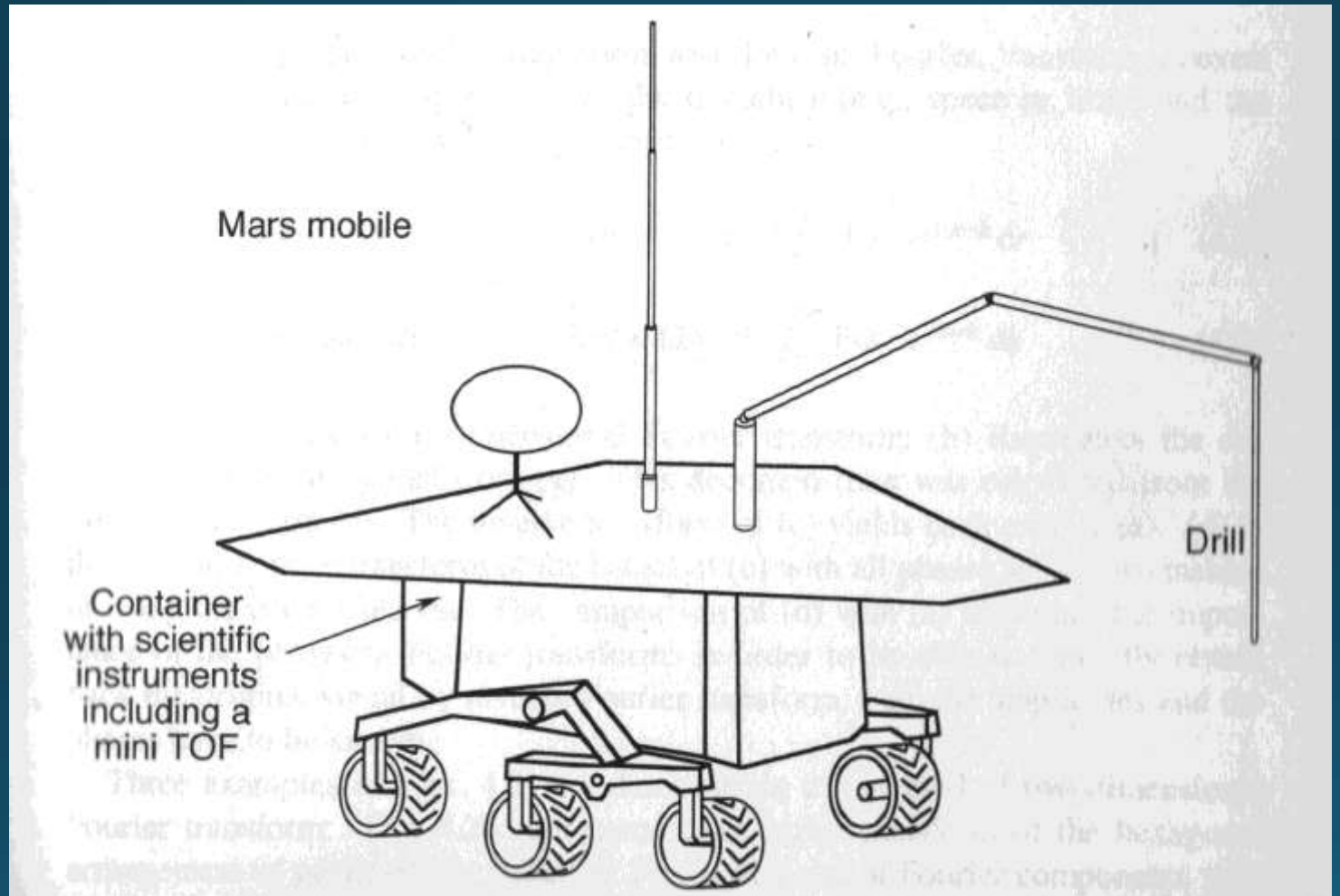



**Fig. 3.32** NASA plans to send rovers outfitted with MS to Mars. This sketch illustrates a mobile with mini-TOF searching for extraterrestrial life. Already now mass-spectrometry is utilized to search for extraterrestrial bacteria in meteors