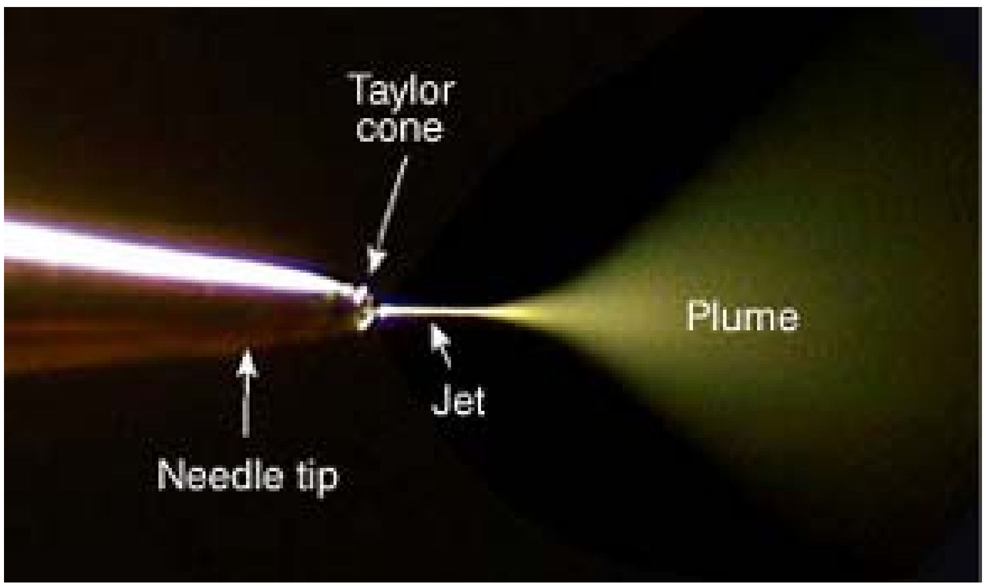
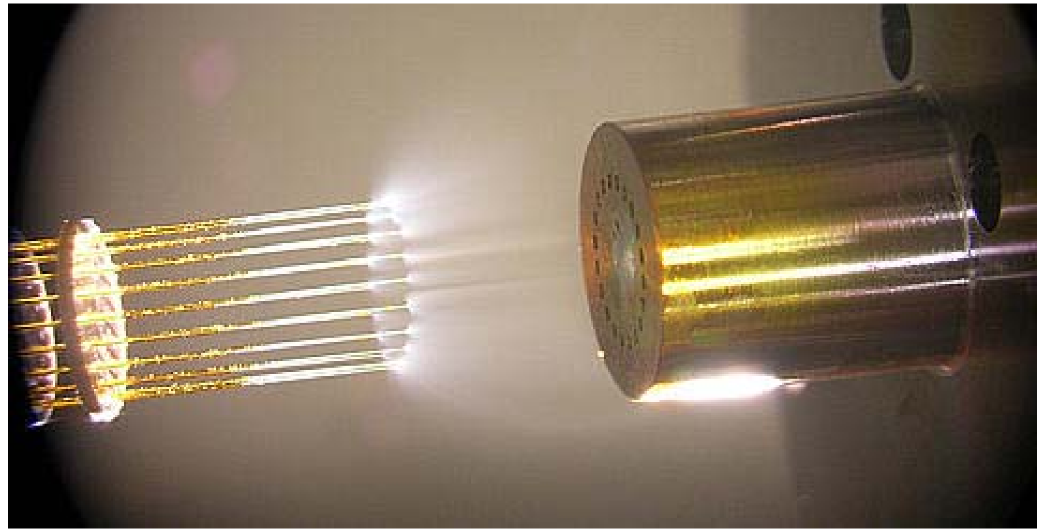
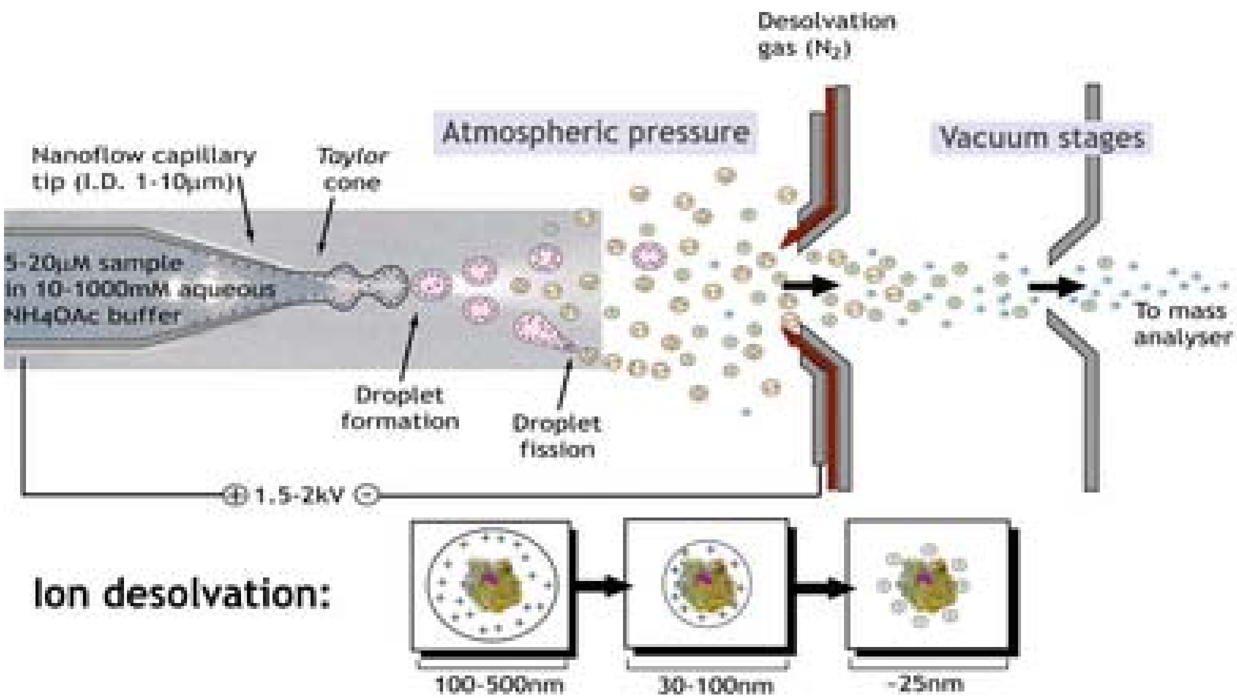
Electrospray Ionization (ESI)

- large, non-volatile molecules (e.g., proteins) with no fragmentation
- Conveniently coupled to liquid separations
- Characterized by multiply charged
- Upper mass range not really limited, but big masses need sufficient mass resolution.
- Extremely soft ionization method → non-covalently bound species (e.g. protein complexes) : native ESI



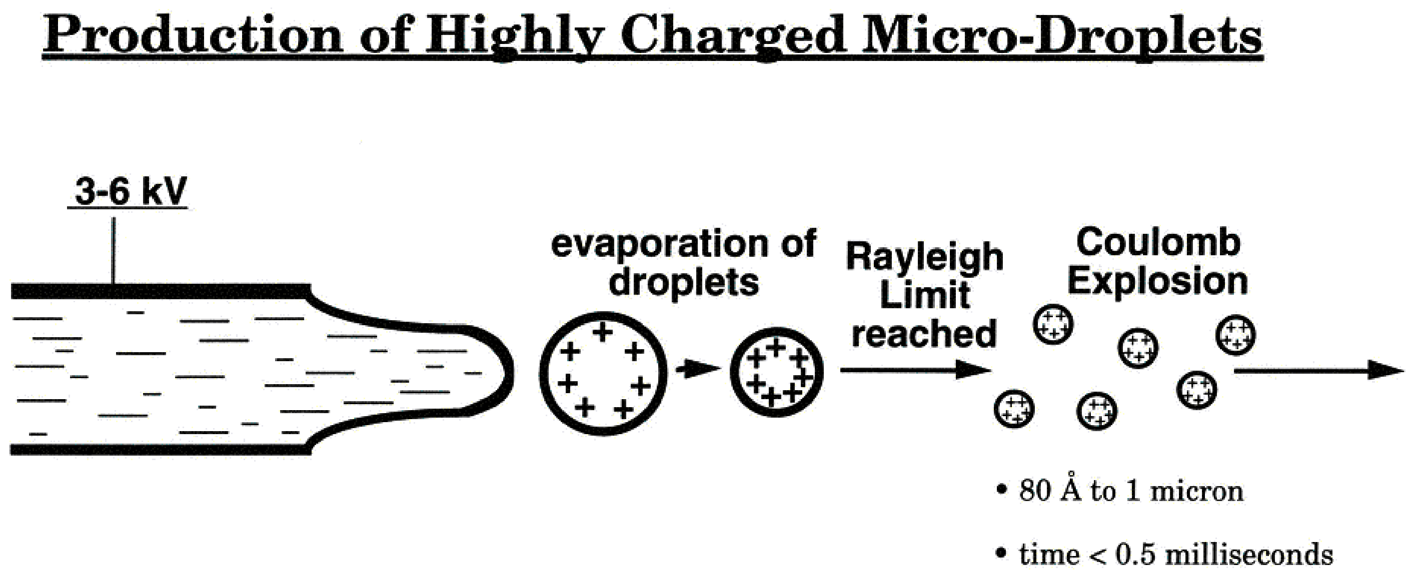
ESI Mechanism





ESI Mechanism

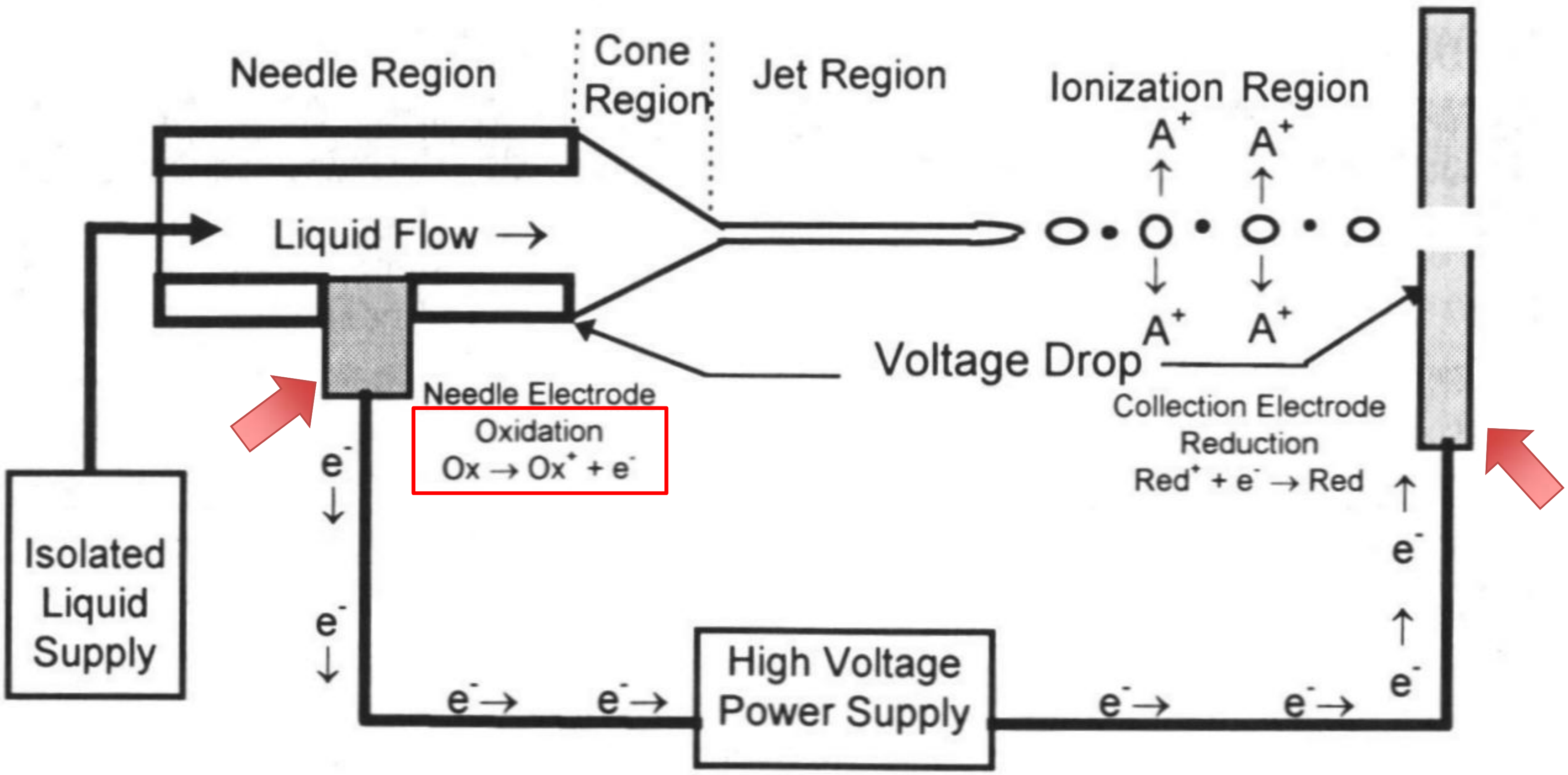
- Evaporation of solvent from the droplets → the charge density increases.
- At the 'Rayleigh limit,' repulsion between ions equal surface tension → '<u>Coulombic explosions</u>' that produce even finer droplets.
- This process of evaporation and explosion repeats until fully desolvated ions are released.



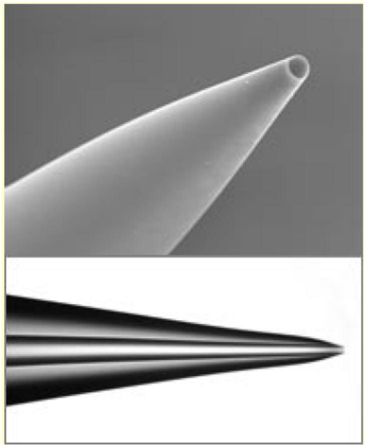
Quiz

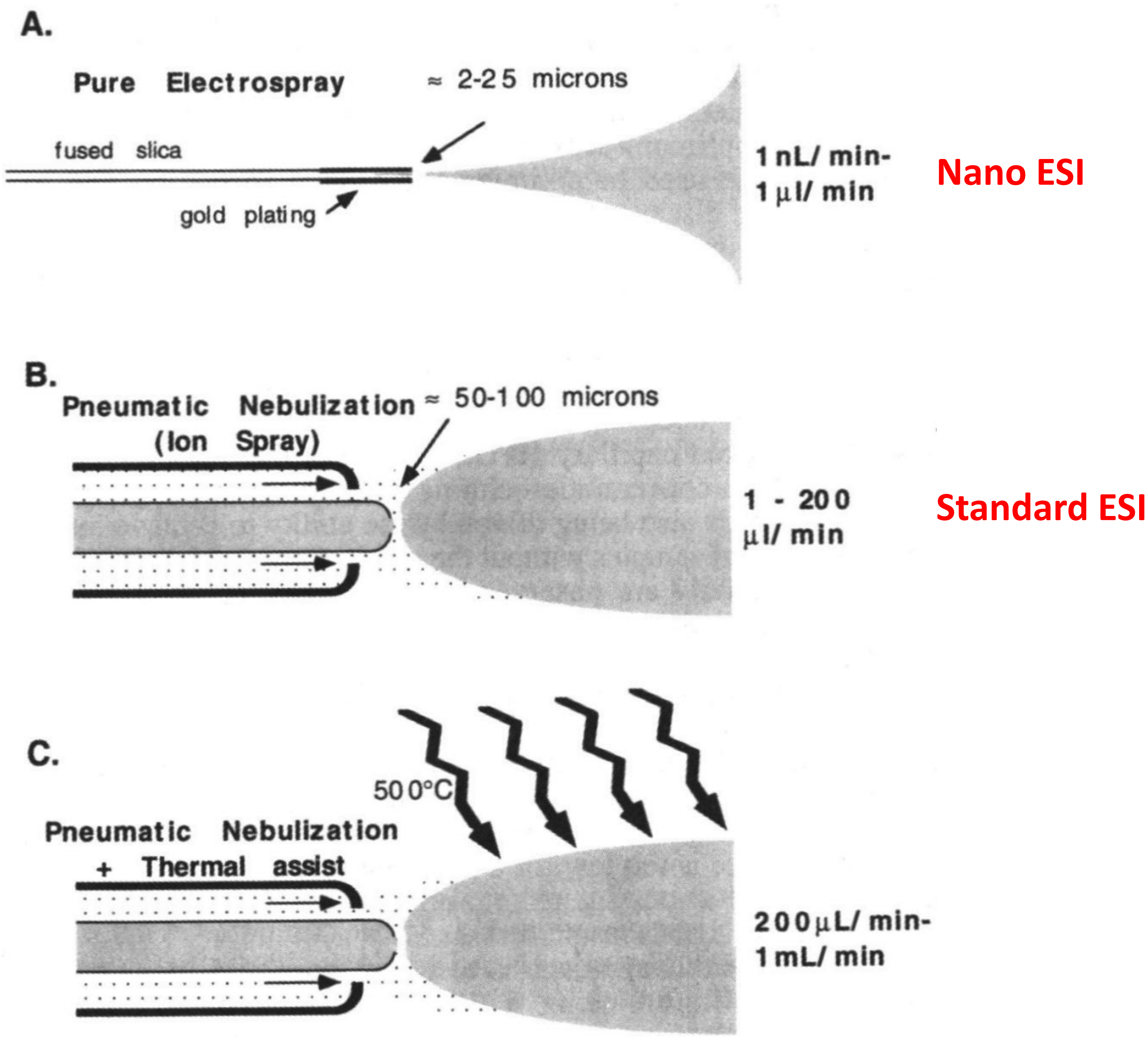
ESI Mechanism –Electrochemical Process

A constant current electrochemical cell



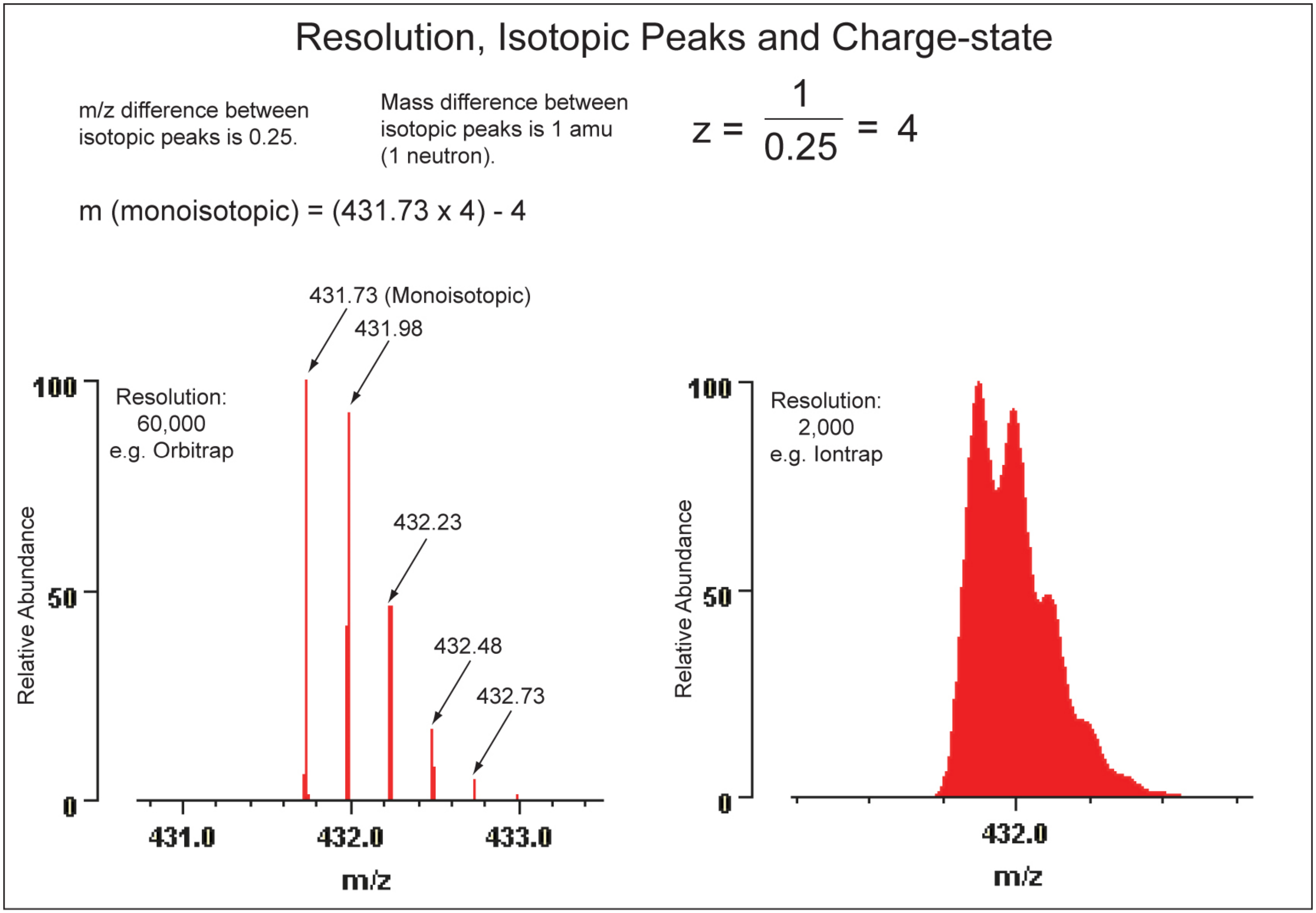
ESI with Various Flow Rates





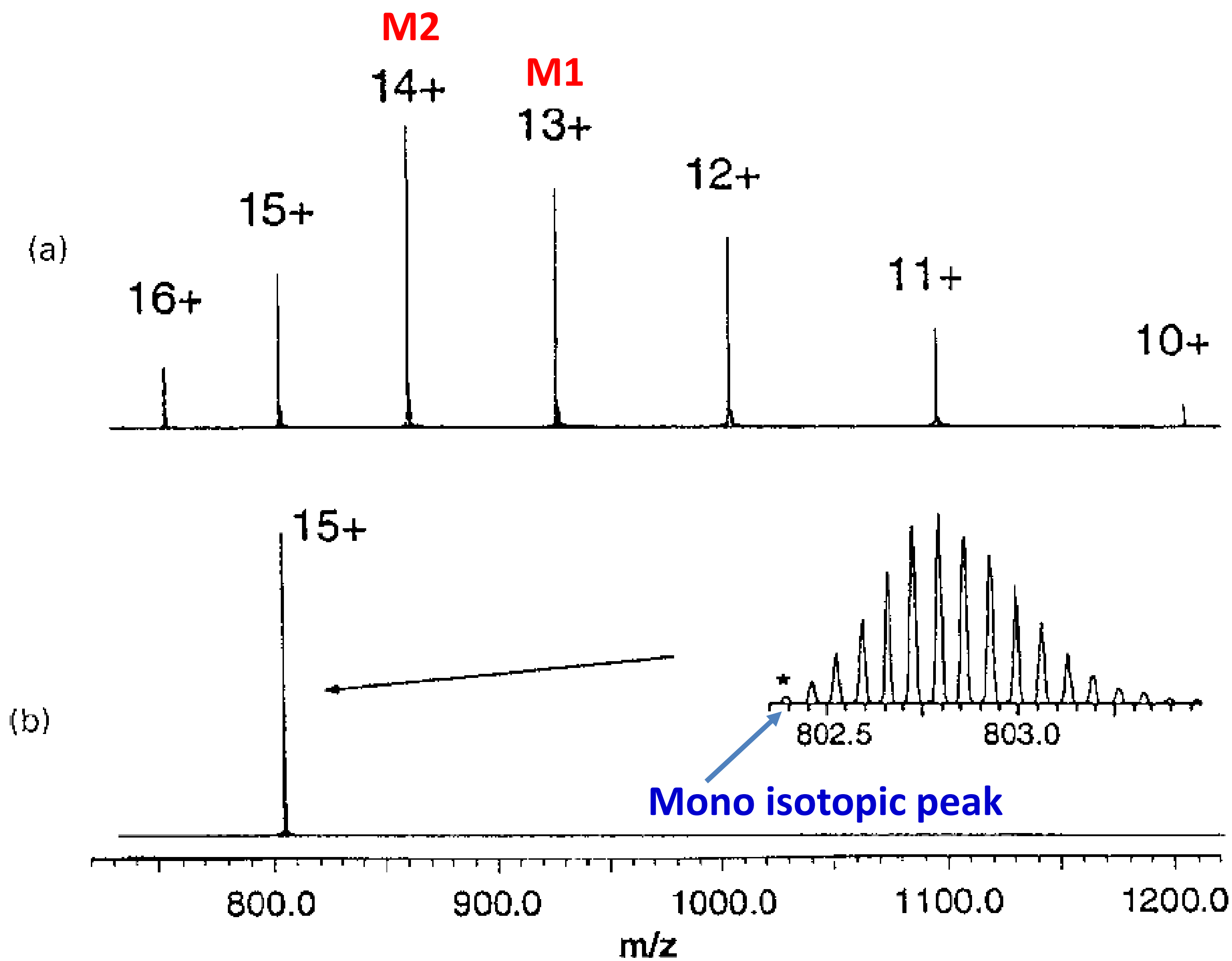
ESI Mass Spectrum – Multiply Charged Ions

How to determine charge state?



High resolution **Low resolution**

ESI Mass Spectrum – Multiply Charged Ions



ESI MS of Proteins

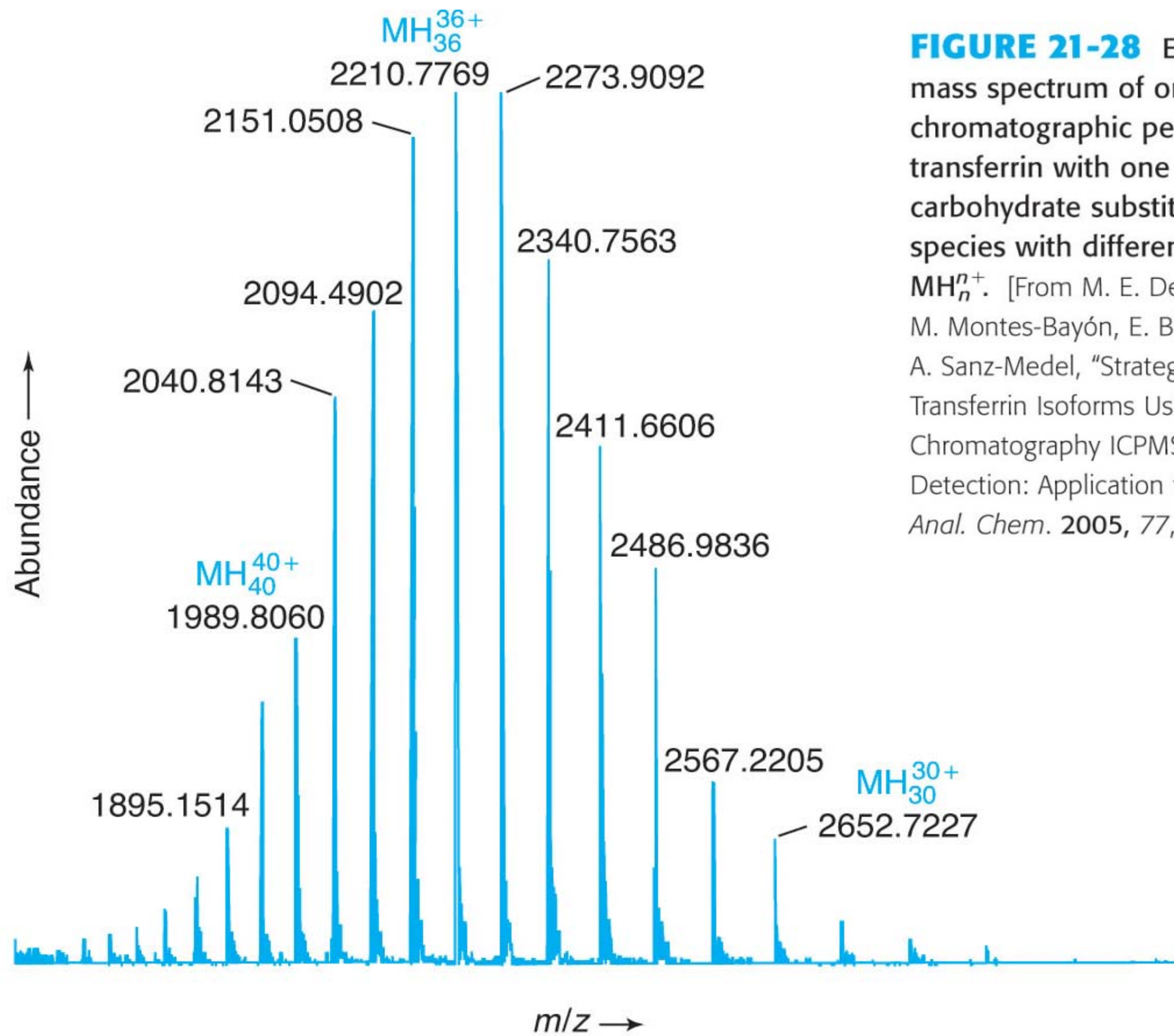


FIGURE 21-28 Electrospray time-of-flight mass spectrum of one anion-exchange chromatographic peak containing the protein transferrin with one particular set of carbohydrate substituents. Peaks arise from species with different numbers of protons, MH_n^{n+}. [From M. E. Del Castillo Busto, M. Montes-Bayón, E. Blanco-González, J. Meija, and A. Sanz-Medel, "Strategies to Study Human Serum Transferrin Isoforms Using Integrated Liquid Chromatography ICPMS, MALDI-TOF, and ESI-Q-TOF Detection: Application to Chronic Alcohol Abuse," *Anal. Chem*. **2005**, *77*, 5615.]

ESI MS of Proteins

Consider a peak at $m/z = m_n$ derived from the neutral molecules plus n protons;

$$m_n = \frac{\text{mass}}{\text{charge}} = \frac{M + n(1.008)}{n} = \frac{M}{n} + 1.008 \quad \Rightarrow \quad \boxed{m_n - 1.008 = \frac{M}{n}}$$

The next peak at lower m/z should have $n+1$ protons;

$$m_{n+1} = \frac{M + (n+1)(1.008)}{n+1} = \frac{M}{n+1} + 1.008 \quad \Rightarrow \quad \boxed{m_{n+1} - 1.008 = \frac{M}{n+1}}$$

$$\frac{m_n - 1.008}{m_{n+1} - 1.008} = \frac{M/n}{M/(n+1)} = \frac{n+1}{n} \quad \Rightarrow \quad n = \frac{m_{n+1} - 1.008}{m_n - m_{n+1}}$$

$$M = n \times (m_n - 1.008)$$

ESI MS of Proteins

TABLE 21-3 **Analysis of electrospray mass spectrum of tetrasialo-transferrin in Figure 21-28**

Observed $m/z \equiv m_n$	$m_{n+1} - 1.008$	$m_n - m_{n+1}$	Charge $= n = \dfrac{m_{n+1} - 1.008}{m_n - m_{n+1}}$	Molecular mass $= n \times (m_n - 1.008)$
2 652.722 7	2 566.212 5	85.502 2	30.013 ≈ 30	79 551.44
2 567.220 5	2 485 975 6	80.236 9	30.983 ≈ 31	79 552.59
2 486.983 6	2 410.652 6	75.323 0	32.004 ≈ 32	79 551.22
2 411.660 6	2 339.748 3	70.904 3	32.999 ≈ 33	79 551.54
2 340.756 3	2 272.901 2	66.847 1	34.001 ≈ 34	79 551.44
2 273.909 2	2 209.768 9	63.132 3	35.002 ≈ 35	79 551.54
2 210.776 9	2 150.042 8	59.726 1	35.998 ≈ 36	79 551.68
2 151.050 8	2 093.482 2	56.560 6	37.013 ≈ 37	79 551.58
2 094.490 2	2 039.806 3	53.675 9	38.002 ≈ 38	79 552.32
2 040.814 3	1 988.798 0	51.008 3	38.990 ≈ 39	79 552.45
1 989.806 0	1 894.143 4		40	79 551.92
				mean = 79 551.78 ± 0.48

SOURCE: *M. E. Del Castillo Busto, M. Montes-Bayón, E. Blanco-González, J. Meija, and A. Sanz-Medel, "Strategies to Study Human Serum Transferrin Isoforms Using Integrated Liquid Chromatography ICPMS, MALDI-TOF, and ESI-Q-TOF Detection: Application to Chronic Alcohol Abuse," Anal. Chem.* ***2005,*** *77, 5615.*

Harris, *Quantitative Chemical Analysis,* 8e

ESI Mass Spectrum – MW Determination from Multiply Charged Ions

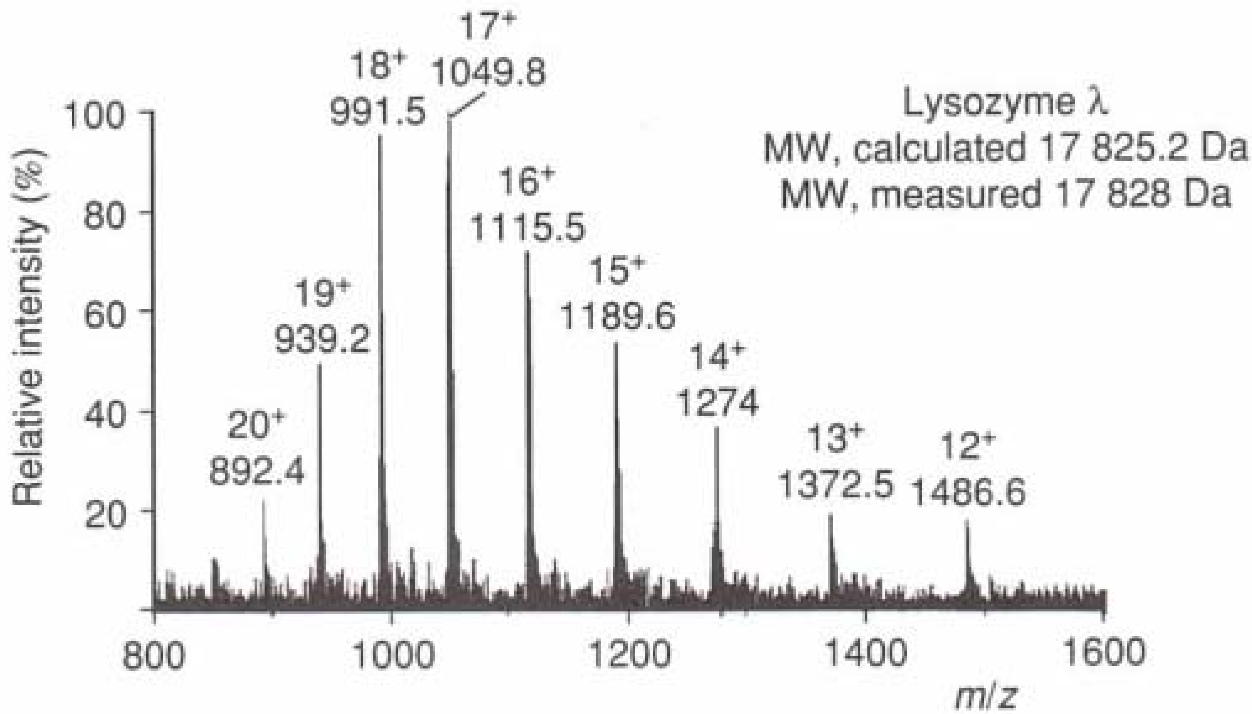


Figure 1.23
ESI spectrum of phage λ lysozyme; *m/z* in Th and the number of charges are indicated on each peak. The molecular mass is measured as being 17 828 ± 2.0 Da.

n_1 = (991.5 – 1.0073)/(1049.8-991.5) = 16.99 ≈ 17
MW = 17(1049.8-1.0073)= 17829 Da

ESI Mass Spectrum – Deconvolution

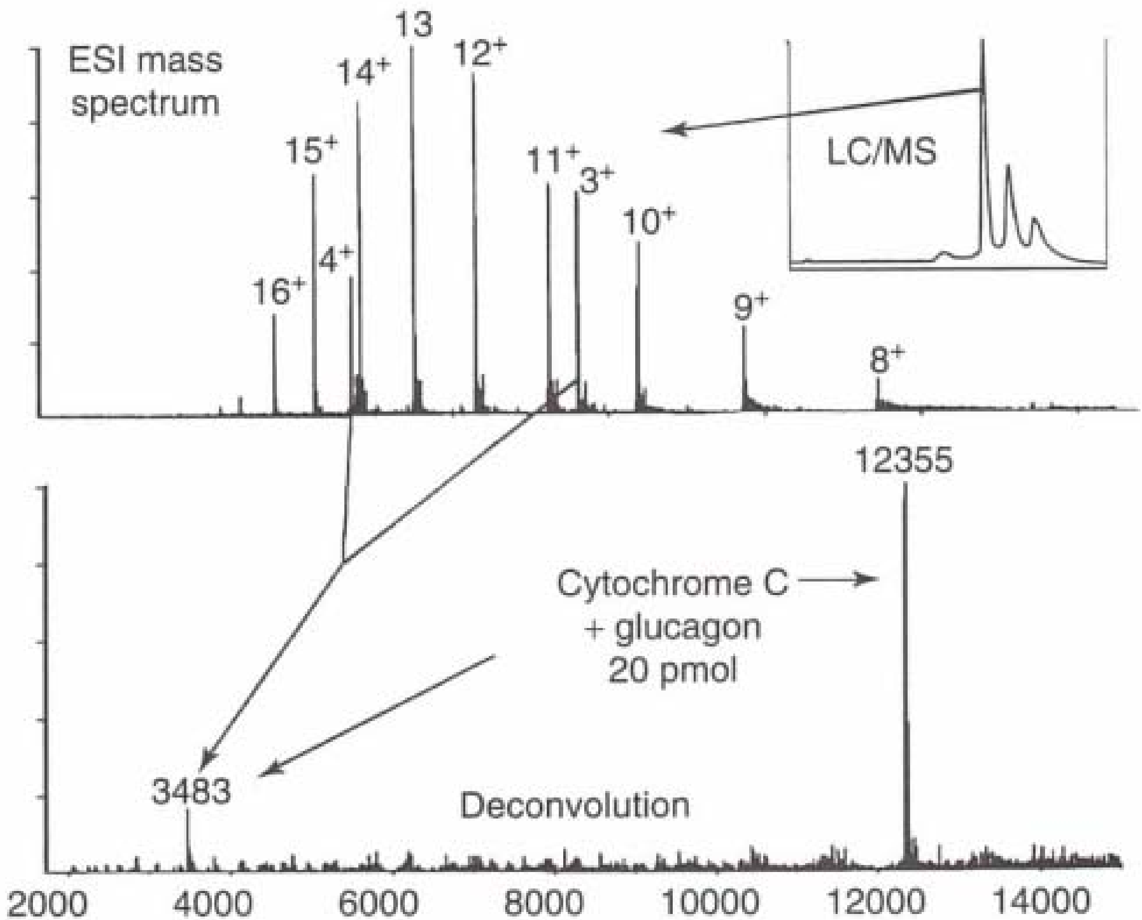


Figure 1.24
Deconvolution of an ESI spectrum of a protein mixture. From Finnigan documentation. Reprinted, with permission.

Atmospheric pressure (AP) MALDI MS

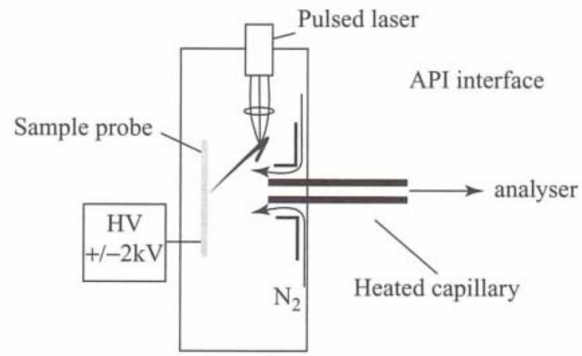
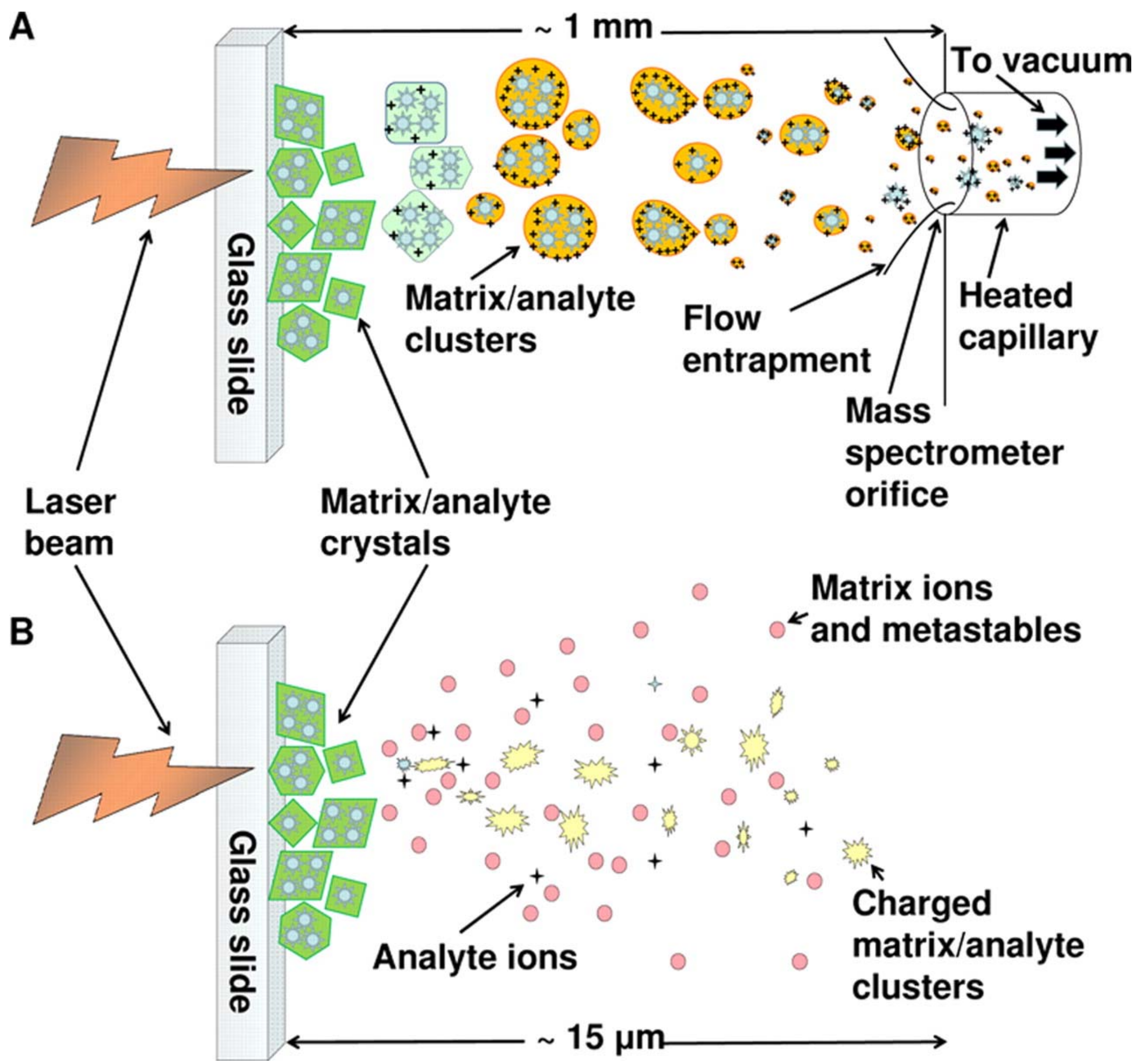


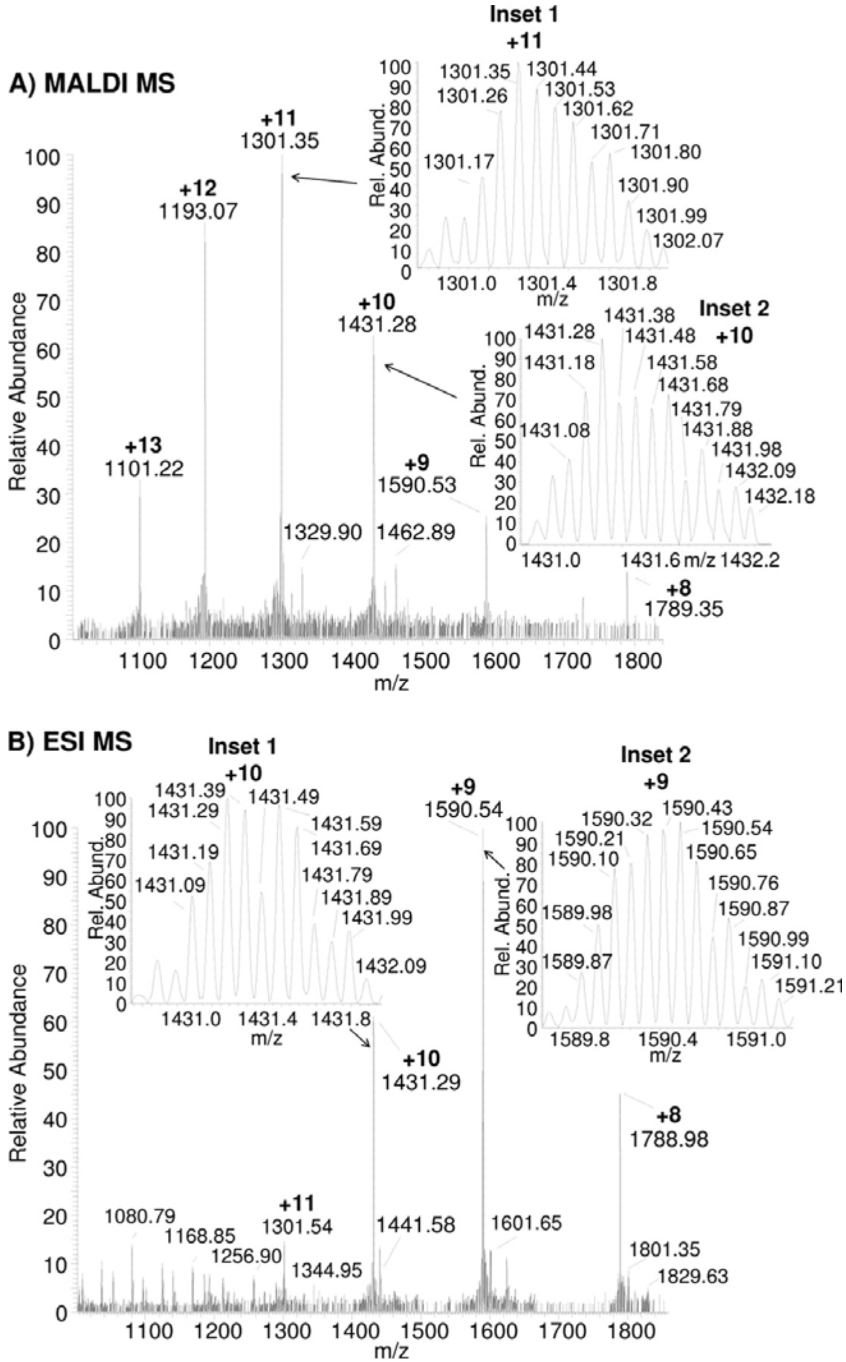
Figure 1.17
Diagram of an AP-MALDI source. Ions are transferred into the mass analyser using the atmospheric pressure interface.

MassTech(www.apmaldi.com)

- High voltage on the plate and dry nitrogen gas around the target plate. → Helps ion transport.
- Ion transfer efficiency is poor compared to **vacuum MALDI**.
- Fast and efficient thermalization of the ion internal E → even softer than vacuum UV or IR MALDI.
- Easily attach to almost any mass spectrometer, interchangeable with other API sources such as ESI.
- Can analyze native biological tissue (NOT vacuum dried) analysis.

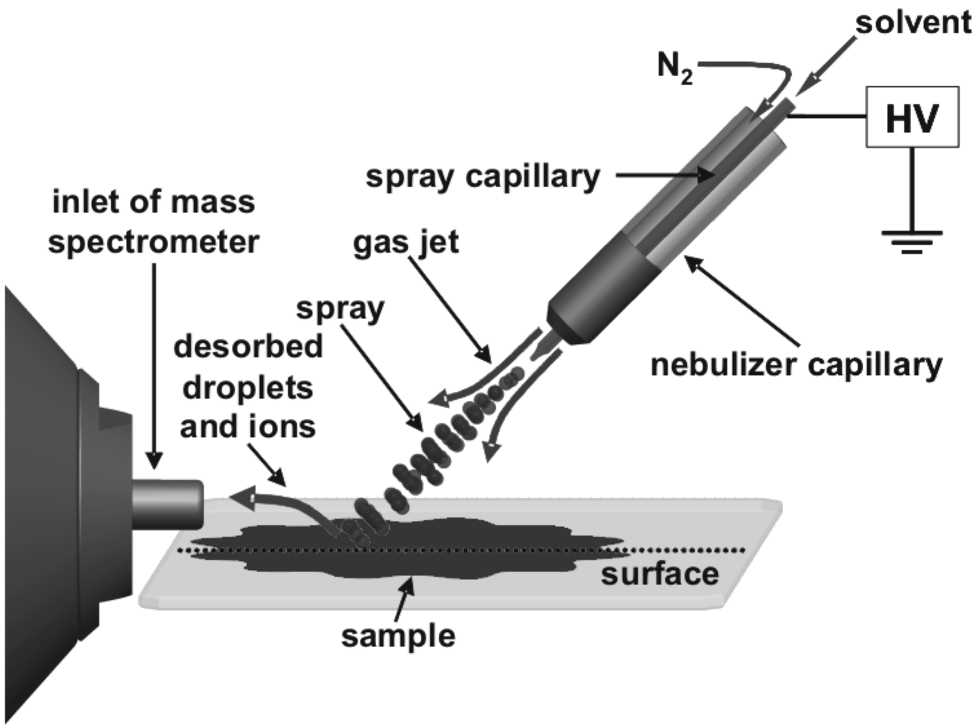
Laserspray Ionization (AP MALDI)





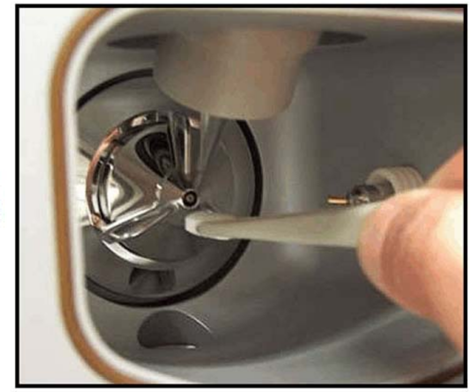
A) MALDI MS
Inset 1
+11
Inset 2
+10
+13
1101.22
+12
1193.07
+11
1301.35
+10
1431.28
+9
1590.53
+8
1789.35
1329.90
1462.89
Relative Abundance
Rel. Abund.
m/z
B) ESI MS
Inset 1
+10
Inset 2
+9
1080.79
1168.85
1256.90
+11
1301.54
1344.95
+10
1431.29
1441.58
+9
1590.54
1601.65
+8
1788.98
1801.35
1829.63

Desorption Electrospray Ionization (DESI)

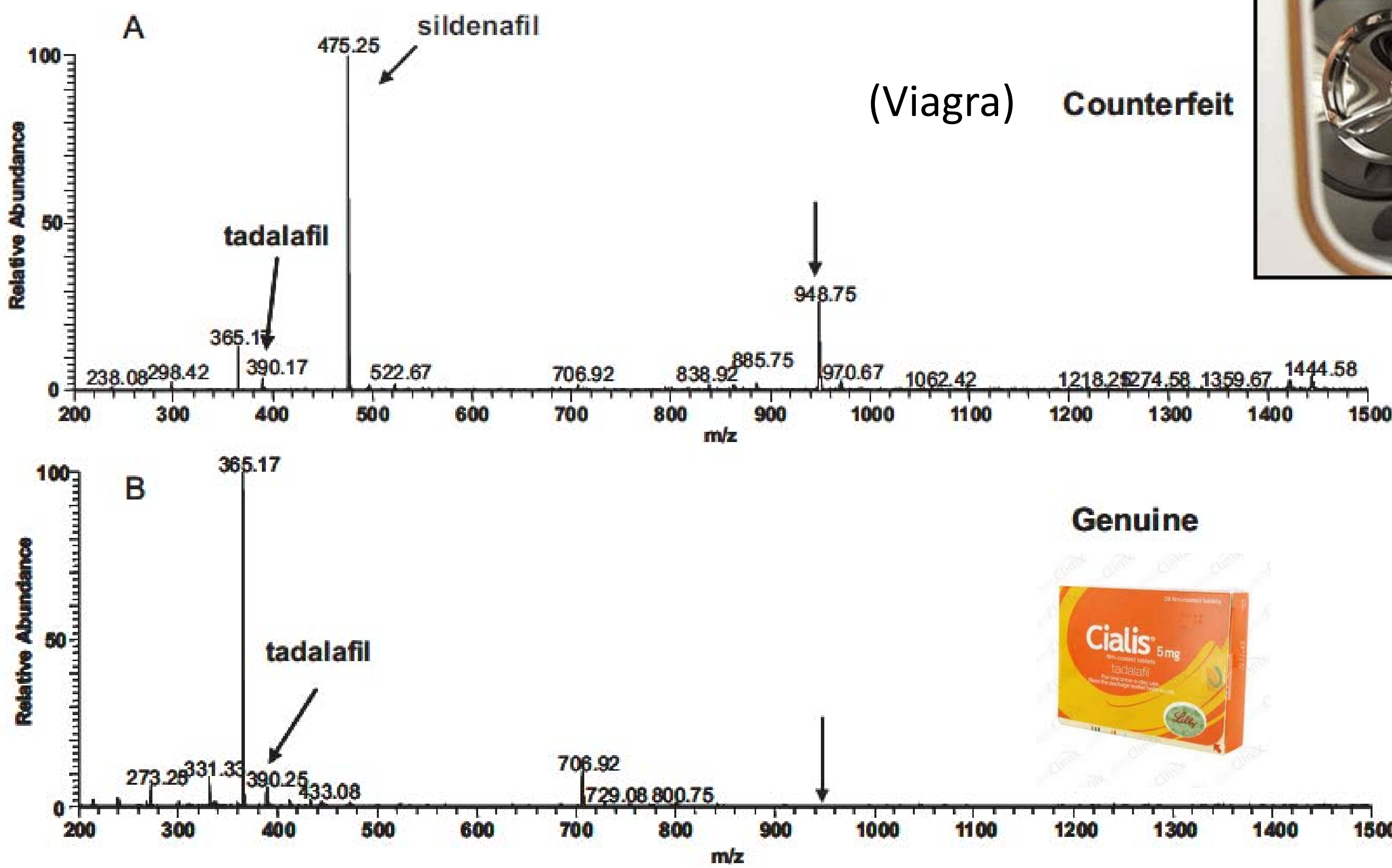


- DESI occurs when analyte particles are solvated by an ionized solvent flow.
- The solvated analyte is ejected from the sample and swept toward the mass analyzer.
- The mechanism and spectra are very similar to ESI.

Desorption Electrospray Ionization (DESI)



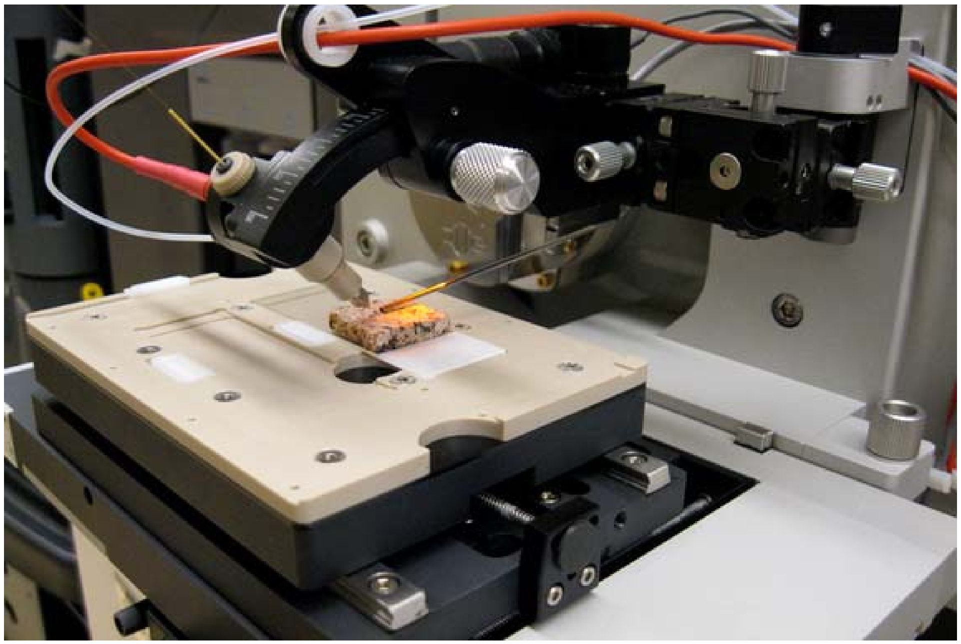
(Viagra) **Counterfeit**



Genuine

Direct tablet analysis for rapid fingerprinting counterfeit and authentic drug tablet formulations.

DESI MS Imaging



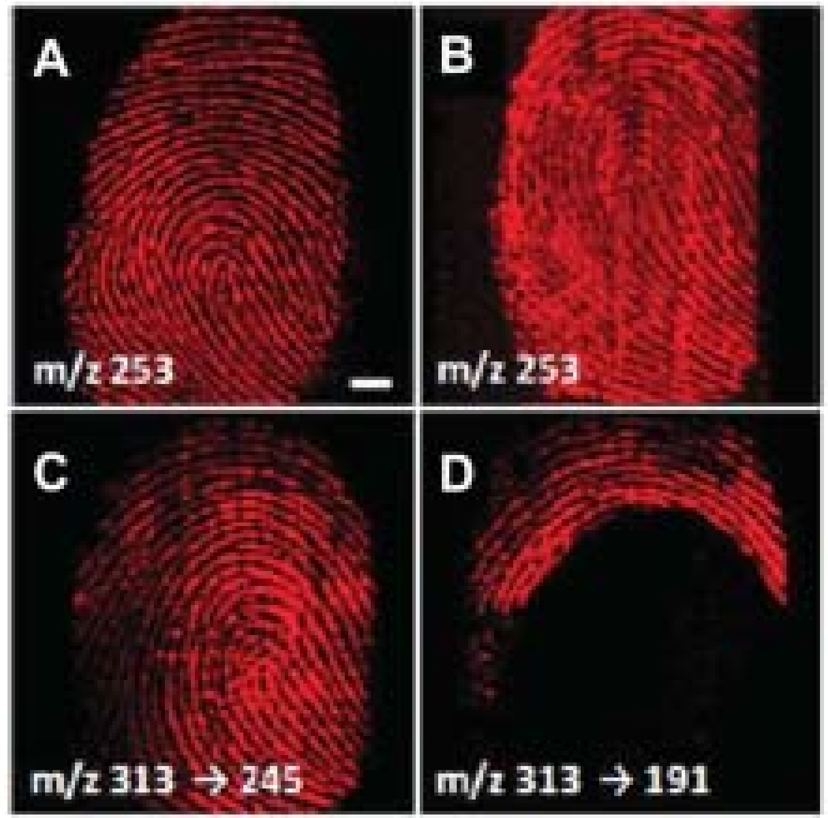


Fig. 10 Virtual DESI image of the fatty acid *cis*-hexadec-6-enoic acid (*m/z* 253) from a LFP blotted on glass (A) and lifted by an adhesive tape (B); Δ9-THC and/or cannabidiol on paper as identified by the MS/MS transition *m/z* 313→245 (C); Δ9-THC distinguished from cannabidiol by the unique MS/MS transition *m/z* 313→191 (D). Adapted from ref. 52.

D. R. Ifa, N. E. Manicke, A. L. Dill and G. Cooks, ***Science,*** 2008, 321, 805

DESI MS Imaging of phospholipids

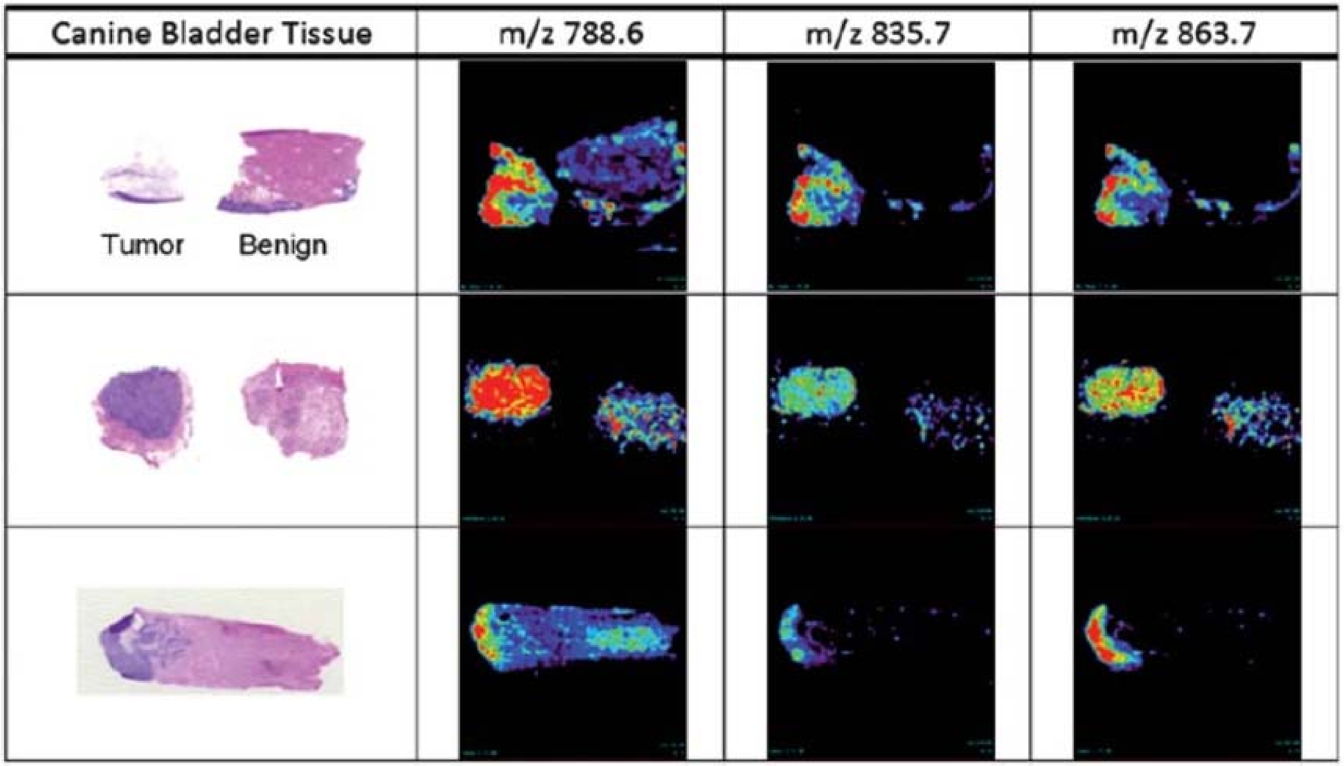
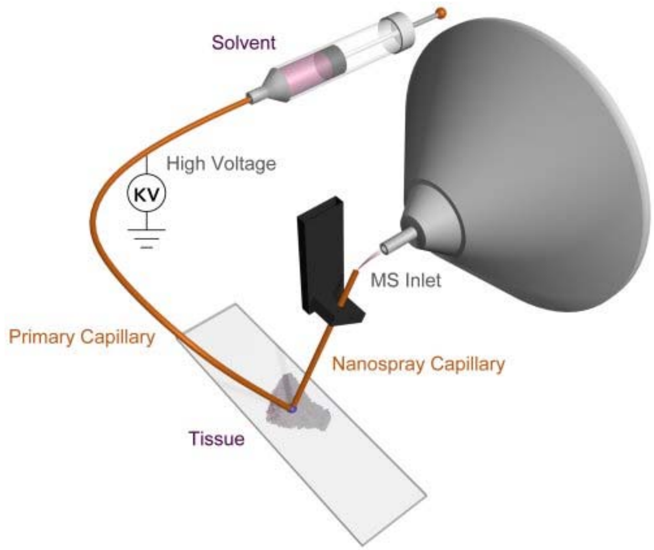
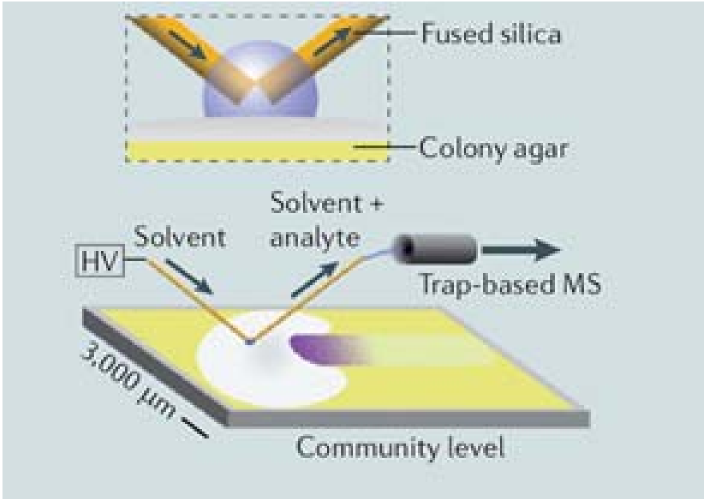


Fig. 9 Negative ion mode tissue imaging of canine bladder tissues including areas of cancer and adjacent normal tissues. H&E-stained tissue sections of the tumor tissue and the tissue adjacent to the tumor were shown on the left panel. Ion images of PS(18:0:18:1) at *m/z* 788.6, PI(16:0/18:1) at *m/z* 835.7, and PI(18:0/18:1) at *m/z* 863.7, indicate that these lipids are more enriched in the tumor as compared to the normal tissues. Adapted from ref. 55.

Dill, D. R. Ifa, N. E. Manicke, A. B. Costa, J. A. Ramos-Vara,D. W. Knapp and R. G. Cooks, ***Anal. Chem.***, 2009, 81, 8758–8764.

Alternative Ionization Source for Direct Tissue Analysis





Nanospray Desorption Electrospray Ionization (nanoDESI) by Laskin, 2010

- Steady-state liquid junction
- Self-aspirating nanospray
- No nebulizing gas

NanoDESI Source for Direct Tissue Analysis