Ionization Methods

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OSU
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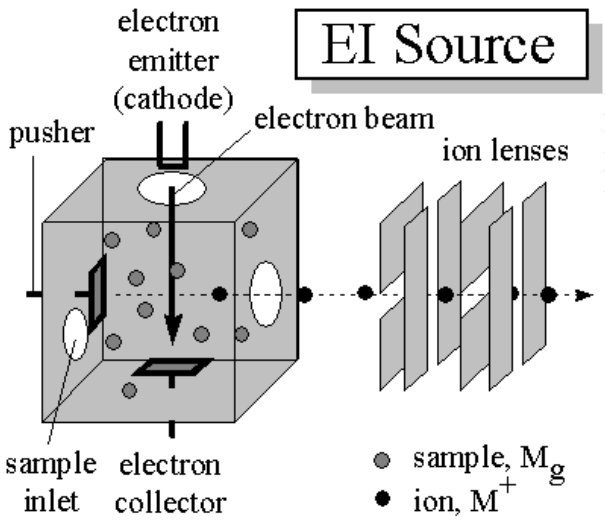
Ionization Methods

Neutral species → Charged species

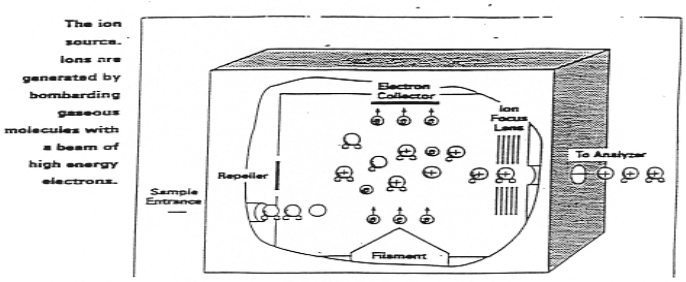
- Removal/addition of electron(s)
  - $M + e^- \rightarrow (M^{+\cdot})^* + 2e^-$
    - electron ionization
- Removal/addition of proton(s)
  - $M + (Matrix)\text{-}H \rightarrow MH^+ + (Matrix)^-$
    - chemical ionization (CI)
    - atmospheric pressure CI (APCI)
    - fast atom bombardment (FAB)
    - Electrospray/nanospray ionization (nESI)
    - matrix assisted laser desorption/ionization (MALDI)
    - desorption electrospray ionization (DESI)
    - direct analysis in real time (DART)
    - native spray

Electron Impact (EI) Ionization



Still widely used in

Forensic
Environmental
Drug Metabolism, etc.



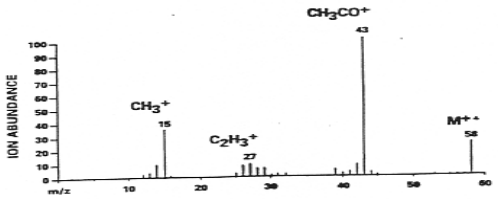
$$M + e^- \rightarrow (M^{+\cdot})^* + 2e^-$$

Unimolecular fragmentation

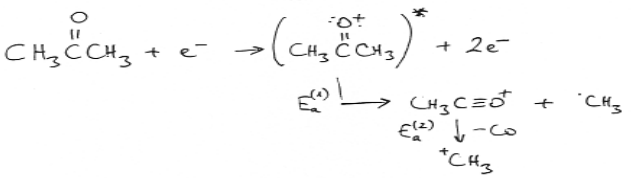
$$(M^{+\cdot})^* \rightarrow \begin{cases} F_{11} \rightarrow F_{12} & \cdots \\ F_{21} \rightarrow F_{22} & \cdots \\ F_{31} \rightarrow F_{32} & \cdots \end{cases}$$

Mass spectrum: the result of consecutive and competitive processes

The Mass Spectrum



The mass spectrum of acetone, CH_3COCH_3, contains many fragment ions as well as the molecular ion at m/z 58.



- molecular ion ($M^{+\cdot}$)
- fragment ions (F^+)
- metastable ions (discussed later)
- base peak (m/z 43)

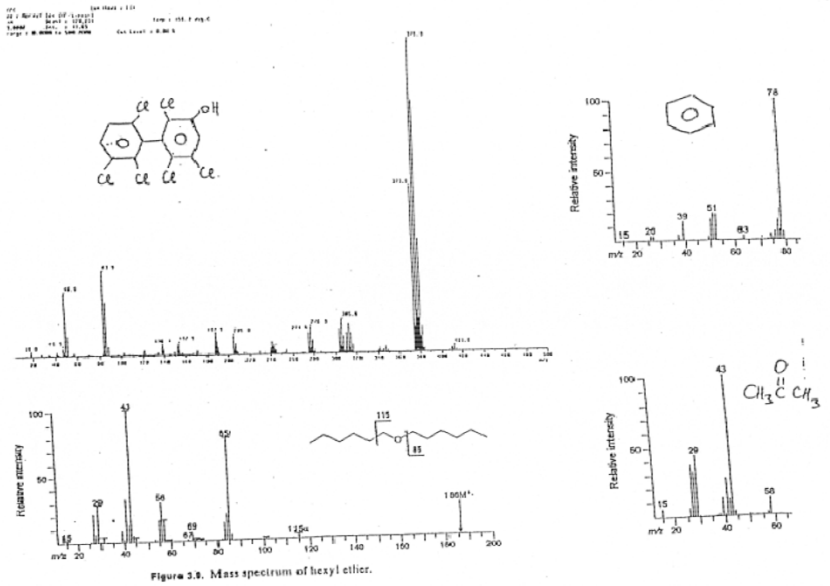
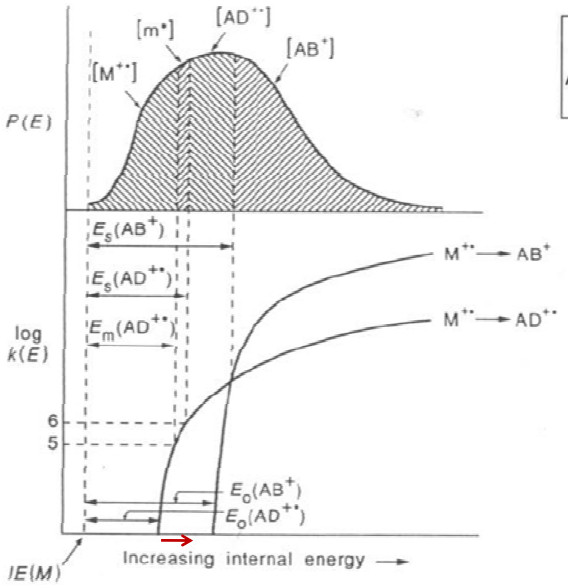
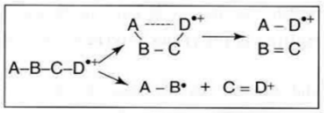


Figure 3.9. Mass spectrum of hexyl ether.

Internal Energy Distribution P(E) and k(E) Curves





Direct bond cleavage

Rearrangement

RRK (simplified)

$k(E) = (1 - E_0/E)^{s-1}$

7

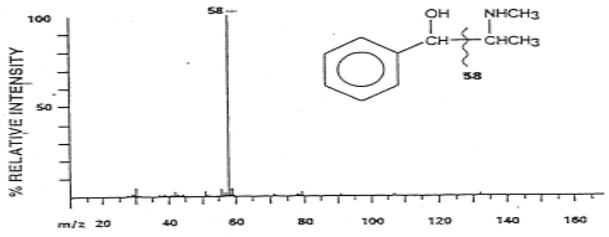
TABLE 1.2
Advantages and Disadvantages of Electron Ionization

Advantages	Disadvantages
Subpicomole to picomole sensitivity.	Limited mass range due to thermal desorption (volatility) requirement.
Availability of vast computer databases, containing over 100,000 compounds.	Possible decomposition by thermal desorption prior to vaporization.
Use of fragmentation pattern as a fingerprint with databases to identify unknowns.	Too much fragmentation, often resulting in no observable molecular ion.
Structural information obtained from fragmentation pattern.	

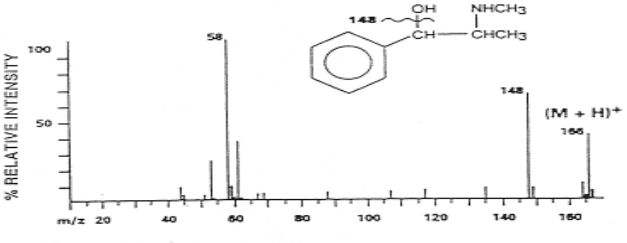
Chemical Ionization

- *Ion-molecule reaction(s) between a reagent gas and the sample at a relatively high pressure*
- Most common reagent gases
  - methane, isobutane, ammonia
- Mechanisms
  - $CH_4 + e^- \rightarrow CH_4^{+\cdot}, CH_3^+, CH_2^{+\cdot}, \ldots$
  - $CH_4^+ + CH_4 \rightarrow CH_5^+ + CH_3$
  - $CH_3^+ + CH_4 \rightarrow C_2H_5^+ + H_2$
  - $CH_5^+ + M \rightarrow [M+H]^+ + CH_4$
  - $C_2H_5^+ + M \rightarrow [M-H]^+ + C_2H_6$
  - $C_2H_5^+ + M \rightarrow [M+C_2H_5]^+$

Figure 11 Electron ionization of ephedrine at 70 eV. No molecular ion is seen (m/z 165).



Methane chemical ionization mass spectrum of ephedrine. The protonated molecule is seen at m/z 166.



Protonation is one type of ionization

$M + AH^+ \rightarrow MH^+ + A$

$CH_3CH_2NH_2 + (NH_3)nNH_4^+ \rightarrow CH_3CH_2NH_3^+ + (n-1)\ NH_3$

The extent of fragmentation depends on the *exothermicity* of the reaction

Proton affinity (PA):

$M + H^+ \rightarrow MH^+ \quad -\Delta H_r = PA$

Proton affinity (PA):

$M + H^+ \rightarrow MH^+ \quad -\Delta Hr = PA$

PAs of common CI reagents (kcal/mol)
methane (131) < water (173) < methanol (185) < $CH_2=C(CH_3)_2$ (197) < ammonia (205)

If the analyte has a much higher PA than that of the unprotonated reagent, the protonation of the analyte will be (very) energetic (fragment rich CI spectra, “semi-CI”)

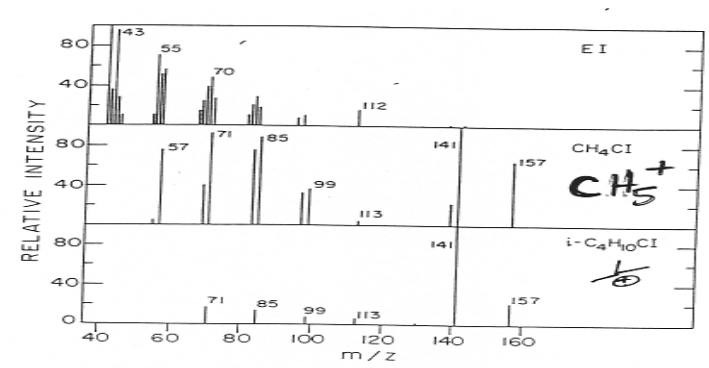


FIGURE 5. Mass spectra of n-decanol. CH4 CI data from Reference 36. i-C4H10 CI data from Reference 33.

PA = 131
CH4

197 = PA

$CH_3CH_2CH_2CH_2CH_2CH_2CH_2CH_2CH_2CH_2CH_2OH$ (H+)

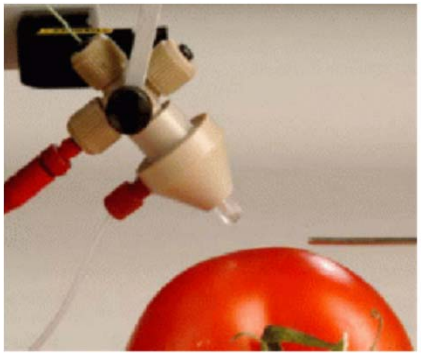
57, 71, 85

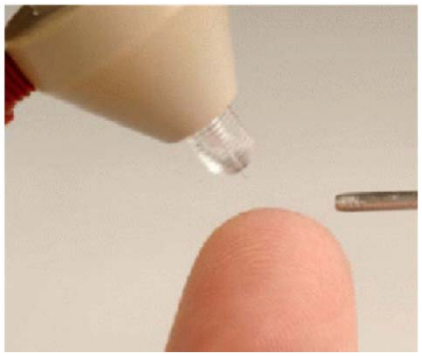
190's = PA

140
17
158
159

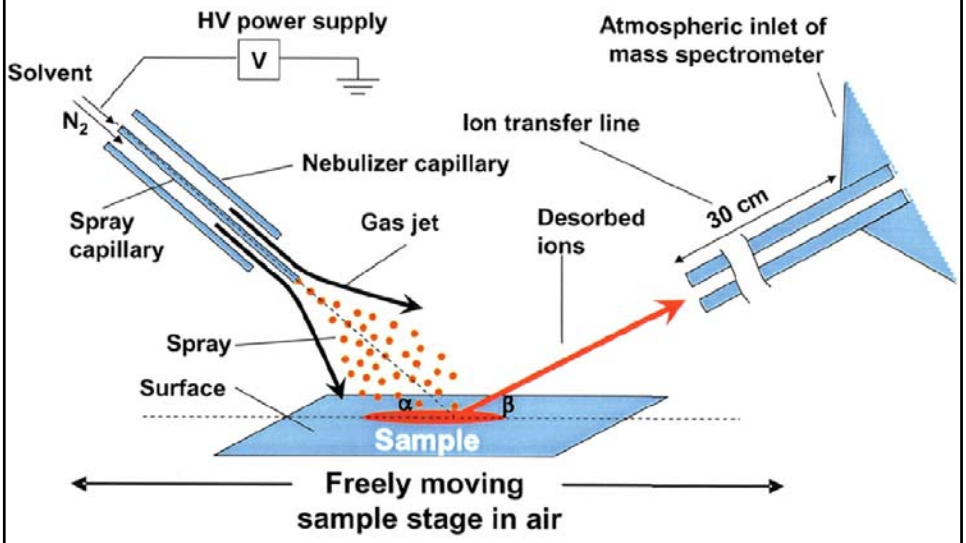
$-H_2O$ 141

Sources still being developed
DESI- desorption ESI
(sample not in solution)





DART



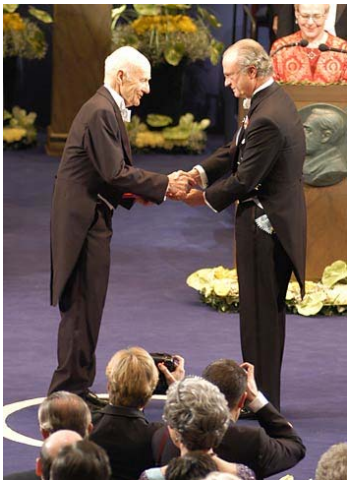
DART
Direct Analysis in Real Time

Penning Ionization M* + S → S+. + M + electron (but also allows MH+, M-H-, etc)

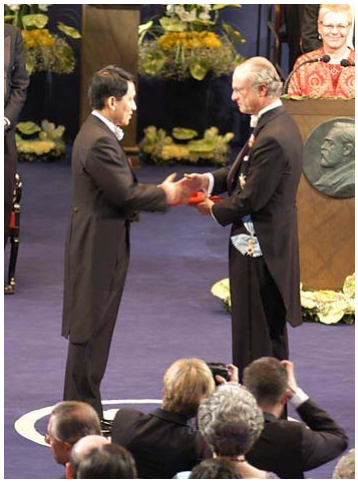
Can we teach elephants to fly?

John Fenn

Yes, of course!!!
Nobel Price in Chemistry, 2002



John B. Fenn



Koichi Tanaka

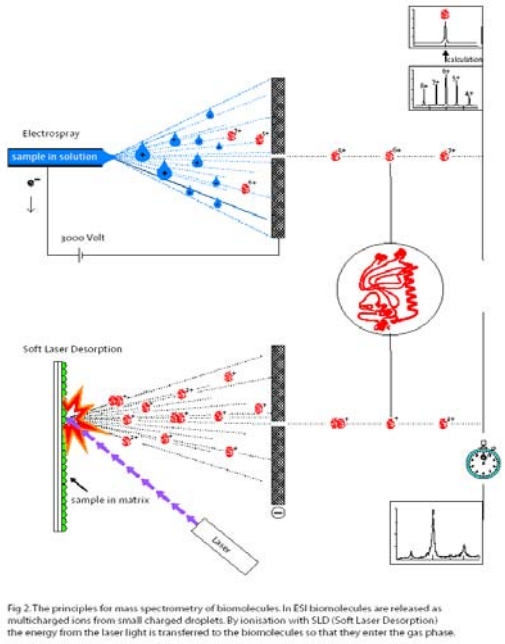


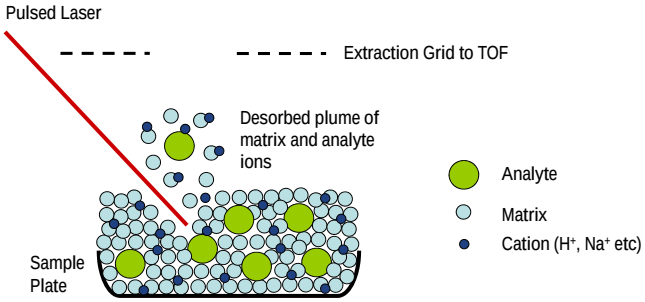
Fig 2. The principles for mass spectrometry of biomolecules. In ESI biomolecules are released as multicharged ions from small charged droplets. By ionisation with SLD (Soft Laser Desorption) the energy from the laser light is transferred to the biomolecules so that they enter the gas phase.

Electrospray Ionization
ESI

Soft Laser Desorption
SLD

Matrix Assited Laser Desorption/Ionization
MALDI

Matrix-Assisted Laser Desorption\ Ionization



MATRIX - A small acidic organic molecule (matrix) is mixed with a low concentration of analyte in a common solvent and allowed to co-crystallize on a sample plate to form a "solid solution" by which the analyte molecules are isolated from each other.

ASSISTED – matrix "assists" the desorption and ionization of the analyte(s)

LASER – Typically a Nitrogen laser (351 nm) or Yag/Nd laser (334 nm)

DESORPTION – Energy from the laser desorbs the matrix into the gas-phase and "carries" the analyte with it.

IONIZATON - Detect [M+H]+ by transferring a proton from the matrix to the analyte

- Choose a matrix based on the molecular weight, solubility and chemical structure of the analyte.
- Excellent for intact molecular weight determination for both polar and non-polar molecules with mass > 500 amu.

MALDI Matrices

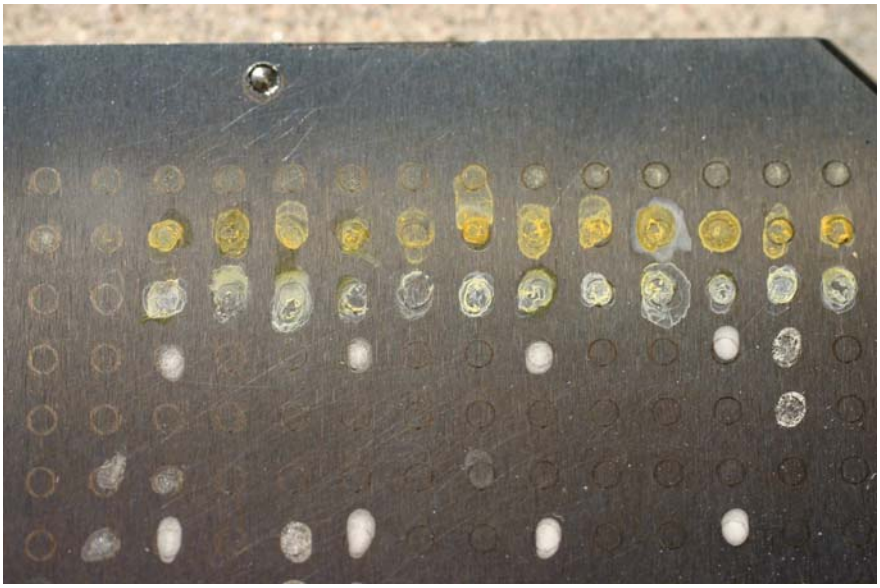
Matrix	Abbrev	Sample Type	
2,5-dihydroxybenzoic acid	DHB	Peptides < 5,000 polymers, dedrimers Good universal matrix "cold matrix"	50% ACN in 0.1% TFA, THF, 2:1 chloroform:MeOH
3,5-dimethoxy-4-hydroxycinnamic acid (Sinapinic acid)	SA	Peptides and Proteins > 10,000 "hot matrix"	50-70% ACN in 0.1% TFA
α-cyano-4-hydroxycinnamic acid	HCCA	Excellent for peptides, digestion products and proteins	50% ACN in 0.1% TFA
Dithranol		Non-polar polymers	THF, Methylene chloride
Indoleacrylic acid	IAA	Non-polar polymers	THF, methylene chloride
3-hydroxypicolinic acid	HPA	DNA and negative ion samples	See me for more specific procedure
Trihydroxyacetophenone	THAP	DNA and negative ion samples	See me for more specific procedure
Nor-harmane		Universal	50% ACN, THF, chloroform

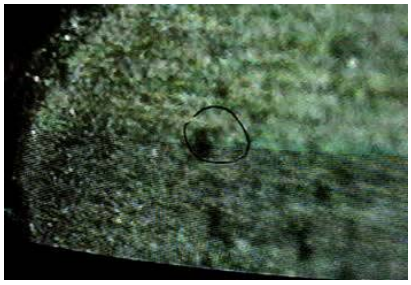
Table 2. PA values (kcal/mol) of MALDI matrices given in the present study and comparison of the same with literature values

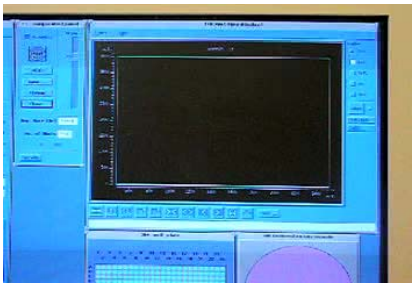
		Present study									
		Apparent free energy at collision energy (lab frame)									
Matrix compound	Reference bases used	2 eV	5 eV	7 eV	10 eV	Proton affinity[a]	Average T_{eff} in K	Jorgensen et al. [31]	Burton et al. [32]	Steenvoorden et al. [34]	Nelson et al. [33]
4HCCA	1-4	201.5	201.0	200.9	200.6	**201.0 ± 0.27 (±0.64)**	817	201.0	183.0	223.0	203.0
GA	2-6	204.7	204.3	204.3	204.3	**204.4 ± 0.17 (±0.36)**	528	-	204.0	204.0	202.9
MSA	3-5,8	205.3	205.2	205.2	205.1	**205.2 ± 0.52 (±1.22)**	399	-	-	-	-
SA	7,9-11	209.3	209.2	209.2	209.2	**209.2 ± 0.38 (±0.89)**	427	212.0	204.0	214.0	210.0
DT	10,12-14	211.5	211.4	211.6	211.6	**211.5 ± 0.77 (±1.81)**	507	-	209.0	-	-
AMT	10-12,14	213.1	213.0	212.9	213.0	**213.0 ± 0.31 (±0.73)**	515	-	-	-	-
THAP	10-12,14	213.5	213.4	213.2	213.0	**213.3 ± 0.65 (±1.53)**	576	-	-	-	210.8
IAA	10-12,14	214.0	213.6	213.3	212.9	**213.5 ± 0.26 (±0.61)**	772	-	215.0	-	-
HPA	9-11,14	214.9	214.7	214.5	214.2	**214.6 ± 0.33 (±0.78)**	714	214.0	-	-	214.5
MBT	10-12,14	214.9	214.9	214.9	214.9	**214.9 ± 0.23 (±0.54)**	655	-	-	-	-
AAMT	10-12,14	215.5	215.7	215.8	215.8	**215.7 ± 0.14 (±0.33)**	619	-	-	-	-
EMT	15-19	218.1	218.0	217.9	217.9	**218.0 ± 0.17 (±0.36)**	432	-	-	-	-
MP	15-19	219.6	219.5	219.5	219.5	**219.5 ± 0.19 (±0.41)**	402	-	-	-	-
HABA	18-21	227.3	226.9	226.7	226.6	**226.9 ± 0.26 (±0.61)**	556	225.0	183.0	-	-
NH	22-25	233.1	233.1	233.0	232.8	**233.0 ± 0.44 (±1.03)**	395	-	-	-	-

[a] ±Average of standard deviation at different collision energies. Values in parentheses are uncertainties at 90% confidence limits [49].

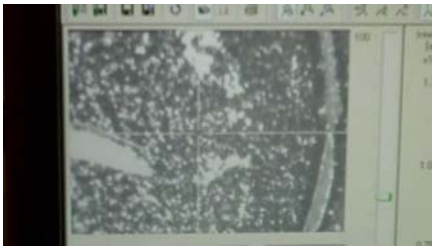
The MALDI Plate with Different Samples in Different Matrices

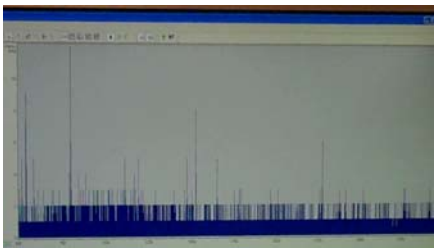




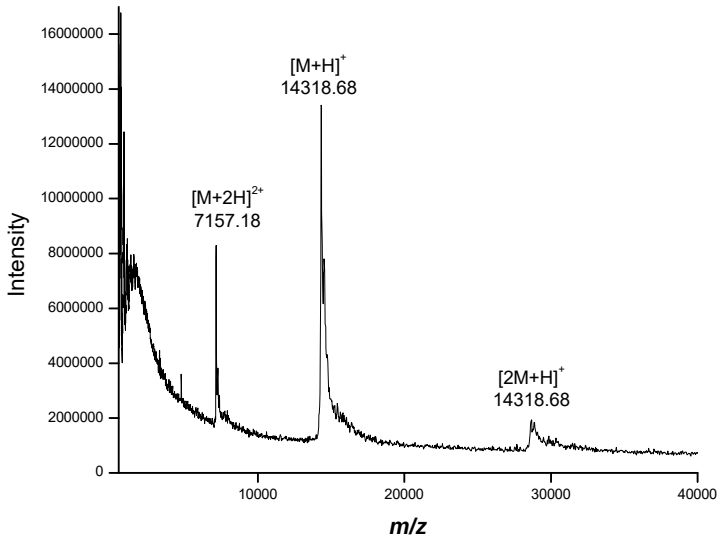


2 GHz time digital converter
1000 Hz laser (acquisition of
1000 spectra in 1 second



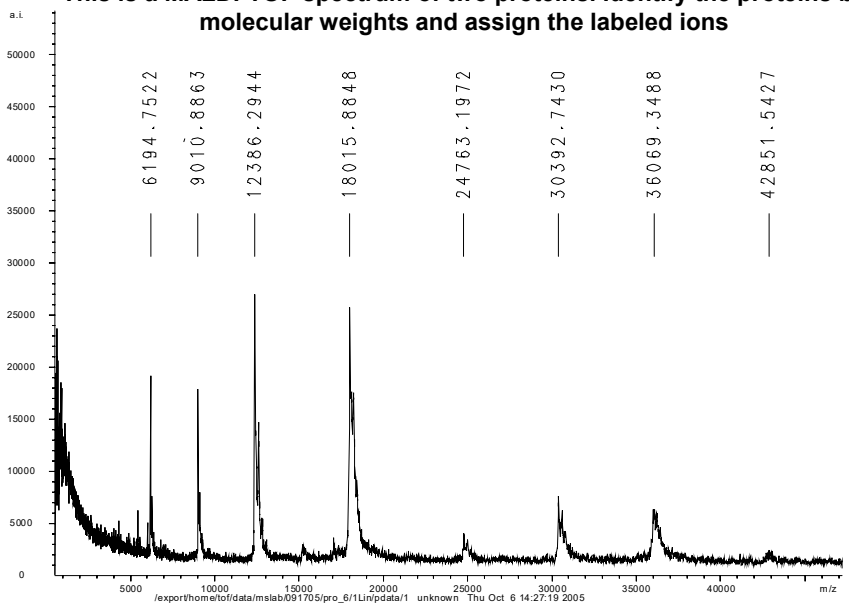


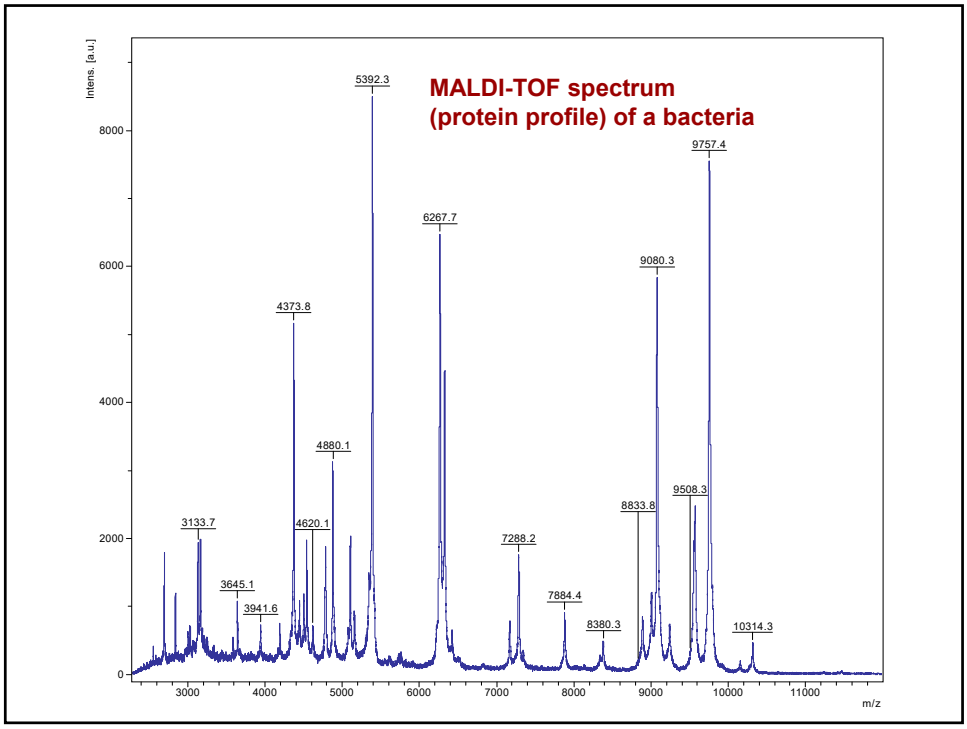
MALDI-TOF spectrum of a pure protein (linear mode)



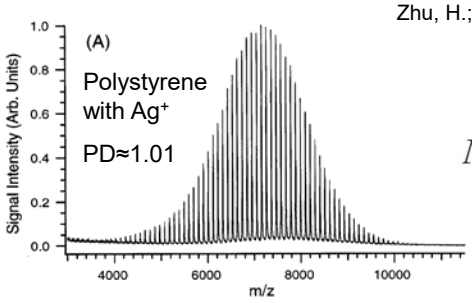
Exercise 2

This is a MALDI-TOF spectrum of two proteins. Identify the proteins by molecular weights and assign the labeled ions





Molecular weight distribution defines polydispersity of polymers

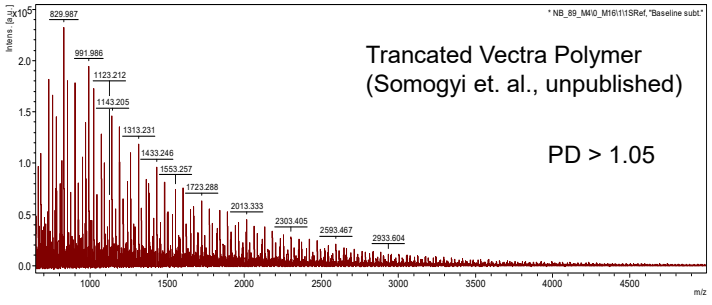


Zhu, H.; Yalcin, T.; Li, L. *JASMS*, **1998**, *9*, 275-281.

$$M_n = \sum (N_i M_i) / \sum N_i$$

$$M_w = \sum (N_i M_i^2) / \sum N_i M_i$$

Polydispersity (PD) = M_w/M_n



Trancated Vectra Polymer
(Somogyi et. al., unpublished)

PD > 1.05

MS is an absolute method for MW determination
(but remember possible degradation!)

Table 1. Molecular weight data for polystyrene standards

Polymer standard	Molecular weight and polydispersity: By classical methods[a]	By MALD[b]
Polystyrene 5050	M_n = 4755 (GPC) M_w = 4992 (GPC) M_n = 4720 (VPO) M_v = 4950 (IV) PD = 1.05 (GPC)	M_n = 5189 (0.5% RSD) M_w = 5329 (0.5% RSD) PD = 1.027 ± 0.001
Polystyrene 7000	M_n = 6770 (GPC) M_w = 6962 (GPC) M_w = 7170 (LLS) M_v = 6943 (IV) PD = 1.03 (GPC)	M_n = 6998 (0.4% RSD) M_w = 7132 (0.4% RSD) PD = 1.019 ± 0.001
Polystyrene 11,600	M_n = 11,356 (GPC) M_w = 11,687 (GPC) M_w = 11,000 (LLS) M_v = 10,720 (IV) PD = 1.03 (GPC)	M_n = 11,074 (0.3% RSD) M_w = 11,187 (0.3% RSD) PD = 1.010 ± 0.001

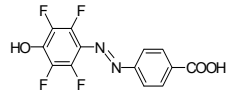
[a]These results are provided by the suppliers; GPC, gel permeation chromatography; VPO, vapor pressure osmometry; IV, intrinsic viscosity; LLS, laser light scattering.
[b]From five trials.

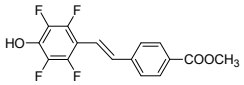
Zhu, H.; Yalcin, T.; Li, L. *JASMS*, **1998**, *9*, 275-281.

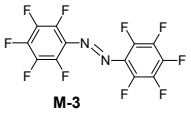
Problems with polymer analysis

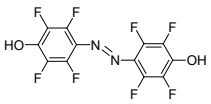
- Sample preparation
  - Several polymers are not well soluble in conventional solvents
  - Solventless technique
  - Cationizing with Na^+, K^+, and Ag^+ by spiking
  - Synthesis of tailor (home) made matrices
- Degradation during ionization (especially with MALDI)
  - Photodissociation in MALDI (MALDI/ESI comparison desirable)
- End group analysis reliable but better to have high resolution/accurate mass (FT-ICR)

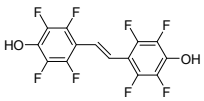
Some Examples for Tailor-Made Matrices

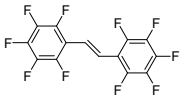

M-1


M-2


M-3


M-4


M-5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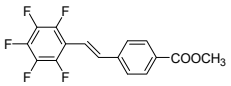
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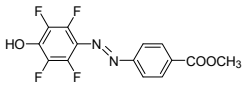
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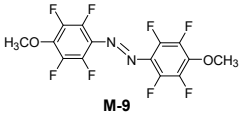
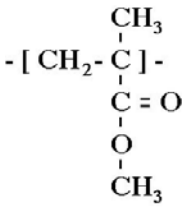

M-9

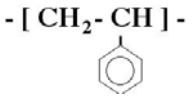
Somogyi, et al., *Macromolecules*, **2007**, *40*, 5311-5321.

Repeating unit masses of polymers

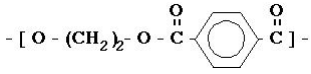
http://www.polymerprocessing.com/polymers/alpha.html



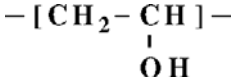
Poly(methyl methacrylate) (PET)
$C_5H_8O_2$ (100.052430)



Polystyrene (PS)
C_8H_8(192.042259)



Poly(ethylene terephtalate) (PET)
$C_{10}H_8O_4$ (192.042259)

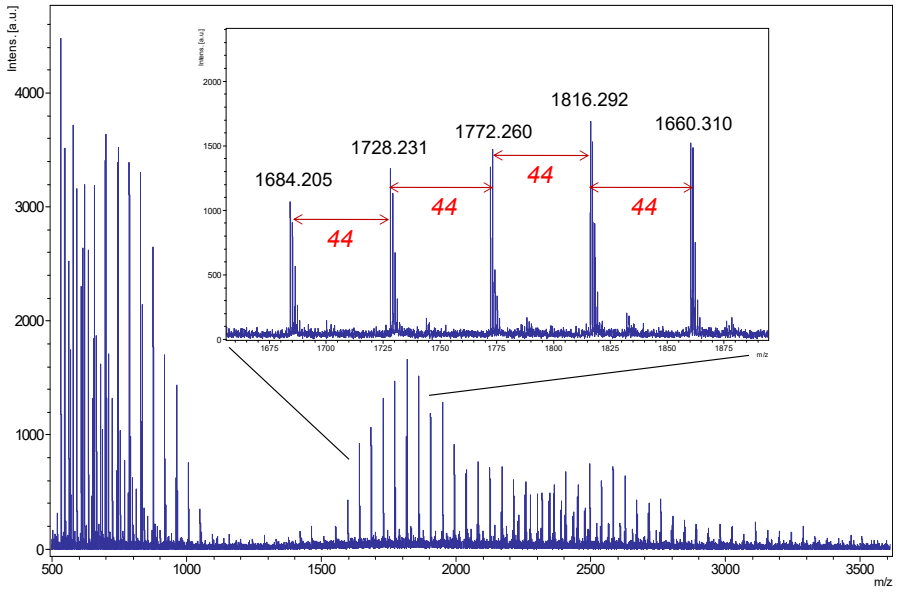


Poly(vinyl alcohol) (PVA)

C_2H_4O (44.026215)

- CH2-CH2-O-
Poly(ethylene glycol) (PEG)

Synthetic Polymer Analysis by MS (MALDI-TOF)



Polymer analysis

Clues to polymer identity (Repeated patterns)

- 1655.7-1639.7 = 16 Possibly Na (23) and K (39) adducts
- 1639.7-1595.7 = 44 Polyethylene glycol (CH_2CH_2O)

Possible formula for 1639.7 peak:

- C 73 O 37 H 148 Na 1
- Hydrogen increases mass by 1
- (Nitrogen rule not broken)
- 1640 = measured
- - 23 = subtract Na
- 1617 = polymer mass
- -1585 = 36 PEG units
- 32 = END GROUP

Must be methoxy-PEG

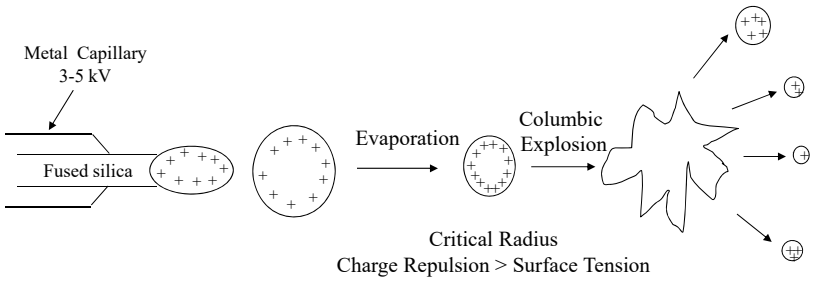
CH_3-(OCH_2CH_2)-OH
15 ----44--- 17

CH_3OH exact mass: 32.0262 u

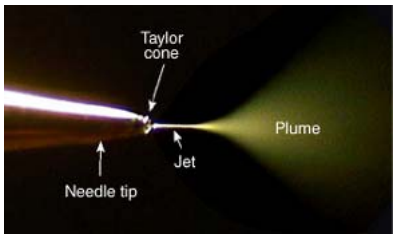
# of Atoms		Exact Mass	Exact Total
C	73	12.000000	876.0000
O	37	15.994914	591.8118
H	148	1.007825	149.1581
Na	1	22.989769	22.9898
			1639.9597

# of Atoms		Polymer unit x 36 Mass	Polymer unit x 36 Total
C	72	12.000000	864.0000
O	36	15.994914	575.8169
H	144	1.007825	145.1268
Na	0	22.989769	0.0000
			1584.9437

Electrospray Ionization (ESI)



- An electric field on the capillary produces a spray of fine charged droplets
- The presence of a drying gas (Nitrogen) and heat evaporates off the solvent leaving a distribution of multiply charged de-solvated ions.
- Soft ionization
- In-line compatible with HPLC



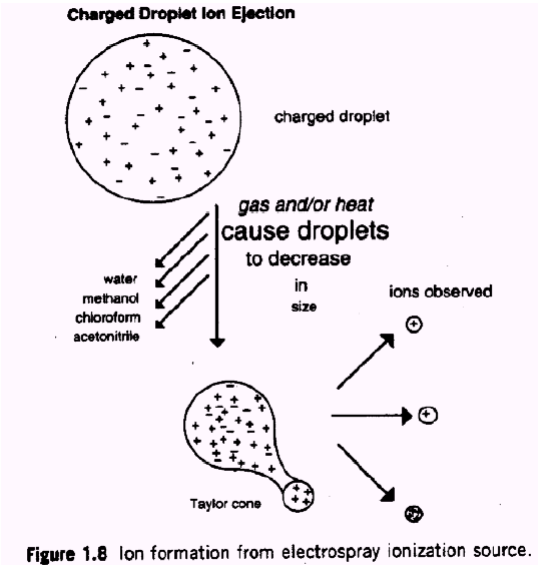


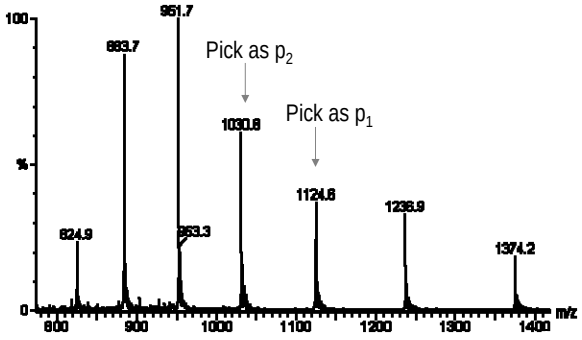
Figure 1.8 Ion formation from electrospray ionization source.

For any peak j charges away $Z_1 = \frac{j(p_2 - M_a)}{p_1 - p_2}$

$Z_1 = \frac{1(1030.8 - 1)}{(1124.6 - 1030.8)}$

$Z_1 = 10.978$ round up to 11

Therefore, p_1 has 11 charges (protons)

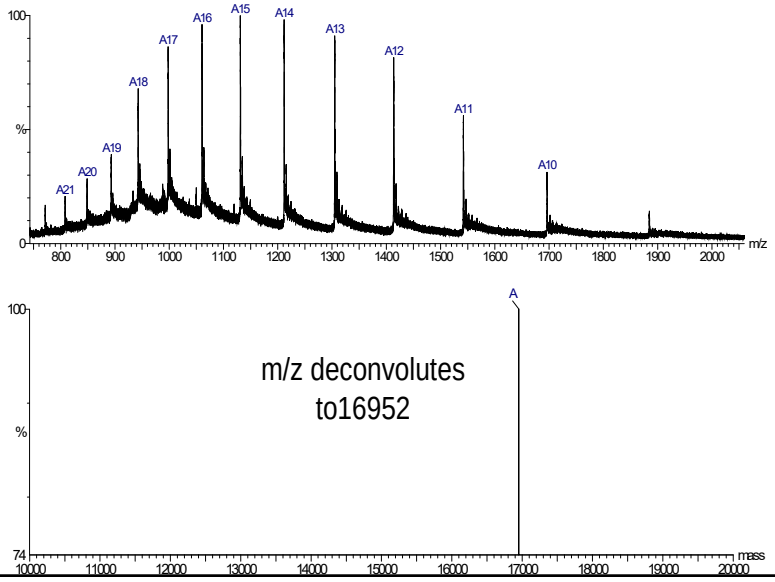


Calculate intact molecular weight $M_r = p_1 z_1 - M_a z_1$

$M_r = (1124.6 * 11) - (1 * 11)$

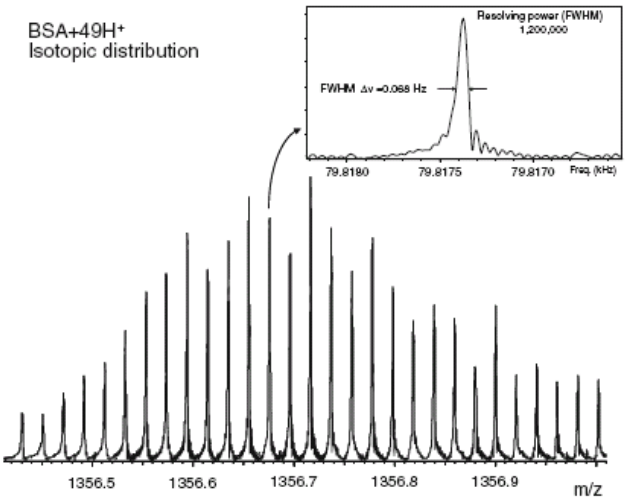
$M_r = 12359.6$ (Actual for Cytochrome C is 12360.1

ESI-MS of Myoglobin



Isotope distribution/charge state by ultrahigh resolution MS

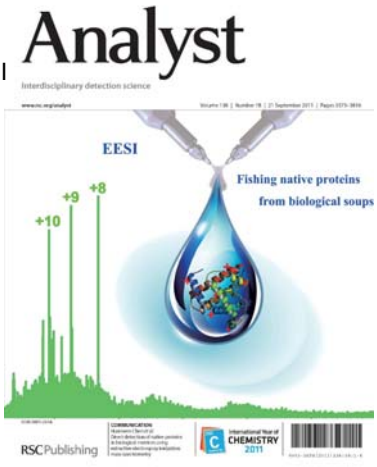
E. Nikolaev et al., Initial Characterization of a New Ultrahigh Resolution FTICR Cell with Dynamic Harmonization, *JASMS*, **2011**, *22*, 1125-1133.



E. Nikolaev and coworkers, Twelve Million Resolving Power on 4.7T FT-ICR Instrument with Dynamically Harmonized Cell – Observation of Fine Structure In Peptide Mass Spectra, *JASMS*, **2014**, *25*, 790-799.

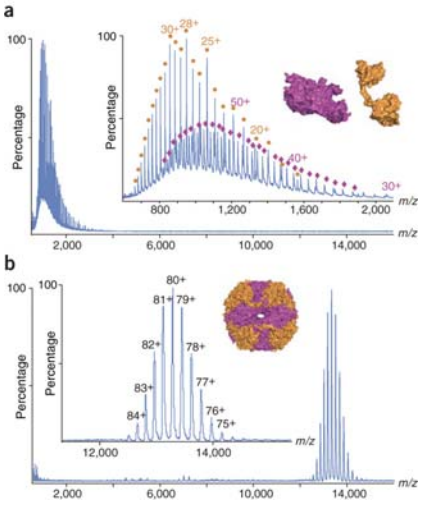
Native (nano)spray

- mimic biological environments
- prevent unfolding
- keep protein complexes intact
- narrower charge distributions than with ESI



(**a**) Denatured urease was electrosprayed from an aqueous 50% (vol/vol) acetonitrile containing 0.1 % (vol/vol) formic acid solution revealing individual charge distributions from the multiply charged (26.6 kDa, orange) and (61.7 kDa, magenta) monomers of urease. (**b**) A mass spectrum of native urease electrosprayed from an aqueous ammonium acetate solution (bottom) displaying multiple ion signals that originate from multiple charged species of the $\alpha_{12}\beta_{12}$ intact urease machinery with a measured mass of 1,063.4 1.0 kDa. Insets are close-ups of the indicated regions. The cartoons are adapted from the X-ray structure of the intact $\alpha_{12}\beta_{12}$ urease.

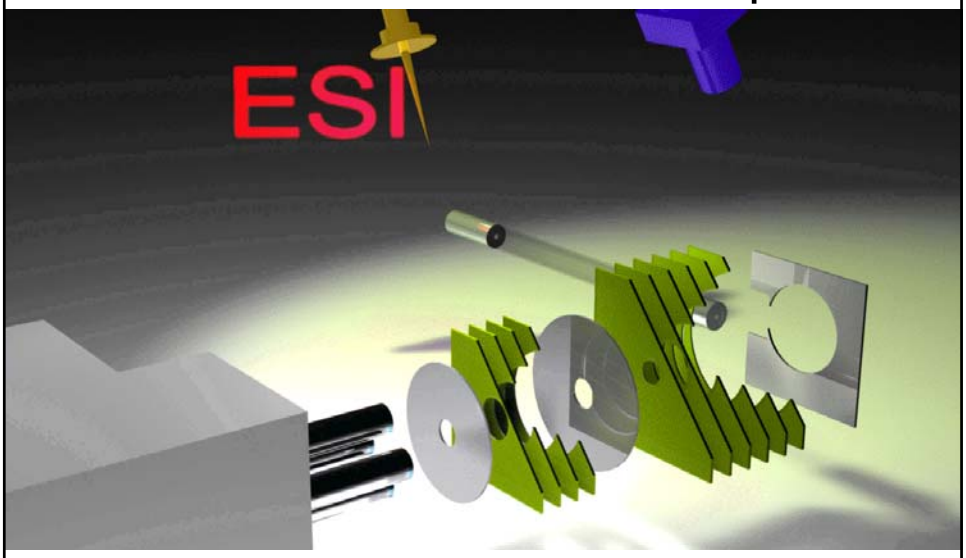
Pinkse, M.W., Maier, C.S., Kim, J.I., Oh, B.H. & Heck, A.J. Macromolecular assembly of *Helicobacter pylori* urease investigated by mass spectrometry. J. Mass Spectrom. 38, 315–320 (2003). | Article | PubMed | ChemPort |



ESI vs MALDI

- Choose MALDI when:
  - Peptides or protein with a MW < 5000 Da (MALDI can detect MW > 100KDa, but not very accurately)
  - Complex mixtures (more than 5 compounds)
  - Very little material with higher salt/buffer concentration
- Choose ESI when:
  - MW > 5000 Da (proteins)
  - Want better mass assignment
  - Want good MS/MS data

Dual ionization mode on the apex-Qe



Apollo™ II with MALDI Option