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User school lecture

Atmospheric pressure ionization techniques

for high resolution mass spectrometry of complex samples

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FT-ICR mass spectrometry

- High accuracy in mass determination
- Sum formula and isotopic fine structure accessible
- Universal, as every molecule has a mass, but… mass spectrometry detects **ions** (mass per charge)

Ionization requests for complex mixtures:

- Universal

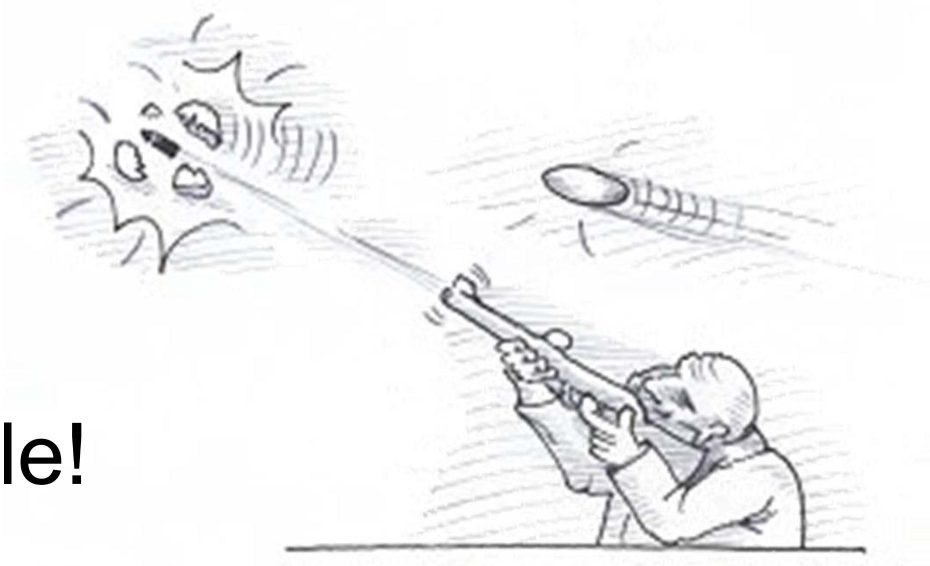
  …mass spectrometric signals reflects composition of neutrals

- Preserve molecular ion information

  …No fragmentation or adduct formation (can be done later…)

- Low “matrix” effects and linear response

Excurse EI: Universal electron impact ionization



Universal and simple!

In high vacuum, no collision and therefore no chemical reaction,

simply: $M + e^{-} \rightarrow M^{\bullet +} + 2e^{-}$

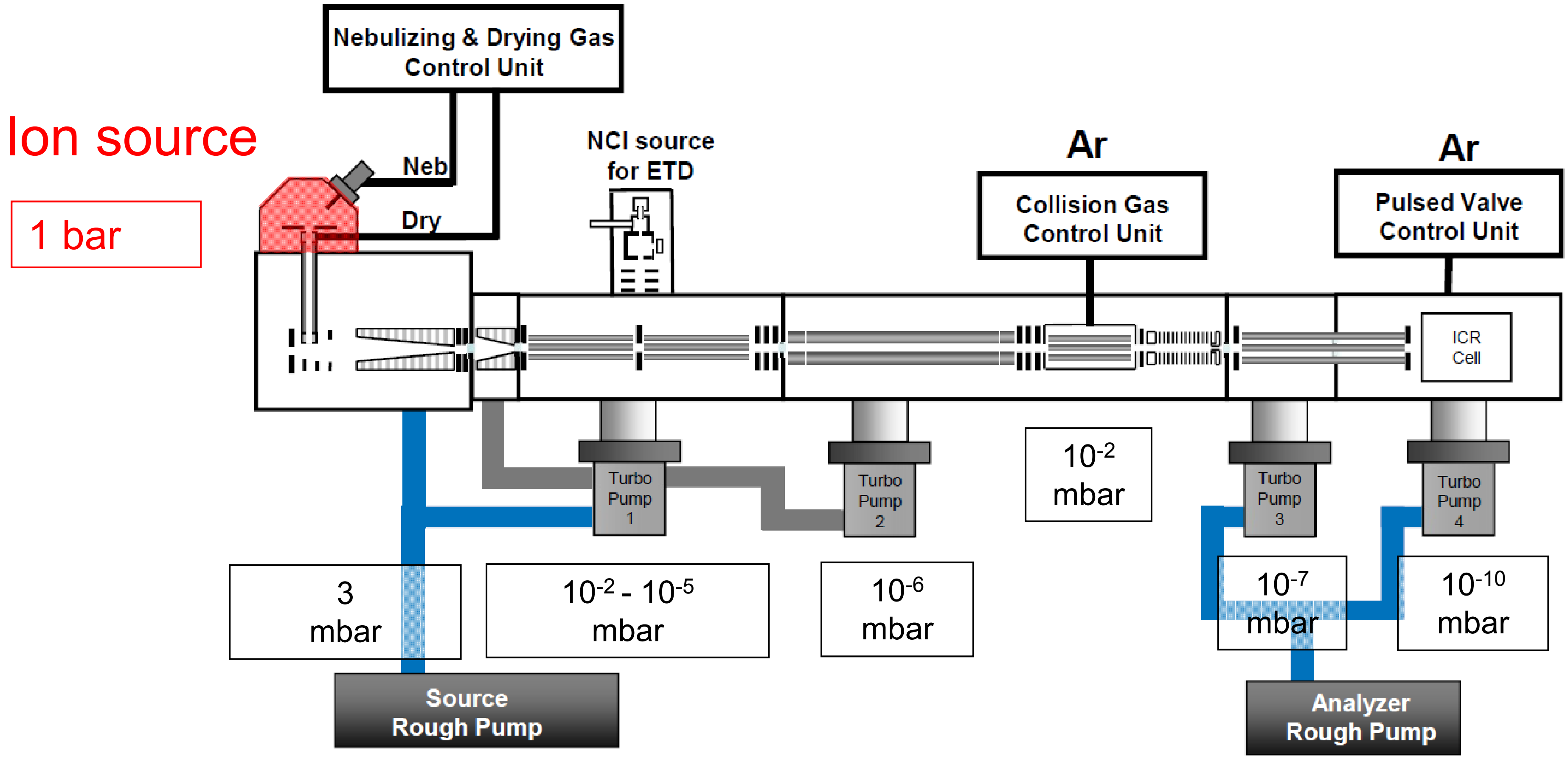
70 eV electron energy

Ionization potential for most organic compounds: 7-12 eV

→ Needs transfer **of neutrals** into **high vacuum**

→ Excess energy often leads to fragmentation (m/z values <200) often gain of structural but loss of molecular ion mass information

- Ions were guided into the ICR-cell by gas flow (ion capillary), and static/alternating fields (lenses, funnels, multipols)
- Neutrals were pumped off (1000 mbar → 10^{-10} mbar)



Atmospheric pressure ionization in general

Common to all atmospheric pressure ionization techniques

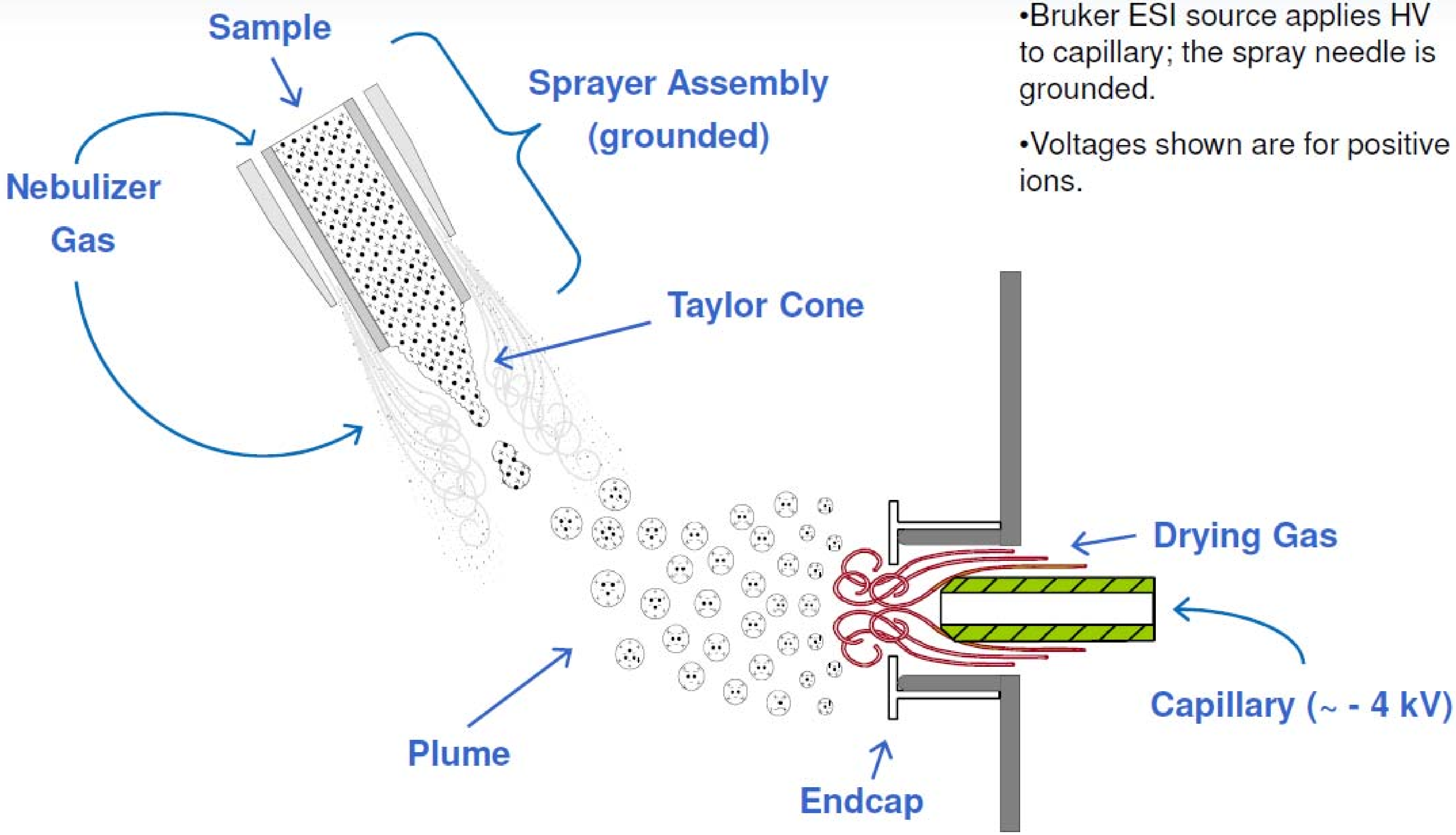
→ High sensitivity obtainable, as
high pressure leads to high density of analyte ions

→ Mean free path (N_2 @ 1013mbar, 273K): ~75nm
Time between collision: ~200ps
Mean residence time in the ion source ~10ms!

→ More than 10 millions of collisions
Reactions can/will occur
Collisional cooling leads to less fragmentation

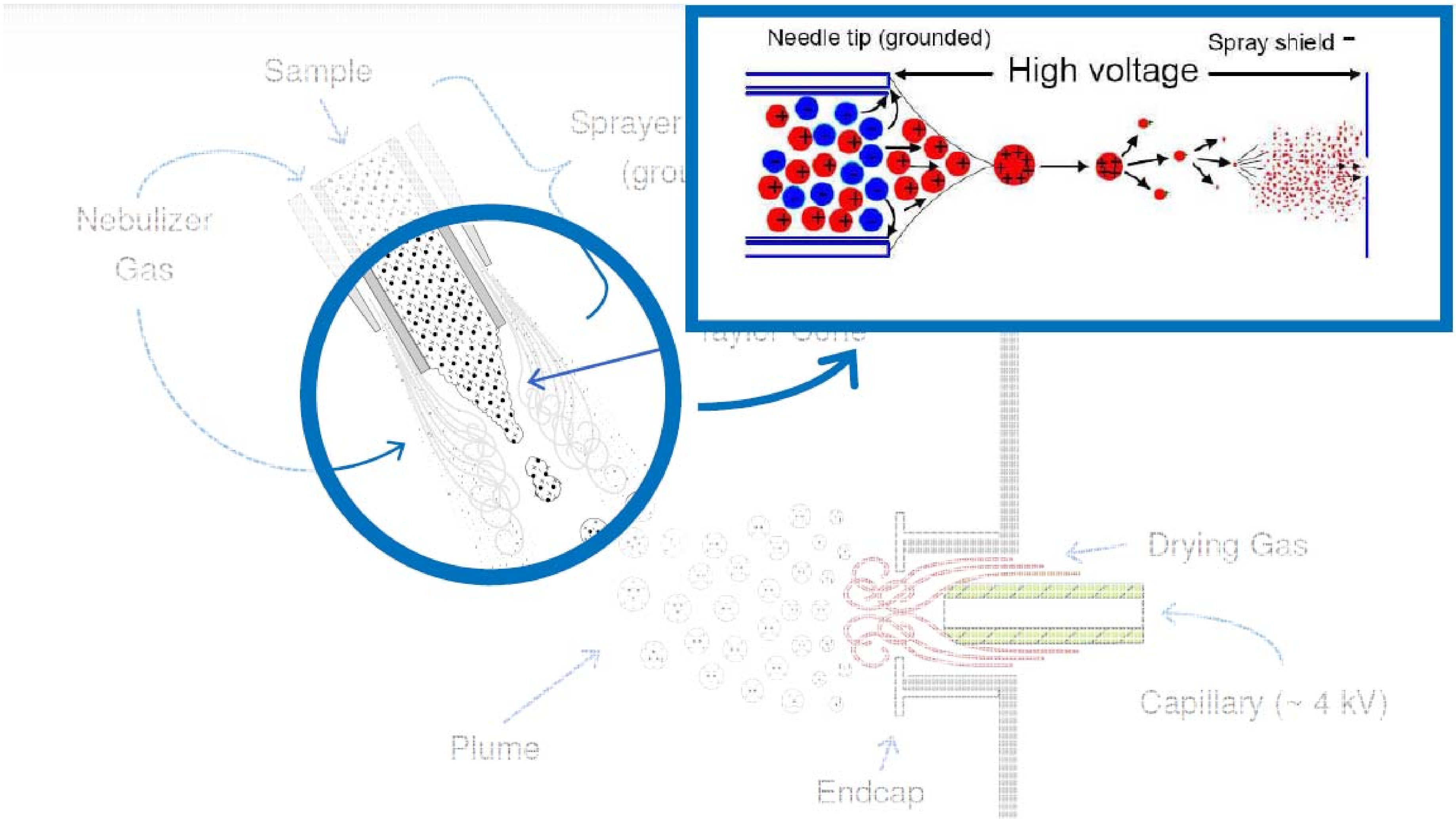
Processes under collision controlled regimes are not as simple as in high vacuum

Electrospray Ionization (ESI)



- Bruker ESI source applies HV to capillary; the spray needle is grounded.
- Voltages shown are for positive ions.

John B. Fenn: Science 1989, Mass Spec Rev 1990, JAMS 1993, Noble prize 2002


Sample
Sprayer
Nebulizer Gas
Needle tip (grounded)
High voltage
Spray shield -
Drying Gas
Capillary (~ 4 kV)
Plume
Endcap

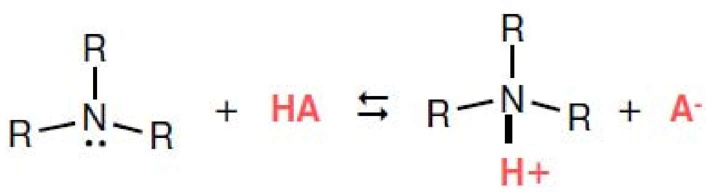
ESI = solution chemistry

Positive Ion Mode

Formation of **protonated** molecular ions

$M + HA \leftrightarrows [M{+}H]^{+} + A^{-}$

Example:

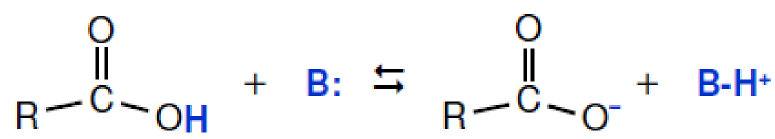


Negative Ion Mode

Formation of **deprotonated** species

$M + B \leftrightarrows [M{-}H]^{-} + BH^{+}$

Example:



HA: Water, methanol, dichloromethane
Common modifiers:

0.1 - 1 %	formic acid
0.1 - 1%	acetic acid
<0.05%	trifluoro acetic acid (toxic!)

B: Water, methanol, etc
Common modifiers:
< 10mM ammonium hydroxide
or buffers e.g. ammonium format
or ammonium acetate

Polar and „acid/base - charged“ molecules are detectable!

ESI = solution chemistry

Pros:

- No evaporation necessary → even nonvolatile and fragile compounds are ionized
- Multiple charged ions available → high molecular weight compounds accessible

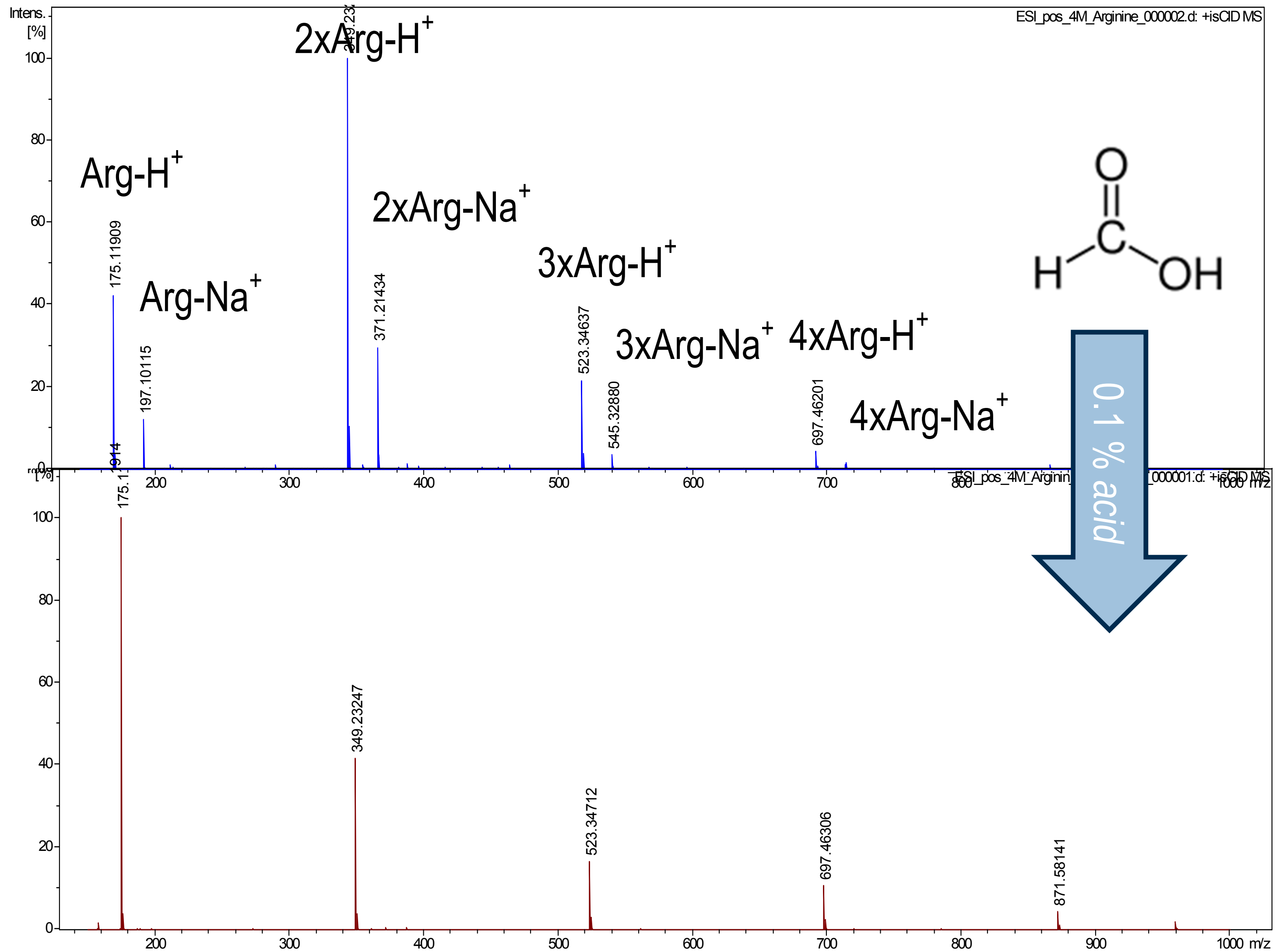
works well for polar compounds
most suitable for peptides, proteins, and lipids

Problems:

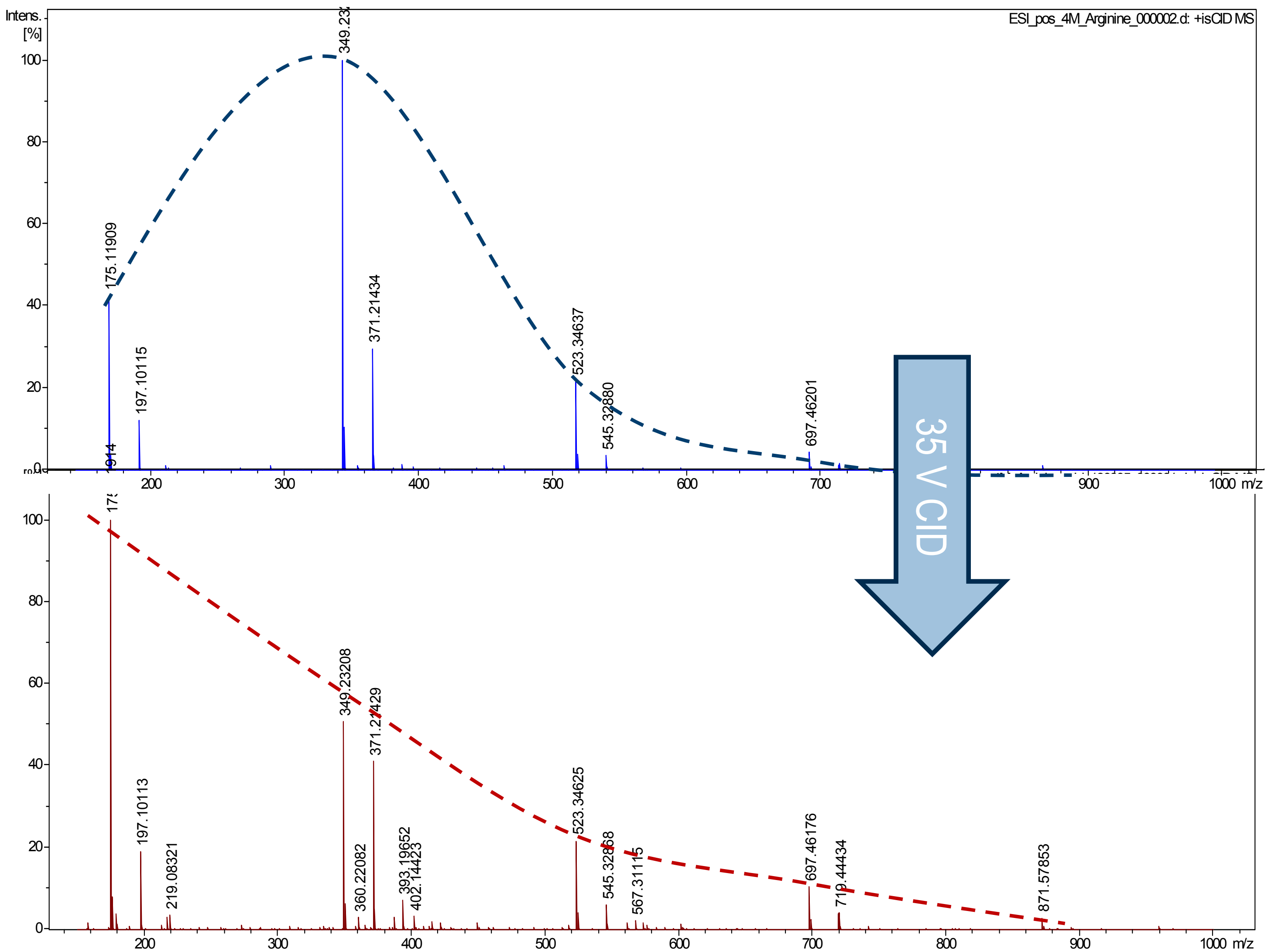
- Molecule-ion-interaction in liquid
- Adduct formation (Na^+, K^+) e.g. from sample or glassware
- Cluster formation ($[M+Na+CH_3CN]^+$, $[M+H+MeOH]^+$, $[M+H+M]^+$…
- High matrix suppression → all analytes compete to limited (surface-)charge
  - → Complex spectra may be obtained (adducts, multiple charge states, etc.)
  - → Quantitative results difficult (use internal standards!)

Interferences and contaminants compare: Keller et al. Anal Chim Acta 2008

ESI+ of Arginine solution ➔ cluster formation and sodium adducts

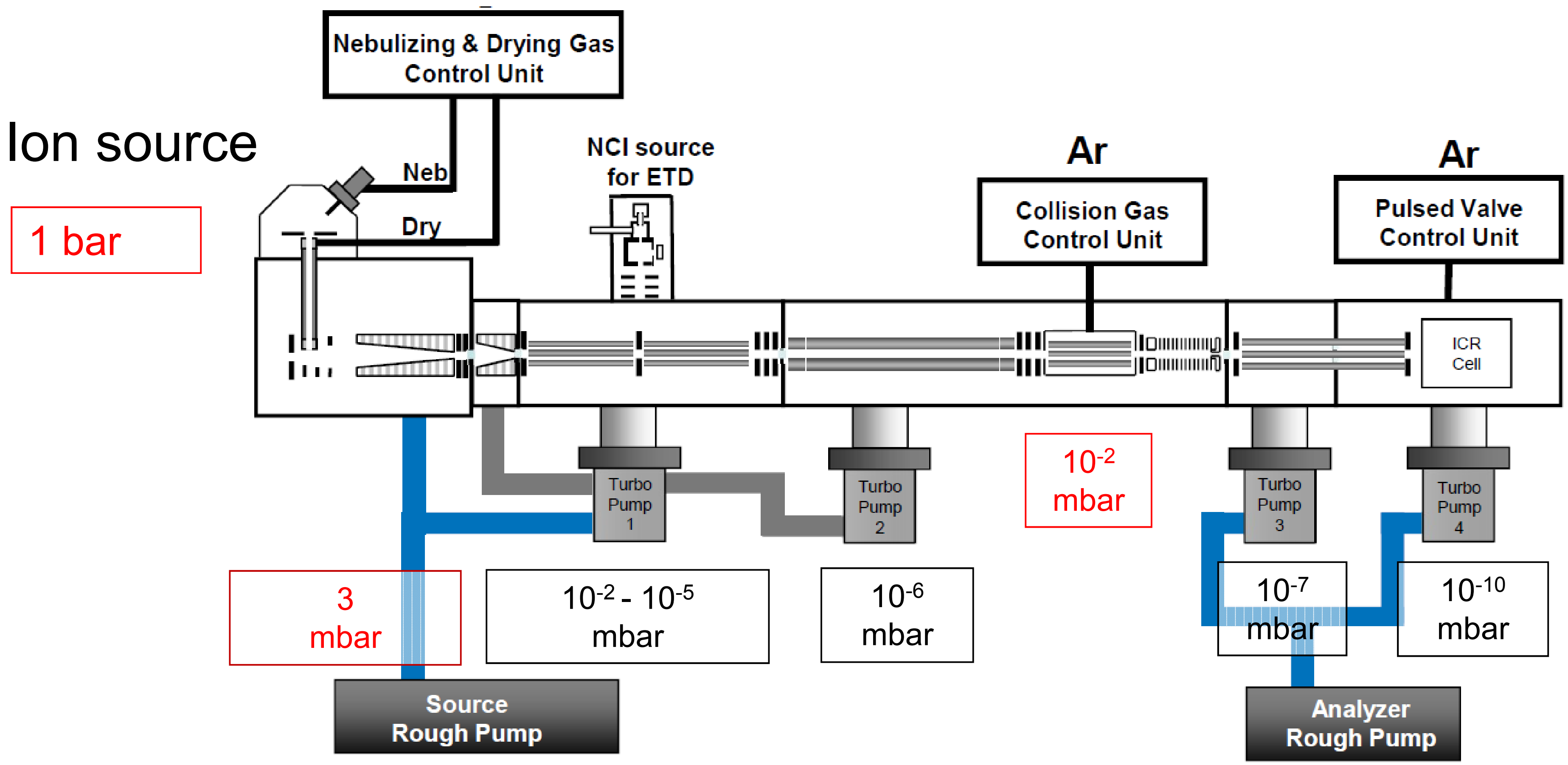


ESI+ of Arginine solution ➔ declustering

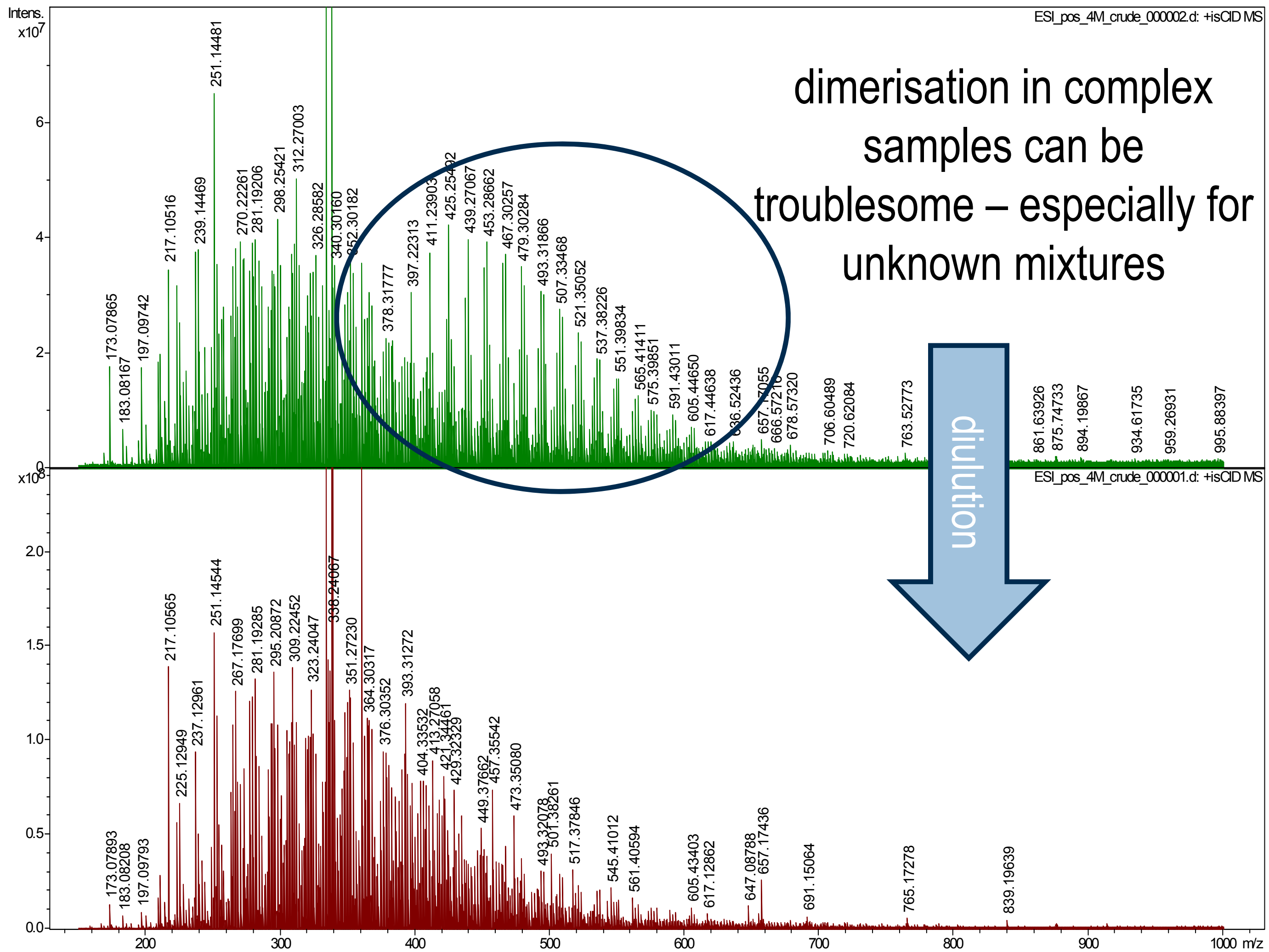


Atmospheric pressure ionization in general

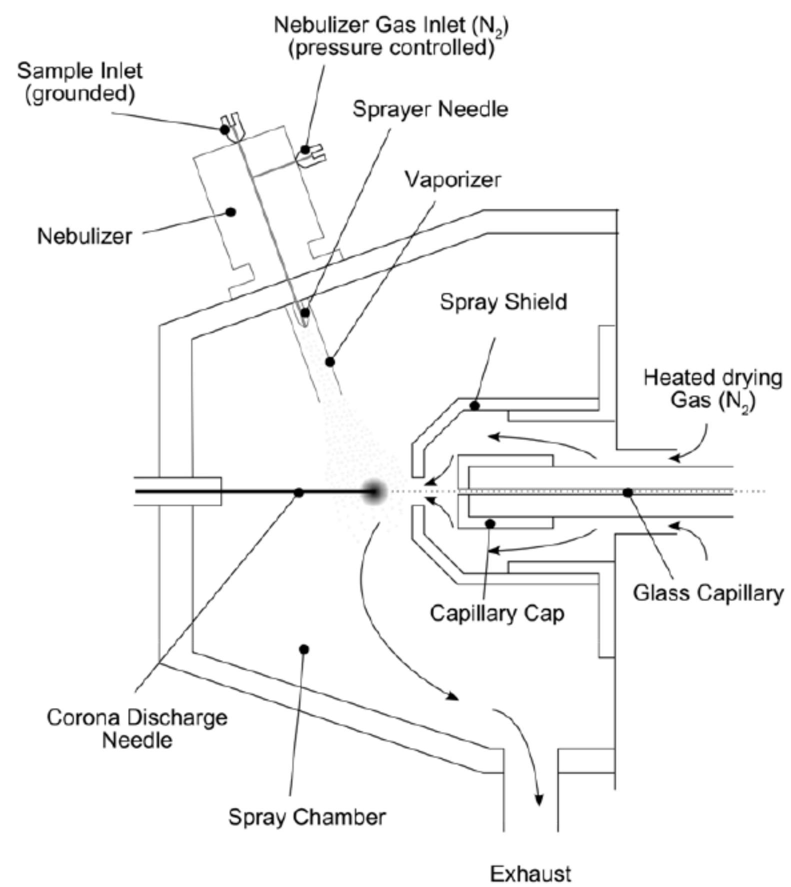
Declustering by: - adjusting dry gas flow,
- enhancing ion collision energy



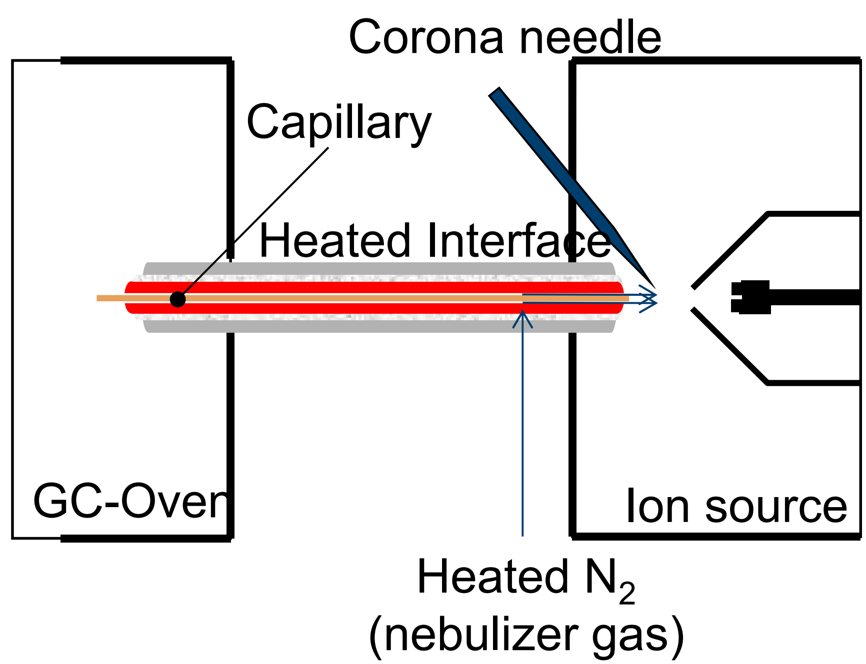
ESI+ of complex samples ➔ effect of concentration



APCI - evaporated liquid

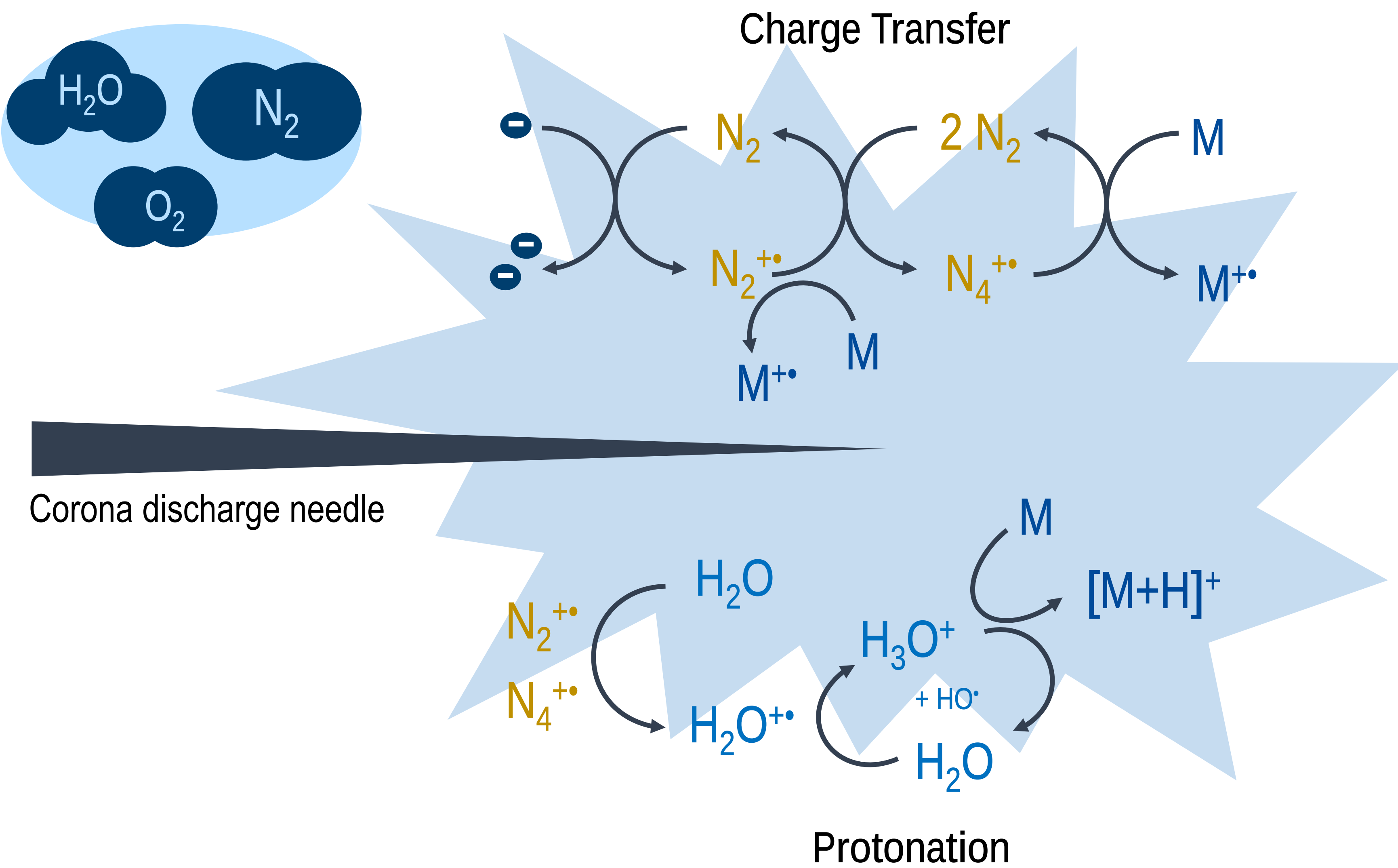


APCI - gas



Horning et al., Anal Chem 1973, , McEwen et al.JASMS 2005, Schiewek et al. ABC 2008

ACPI = gas-phase chemistry



ESI **APCI** APPI APLI

Ionization energy

Molecule	IE (eV)
Nitrogen	15.58
Carbon dioxide	13.78
Water	12.62
Acetonitrile	12.20
Oxygen	12.07
Methanol	10.84
Isopropanol	10.17
n-Hexane	10.13
Heptane	9.93
Isooctane	9.80
Benzene	9.24
Furan	8.88
Toluene	8.83
Anisole	8.20
Naphthalene	8.14
Triethylamine	7.53

Proton affinity

Molecule	PA kJ/mol
Oxygen	422
Nitrogen	495
Carbon dioxide	541
n-Hexane	672
n-Heptane	677
n-Octane	684
Water	691
Benzene	750
Methanol	754
Toluene	784
Isopropanol	793
Acetonitrile	794
Naphthalene	803
Furan	804
Anisole	840
Triethylamine	982

webbook.nist.gov, Hunter&Lias J Phys Chem Rev Data 1998, Hunter&East J Phys Chem 2002

APCI = gas-phase chemistry

Pros:

- No formation of Na and K adducts
- Higher linear dynamic range and less matrix effects (compared to ESI)
- Ideal for hyphenation to gas and liquid chromatography

works well for intermediate polar compounds
most suitable for "small molecules" (metabolomic, petroleomic)

Problems:

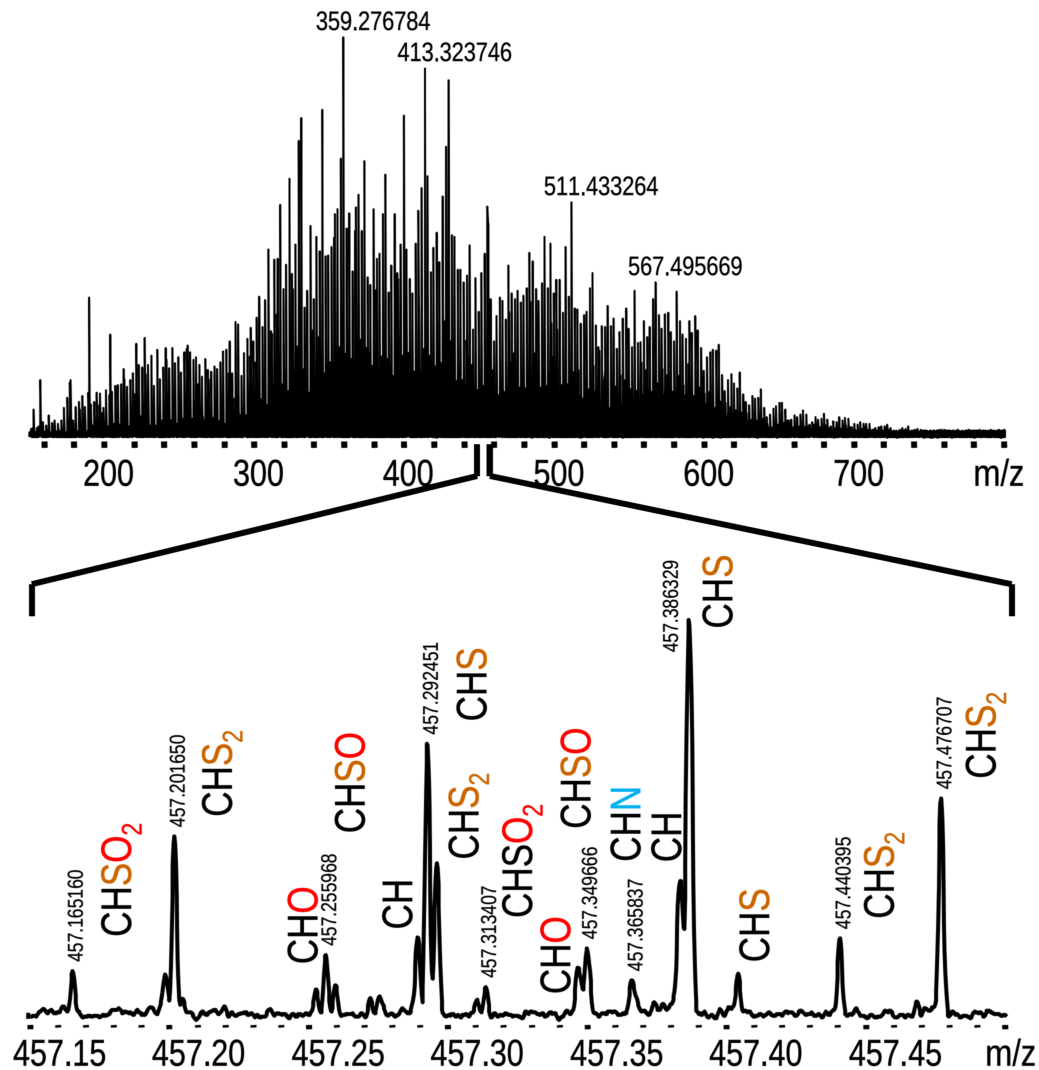
- Lossless evaporation needed → not applicable for thermo-labile components
- Molecule-ion-interaction in gas-phase
- Complex gas-phase chemistry (reactants, solvent, concentration, reaction time …)
- Corona current needs to be tuned, position of sprayer and needle is critical
- Adduct formation with oxygen

Example: Fingerprinting asphaltene pyrolysis products

APCI:

CHS-, CHS_2-, $CHSO_x$- and CH-species dominating

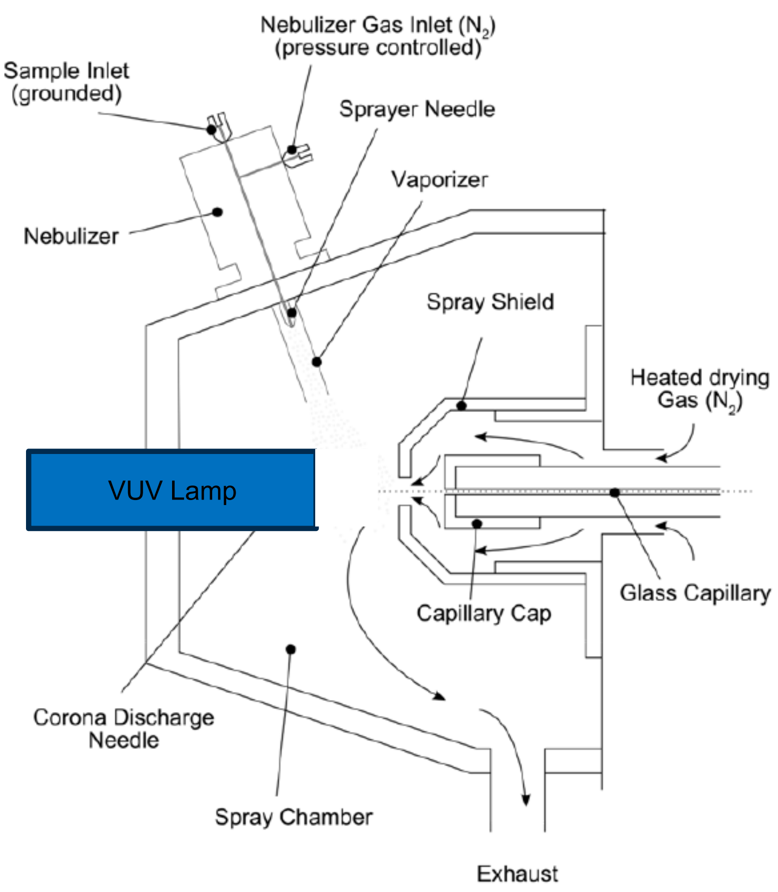
> 3,500 distinct peaks



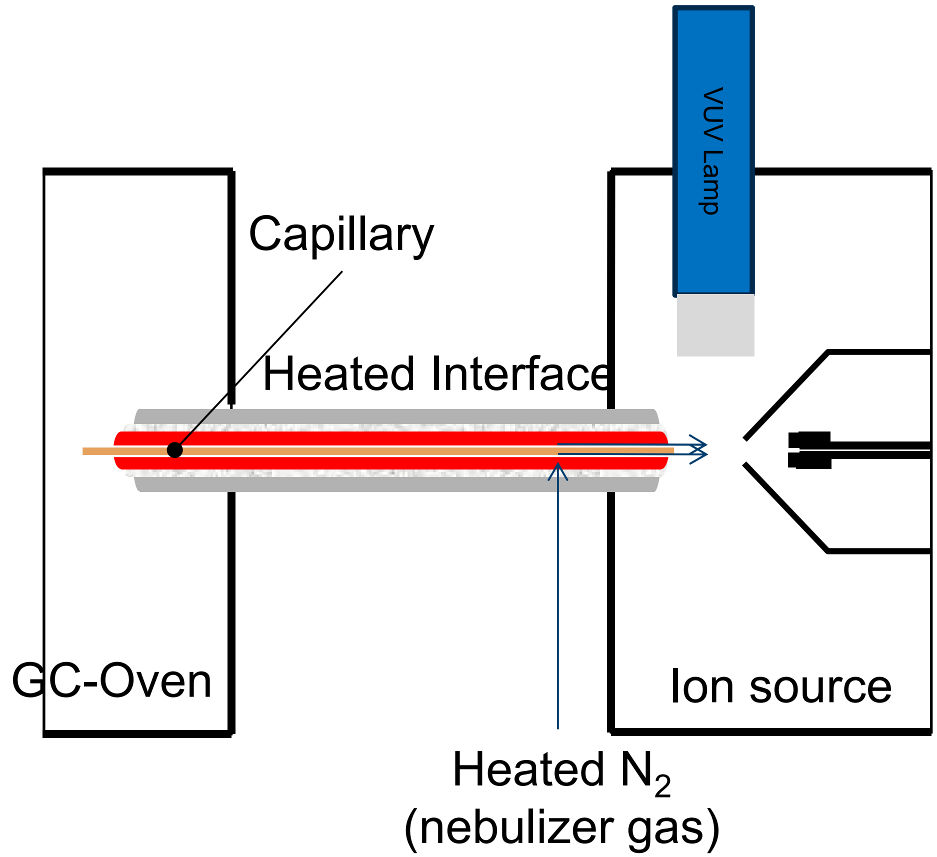
Pyrolysis GC/EI-MS results:

Mainly alkanes and alkenes, and only minor concentration of alkylated benzothiophenes

APPI - liquid

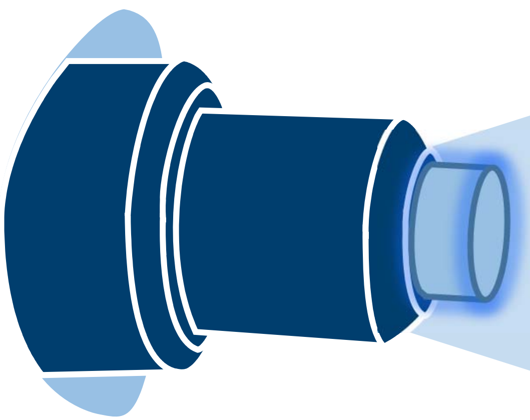


APPI - gas



Horning et al., Anal Chem 1973, McEwen et al. JASMS 2005, Benter et al. ABC 2008

APPI = direct photon/molecule reaction



Direct Photo Ionisation

$M \xrightarrow{h\nu} M^{+\bullet} + e^{-}$ *if IE(M) < hv*

$S \xrightarrow{h\nu} S^{+\bullet} + e^{-}$

Gaseous Phase Reactions

$S^{+\bullet} + M \longrightarrow S + M^{+\bullet}$ *if IE(M) < IE(S)*

$S^{+\bullet} + M \longrightarrow [S\text{-}H]^{\bullet} + [M+H]^{+}$ *if PA(M) > PA(S)*

+ gas-phase chemistry (by dopant or solvent or matrix)!!!

Ionization energy

Molecule	IE (eV)
Nitrogen	15.58
Carbon dioxide	13.78
Water	12.62
Acetonitrile	12.20
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Furan	8.88
Toluene	8.83
Anisole	8.20
Naphthalene	8.14
Triethylamine	7.53

webbook.nist.gov, Short et al. JASMS 2006

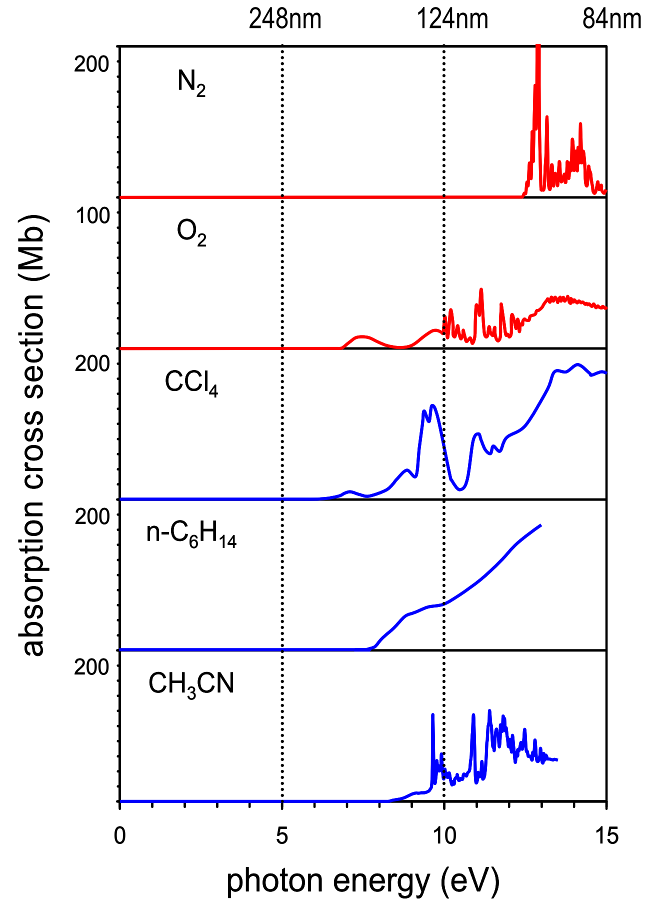
Direct PI

$M + h\nu \rightarrow M^{\bullet +} + e^-$

Krypton lamp (116…124nm)
10eV …10.6eV

Xenon lamp (148nm)
8.4eV

Common solvents (and oxygen) are not ionized, but may strongly absorb photons



APPI = direct photon/molecule reaction and gas-phase chemistry

Pros:

- Higher linear dynamic range and less matrix effects (compared to APCI)
- Low background noise
- Ideal for hyphenation to gas chromatography

works well for compounds with comparable low IP
most suitable for “small and even non-polar molecules”

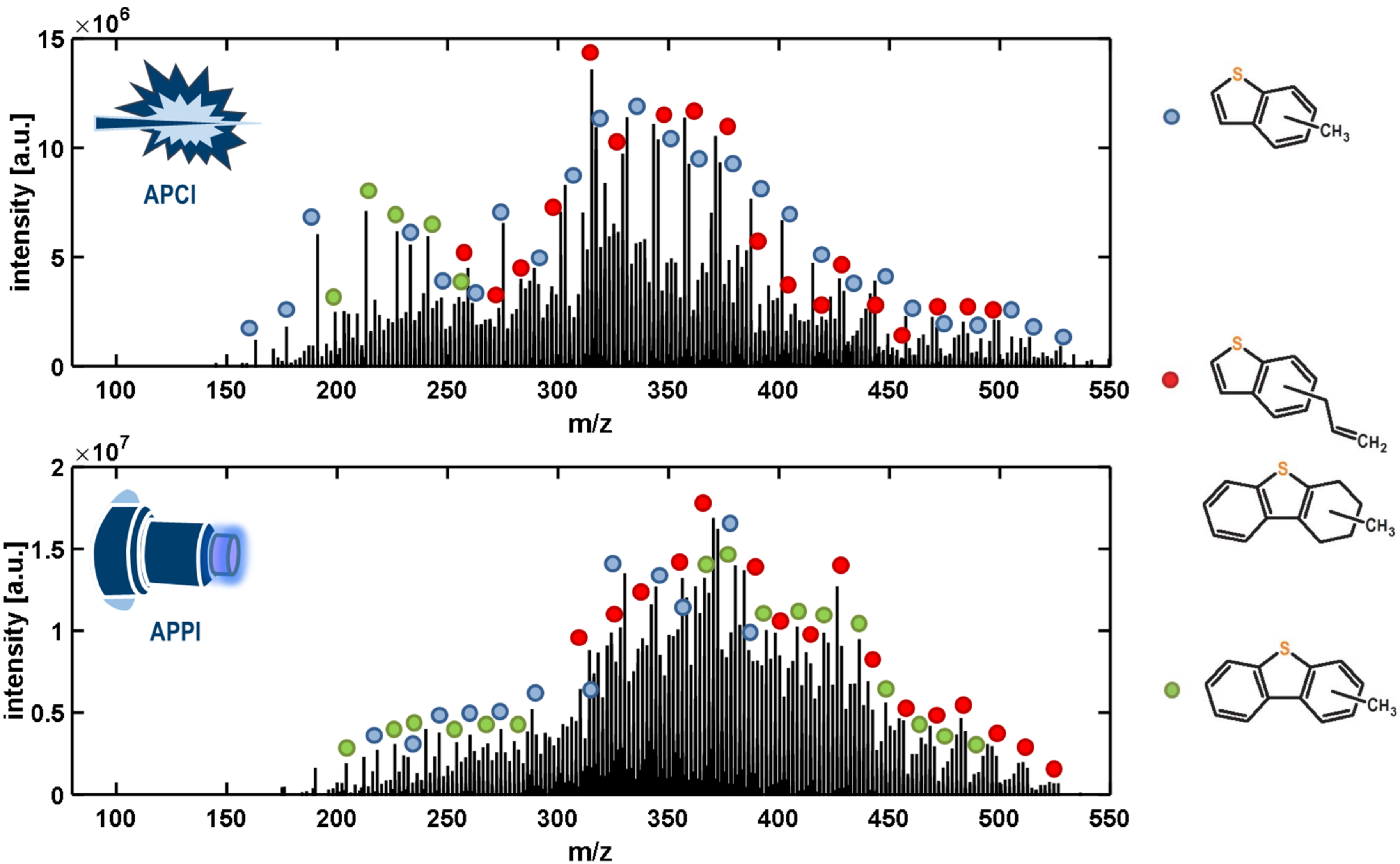
Problems:

- Lossless evaporation needed → not applicable for thermo-labile components
- Still molecule-ion-interaction occurs (formation of radical cation vs. protonation)
- Dopants and solvents may lead to complex gas-phase chemistry
- Adduct formation with oxygen
- Lamp has a limited lifetime

Syage_et al. Am Lab 2000, Kauppila et al. Anal Chem 2002, Hanold et al. Anal Chem 2004, Kauppila, Syage, Benter Mass Spec Rev. 2017

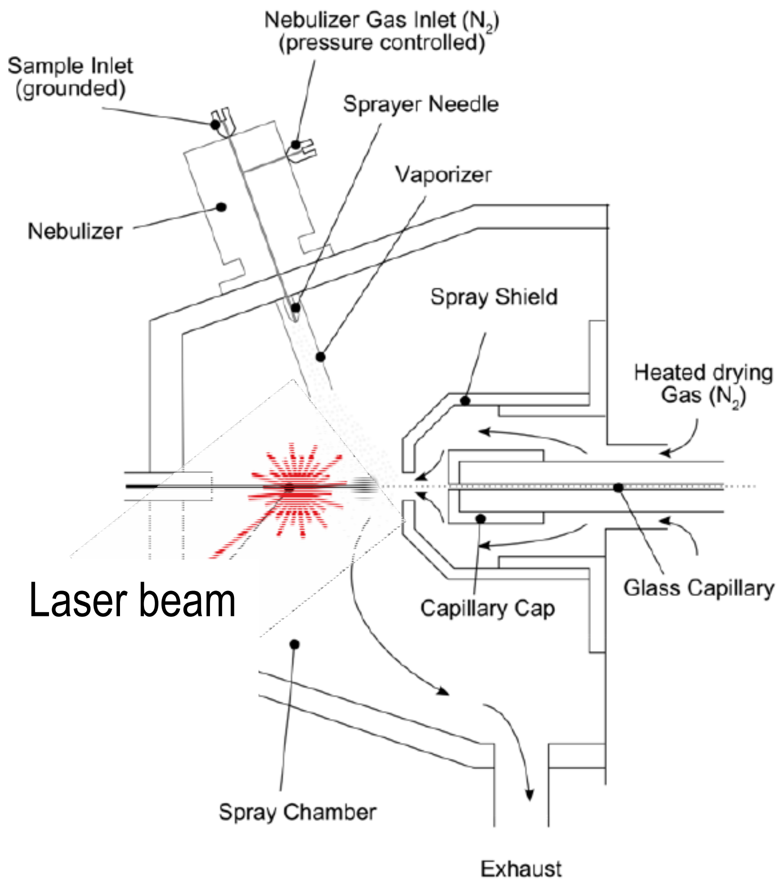
ESI APCI **APPI** APLI

Example:Fingerprinting asphaltene pyrolysis products

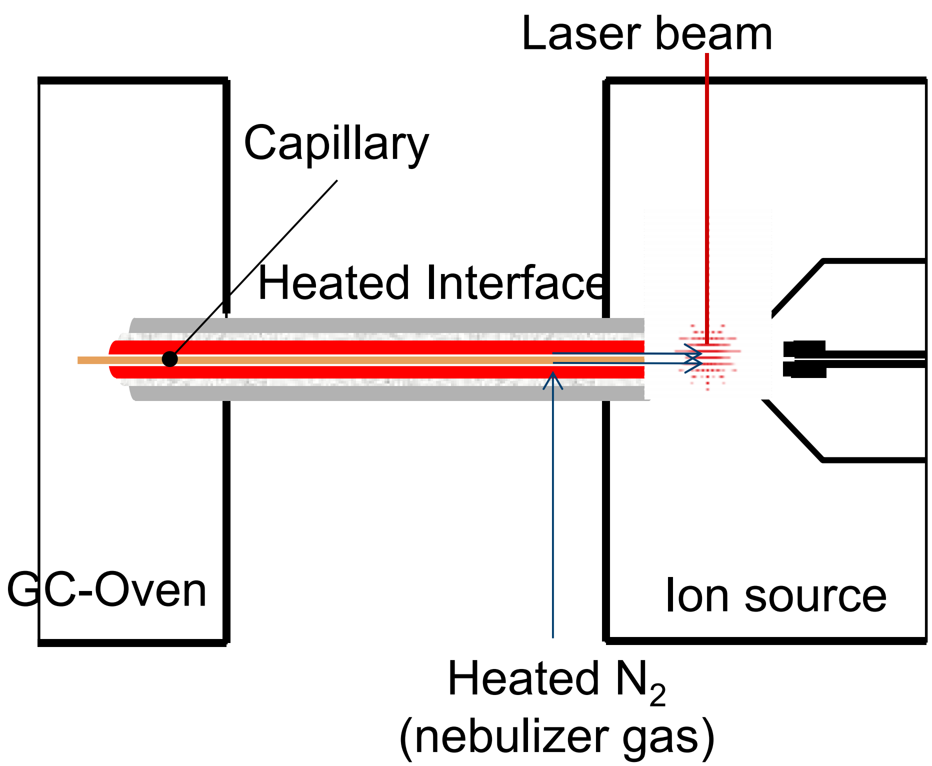


Rüger et al ASMS2018

APLI - evaporated liquid



APLI - gas



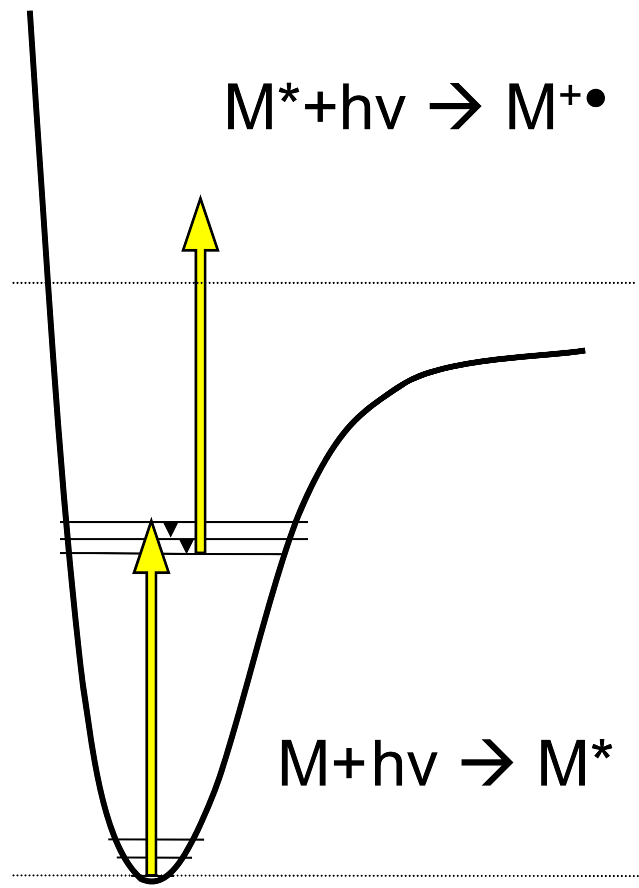
Schmidt et al. Anal Chem 1999, Kersten et al. JASMS 2011

APLI (REMPI) uses UV photons
248nm (5.00 eV)
266nm (4.66 eV)

High photon density (~10^7 W/cm^2)
Short pulses (6ns…10ns)

Two photon “resonant “process

Selective ionization of **aromatic and polyaromatic compounds**



1. UV photon adsorbed
2. Lifetime of intermediate longer than pulse length (2. photon)
3. 2 x hv > IE

Boesl et al. Int J Mass Spec Ion Process 1994, Zimmermann et al RCM 1996

ESI APCI APPI **APLI**

APLI = direct photon/molecule reaction

Pros:

- Higher linear dynamic range and less matrix effects (compared to APPI)
- Compatible to common LC-solvents
- High selectivity and extreme high sensitivity can be reached

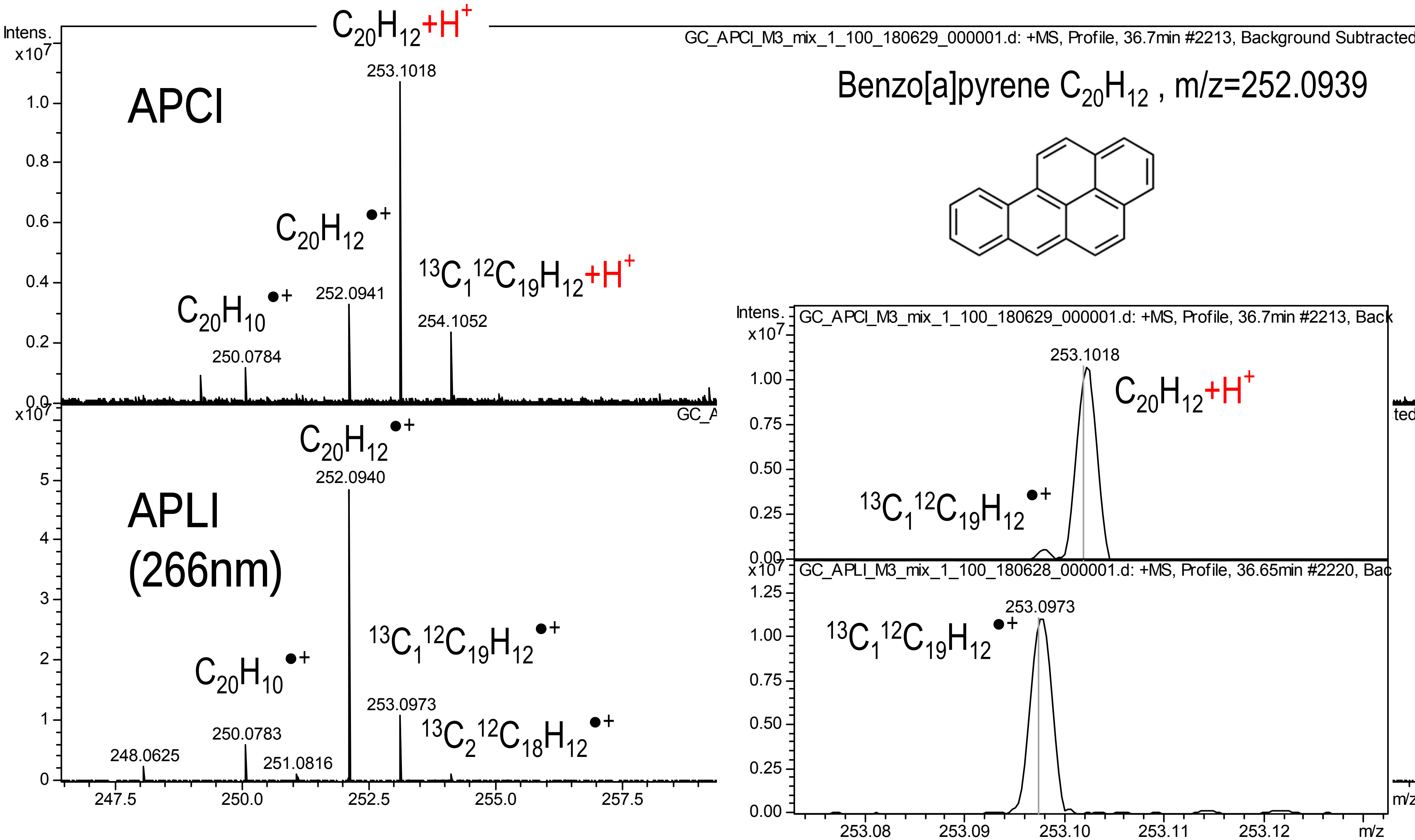
works well for compounds with aromatic core (low IE)
most suitable for polycyclic aromatic hydrocarbons
and derivatives

Problems:

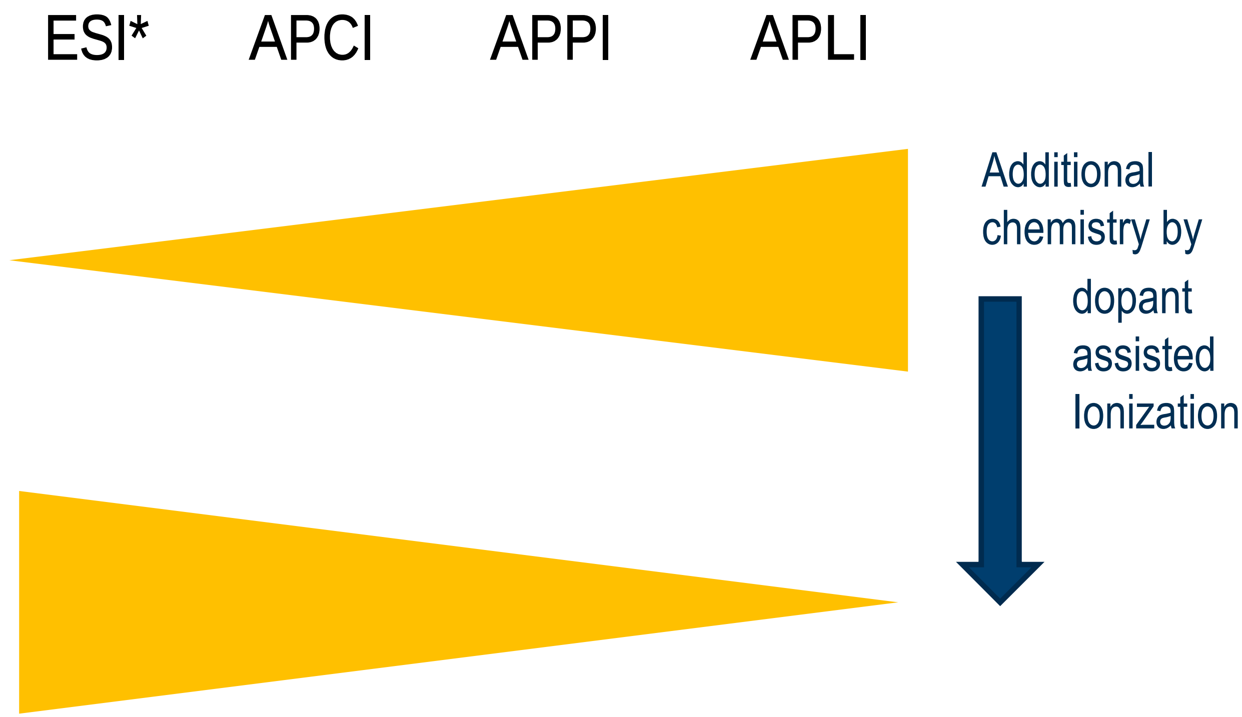
- Lossless evaporation needed → not applicable for thermo-labile components
- Ionization efficiency depends strongly on compound structure, laser wavelenght, and laser fluence
- Laser safety

Constapel et al. RCM 2005, Schiewek et al Anal Chem 2007, Brinkhaus et al. ABC 2017

ESI APCI APPI **APLI**



Summary



Direct formation of radical cations by charge transfer or photoionization

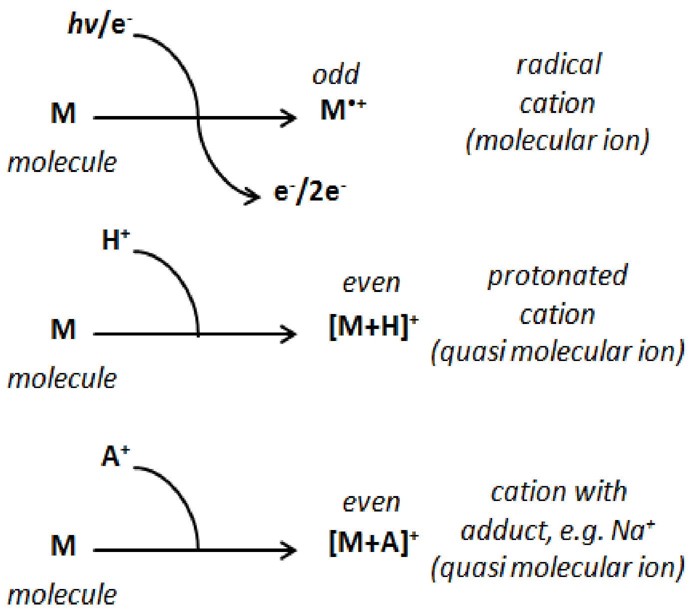
Proton transfer and chemistry in gas or liquid phase

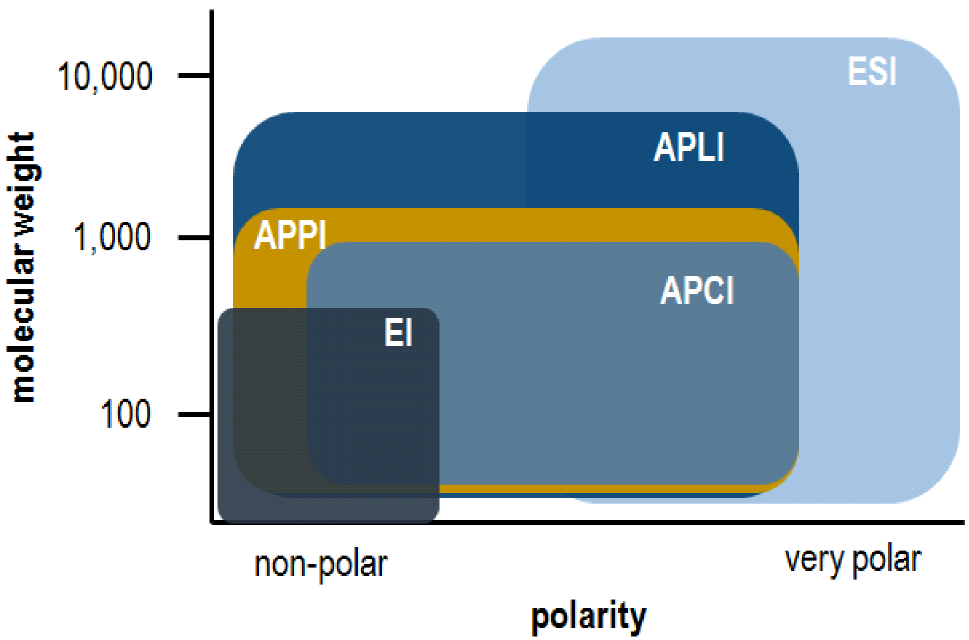
Reactions can/will take place and depend on:

- reactant concentration
- reaction enthalpies and rates
- fluid dynamics (flows and source design)

Ionization method determines the detectable components…

- molecular weight (vaporizable?)
- polarity (internal acidic/basic ions?) and reactivity
- Ionization potential and proton affinity





Ionization requests for complex mixtures

- Universal

  …mass spectrometric signals reflects composition of neutrals

→ **NO**

Ionization efficiency varies extremely by:

- The applied technique
- Physical and chemical properties of the targeted compounds
- Solvent, background and matrix components and modifiers

Ionization requests for complex mixtures

- Preserve molecular ion information

  …No fragmentation or adduct formation

- Low “matrix” effects and linear response

→ YES (mostly…)

Think about:

- Diluting the sample
- Using pure solvents and gases
- Optimizing solvent composition and instrumental settings
- Clean-up and separation steps before mass spectrometric analysis
- Analyzing the same sample with different dilution ratios
- Spiking the sample or adding isotope labeled standards
- Checking the ratio of radical cation / protonated species
- ….

Rem: Additional fragmentation and adduct formation may happen
during ion transport from atmospheric pressure to high vacuum

Co-funded by the Horizon 2020
program of the European Union

General reading

Jürgen H. Gross:
Mass spectrometry - A Textbook, Springer International Publishing (2017)

John B. Fenn:
Electrospray Wings for Molecular Elephants (Nobel Lecture). Angew. Chem. Int. Ed. 42, 3871-3894 (2003):

Ralf Schiewek, Matthias Lorenz, Ronald Giese, Klaus Brockmann, Thorsten Benter, Siegmar Gäb, Oliver J. Schmitz:
Development of a multipurpose ion source for LC-MS and GC-API MS. Anal. Bioanal. Chem. 392, 87–96 (2008)

Tiina J. Kauppila, Jack A. Syage, Thorsten Benter:
Recent Developments in atmospheric pressure photoionization-mass spectrometry. Mass Spec. Rev. 36, 423-449, 2017