Exploding Nanodroplets: Applications to Frontier Studies in Mass Spectrometry, Ion Solvation, and the Chemistry of Molecular Clusters

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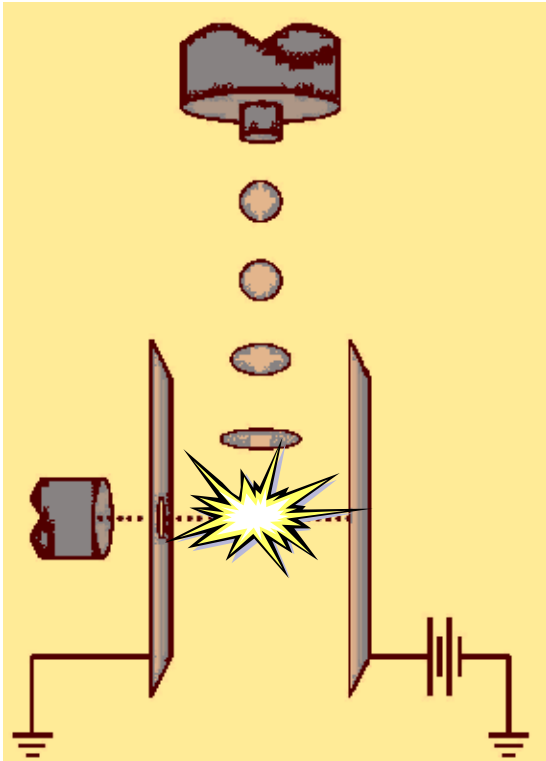

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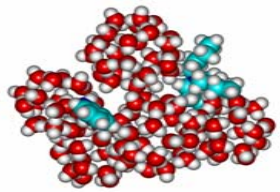
Abstract

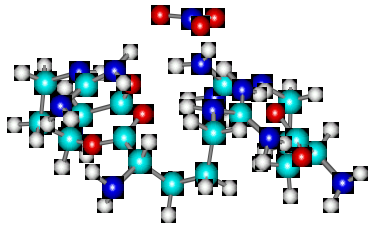
The development of new methods to achieve transfer of intact molecules and molecular complexes from solids and liquids into the vapor phase invariably ushers in a broad range of new applications. This has certainly been the case with electrospray ionization, recognized by the 2002 Nobel Prize in Chemistry. We have undertaken a systematic study of the life history of a charged droplet, from formation at the ESI tip to the completely desolvated ions that are eventually formed. The final stages of ion desolvation reveal details of the specific interaction of solvent molecules with charged functional groups. These investigations have led to the development of a new method for the controlled extraction of desolvated ions from *neutral* droplets in a process we call field induced droplet ionization or FIDI for short. In addition, these studies have led to the development of "soft sampling" methodology that facilitates observation of molecular clusters and non-covalent complexes. Of particular interest are clusters that have unique stability, such as the homochiral serine octamer, and the controlled covalent coupling of the components of molecular clusters, the formation of ATP from anionic salt clusters of AMP being an example.

Overview

solution



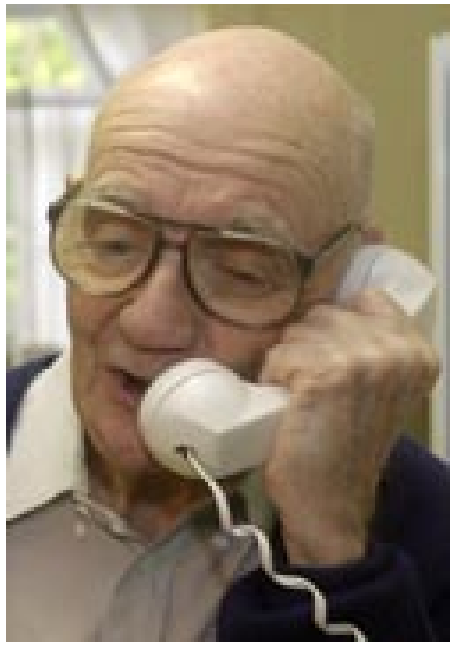
nanodroplet



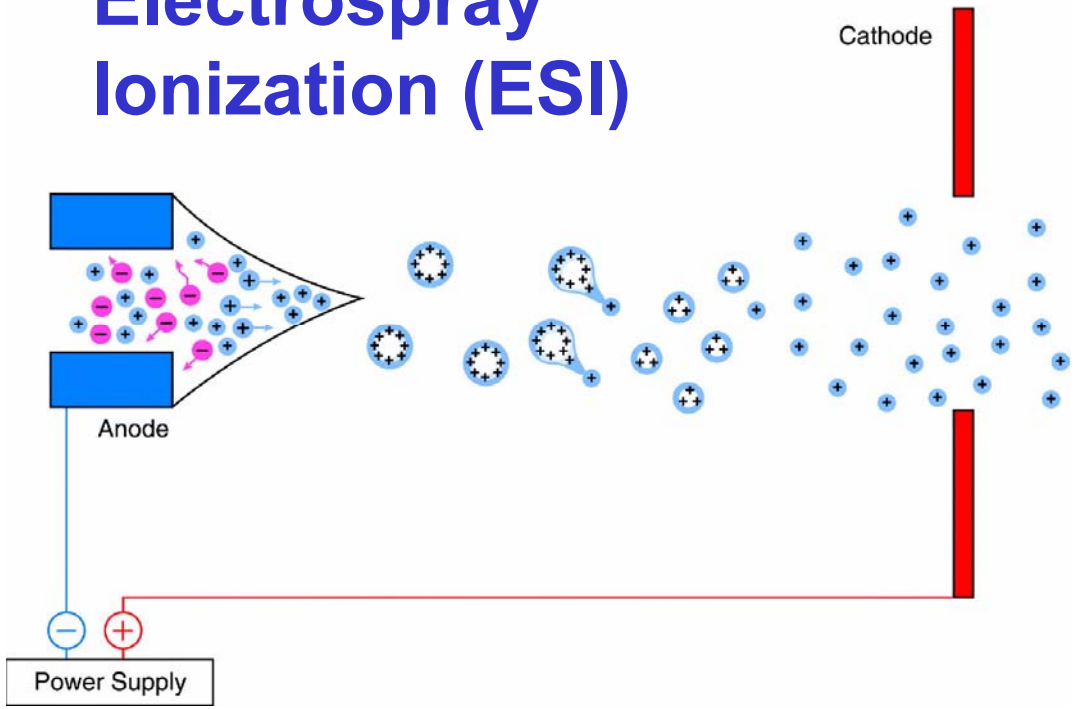
gas phase clusters

1. Electrospray Ionization: Dynamics of Droplet Evaporation and Discharge Processes
2. Field Induced Droplet Ionization (FIDI): A New Route to Gas Phase Biomolecules and Clusters
3. Structure and Reactivity of Molecular Clusters: Role of Cluster Formation in Homochirogenesis and Abiogenic Synthesis

2002 Nobel Prize in Chemistry



Electrospray Ionization (ESI)



JOHN B. FENN, for his development of soft desorption ionization methods for mass spectrometric analyses of biological macromolecules (electrospray ionization)

Rayleigh Model for Droplet Discharge Dynamics

"On the Equilibrium of Liquid Conducting Masses Charged with Electricity", Lord Rayleigh, F.R.S, Proc. Roy. Soc. 184-186 (1882).

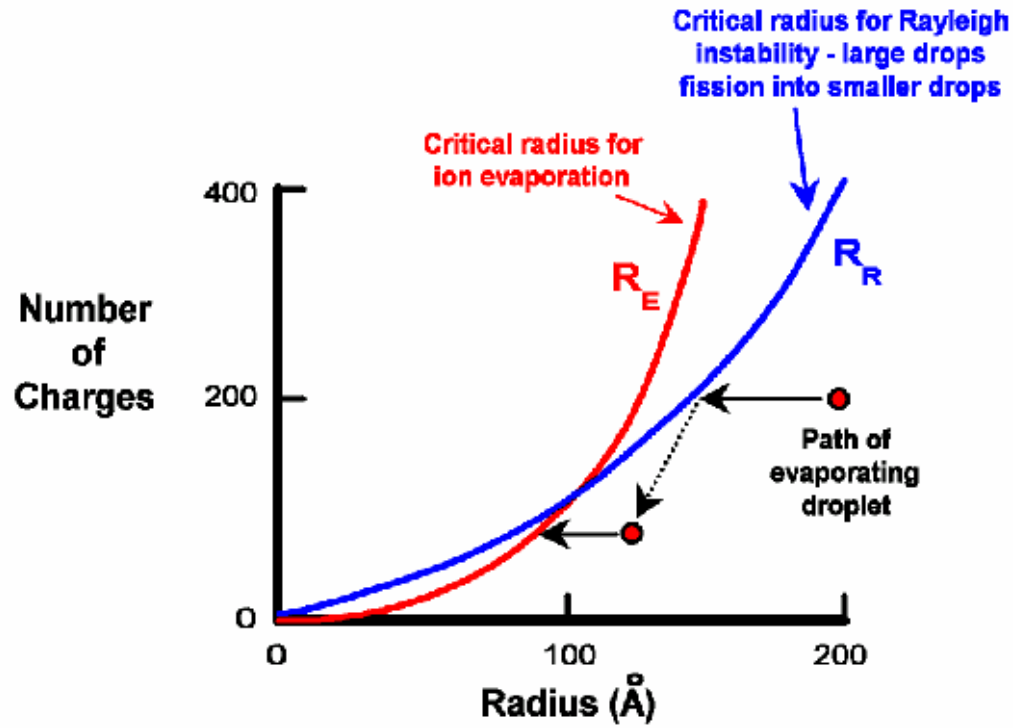
Theoretical Limit for Droplet Stability (Rayleigh Limit):

$$Q_R = 8\pi\sqrt{\gamma\varepsilon_0 r^3}$$

Limit for Ion Desorption:

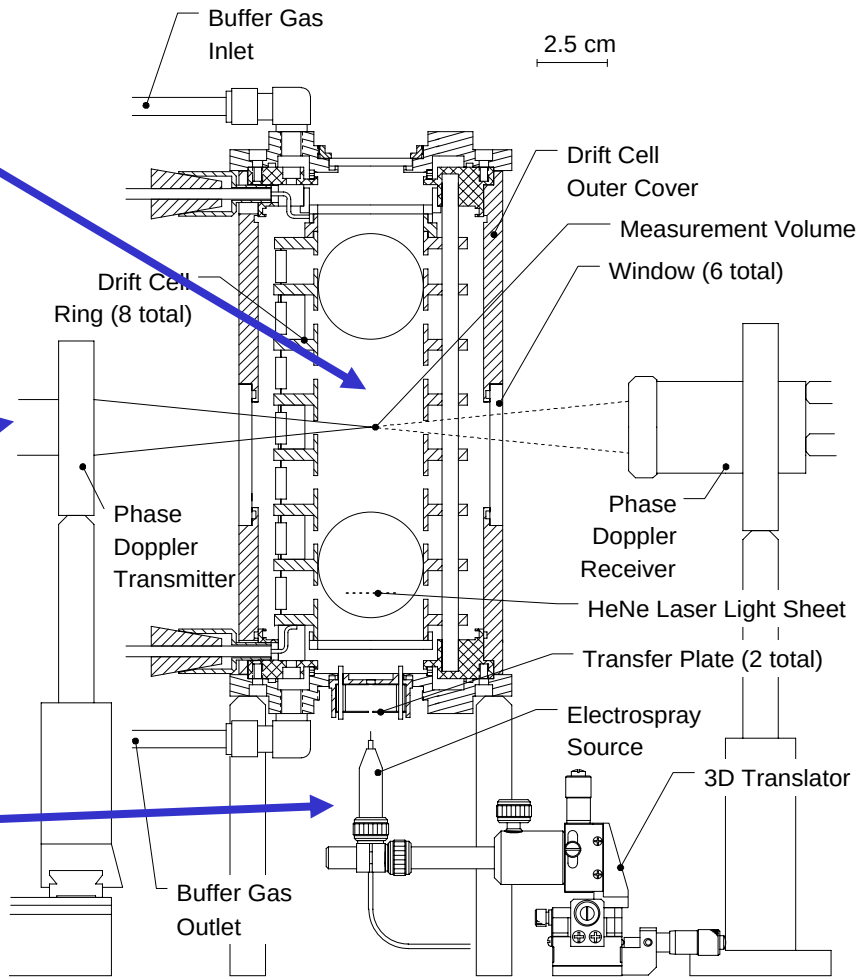
$$Q_E = 4\pi\varepsilon_0 E_c r^2$$

Q: Droplet Charge
r: Droplet Radius
γ: Bulk Liquid Surface Tension
ε_0: Permittivity of Free Space
E_c: Critical Field for Ion Desorption



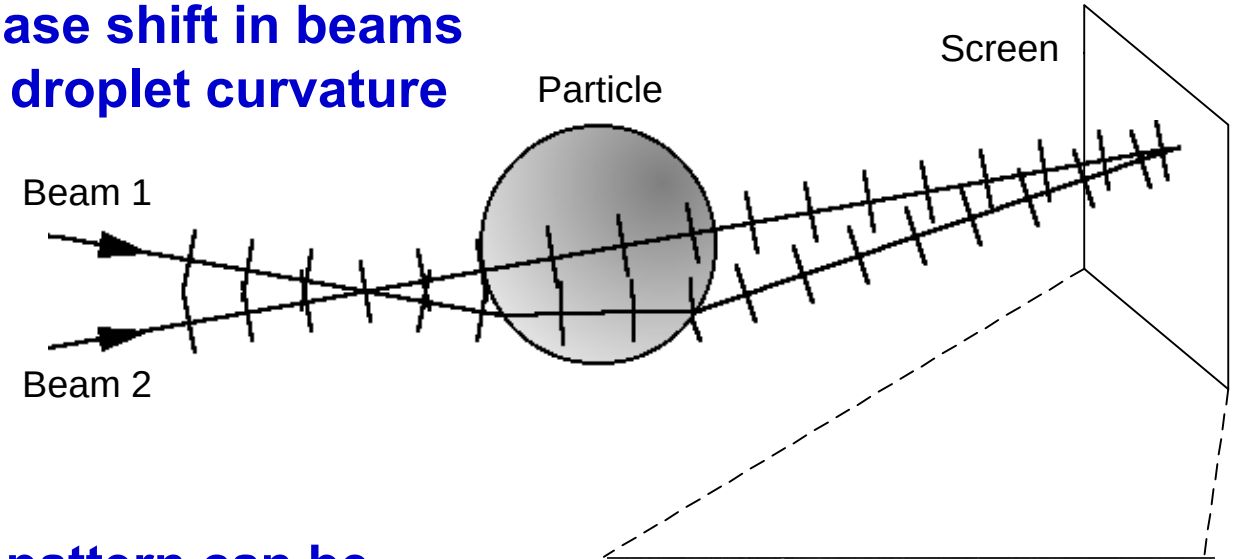
Studies of Charged Droplet Evaporation and Discharge Processes

- Uniform electric field drift cell (typically 50 V/cm; 1 atm. dry N_2).
- Phase Doppler anemometer for measuring size and velocity of droplet; charge is calculated.
- Electrospray source: 50 µm inner diameter steel capillary, flow rate 0.2-1 µl/min.

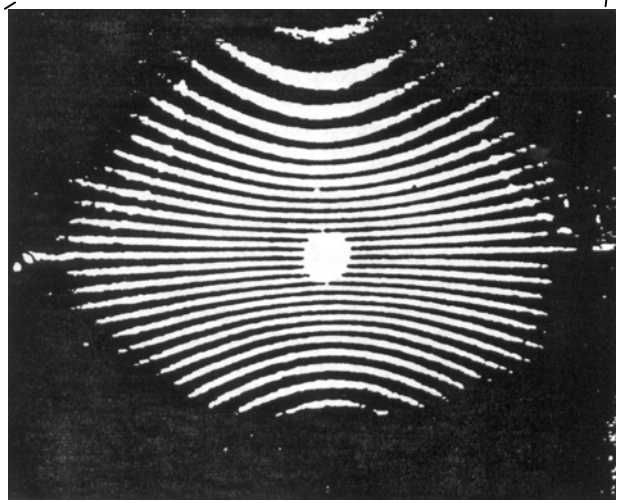


Phase Doppler Anemometry

Relative phase shift in beams caused by droplet curvature



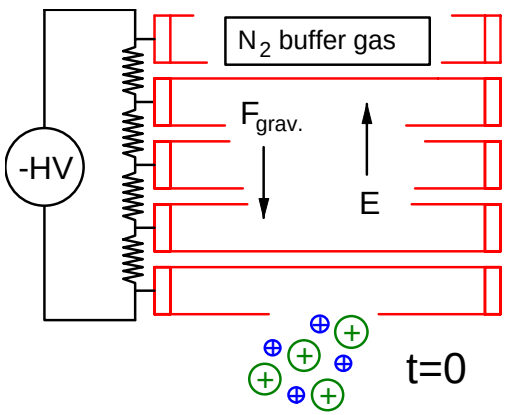
- **The fringe pattern can be analyzed to determine the droplet diameter.**
- **The fringe pattern sweeps past the screen at a velocity that is proportional to the vertical component of particle velocity.**

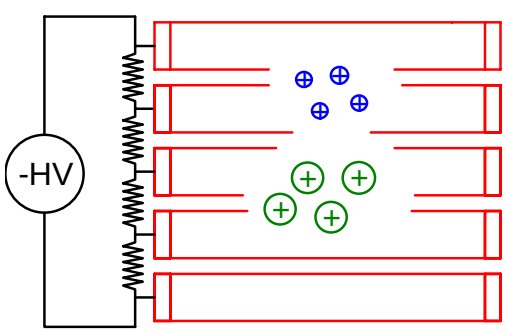


Scattered Fringe Pattern

A measurement of droplet's size and velocity in a constant electric field can be used to obtain its charge.

Drift Cell:





For a droplet in a constant electric field:

$$v_d = \left[\frac{C_C\left(qE - m_p g\right)}{\frac{\pi}{8} C_D \rho_p D_p^2} \right]^{\frac{1}{2}}$$

Solving for q:

$$q = \frac{\pi C_D \rho_p D_p^2 v_d^2 + 8 C_C m_p g}{8 C_C E}$$

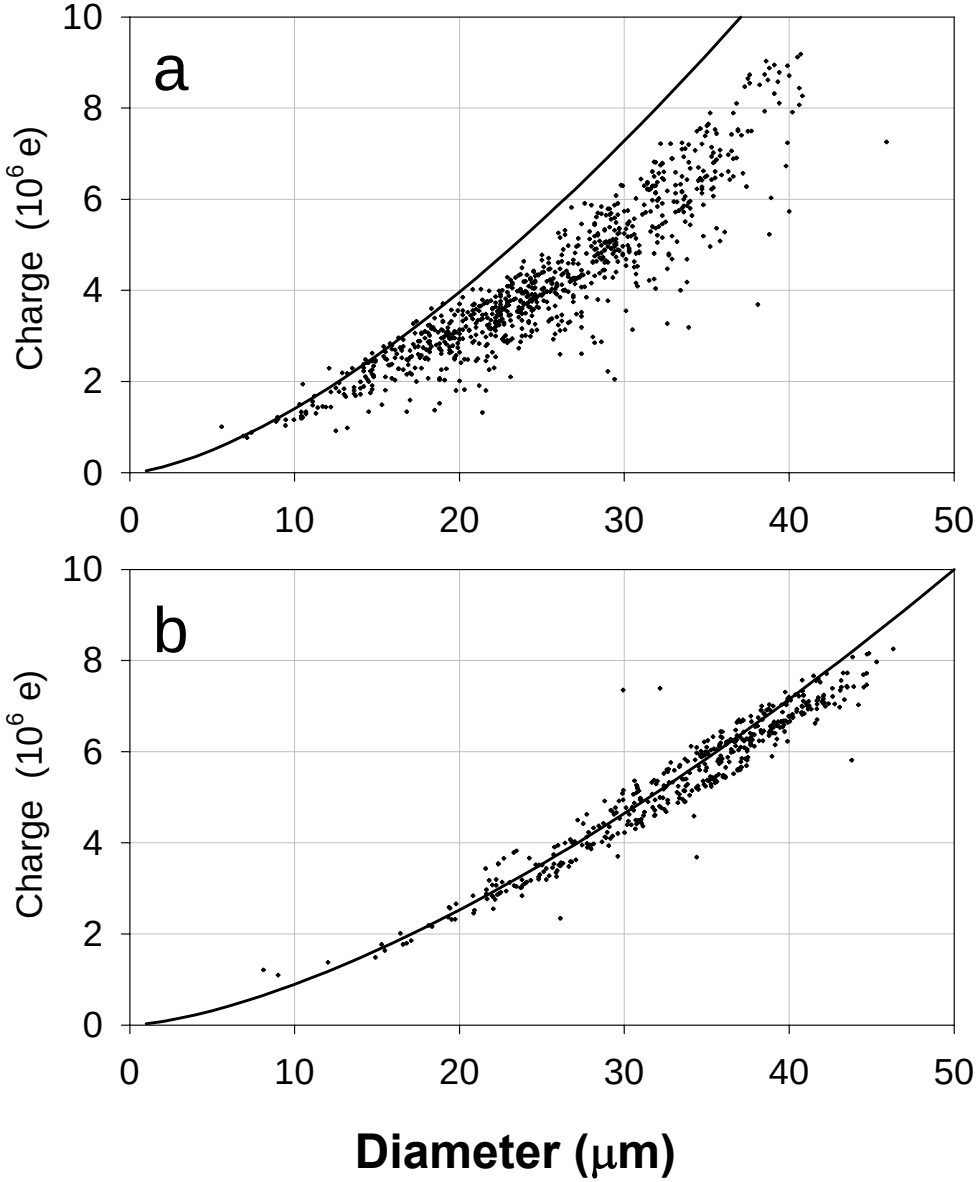
q:	droplet charge
E:	electric field strength
m_p:	droplet mass
ρ_p:	droplet density
D_p:	droplet diameter
C_C:	slip correction factor
C_D:	drag coefficient.

Charge-Size Correlations of Electrospray Droplets

Solid Line Represents Rayleigh Limit

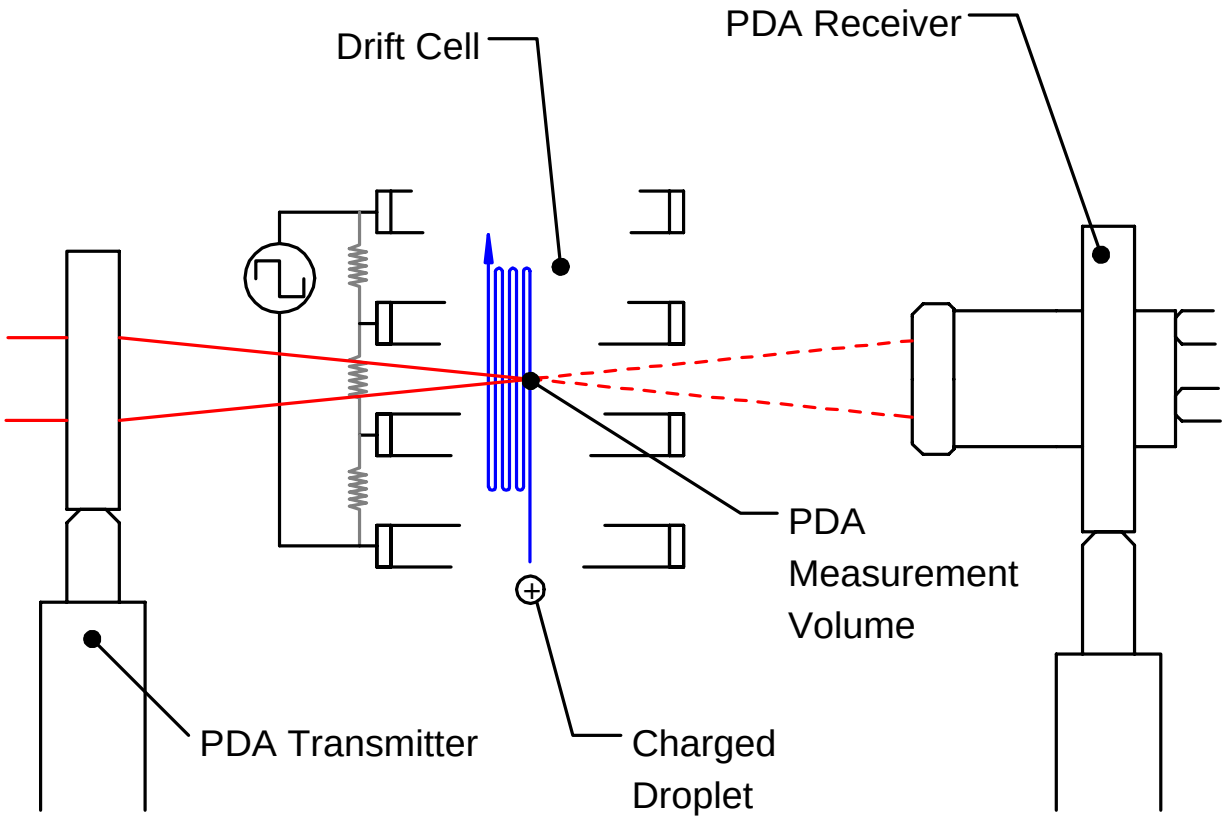
Water / 10^{-4} M NaCl

Acetonitrile



Droplet Evaporation and Discharge Dynamics in Electrospray Ionization. J. N. Smith, R. C. Flagan, and J.L. Beauchamp, *J. Phys. Chem. A*, 106, 9957-9967 (2002).

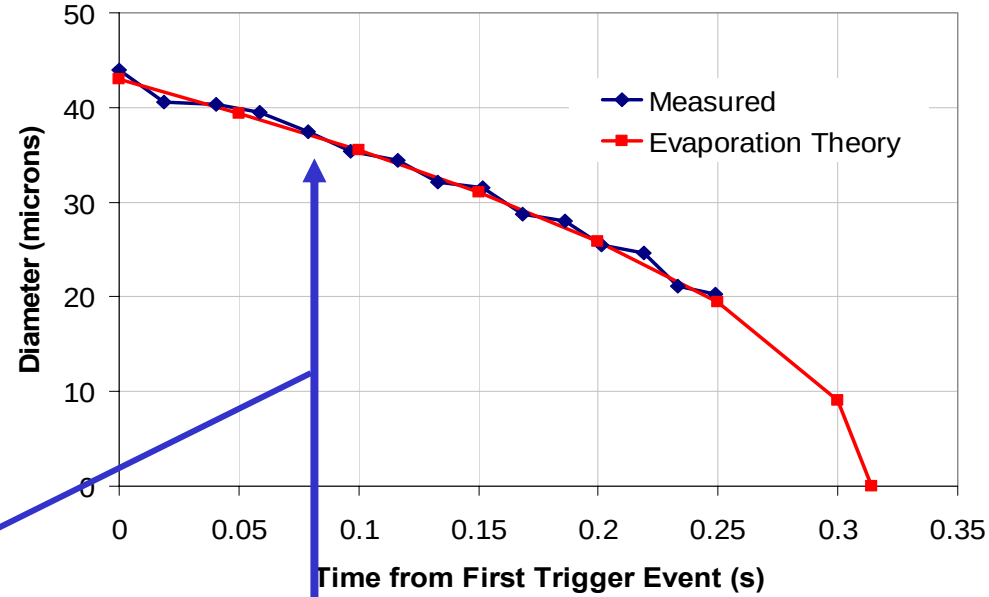
Droplet Ping-Pong: Charting the Dynamics of Droplet Evaporation and Discharge Processes

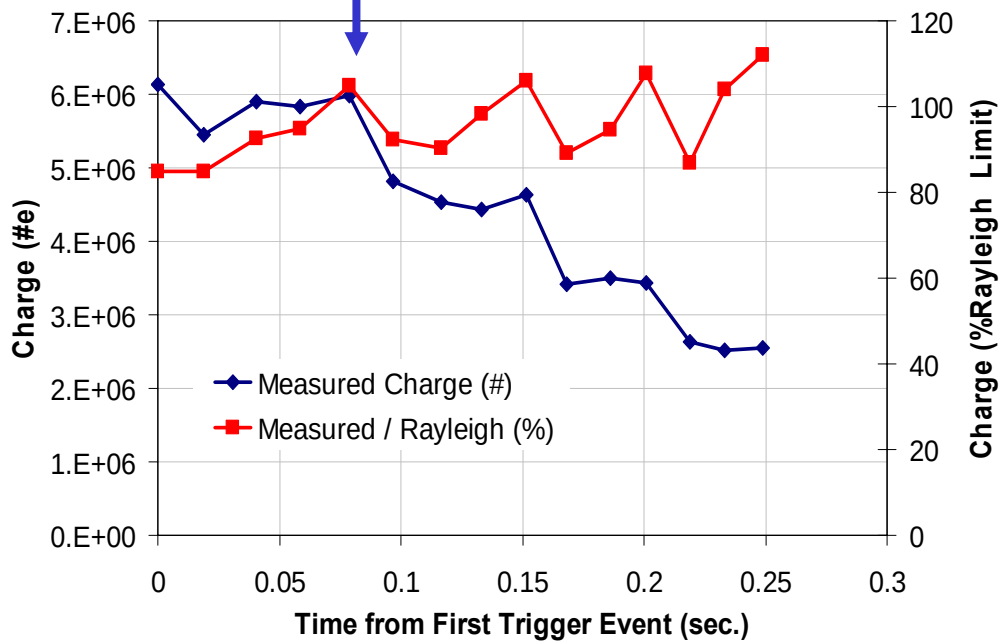


Repeated Reversal of the Drift Field Permits Multiple Measurements of the Size and Charge of a Single Droplet

Droplet Ping-Pong

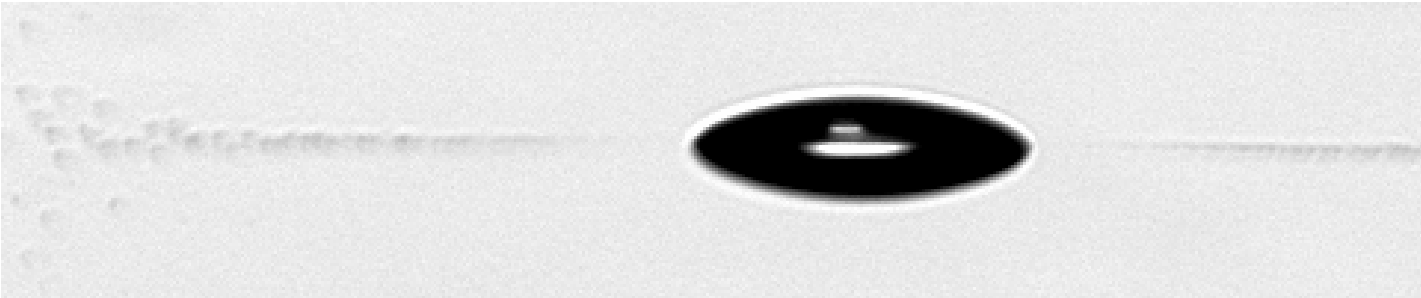
Evaporation and Discharge of a Positively Charged Methanol Droplet



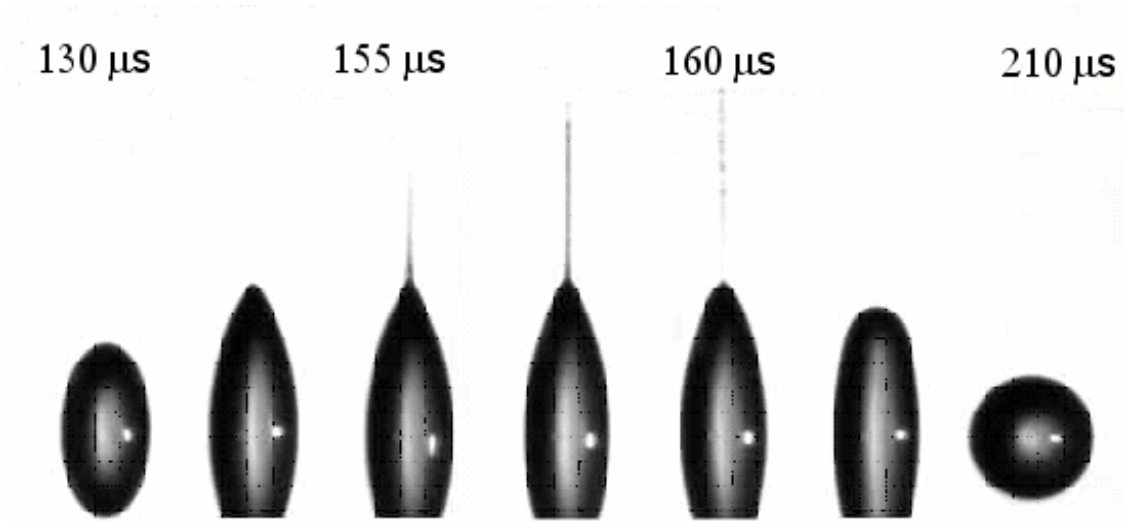


Note that at the time of observed discharge events (in which 15-20% of the charge is lost) there is no measurable decrease in droplet diameter!

Photographs of Droplet Discharge Processes



Ethylene Glycol Rayleigh Discharge



D. Duft, T. Atchtzehn, R. Muller, B. A. Huber, T. Leisner, *Nature* 421, 6919 (2003).

A Prediction Ahead of its Time!

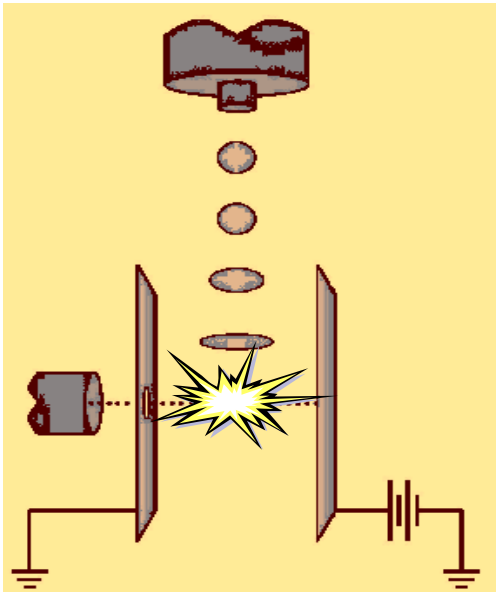
***"On the Equilibrium of Liquid Conducting Masses Charged with Electricity"*, Lord Rayleigh, F.R.S, Proc. Roy. Soc. 184-186 (1882).**

If $T > \frac{Q^2}{16\pi a_0^3}$, the spherical form is stable for all displacements. When Q is great, the spherical form is unstable for all values of n below a certain limit, the maximum instability corresponding to a great, but still finite, value of n. Under these circumstances the liquid is thrown out in fine jets, whose fineness, however, has a limit.

Field Induced Droplet Ionization (FIDI)

A New Route to Gas Phase Biomolecules and Molecular Clusters (From Neutral Droplets)

Bug Zapper

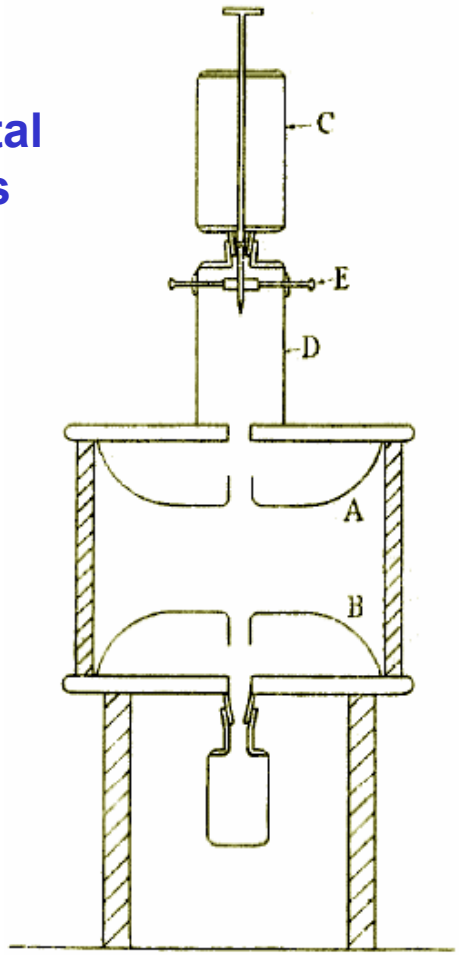


Droplet Zapper

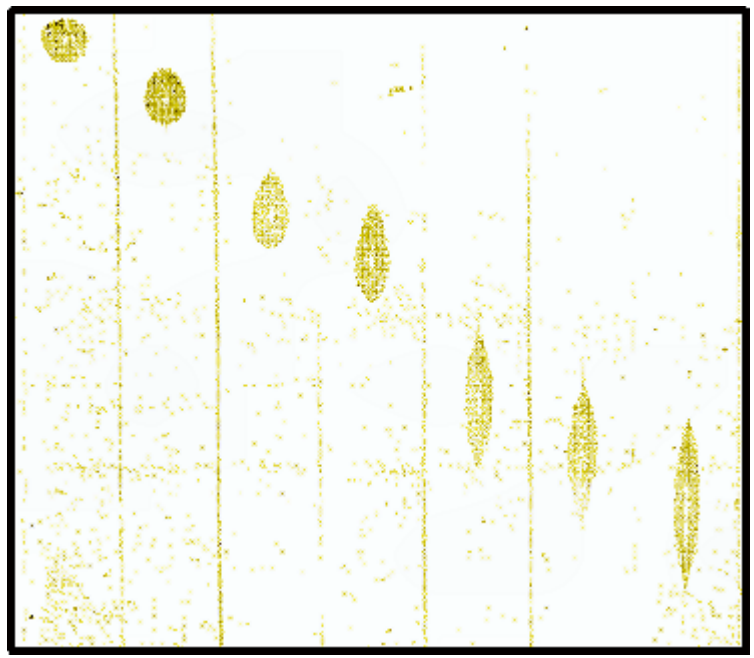
"Some Investigations on the Deformation and Breaking of Water Drops in Strong Electric Fields"

W. A. Macky, *Proc. Roy. Soc. A* 133, 565 (1931)

Experimental Apparatus



2.5 mm Drop at 8.7 kV/cm



Early Experimental and Theoretical Investigations of Liquid Droplets in Strong Electric Fields

Electric field necessary to induce instability and discharge event

Surface tension of liquid

$$E^2 = c\frac{\sigma}{4\pi\varepsilon_0 r}$$

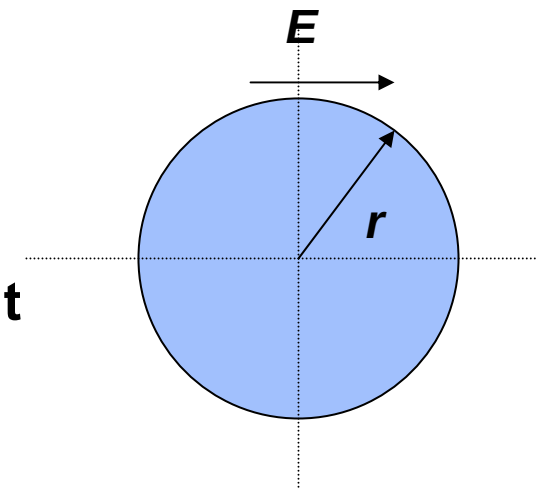
Droplet Radius

J. Zeleny, *Phys. Rev.* 10, 1 (1917).
C. T. R. Wilson, G. I. Taylor, *Proc. Cambridge Philos. Soc.* 22, 728 (1925).
J. J. Nolan, *Proc. R. Irish Acad. Sect. A* 37, 28 (1926).
W. A. Macky, *Proc. Roy. Soc. A* 133, 565 (1931).
G. Taylor, *Proc. R. Soc. London Ser. A* 280, 383 (1964).
G. Taylor, *Proc. R. Soc. London Ser. A* 291, 159 (1966).

Liquid Droplets in Electric Fields

r **= droplet radius**

ε = dielectric constant



Electric field inside the droplet:

$$E_{in} = \frac{3E}{\varepsilon + 2}$$

Outside the droplet the potential is equivalent to the applied field E plus the field of an electric dipole at the origin with dipole moment

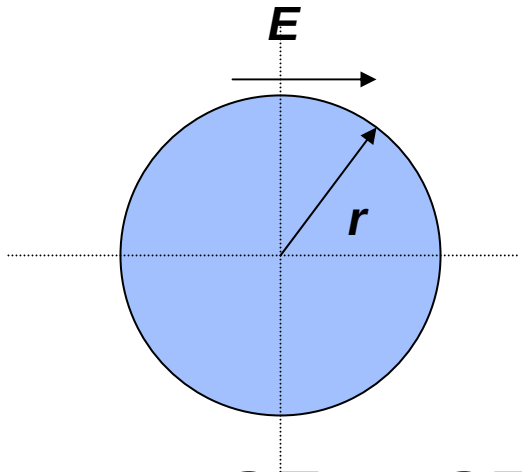
$$\mu = \left(\frac{\varepsilon - 1}{\varepsilon + 2} \right) r^3 E$$

(Reference: Jackson, Classical Electrodynamics, p. 113)

Liquid Droplets in Electric Fields

r **= droplet radius**

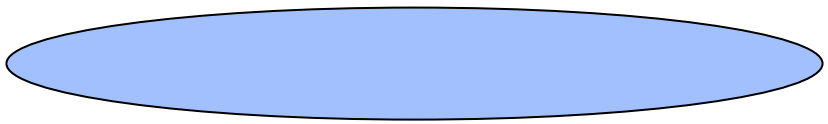
ε = dielectric constant



Electric field inside the droplet:

$$E_{in} = \frac{3E}{\varepsilon + 2} \approx \frac{3E}{\varepsilon} \text{ for large } \varepsilon$$

Stretched droplet:

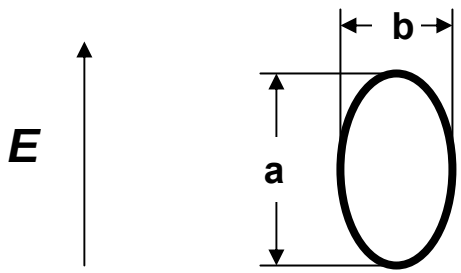


$$E_{in} \approx E$$

(Reference: Jackson, Classical Electrodynamics, p. 113)

Early Experimental and Theoretical Investigations of Liquid Droplets in Strong Electric Fields

Small displacements from spherical geometry are proportional to the square of the applied field:



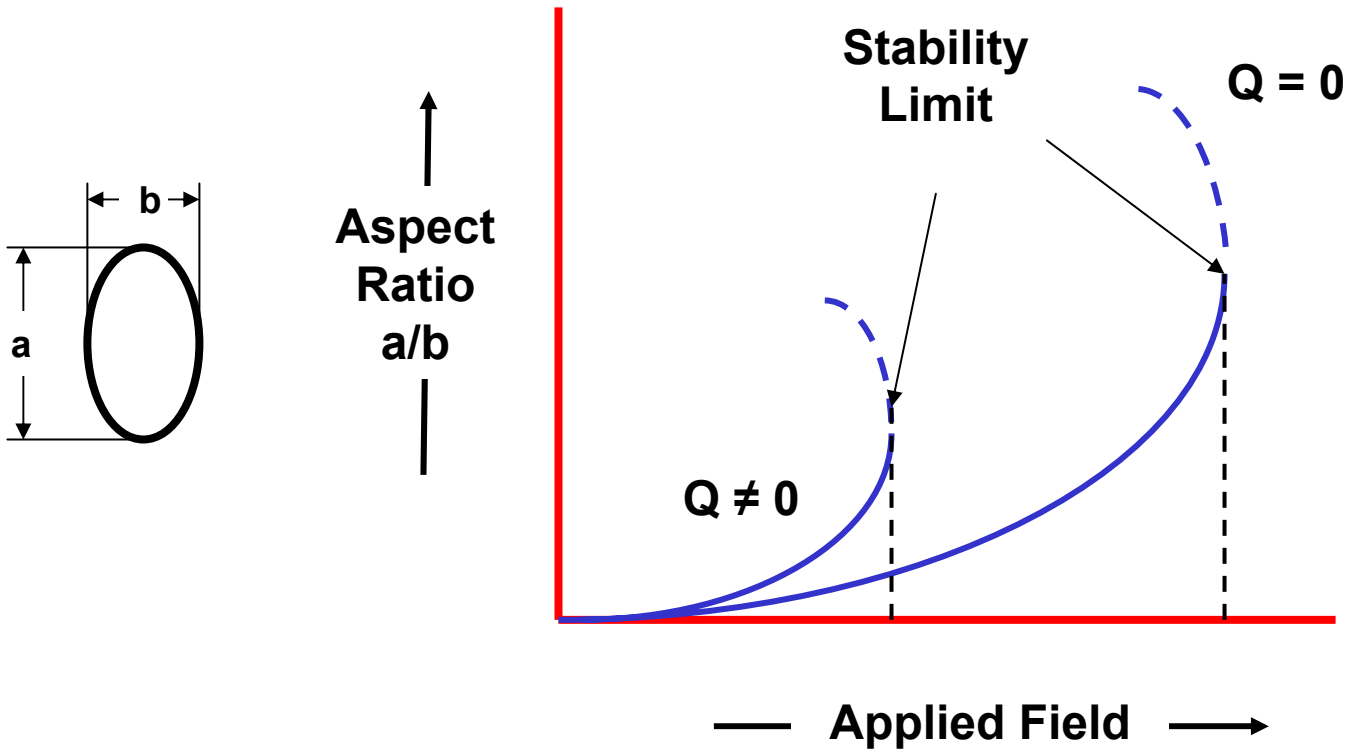
Droplet Radius

$$D = \frac{a-b}{a+b} \approx \frac{9rE^2}{16\sigma}$$

Surface tension of liquid

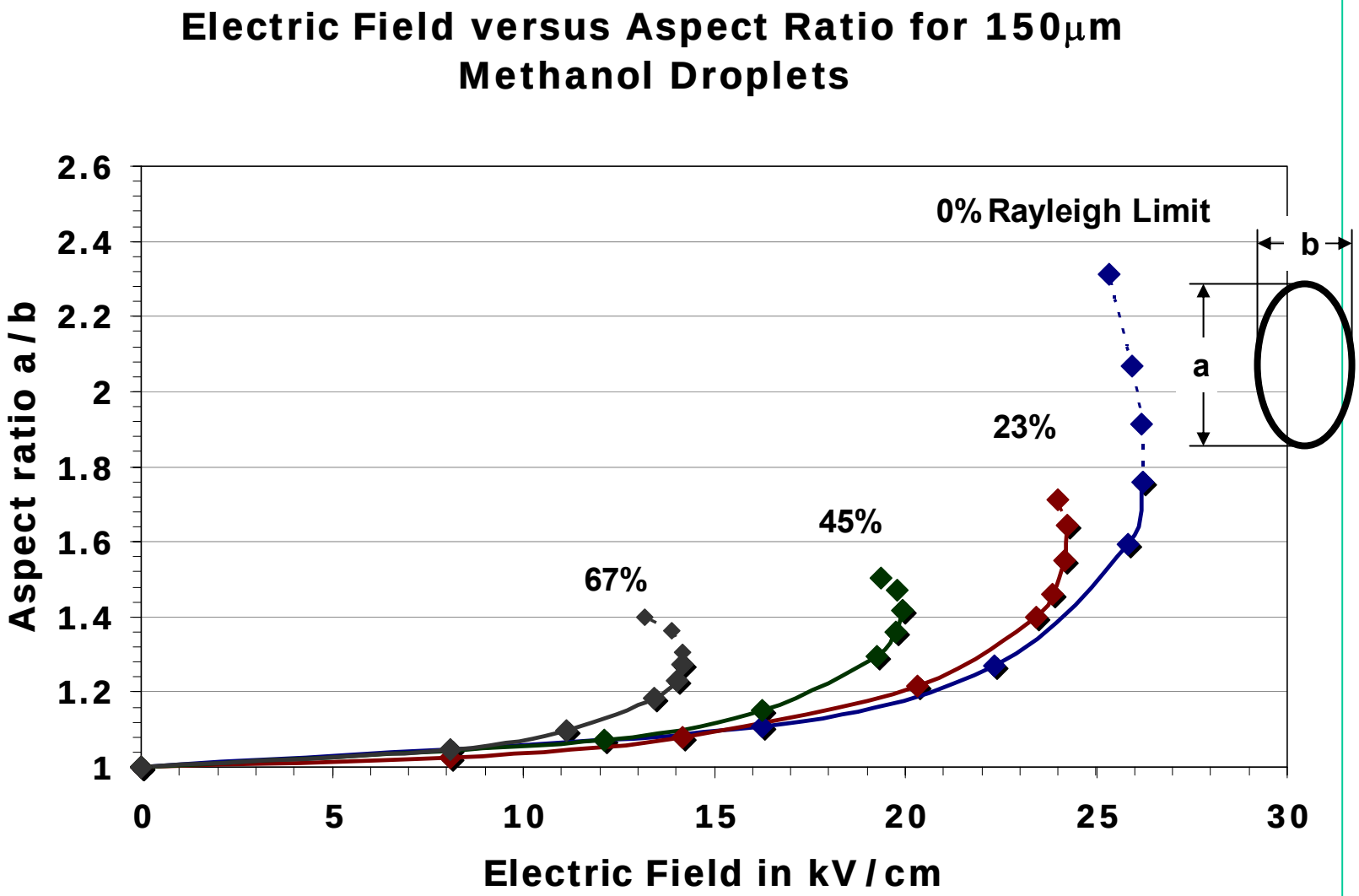
J. Zeleny, *Phys. Rev.* 10, 1 (1917).
C. T. R. Wilson, G. I. Taylor, *Proc. Cambridge Philos. Soc.* 22, 728 (1925).
J. J. Nolan, *Proc. R. Irish Acad. Sect. A* 37, 28 (1926).
W. A. Macky, *Proc. Roy. Soc. A* 133, 565 (1931).
G. Taylor, *Proc. R. Soc. London Ser. A* 280, 383 (1964).
G. Taylor, *Proc. R. Soc. London Ser. A* 291, 159 (1966).

Droplet Distortion in Strong Electric Fields



As the strength of the electric field is increased, the droplet distorts into a prolate shape aligned with the field direction. At a critical field the droplet becomes unstable and develops conical tips that lead to formation of a cone-jet. This happens at a lower field when the droplet is charged.

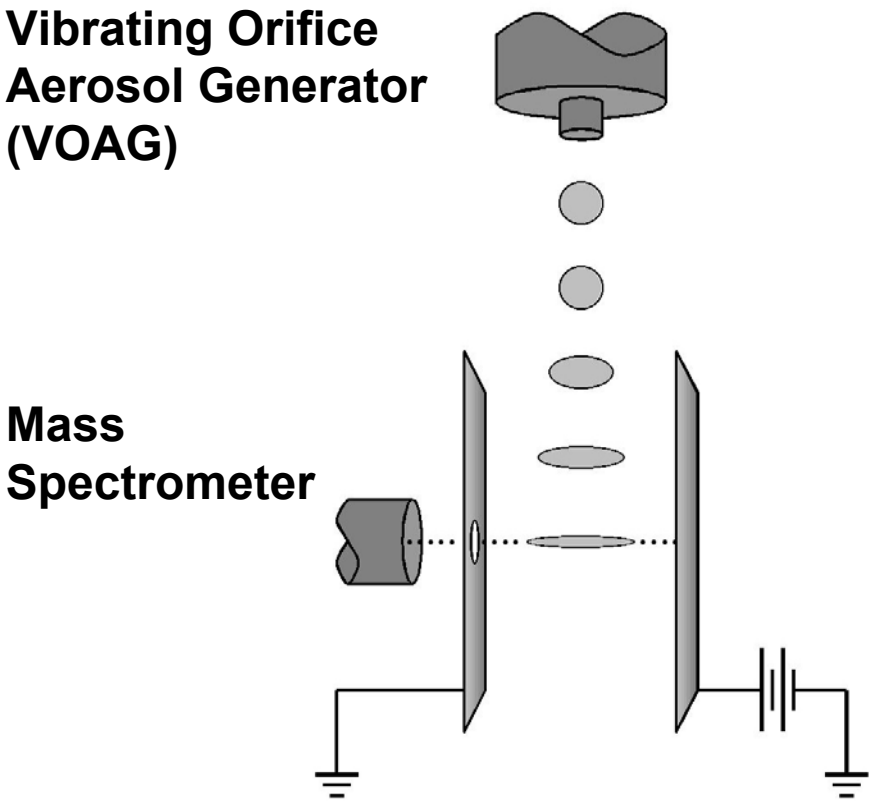
Droplet Distortion in Strong Electric Fields



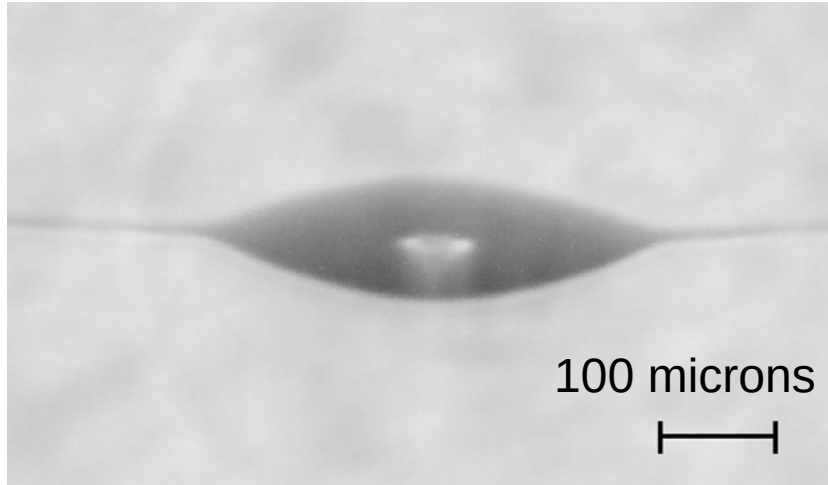
O. A. Basaran and L.E. Scriven, "Axisymmetric shapes and stability of charged drops in an external electric field," Phys. Fluids A, 1,799-809 (1989)

Field Induced Droplet Ionization (FIDI)

Neutral Droplet



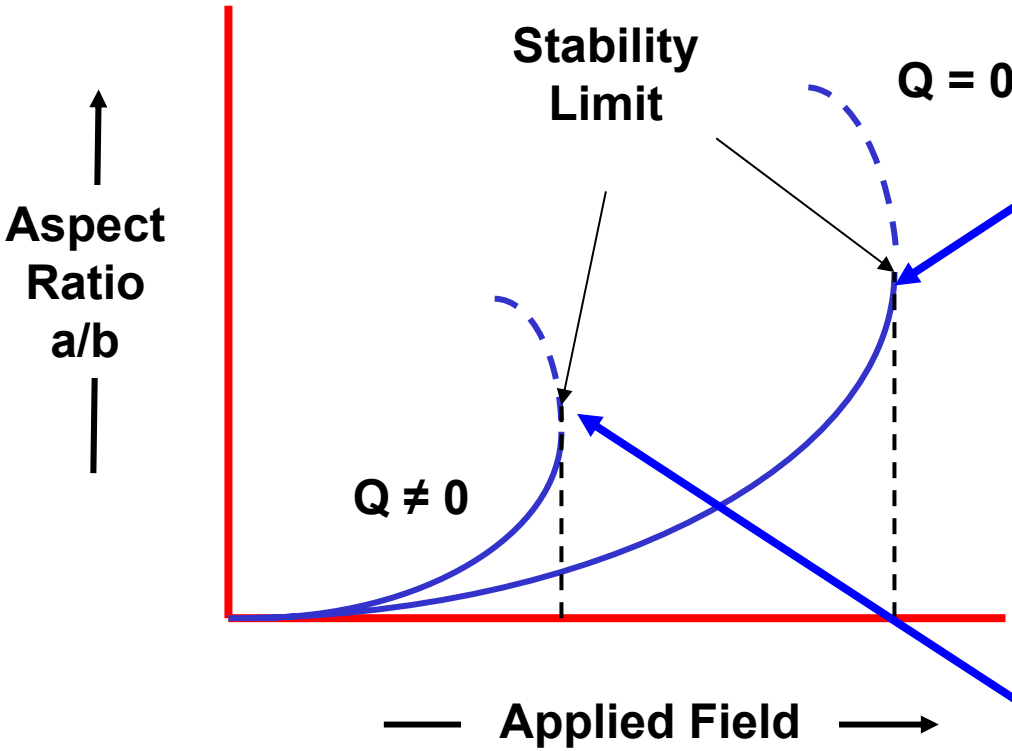
Experimental Arrangement

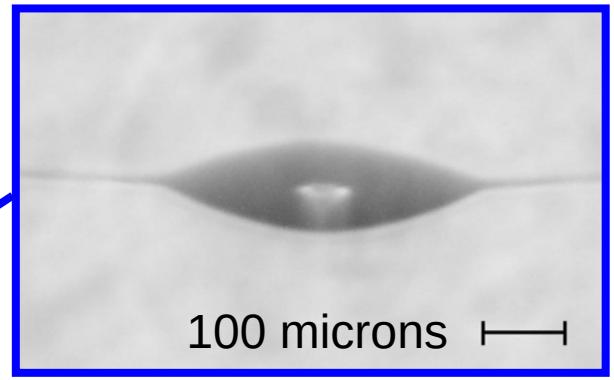


170 Micron Diameter Methanol Droplet at 22 kV/cm

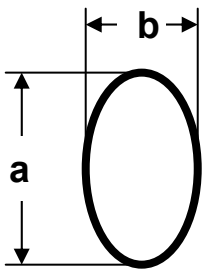
Theory: 170 micron neutral methanol droplet becomes unstable at 24 kV/cm and a/b = 1.8

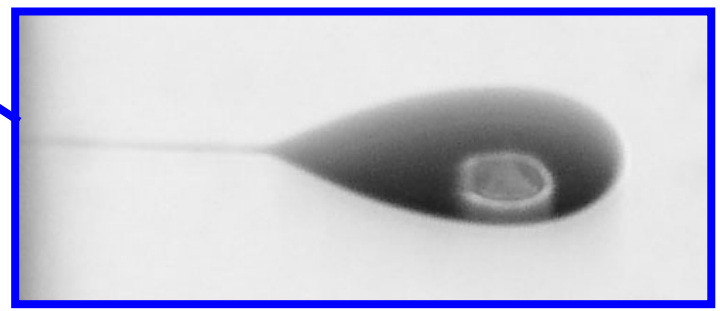
Field Induced Droplet Ionization Mass Spectrometry. Ronald L. Grimm and J.L. Beauchamp, *J. Phys. Chem. B*, **107**, 14161-14163 (2003).





170 micron diameter neutral methanol Droplet at 22 kV/cm: opposing positive and negative jets develop

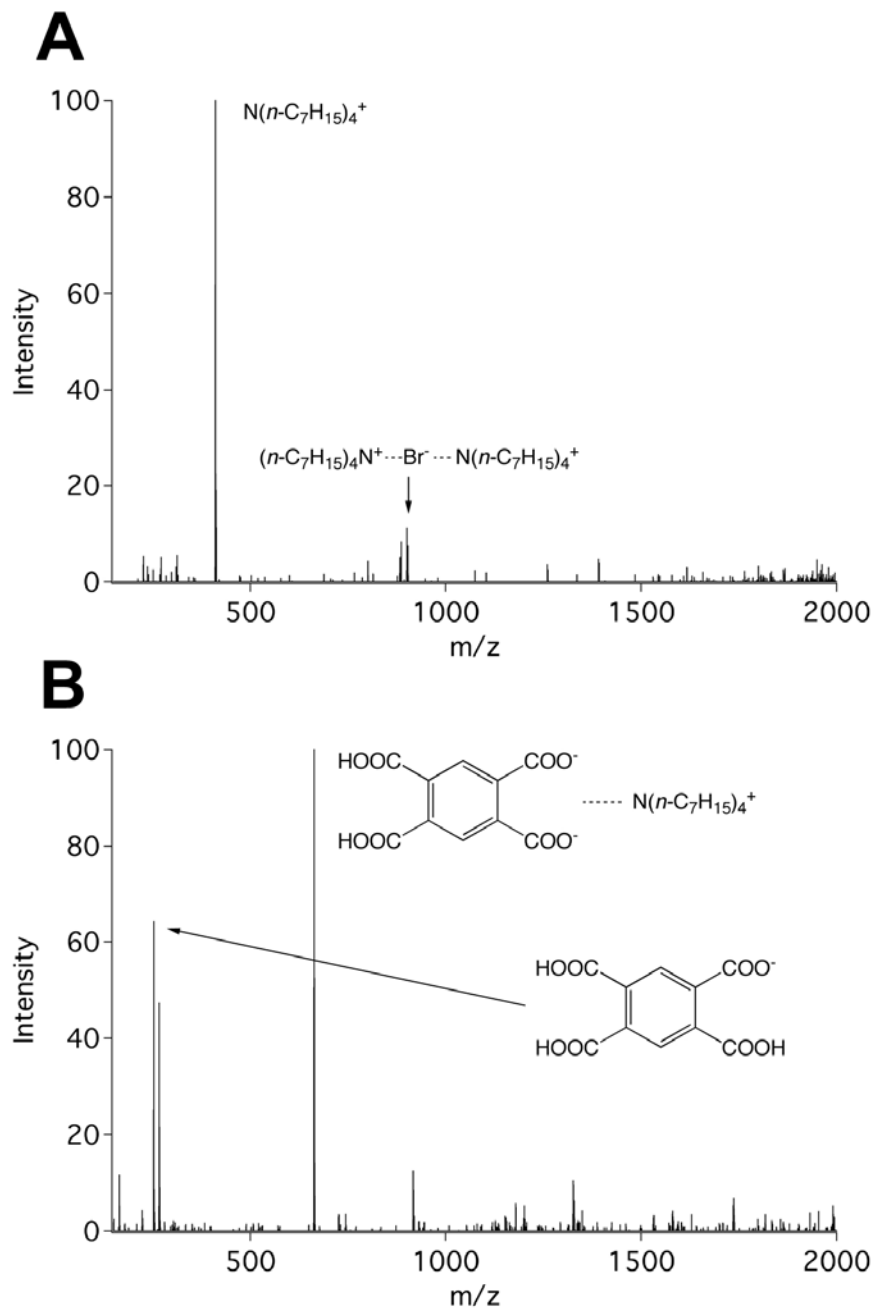




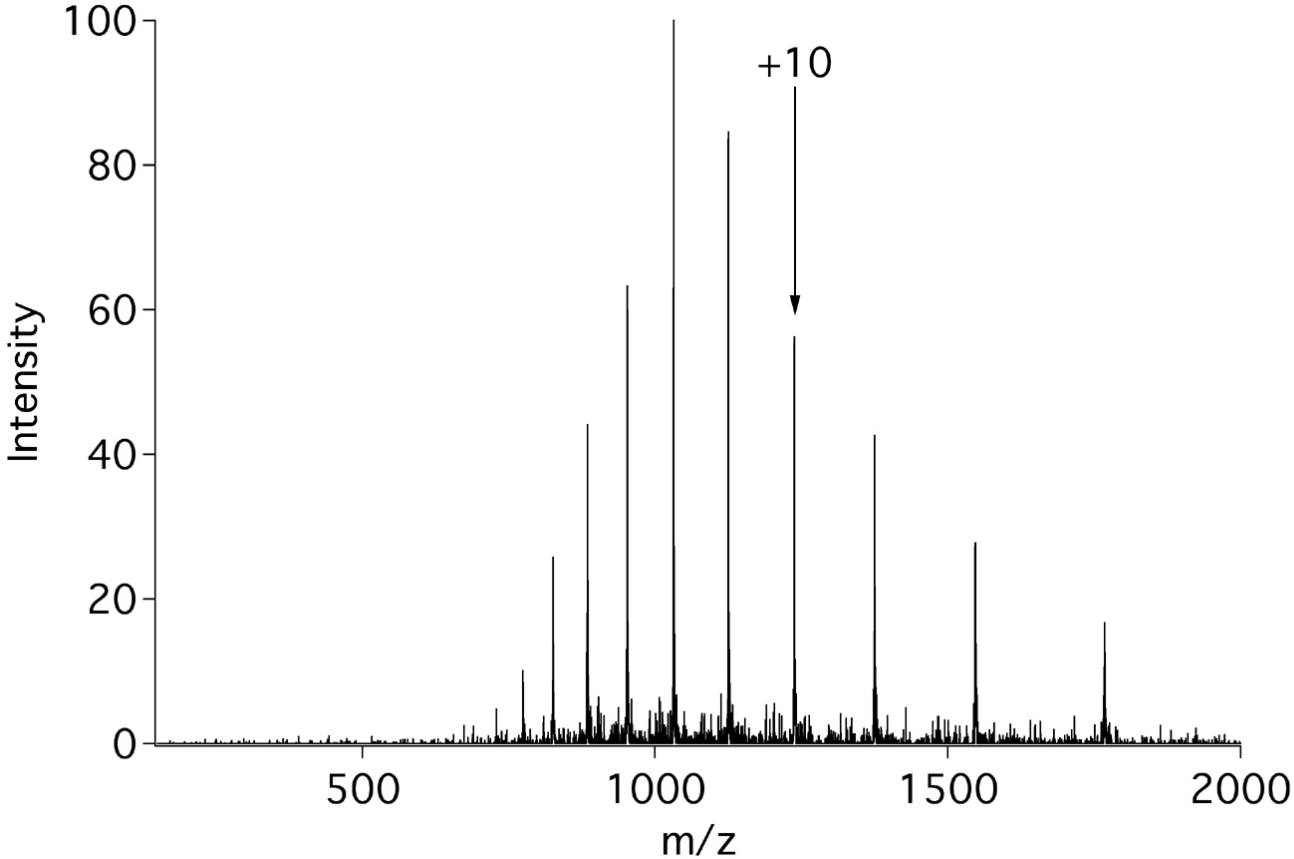
275 micron diameter charged methanol droplet at 20 kV/cm: A single positive jet develops.

Field Induced Droplet Ionization (FIDI)

Positive ion (A) and negative ion (B) mass spectra of a methanol droplet containing an alkyl ammonium salt and an organic acid.



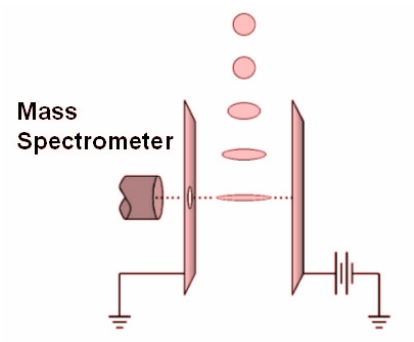
Field Induced Droplet Ionization (FIDI)



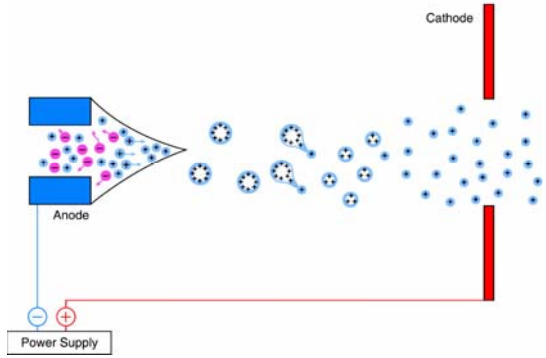
Mass spectrum of a 20 μM solution of horse heart cytochrome-c in 80 % methanol, 20 % water, and 0.1 % acetic acid. The broad distribution of charge states acquired with the FIDI-MS technique resembles the ESI-MS of the same solution.

Field Induced Droplet Ionization (FIDI) Differs from Electrospray Ionization

FIDI



ESI



FIDI adapts to any type of MS – quadrupole, ion trap, TOF, FT-ICR - can be combined with IMS for multidimensional analysis of complex mixtures

Simultaneous analysis of positive and negative ions

Sample individual aerosol droplets “on the fly” – easily interfaced with microfluidic devices

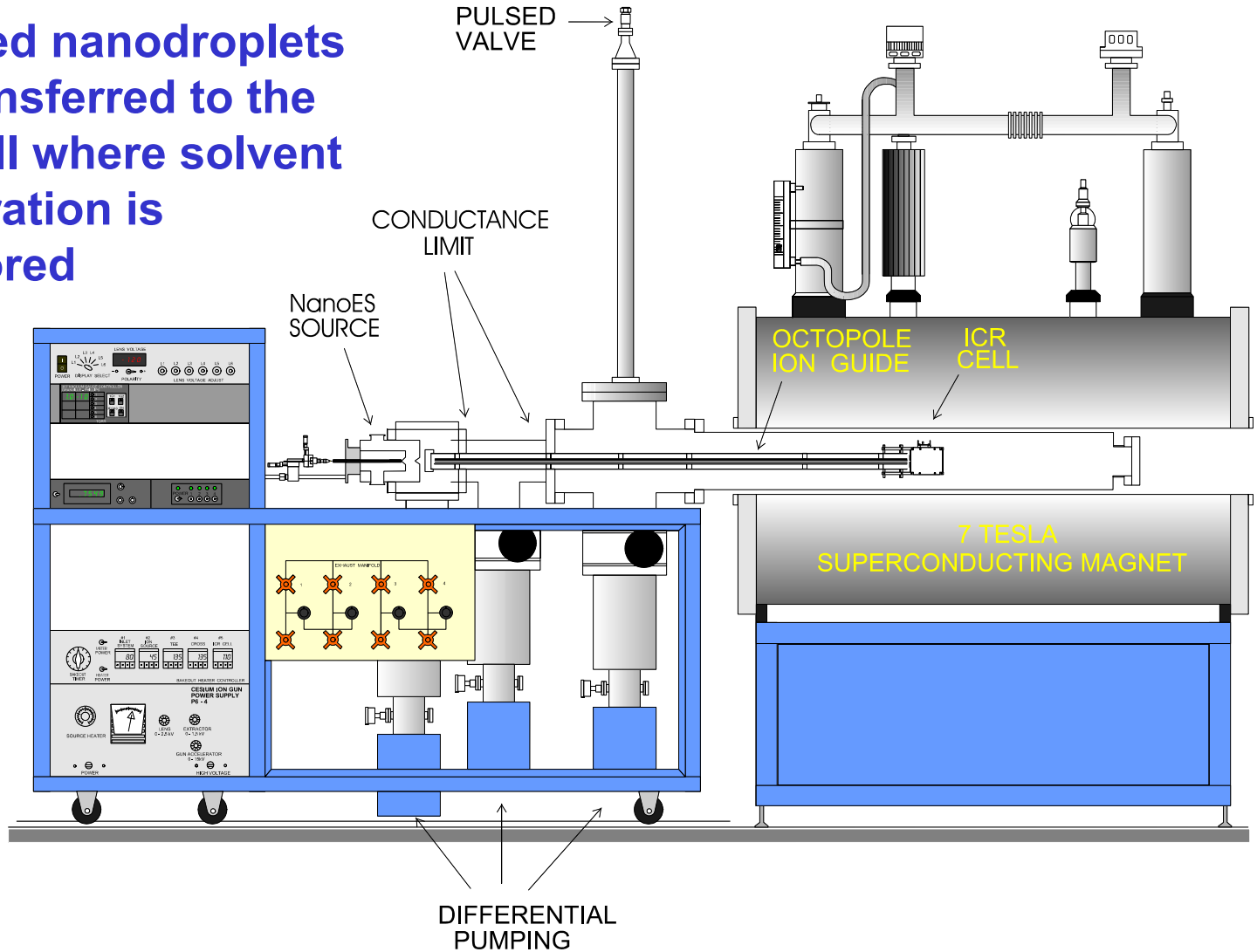
Continuous or repeated sampling from trapped or suspended droplets

Tolerant of high salt/biopolymer concentrations – no clogging of capillary

Cute trick department: drop hop experiment; rotating droplets; droplet oscillators; lyse cells in high electric fields

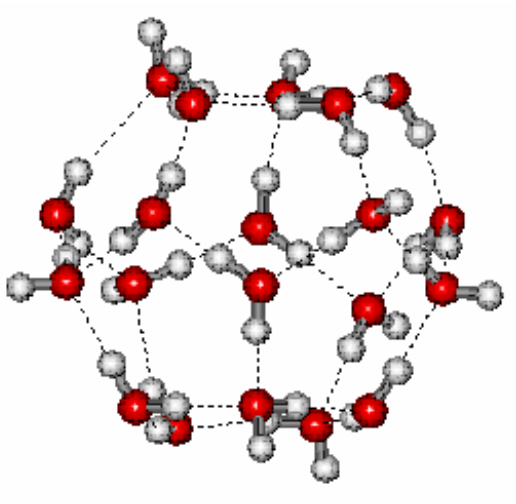
Final Stages of Ion Desolvation in ESI/FIDI External Ion Source FT-ICR Mass Spectrometer

Charged nanodroplets are transferred to the ICR cell where solvent evaporation is monitored

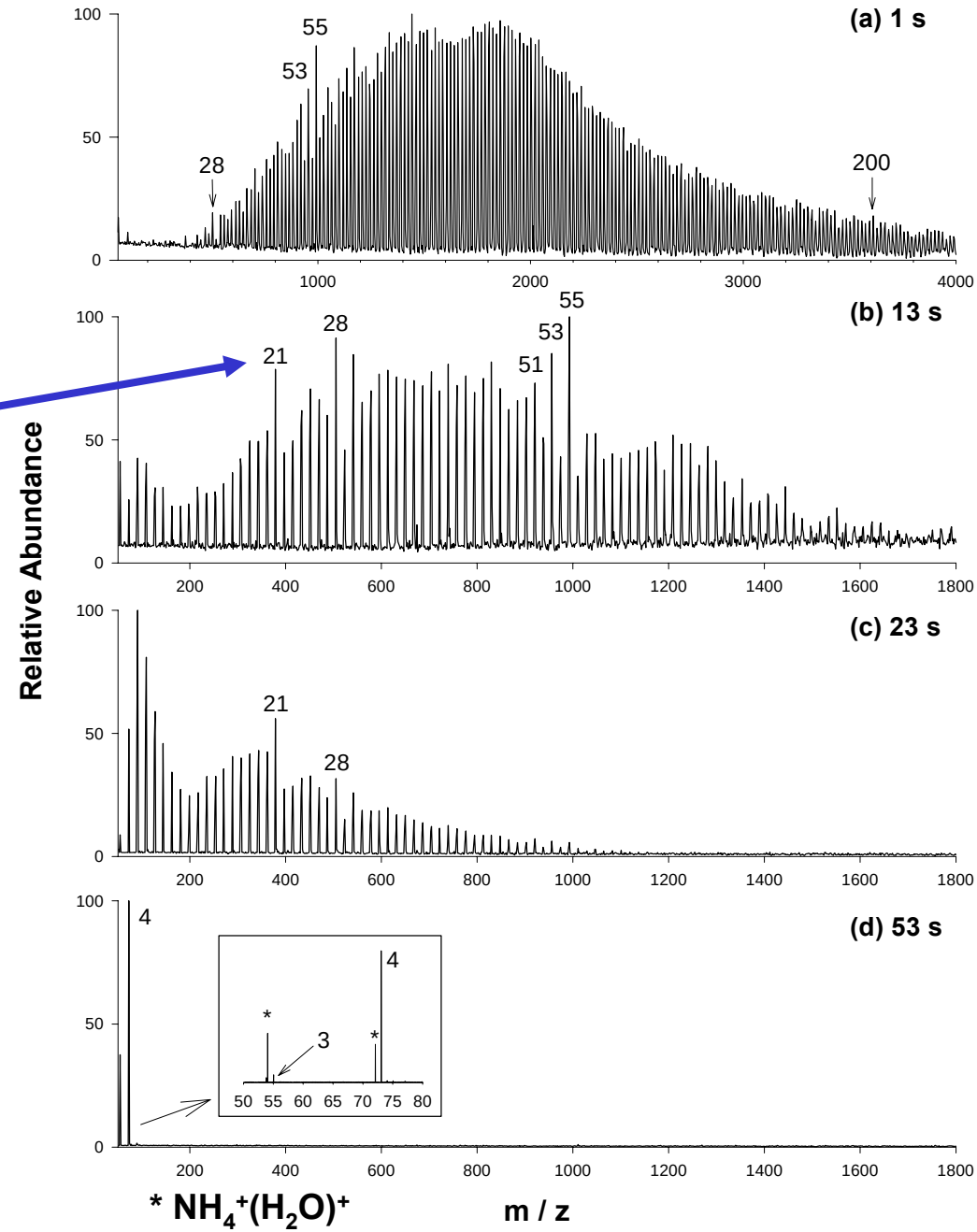


Final Stages of Ion Desolvation in ESI/FIDI

Clusters with special stability are observed (e.g. n = 21, 28)



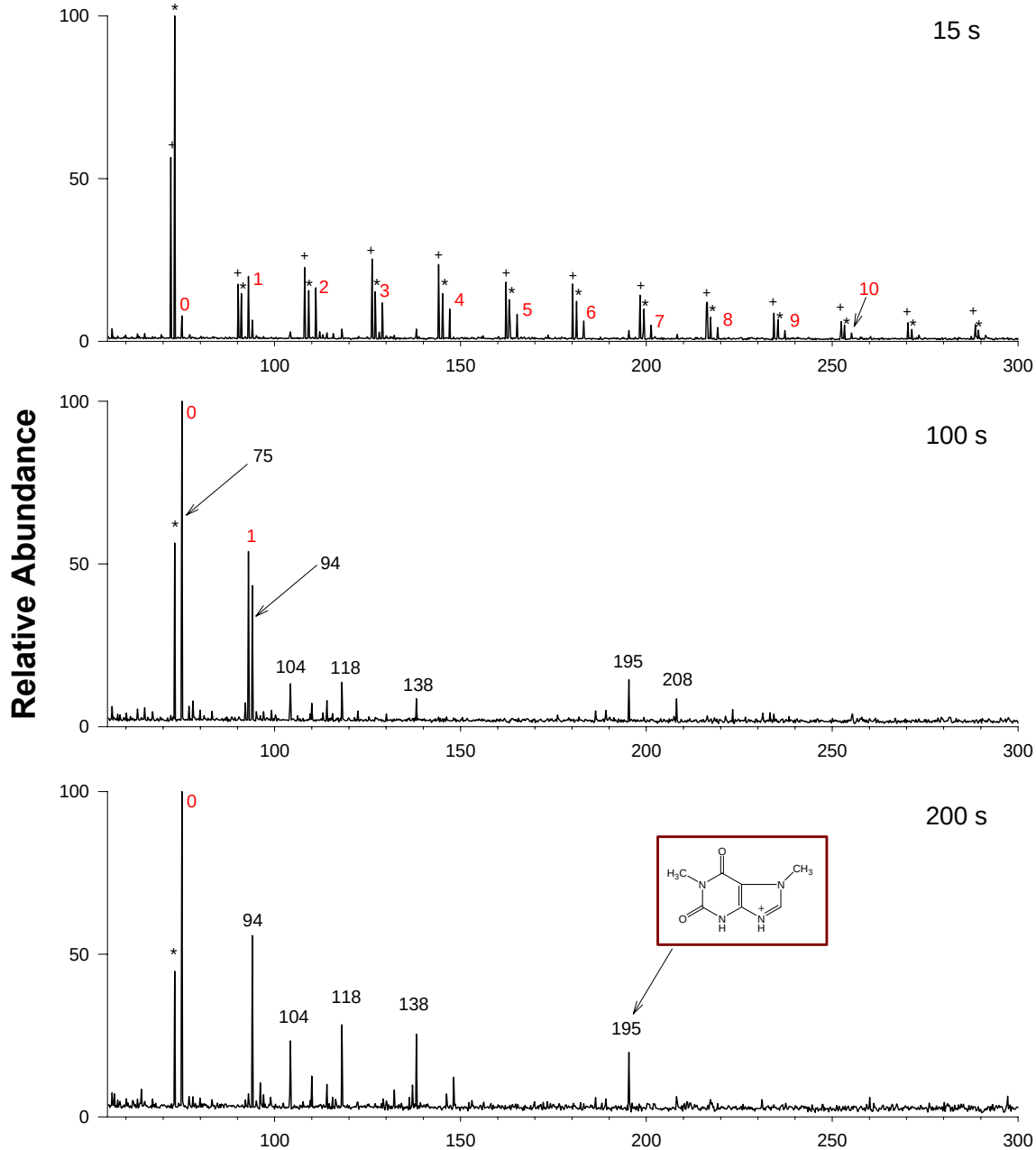
Pentagonal dodecahedron



“Real” Freeze Dried Coffee

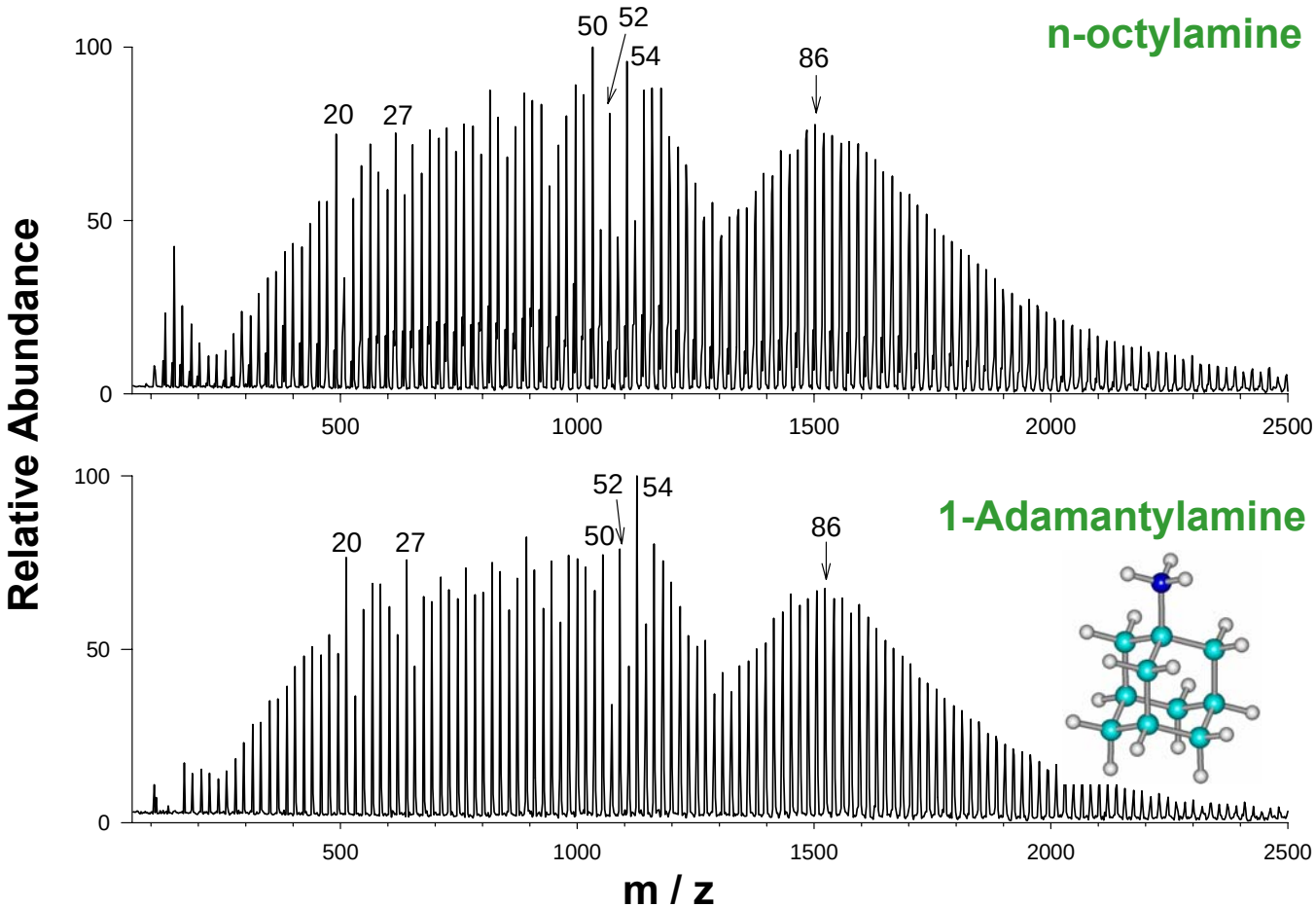
Peet’s Coffee (Major Dickason’s Blend)

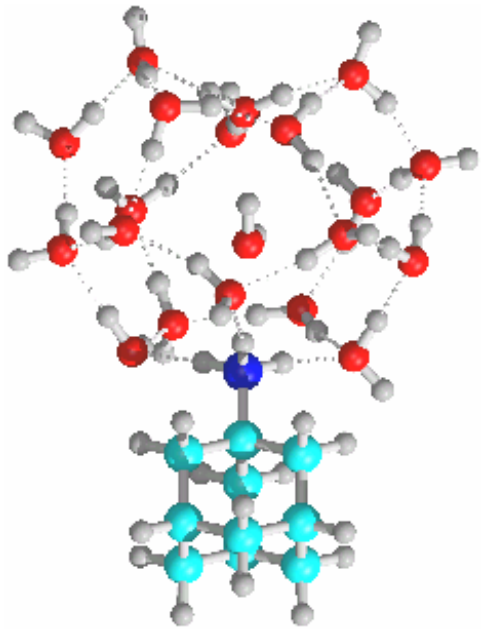
These experiments provide “soft sampling” conditions ideal for observing and studying molecular clusters and noncovalent complexes extracted from solution



* $H^+(H_2O)_m$ + $NH_4^+(H_2O)_m$

Cluster Distribution after 8 Seconds of Evaporation of Water from Solvated Protonated Primary Amines

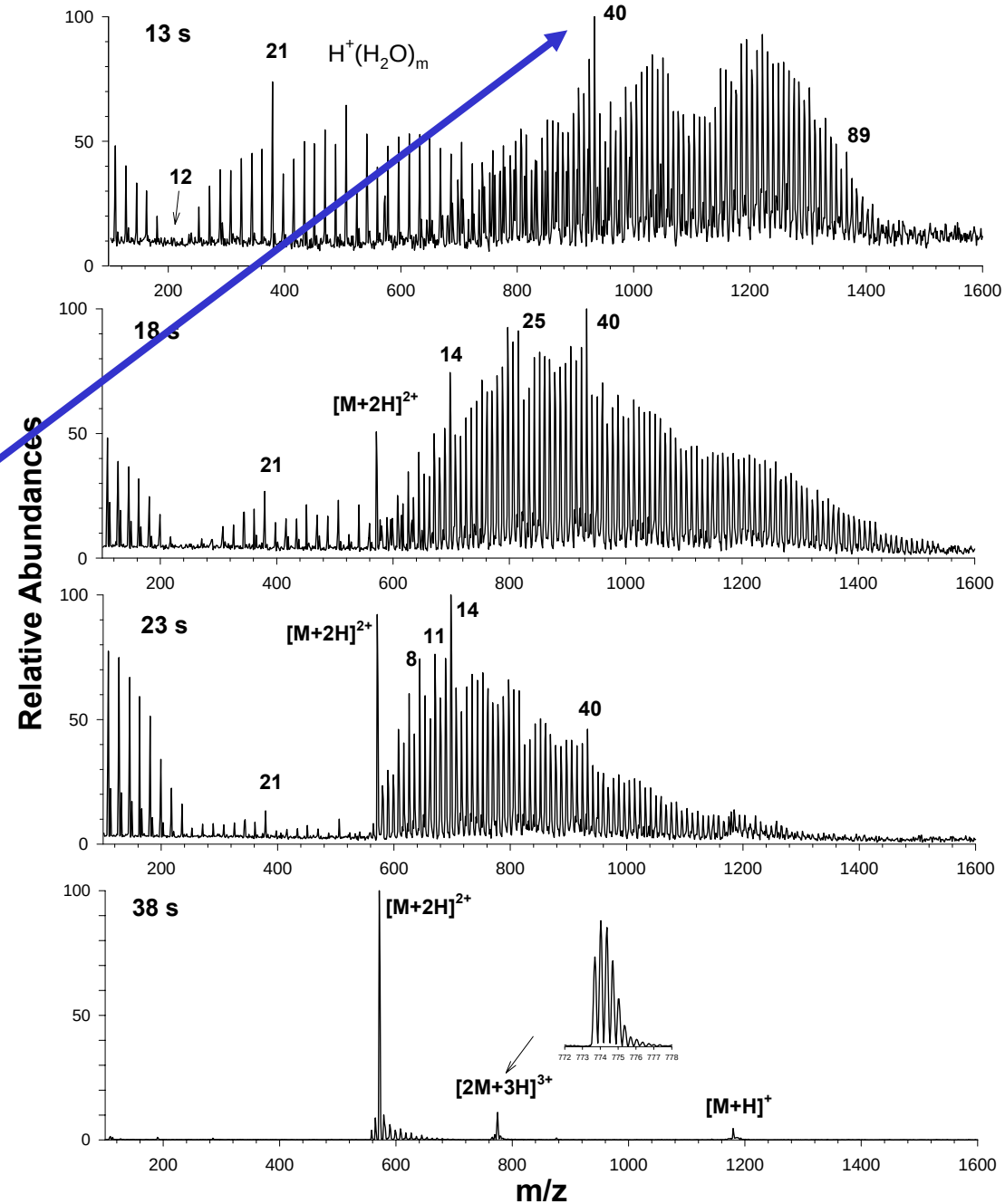




Proposed Structure of Water Cluster of 1-Adamantylamine with 20 Water Molecules (PM3 Calculation)

Solvation of both protonated primary amines is identical, even though the hydrocarbon portion of the molecules is very different.

Evaporation of Water from Water Clusters of Doubly Protonated Gramicidin S in FT-ICR



Note: Solvated peptide with 40 water molecules has special stability

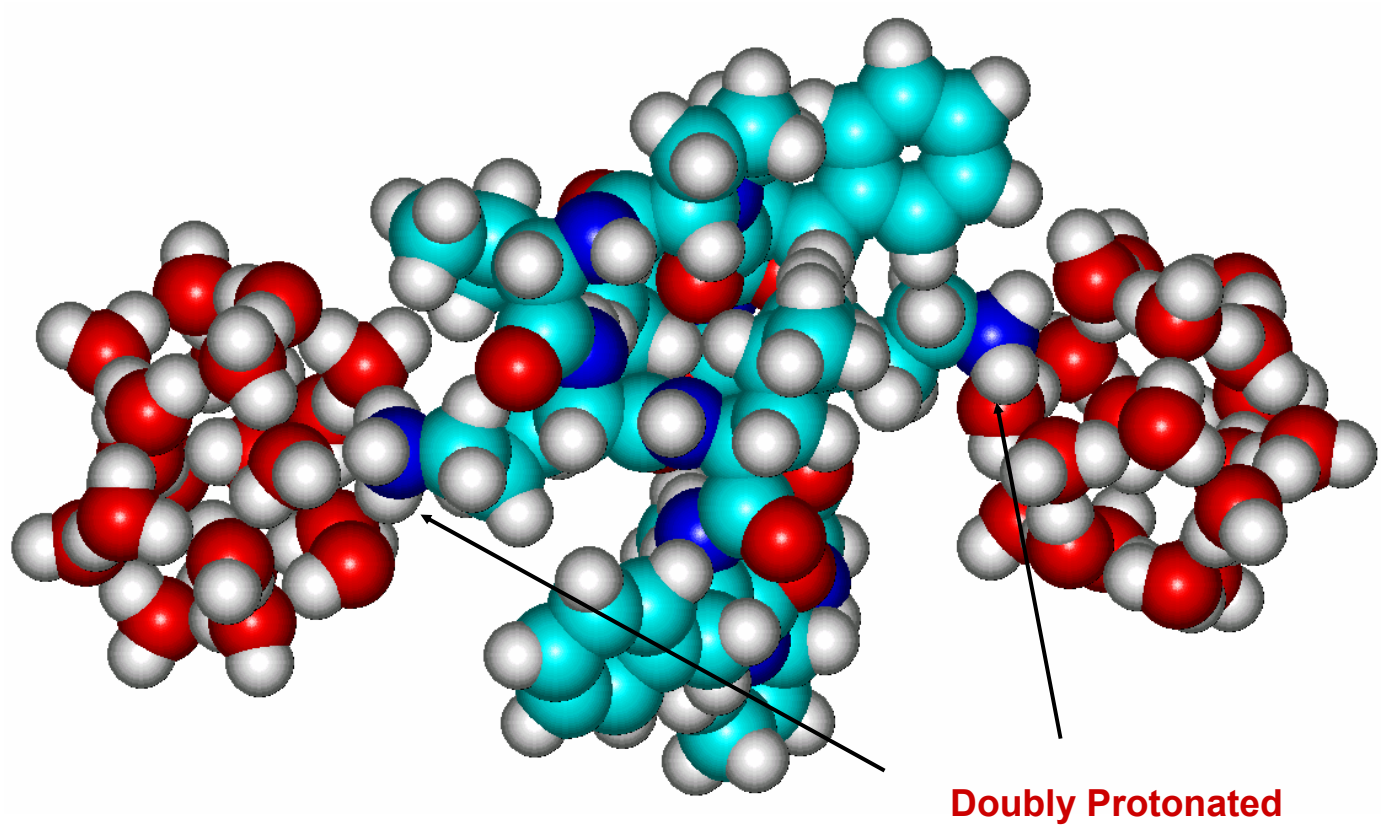
Freeze-Dried Biomolecules. Sang-Won Lee, Patrick Freivogel, Thomas Schindler, and J.L. Beauchamp, *J. Am. Chem Soc.*, 120, 11758-11765 (1998).

A Proposed Structure of Gramicidin S with 40 Water Molecules

Pro - Val - Orn - Leu - D-Phe

D-Phe - Leu - Orn - Val - Pro

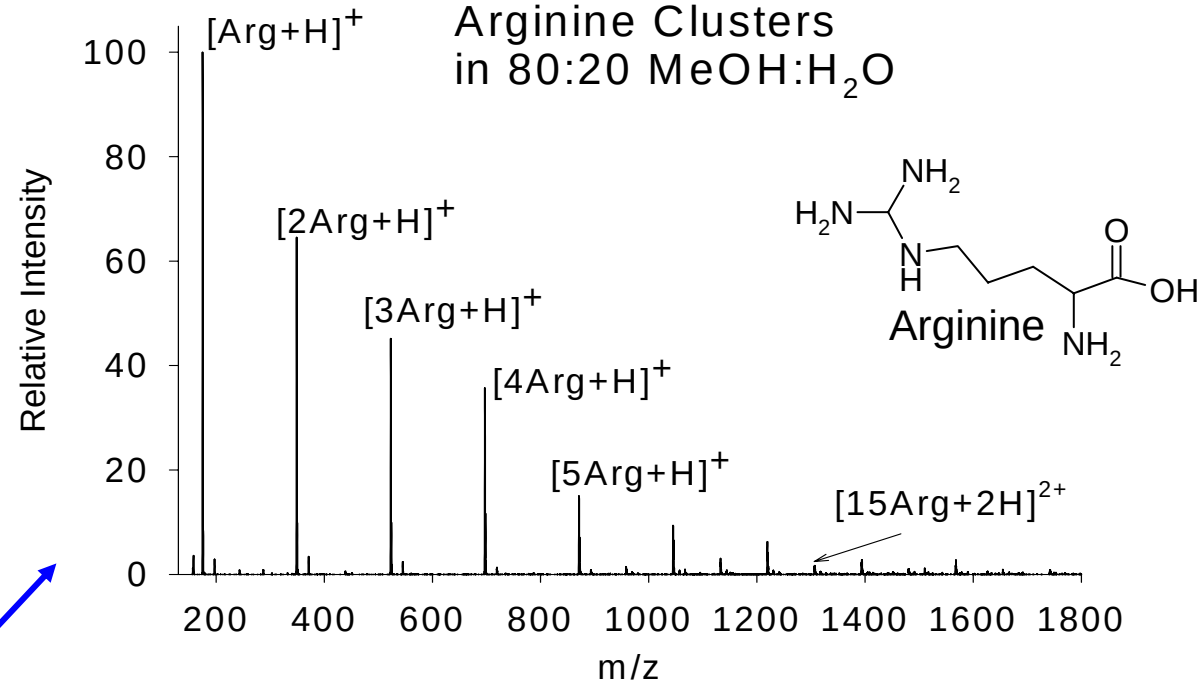
(Orn ≡ $-CH_2CH_2CH_2NH_2$)



Doubly Protonated

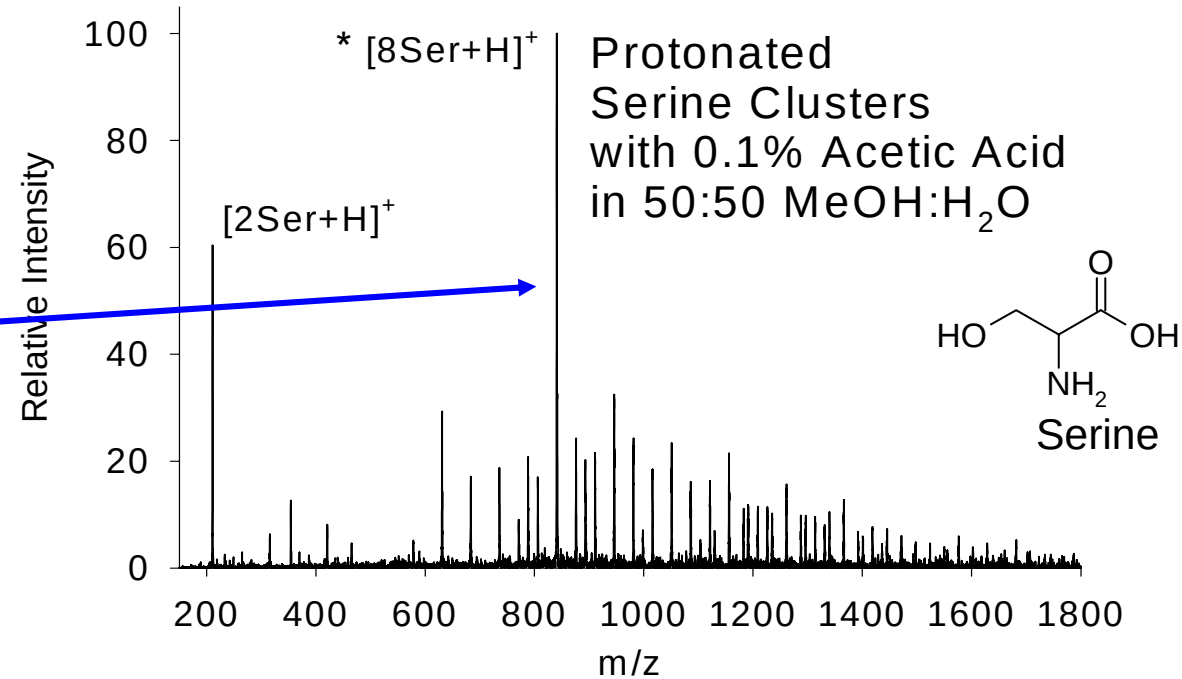
(Protons on Ornithines)

Soft Sampling Conditions Facilitate the Observation of Molecular Clusters



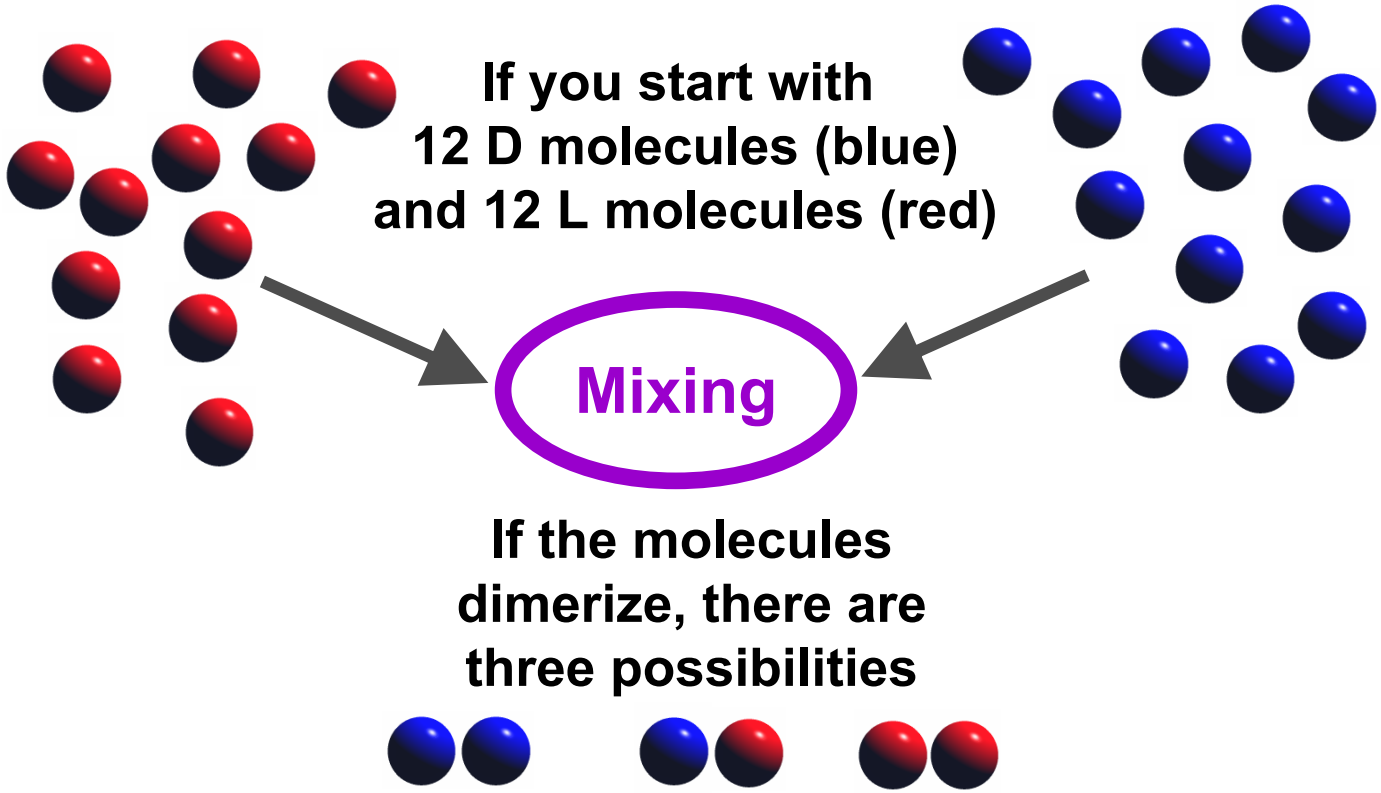
Non-specific clustering

"Magic Numbers"



*The peak is also composed of clusters of the form $[n(8Ser+H)]^{n+}$

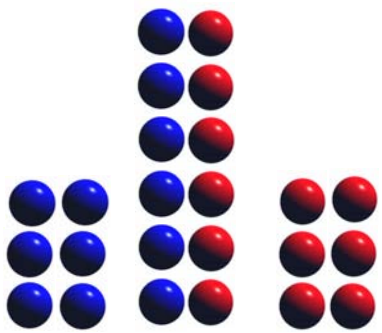
Methodology for Identifying Preference for Homochirality in Molecular Clusters



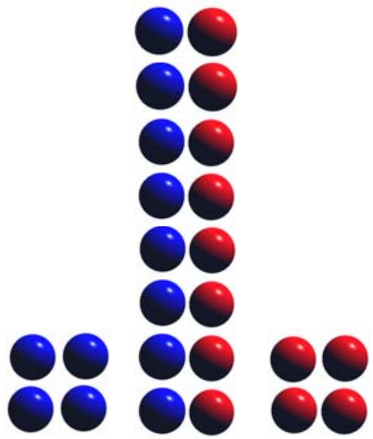
Methodology for Identifying Preference for Homochirality in Molecular Clusters

Consider the case of a dimer:

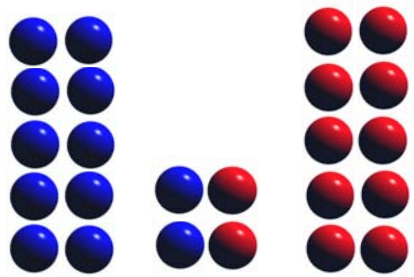
In the absence of chiral discrimination, mixing will follow a binomial distribution.



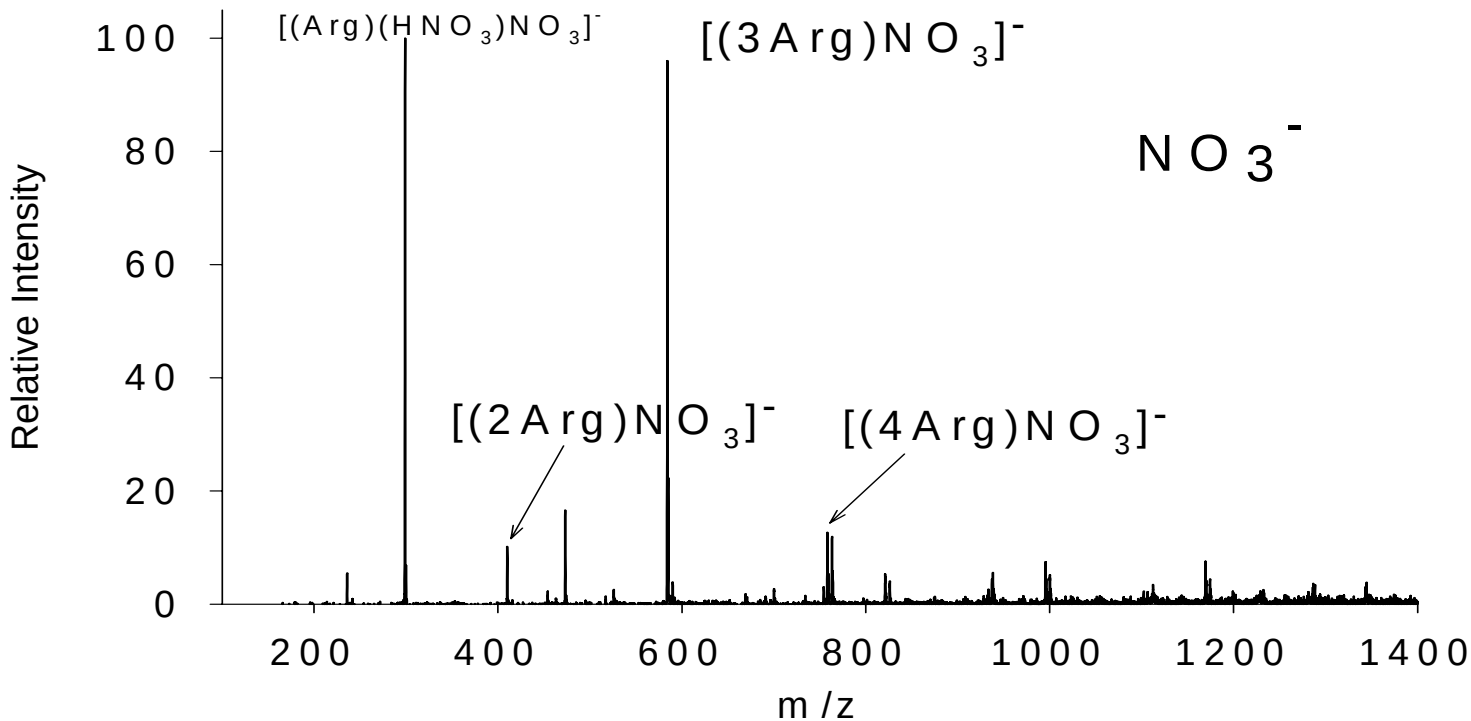
A heterochiral bias will result in a preference for the mixed cluster.



A bias towards homochirality will result in a bimodal distribution.

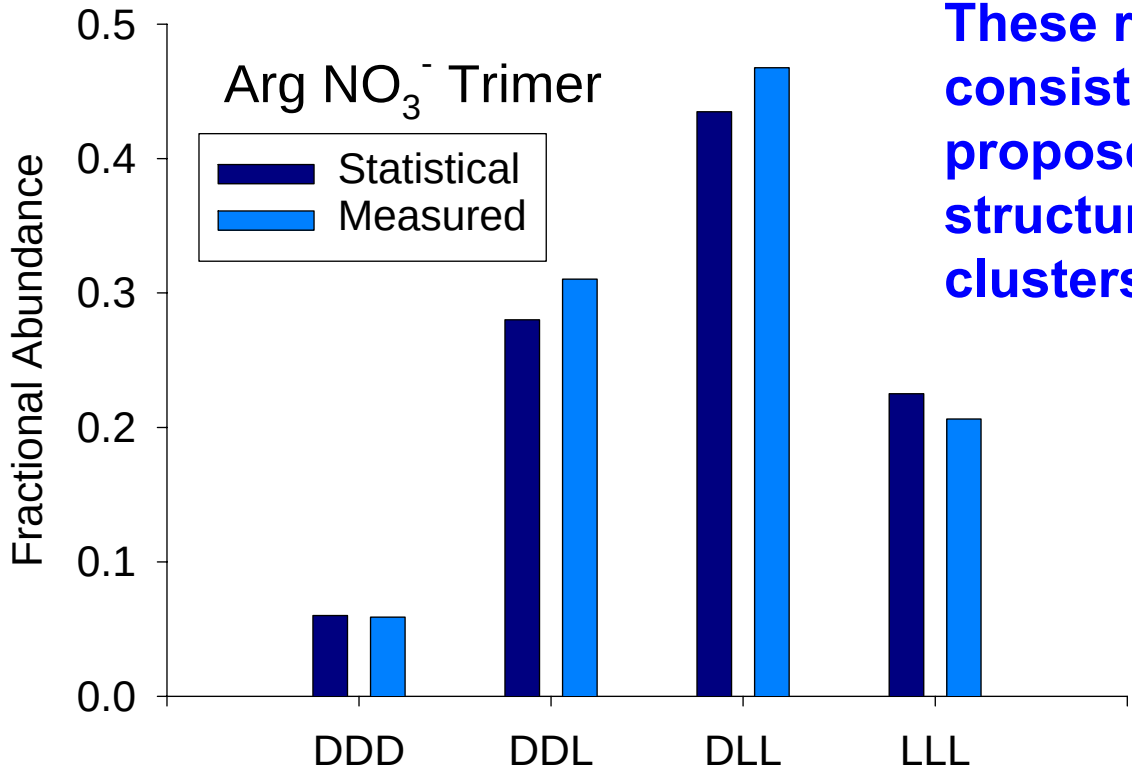


Arginine Forms an Unusually Stable Trimer with a Negative Ion Such as Cl^- or NO_3^- Attached



Unusually stable clusters are the best candidates for observation of chiral discrimination.

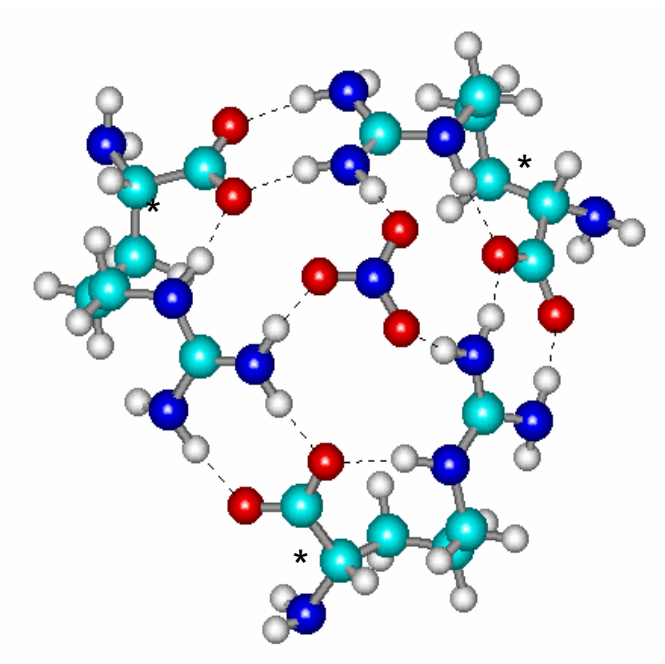
Anionic Arginine Trimers Have No Preference for Homochirality



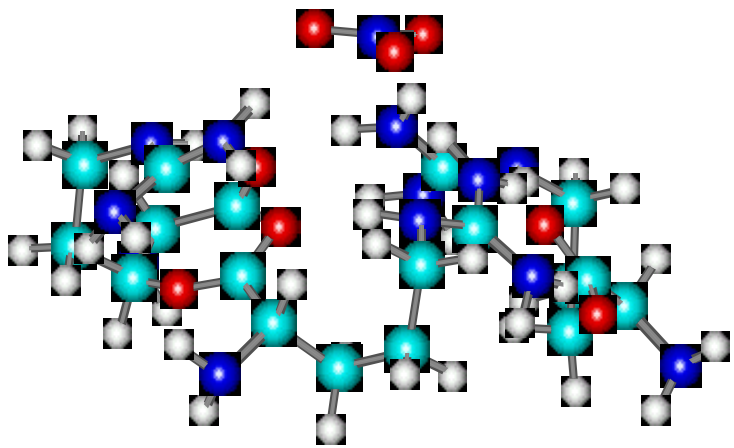
These results are consistent with the proposed structure for these clusters.

Salt Bridge Stabilization of Charged Zwitterionic Arginine Aggregates in the Gas Phase. Ryan R. Julian, Robert Hodyss and J.L. Beauchamp, *J. Am. Chem. Soc.*, 123, 3577-3583 (2001).

Structure of Anionic Arginine Trimer Capped by Nitrate Ion



Top View

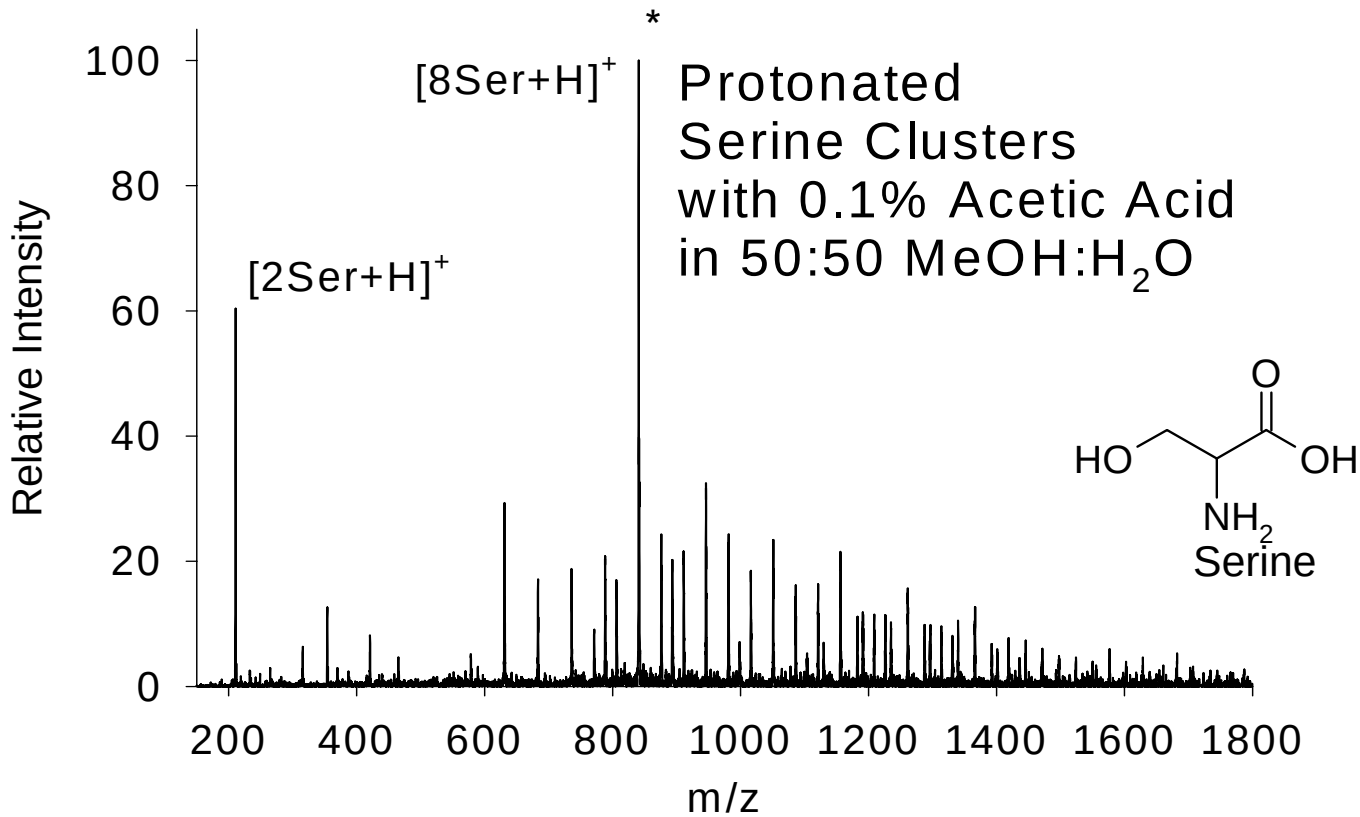


Side View

*Indicates chiral center

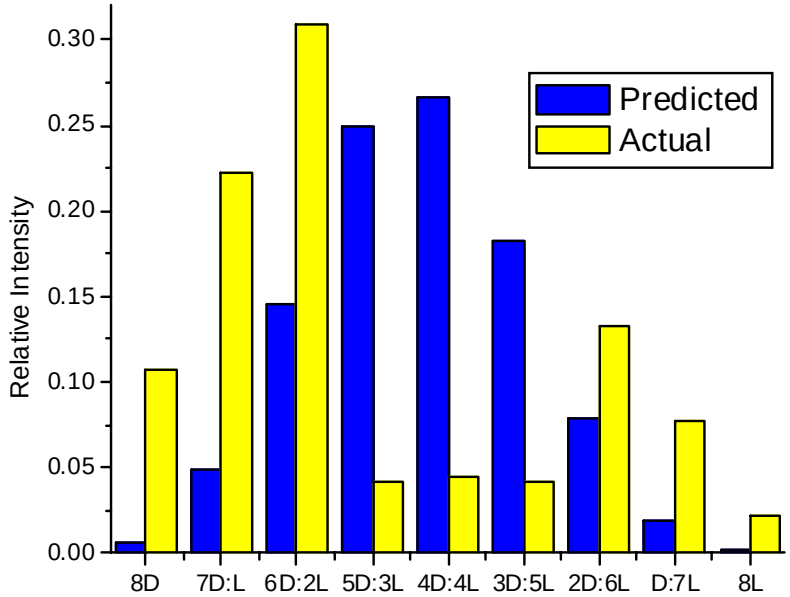
The stability of this cluster is determined by salt bridge interactions between adjacent zwitterionic arginines.

Serine Forms an Unusually Stable Octamer
Is it Homochiral?

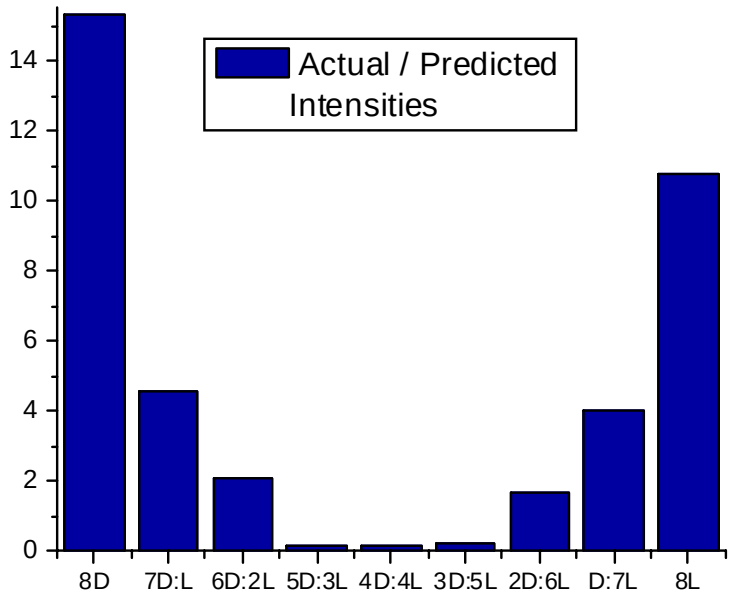


*The peak is also composed of clusters of the form $[n(8Ser+H)]^{n+}$

The protonated serine octamer exhibits a strong preference for homochirality



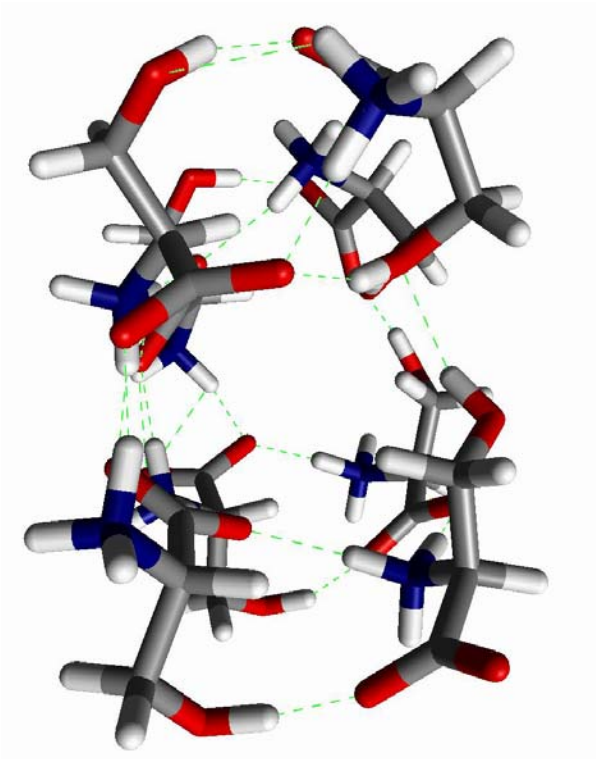
(Predicted results refer to statistical distribution)

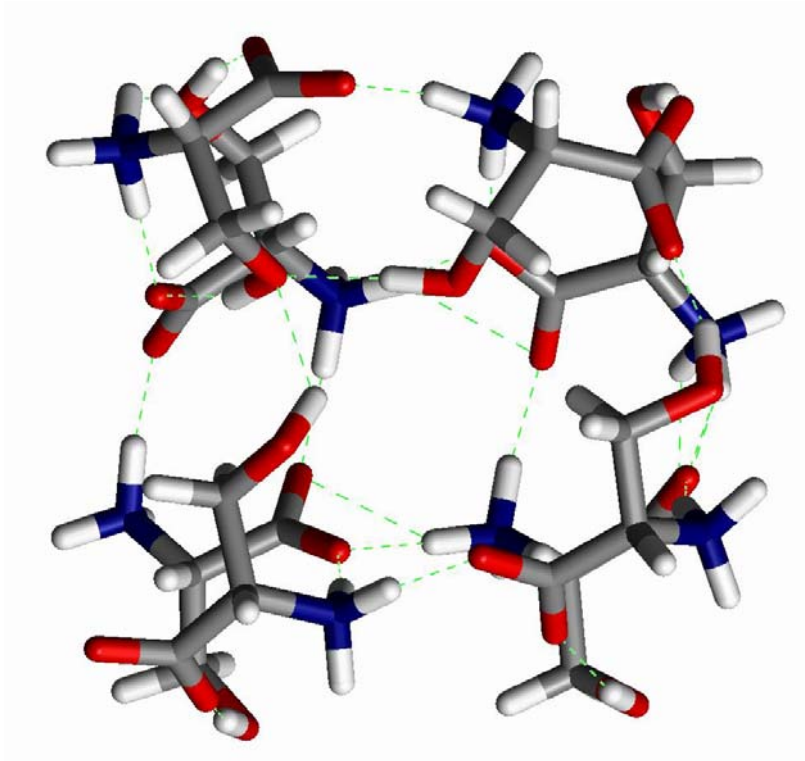


Ryan R. Julian, Robert Hodyss, Brian Kinnear, Martin F. Jarrold, and J. L. Beauchamp*

J. Phys. Chem. B **2002,** *106,* 1219-1228

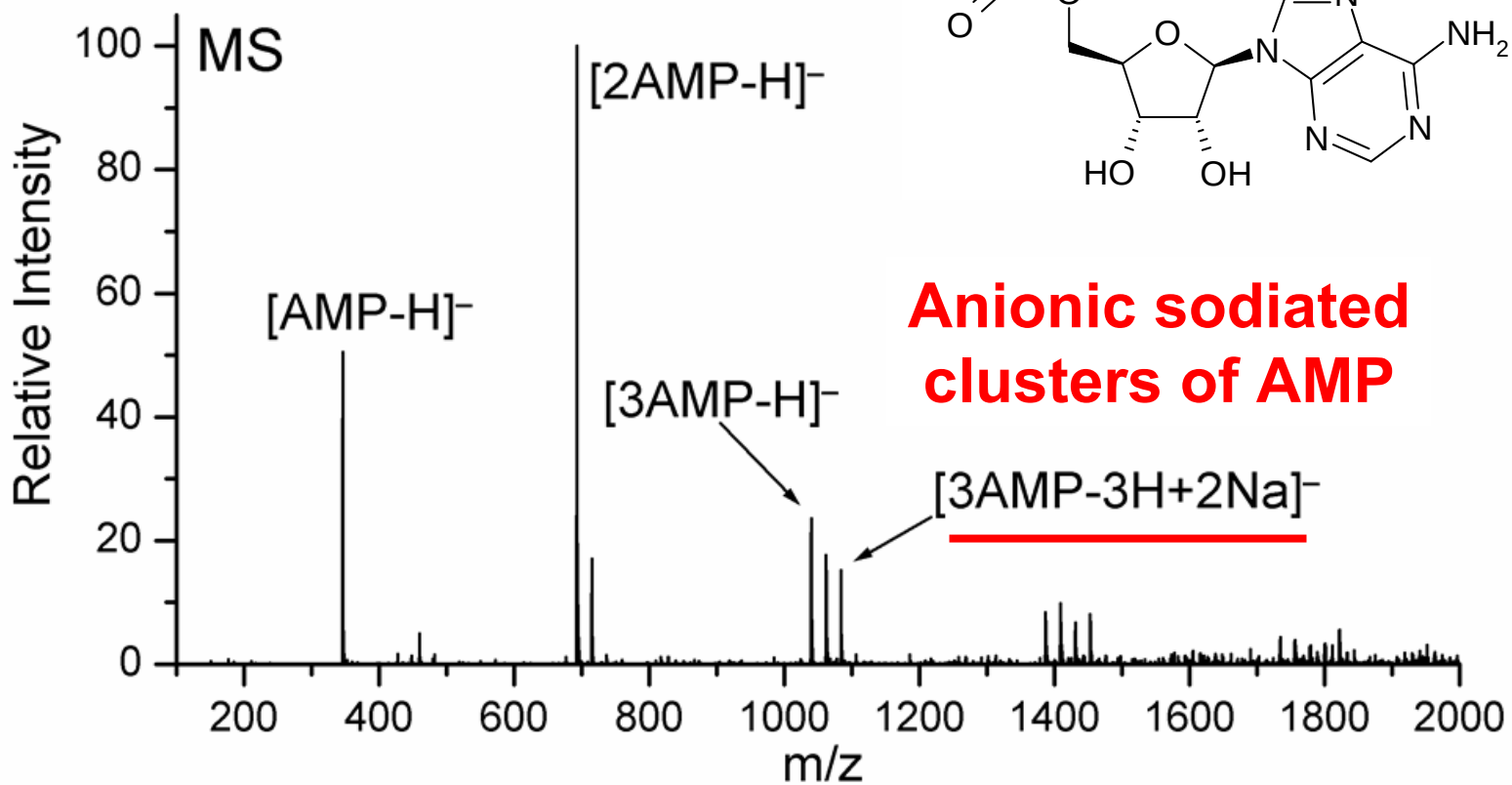
Homochiral Serine Octamer: A Compact Cubic Structure of Zwitterionic Serines

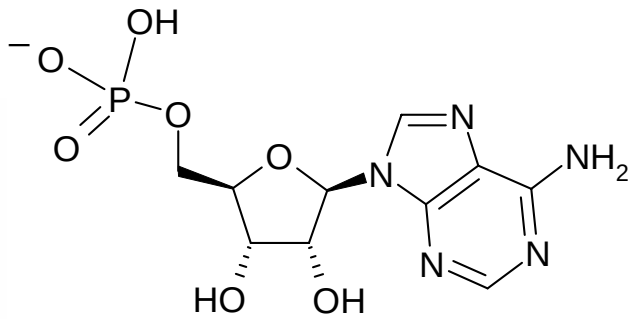




Two views of protonated octamer; calculated cross section agrees with ion mobility data within 1 %.

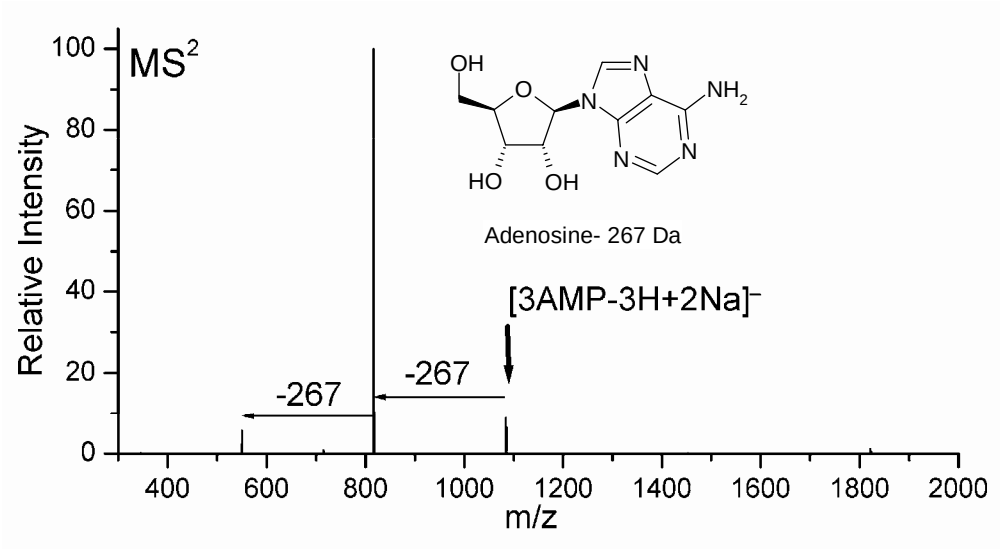
Chemical Synthesis In Noncovalent Molecular Clusters: formation of ATP from AMP

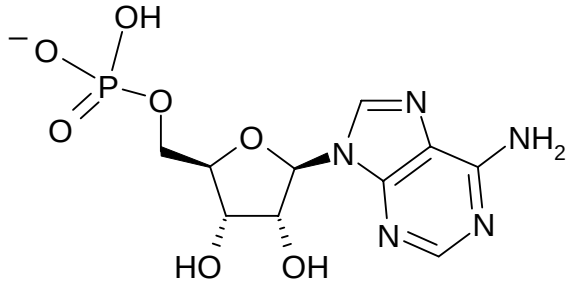




Anionic sodiated clusters of AMP

Two Step Synthesis of ATP from 3 AMP

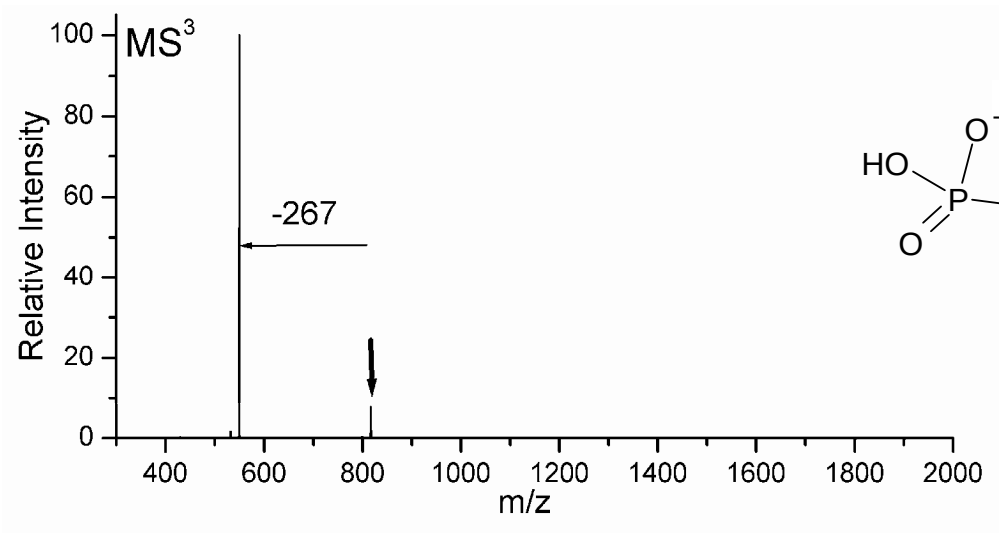


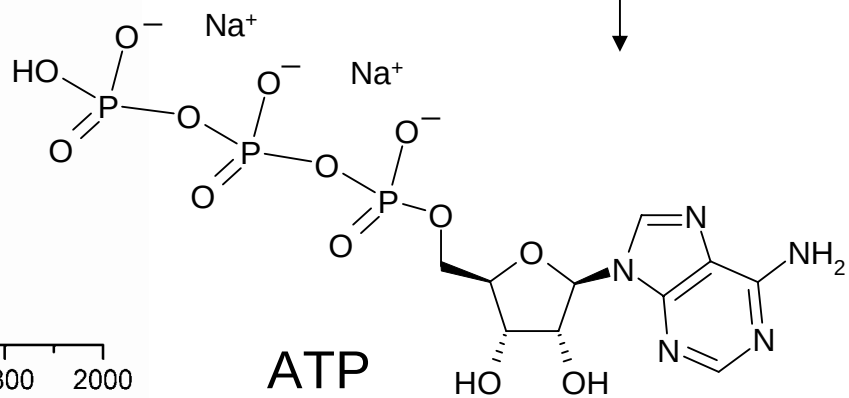


[3AMP-3H+2Na]−

2 Adenosine

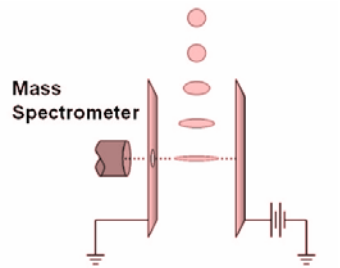
CAD





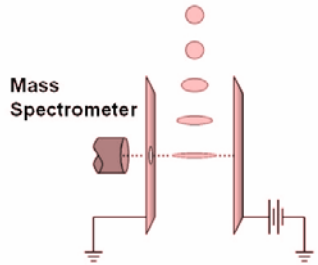
ATP

Abiotic Synthesis of ATP from AMP in the Gas Phase: Implications for the Origin of Biologically Important Molecules from Small Molecular Clusters. Ryan R. Julian and J.L. Beauchamp, *Intl. J. of Mass Spec.,* 227, 147-159 (2003).



Field Induced Droplet Ionization (FIDI)

Cute Tricks and Future Developments

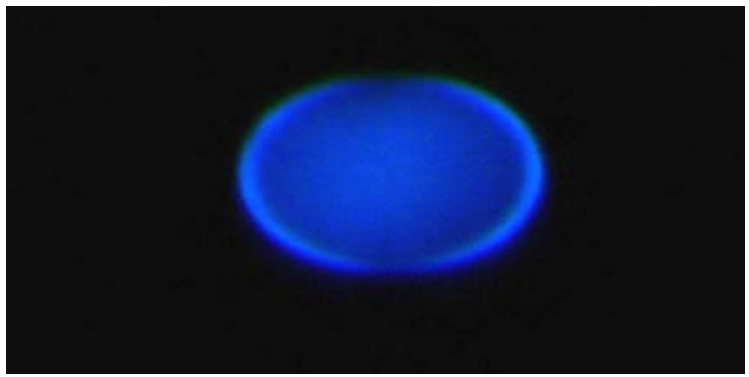


- Use fluorescent probes to track ion motion in droplets
- "Drop Hop Analysis" - Sampling of single drops from microfluidic devices
- Studies of trapped droplets using electrodynamic balances, optical traps, and acoustic levitation; continuous or repeated sampling of individual drops
- Droplets are harmonic oscillators: resonant excitation using tailored electric fields is possible
- Spinning droplets - minicentrifuge
- Unique analytical applications, e.g. sampling of organics in aerosols and particulates; rapid characterization of biological aerosols; single cell analysis in conjunction with flow cytometry

Field Induced Droplet Ionization (FIDI)

Fluorescent Imaging of Molecules

E = 0 kV/cm

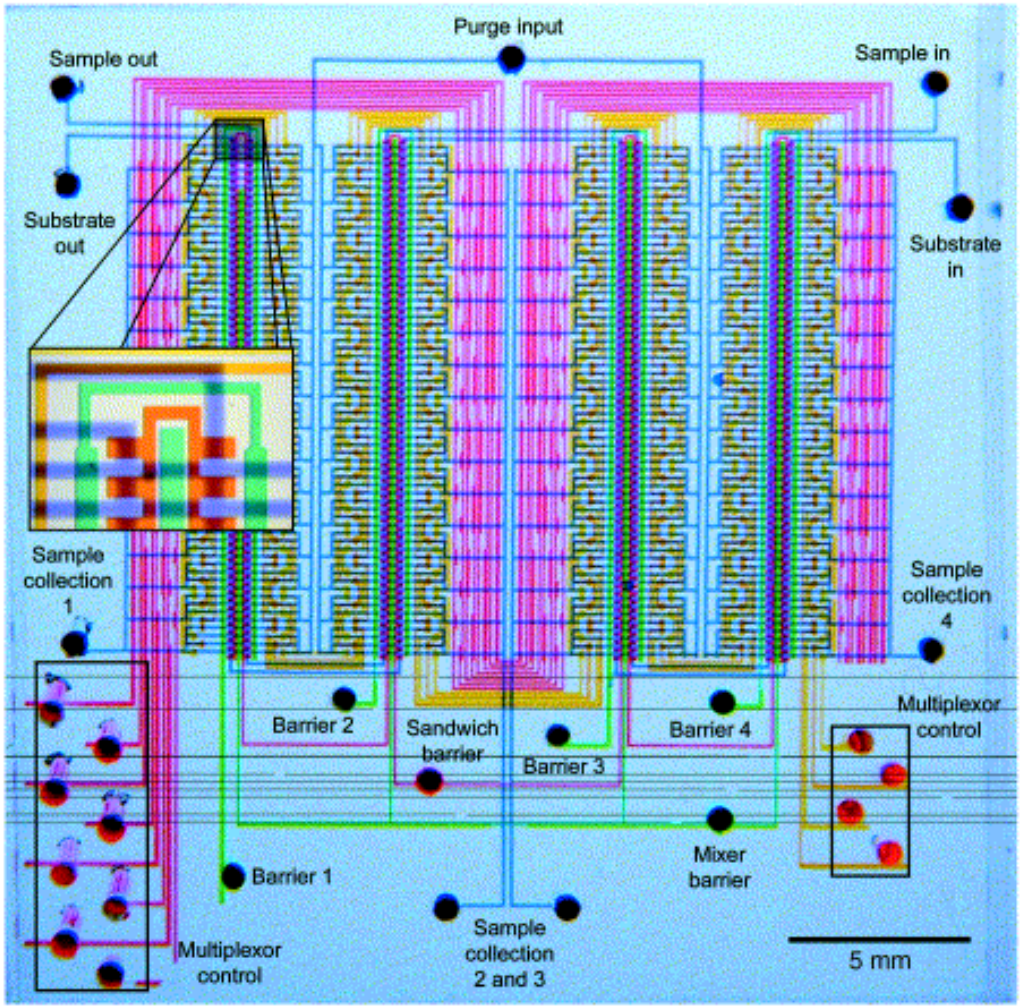


E = 22 kV/cm

a/b = 1.6

150 micron methanol droplets with 10 μM quinine and 0.01% sulfuric acid illuminated with a nitrogen laser.

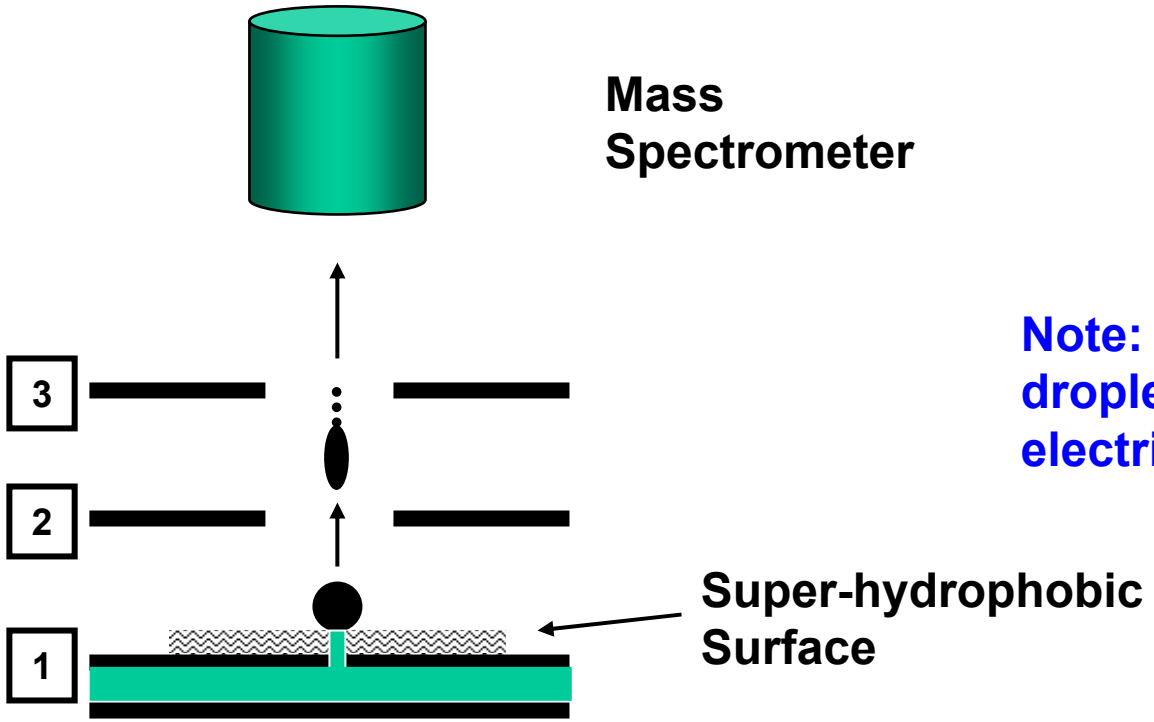
Coupling MS with Microfluidic Devices



LSI (large-scale integration) microfluidic comparator chip. The chip has 2056 active valves and features an addressable storage array that enables 256 distinct subnanoliter reactions.

T. Thorsen, S.J. Maerkl and S.R. Quake, Microfluidic large-scale integration. *Science* **298** (2002), pp. 580–584

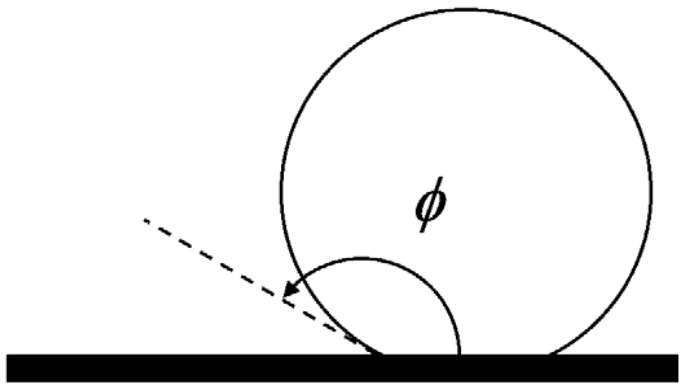
FIDI: Sampling Droplets from Surfaces

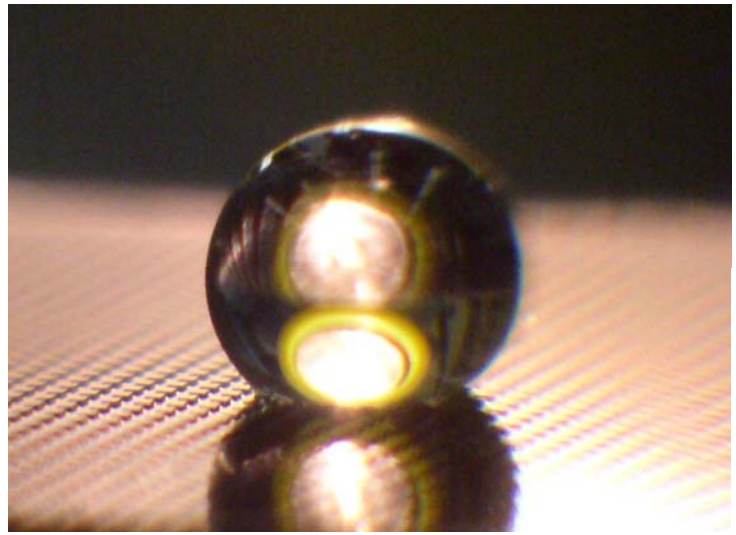


Note: FIDI with charged droplets requires lower electric field strengths.

Electrode 1 is a super-hydrophobic surface. When a sufficiently high potential is applied to electrode 2 the droplet is detached from the surface and moves upward into a region of higher field between electrodes 2 and 3 where FIDI occurs. Ions are sampled by a mass spectrometer.

FIDI: Sampling Droplets from Surfaces

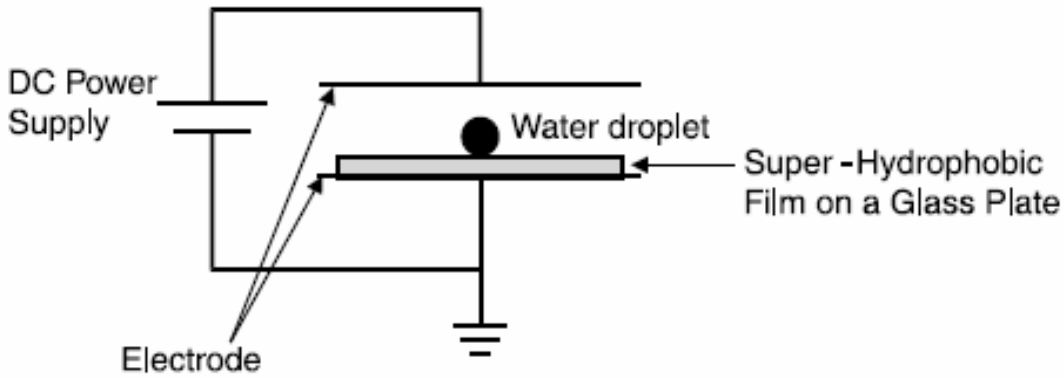


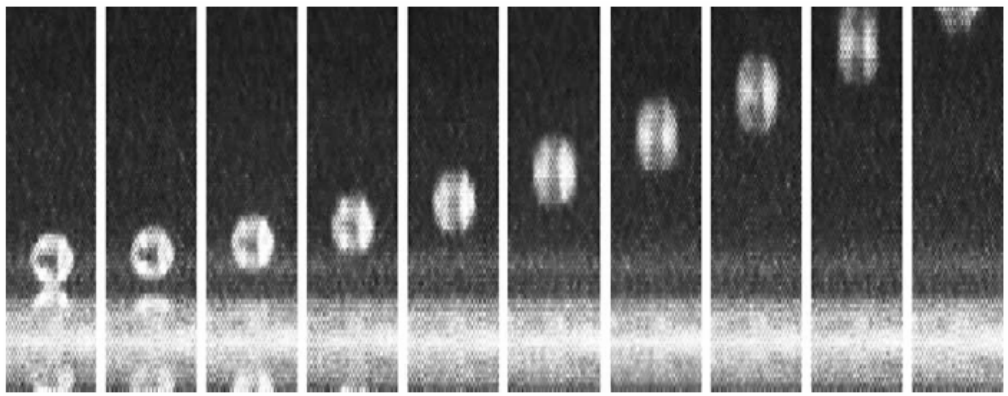


Schematic depiction of the contact angle, and a droplet on a super-hydrophobic surface. 2 µL water sitting on Si pillars of 100 µm on a side, separated by 100 µm. Surface is coated with Teflon. Contact angle is ~140°.

Yoshimitsu, Z., Nakajima, A., Watanabe, T., and Hashimoto, K. *Langmuir* **2002**, 18, 5818-5822.

FIDI: Sampling Droplets from Surfaces

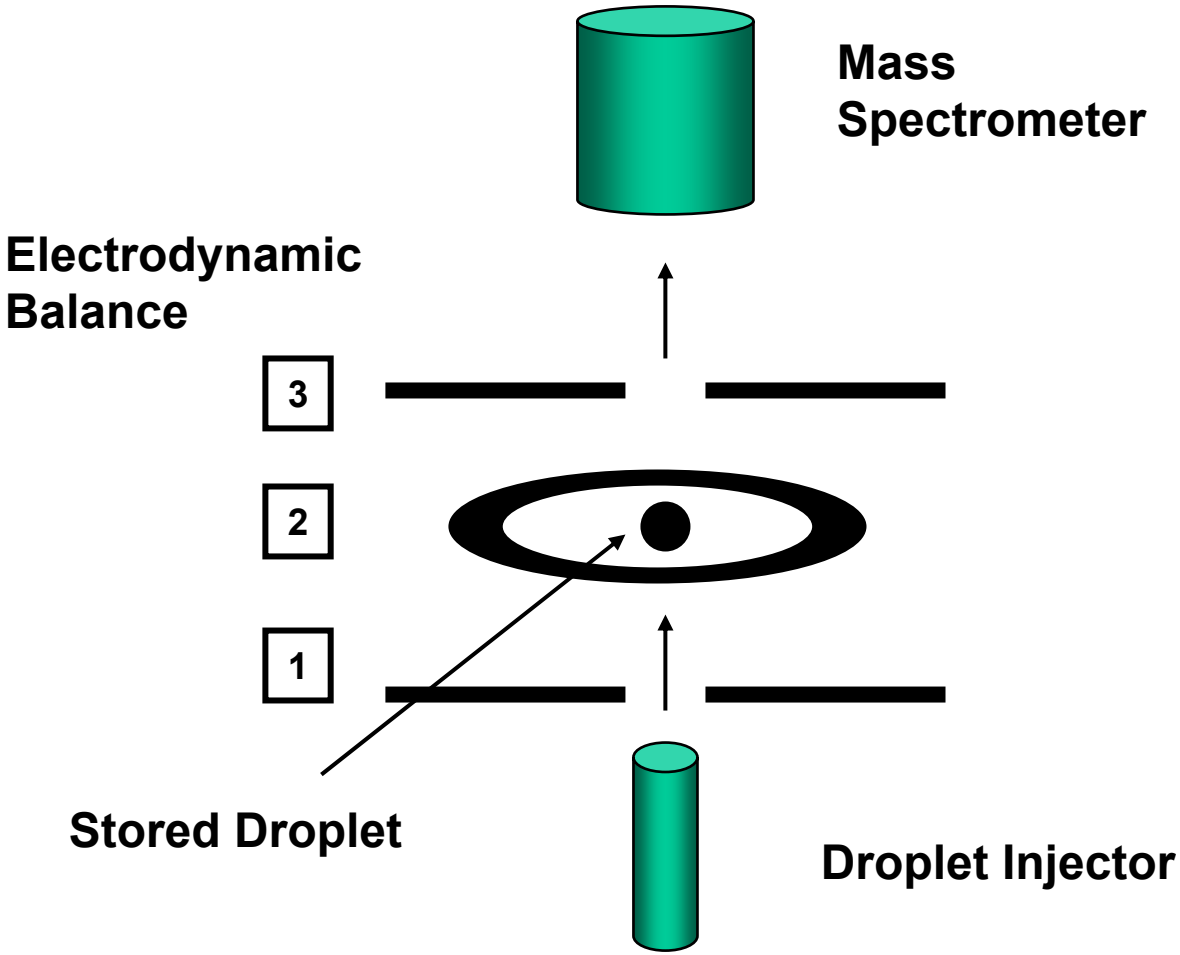




5 mg droplet jumping in a 9.0 kV/cm electric field

Takeda K., Nakajima, A., Hashimoto, K., and Watanabe, T.
Surf. Sci. **2002**, 519, L589-L592.

FIDI: Sampling Droplets from Trap



A charged droplet is injected into the electrodynamic balance where it is confined by the application of rf and dc voltages to the ring electrode and plates 1-3. To sample ions, the three electrodes are biased such that a strong electric field along the axis of the trap leads to field induced droplet ionization.

Summary

ESI and FIDI provide a convenient route to gas phase biomolecules, molecular clusters, and non-covalent complexes. Species are sampled directly from condensed phase.

Non-covalent Complexes: Clusters may have had a role in homochirogenesis and abiogenic synthesis. Observing chemical reactions between cluster components rather than dissociation is of considerable interest.

Molecular Machines: Biomimetic approaches to the design of reagents to manipulate peptides and proteins may provide new tools for studies in proteomics.

Ryan Julian

Heather Cox

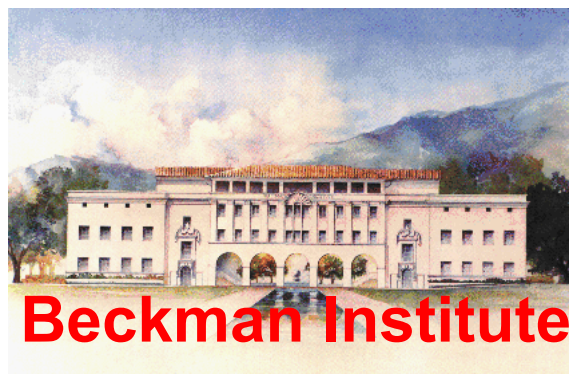
Sang-Won Lee

Jim Smith

Ron Grimm

Rob Hodyss

Acknowledgments

NSF

NASA/JPL