

The Nanopore Ion Source:

Mechanisms of Direct Ion Emission
from Aqueous Solutions into Vacuum

by

Nicholas Drachman

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the Department of Physics as satisfying the
dissertation requirement for the degree of
Doctor of Philosophy

Date.....
Derek Stein, Advisor

Recommended to the Graduate Council

Date.....
Thomas Powers, Reader

Date.....
Matthias Kuehne, Reader

Approved by the Graduate Council

Date.....
Thomas A. Lewis
Dean of the Graduate School

Curriculum Vitae

Nicholas Drachman

Education

PhD in Physics, Brown University, 2024

B.S. in Physics, Boston College, 2014

Awards & Honors

GFSD Fellowship, 2021-2024

Ruth and William Silen M.D. Award, First Prize, 2019

IMSD Fellowship, 2018

Brown DIAP Fellowship, 2017

Boston College Undergraduate Summer Research Fellowship, 2012 & 2013

Work Experience

Graduate Research Fellow, NIST, Physical Measurement Lab, Cooksey Group, 9/2021 to 8/2024

Graduate Researcher, Brown University, Physics Dept., Stein Lab, 9/2017 to Present

Research Specialist, University of Pennsylvania, Radiology Dept., 3/2015 to 5/2017

Undergraduate Research Fellow, Boston College, Physics Dept., Naughton Lab, 4/2013 to 5/2014

Undergraduate Research Fellow, Boston College, Physics Dept., Ren Lab, 4/2012 to 11/2012

Publications

- **Drachman, N.**, Lepoitevin, M., Szapary, H., Wiener, B., Maulbetsch, W., Stein, D., “Nanopore ion sources deliver single amino acid and peptide ions directly into high vacuum”, *Nature Communications*, 15, 7709 (2024).
- Cooksey, G., Iavarone-Garza, S., **Drachman, N.**, Patrone, P., “Accuracy and dynamics of flow measurements to 1 nL/min using an optofluidic flow meter”, *Measurement: Sensors*, (2024) *Accepted, in press*.
- **Drachman, N.**, Vietorisz, J., Winchester, A.J., Vest, R., Cooksey, G.A., Pookpanratana, S., Stein, D., “Photolysis of the peptide bond at 193 nm and 222 nm”, *Under review*.
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Presentations & Conference Proceedings

- **Drachman, N.**, Stein, D., “The nanopore ion source emits amino acid and peptide ions directly into vacuum from a nanoscale aqueous meniscus” Oral presentation, *American Society of Mass Spectrometry*, 2024, San Diego, CA.
- **Drachman, N.**, Cooksey, G., Patrone, P., Sadeghi, J., Stein, D., “Dynamic Measurements of sub-nL/min flows with an optofluidic flowmeter” Oral presentation, *APS March Meeting*, 2023, Las Vegas, NV.
- **Drachman, N.**, “Nanopore ion sources deliver ions directly into high vacuum — applications to single molecule protein sequencing”, Invited Oral presentation, *Nanopore Weekly Meeting*, 2023, virtual.
- **Drachman, N.**, Vietorisz, J., Stein, D., “UV photodissociation of peptides for Nanopore MS sequencing”, Oral presentation, *Single Molecule Protein Sequencing Conference*, 2022, Delft, Netherlands.
- **Drachman, N.**, “Physical properties of the nanopore ion source”, Oral presentation, *APS March Meeting*, 2022, Chicago, IL.
- **Drachman, N.**, “Nanopore mass spectrometry for single molecule protein sequencing - the role of low flow rates”, Invited oral presentation, *NIST Physical Measurement Lab Seminar*, 2021, virtual.
- **Drachman, N.**, Stein, D., “A single-molecule mass spectrometer for protein sequencing”, Oral presentation, *APS March Meeting*, 2021, virtual.
- **Drachman, N.**, Stein, D., “Single-Molecule Sensitivity in Mass Spectrometry Using Nanopore Ion Sources”, Oral presentation, *APS March Meeting*, 2020, Denver, CO. (cancelled due to COVID19)
- **Drachman, N.**, Weiner, B., Stein, D., “Designing a Nanopore Mass Spectrometer for Protein Sequencing”, Oral presentation, *Single Molecule Protein Sequencing Conference*, 2019, Jerusalem, Israel.
- **Drachman, N.**, Lepoitevin, M., Weiner, B., Szapary, H., Stein, D., “Towards Single-Molecule Protein Sequencing via Nanopore Mass Spectrometry”, Oral presentation, *New England Science Symposium*, 2019, Harvard Medical School, Boston, MA.
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- **Drachman, N.**, Kadlecck, S.J., Rizi, R.R., “Analyzing Reaction Dynamics with Hyperpolarized ^{13}C -NMR”, Poster. *International Society for Magnetic Resonance in Medicine Conference*, 2017, Honolulu, HI.
- **Drachman, N.**, “Improving pH Imaging by Monitoring Organic Reactions with Hyperpolarized ^{13}C -NMR”, Invited oral presentation, University of Cincinnati, *Center for Pulmonary Imaging Research Seminar*, 2016, Cincinnati, OH.
- **Drachman, N.**, Kadlecck, S., Pourfathi, M., Xin, Y., Profka, H., and Rizi, R.R., “In Vivo pH and Metabolite MR Imaging Using Hyperpolarized ^{13}C -Pyruvate”, Oral presentation, *International Society for Magnetic Resonance in Medicine Conference*, 2016, Singapore.
- **Drachman, N.**, Kadlecck, S., Xin, Y., and Rizi, R.R., “pH Dependent Kinetics of the Decarboxylation of Pyruvate for pH Mapping Experiments”, Poster. *International Society for Magnetic Resonance in Medicine Conference*, 2016, Singapore.
- **Drachman, N.**, Kadlecck, S., Pourfathi, Xin, Y., Profka, H. and Rizi, R.R., “Simultaneous Measurement of pH and Metabolism in Injured Rat Lungs Using Hyperpolarized ^{13}C Carbon MRF”, Poster, *American Thoracic Society Conference*, 2016, San Francisco, CA.
- **Drachman, N.**, “In Vivo pH and Metabolite MR Imaging Using Hyperpolarized ^{13}C -Pyruvate”, Oral presentation. *The Fourth International Workshop on Hyperpolarized Carbon-13 and Its Applications in Metabolic Imaging*, 2016, Philadelphia, PA.
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- **Drachman, N.**, Calm, Y., Naughton, M.J., “Investigation of Plasmonic and Optical Properties of Ag Nanowires”, Poster, *APS New England Meeting*, 2014, Boston, MA.

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Chapter 1

Introduction

“The trick of reason is to get the imagination to seize the actual world – if only from time to time.”

– Annie Dillard

1.1 Proteins and their analysis

A protein is a folded chain of amino acids. The sequence of amino acids in the chain determines how the protein folds and forms its 3-dimensional structure (Fig. 1.1.1) [1]. The protein’s structure in turn determines its biological function [2].

Proteins play a central role in virtually every biological process. The hemoglobin that carries oxygen and CO₂ in red blood cells, the sodium-potassium pumps that maintain the resting potential across cell membranes, the histones that pack 2 meters of DNA into every 10 μ m wide cell nucleus, and the kinases that walk along microtubules to transport material through the cell are all examples of proteins. The ability to characterize and quantify the protein content of individual cells, i.e. determine how much of each protein is present and distinguish between different proteoforms, would have a significant impact on every corner of biology and medicine.

Proteomics, the large-scale study of proteins, represents the next logical step in our understanding of biological systems following genomics and transcriptomics [4]. These fields correspond to the three key molecular players in the central dogma of molecular biology: DNA is transcribed to RNA, which is then translated into proteins [5]. Genomics, the study of an organism’s complete set of DNA, and transcriptomics, the study of all RNA transcripts, have seen remarkable advances in

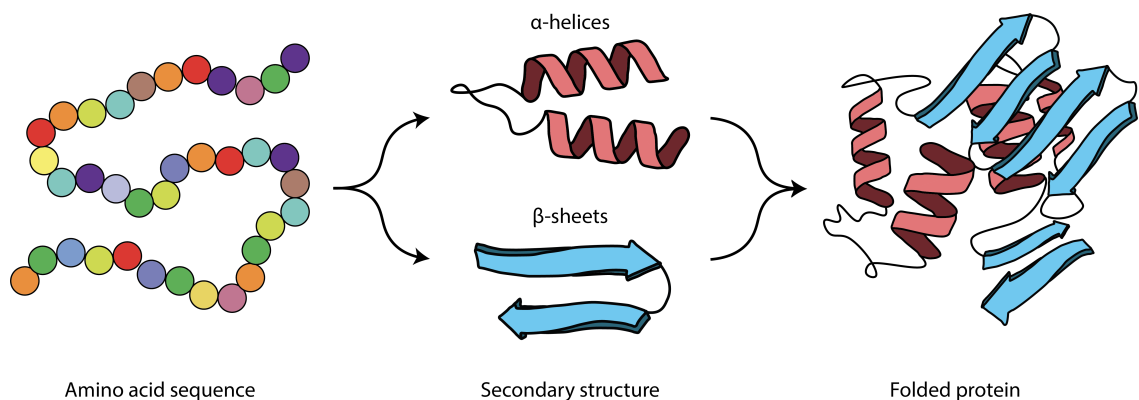


Figure 1.1.1: **Protein structure.** Diagram illustrating the hierarchical, folded structure of proteins. The linear amino acid sequence (left) is the primary structure which folds into secondary structures such as α -helices and β -sheets (middle), which further fold into the complex three-dimensional structure of the fully folded protein (right) [3].

recent decades [6]. Thanks to next generation sequencing techniques, the cost of sequencing an entire human genome has dropped from $\sim$ \$1 billion to less than \$1000 in the past 20 years [7, 8]. Transcriptomics has seen similar advances thanks to RNA-seq techniques [9]. However, protein sequencing has lagged far behind, constrained by the complexity of protein analysis and still relying on variations and combinations of decades-old technologies, Edman degradation [10, 11] and mass spectrometry [12, 13]. While some information about the proteome can be inferred from genomic and transcriptomic data, these often do not correlate with actual protein content, and say nothing about the presence of various proteoforms which result from post-translational modifications or alternative splicing [14, 15, 16]. To illustrate this problem, consider that the human genome encodes about 20,000 unique proteins, but the human proteome contains an estimated 6 million unique proteoforms [12].

Proteomics faces several unique challenges that set it apart from genomics and transcriptomics. First, proteins are composed of 20 different amino acids, compared to the four nucleotides that make up DNA and RNA. This significantly expands the potential diversity of molecules to be analyzed and makes protein sequencing inherently more complex. Second, unlike DNA and RNA, which can be amplified using techniques like PCR (polymerase chain reaction) and RT-PCR (reverse transcription PCR), there is no analogous method to amplify proteins. This limitation means that proteomic analyses must be extremely sensitive to detect low-abundance proteins, as there is currently no good way to increase the amount of sample material. This is related to the so-called dynamic range problem in proteomics, where the concentration of proteins in a sample can vary by as much as 9 orders of magnitude [17], making it difficult to detect low-abundance proteins in a background

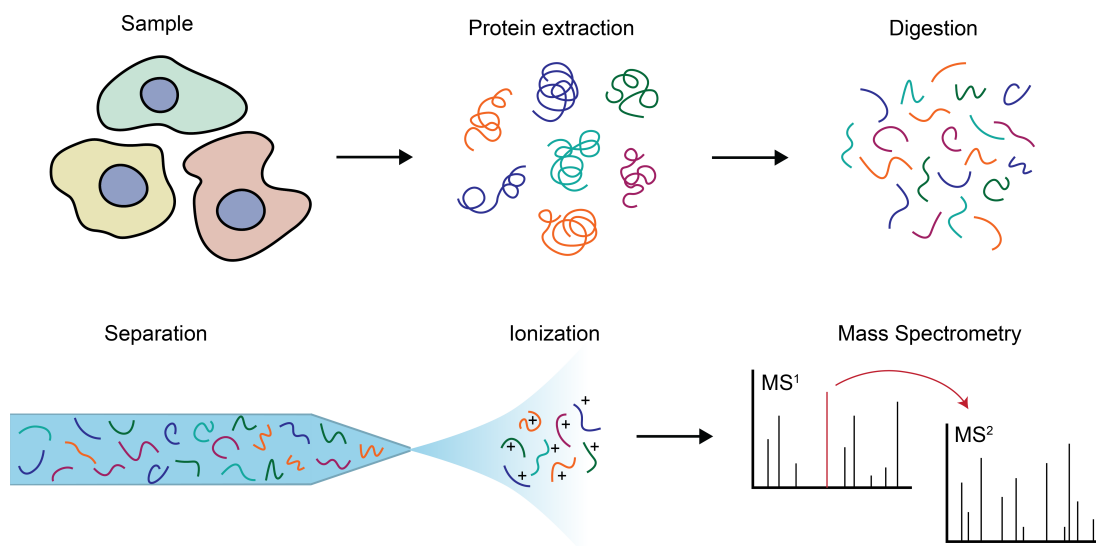


Figure 1.1.2: **Proteomics workflow.** Illustration of a standard ESI-MS-based proteomics workflow.

of highly abundant ones. Finally, the proteome is extremely dynamic, varying both spatially and temporally within an organism and even within a single cell [18].

1.1.1 Mass spectrometry-based proteomics

Figure 1.1.2 shows the workflow of a typical proteomics workflow [19, 12, 20]. Biological samples are prepared by lysing cells or tissues to extract their protein content. The proteins are then digested into smaller peptides using enzymes like trypsin, which cut the protein chain at the site of specific amino acids [21]. The peptides are separated based on their hydrophobicity using high-performance liquid chromatography (HPLC). This separation step reduces the complexity of the mass spectra, and the peptides' retention time through the HPLC column provides an additional measure with which to identify them [22]. Next, the peptides are converted into charged particles (ions), typically using a technique called electrospray ionization (ESI), and introduced into a mass spectrometer [23]. The mass spectrometer measures the mass-to-charge ratio (m/z) of these ionized peptides. To gather more detailed information, selected peptides from the first mass spectrum (MS^1) are fragmented into smaller pieces and analyzed again (MS^2), a process known as tandem mass spectrometry. Finally, The spectra are algorithmically matched to spectral libraries to determine the peptides' identities [24]. The identified peptides can then be mapped back to their corresponding proteins by comparing the peptide's sequence to theoretical peptides derived from the digestion of known proteins, allowing researchers to determine which proteins were present in the original sample [25].

The subsections below will provide a more detailed explanation of the mass spectrometry, ion-

ization, and fragmentation steps of this workflow to set the stage for the work presented in this thesis.

1.1.1.1 Mass spectrometry

Mass spectrometry (MS) has emerged as the central technology in proteomics thanks to its unmatched ability to identify amino acids and peptides by their mass-to-charge ratio (m/z). At its core, MS involves three components: an ion source, a mass analyzer, and a detector. The ion source converts peptides in solution into gas-phase ions which can be transferred into the mass spectrometer (this is discussed in far more detail below), the mass analyzer separates ions based on their m/z ratios, and the detector measures the transmitted ion current downstream of the mass analyzer. Commonly employed detectors like electron multipliers and microchannel plates are often capable of single ion sensitivity [26]. The mass analyzer and detector both operate under high vacuum, typically at pressures below 10^{-6} torr. The vacuum is essential to prevent collisions between ions and gas molecules, which would deflect their trajectories and destroy the ability to discriminate ions by their m/z .

Common mass analyzers used in proteomics include time-of-flight (TOF) [27], Orbitrap [28], and quadrupole [29], each of which has unique strengths and limitations. TOF analyzers separate ions based on their flight time through a vacuum chamber and generate spectra with high speed and high mass accuracy. In an orbitrap mass analyzer, ions orbit a central electrode which measures their oscillation frequency from the generated image current. Long recording times enable orbitraps to measure m/z with very high accuracy and a large mass range. Quadrupole mass analyzers filter ions by their m/z ratios using oscillating electric fields and are often used in combination with other analyzers (see § 2.2.2 for a more detailed discussion of quadrupole mass analyzers). Modern proteomics workflows frequently employ hybrid instruments, such as Q-TOF (quadrupole-TOF) [30] or Q Exactive (quadrupole-Orbitrap) [31], which combine the strengths of multiple analyzer types. Recent innovations like trapped ion mobility spectrometry (TIMS) add an extra dimension of separation, further enhancing the depth and accuracy of proteomic analyses [32]. The choice of mass analyzer and acquisition strategy depends on the specific requirements of the proteomics experiment, balancing factors like speed, sensitivity, resolution, and mass accuracy.

1.1.1.2 Ionization methods

Since mass spectrometers can only detect charged particles, peptides and proteins must first be ionized and transferred from solution into the gas phase before entering the high vacuum environment of

the mass spectrometer. The history of mass spectrometry dates back more than a century, with early work by J.J. Thomson [33], but it was the development of “soft” ionization methods like electrospray ionization (ESI) [34, 23] and matrix-assisted laser desorption/ionization (MALDI) [35] in the 1980s that paved the way for its application in proteomics. Prior to this, “hard” ionization techniques like electron ionization or fast atom bombardment were used to ionize samples by depositing large amounts of energy that would shred large complex molecules like proteins. ESI generates gas-phase ions by electrifying a liquid sample at the tip of a sharpened capillary, creating a plume of charged droplets that evaporate in a background gas to eventually yield ions. MALDI involves mixing the analyte with a matrix compound that absorbs light at a particular wavelength, then irradiating the mixture with a laser to vaporize and ionize the sample. The advent of ESI and MALDI solved the problem of ionizing a peptide without destroying it, revolutionizing proteomics by unleashing the full capabilities of mass spectrometry [36]. However, the efficiencies of these ionization techniques remain relatively low, limiting the sensitivity of MS-based proteomic workflows. The development of a new ionization method related to ESI which dramatically improves the ion transmission efficiency is the focus of much of this thesis, so electrospray ionization will be described in far more detail below.

1.1.1.3 Fragmentation methods

The first protein sequencing technique was based on Edman degradation, developed in 1950, a chemical reaction in which peptides are sequentially cleaved at the N-terminus to identify amino acids one by one, making it a slow but reliable method for protein sequencing in solution [10]. Tandem mass spectrometry (MS/MS) is now the standard approach for *de novo* protein sequencing, where peptide ions from an initial mass spectrum (MS^1) are isolated in an ion trap and fragmented by one of several methods to generate a fragment mass spectrum (MS^2) [37]. The fragmentation process breaks the peptide backbone between amino acid residues, generating a series of fragment ions that differ by one amino acid each. By analyzing the mass differences between these fragments, the amino acid sequence can be reconstructed [38].

There are several dissociation techniques commonly used in tandem mass spectrometry. Collision-induced dissociation (CID) is one of the most straightforward and widely used fragmentation techniques. It is an ergodic process, where energy is distributed across all bonds in the peptide following collisions with neutral gas molecules, leading to fragmentation predominantly along the backbone peptide bonds [39, 40]. Electron-based methods, such as electron capture dissociation (ECD) and electron transfer dissociation (ETD), break backbone bonds predominantly along the N- C_α bond

while preserving non-covalent interactions, making ECD particularly useful for preserving labile post-translational modifications while fragmenting the peptide and probing higher order structure [41]. There are several light based fragmentation methods, such as infrared multi-photon photodissociation (IRMPD) and ultraviolet photodissociation (UVPD), in which the absorption of laser light causes bond cleavages [42, 43]. IRMPD works by incrementally heating up peptide ions with each infrared photon absorbed, resulting in ergodic fragmentation patterns similar to those observed in CID [42]. In the case of UVPD, a single photon carries enough energy to break the peptide bond [44]. UVPD stands out among all of these dissociation methods by fragmenting peptides through a combination of ergodic and non-ergodic pathways, producing a particularly diverse fragmentation pattern which enables high sequence coverage [43, 45, 46].

1.1.2 The next frontier: single cell proteomics

Tissues are composed of many diverse cell types that interact dynamically, and individual cells can exist in multiple possible states which differ in protein expression patterns that drive biological processes [47]. Conventional MS-based proteomics methods, which rely on bulk samples containing thousands or millions of cells, provide a broad overview of the protein content within a sample but obscure the cellular heterogeneity underlying these processes [48, 49]. Several groups are now developing single-cell proteomics techniques to address this problem [50]. Recent advances in sample preparation and handling have led to the accurate quantification of more than 3,000 proteins from a single cell, demonstrating the power of these methods [51]. However, major obstacles remain which are fundamentally linked to the minuscule samples involved in single-cell analysis. Some low abundance proteins may be present in copy numbers as low as a few dozen within a single cell [52], pushing up against fundamental sensitivity limitations in MS-based workflows. Innovations like miniaturized sample handling, improved ionization efficiency, and multiplexing techniques (e.g., SCoPE-MS) have made significant strides toward addressing these challenges [53, 50, 51], but further optimization is needed to reduce sample loss and enhance sensitivity. One of the key limitations to sensitivity, which this thesis covers in depth, is the inefficient transfer of ions into the mass spectrometer, which reduces the number of detectable proteins [54, 55, 56].

1.1.3 The final frontier: Single molecule protein sequencing

Single molecule protein sequencing is the next frontier in proteomics, with various approaches currently under intense development but not yet realized [57, 58]. These approaches seek to analyze

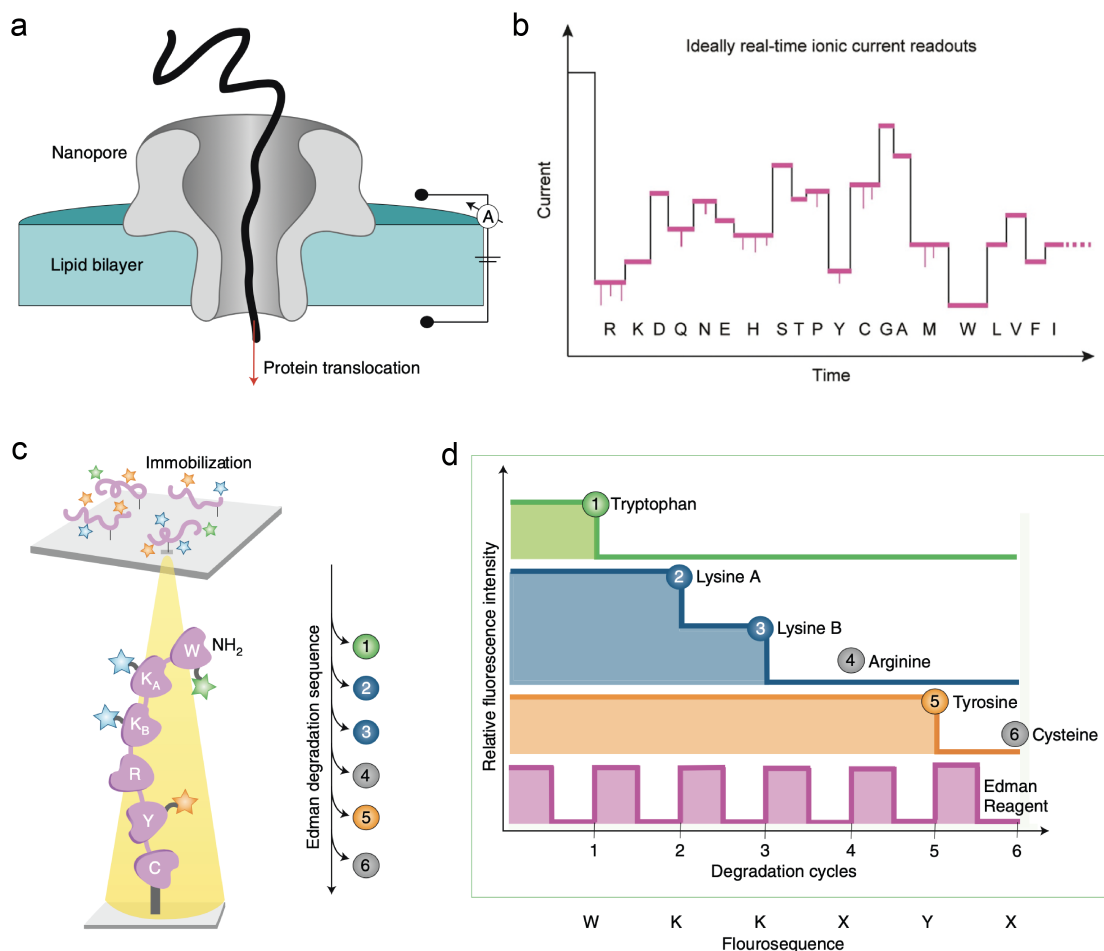


Figure 1.1.3: **Approaches to single molecule protein sequencing** **a** Schematic of a standard nanopore sequencing approach. Adapted from Ref. [57]. **b** Idealized plot of the ionic current through the nanopore during a protein translocation, showing characteristic current blockages for each amino acid. Adapted from Ref. [59]. **c** Illustration of the fluorosequencing approach to single molecule protein sequencing. Adapted from Ref. [60]. **d** Idealized fluorescence data from a protein fluorosequencing measurement. Adapted from Ref. [60].

proteins at the level of individual molecules, pushing detection limits to the theoretical minimum. By eliminating the need for ensemble measurements, single-molecule sequencing techniques could enable truly comprehensive single-cell proteomics, vastly extend the measurable dynamic range currently achievable with MS [17], and provide unprecedented insights into molecular biology [8].

Several approaches at single molecule protein sequencing are under development which have done away with MS altogether, as MS is generally not considered a single molecule technique. These single molecule approaches can broadly be divided into fluorescence and nanopore based methods, with some methods combining aspects of both. The nanopore methods (Fig. 1.1.3a & b), which are inspired by the tremendous success of nanopore DNA sequencing [61, 62], rely on threading

denatured proteins or peptides through a nanometer sized pore while measuring an ionic current through the pore [63, 64, 65, 66, 67, 68]. As each amino acid has a slightly different size and shape, they each block a characteristic amount of current as they pass through the pore, enabling their identification. However, unlike DNA which is made up of just four different bases, proteins are made of 20 different amino acids (plus hundreds of unique post-translational modifications [69]), so achieving sufficient contrast in the picoampere-level measurement signal to distinguish all the amino acids has been a significant challenge [70]. Fluorescence based approaches (Fig. 1.1.3c & d) typically involve labeling specific amino acids with fluorescent tags and then sequentially removing amino acids from the end of the peptide one at a time using a modified Edman degradation process [60, 71, 72]. While these fluorosequencing approaches have shown promise for protein identification, they have been limited to fingerprinting rather than full sequencing because of the difficulty of multiplexing 20 distinct fluorescent tags specific to just one amino acid each [73]. So far, no approach has succeeded at true single-molecule protein sequencing.

1.1.4 Sequencing single proteins by nanopore mass spectrometry

We envision an alternative approach to sequencing that combines the advantages of nanopores with those of mass spectrometry. As pointed out in § 1.1.1.1, MS detectors are often sensitive to single ions, so there is no fundamental reason that MS cannot be used as a single molecule measurement technique. If an ion source and mass analyzer could be designed such that every emitted ion is measured, MS could be applied to the analysis of single molecules. Figure 1.1.4 illustrates the approach. The basic idea is to drive a single denatured protein through a nanopore ion source that compels its constituent amino acids to pass in sequential order, break off the individual amino acids using UV light, and finally deliver those amino acids directly into the high vacuum of a mass spectrometer where they can be identified by their mass-to-charge ratios. There are multiple technical challenges that must be overcome to achieve this vision, chief among them are the ability to emit amino acid ions directly into vacuum from a nanoscale emitter and the ability to fragment peptides within the tip of the emitter. This thesis will attempt to address both of these challenges.

1.2 Electrospray

The primary focus of this thesis is the development and physical characterization of a nanopore ion source as an alternative to conventional electrospray ionization. To fully appreciate the motivations for and advantages of the nanopore ion source, it is essential to first understand the history, physics,

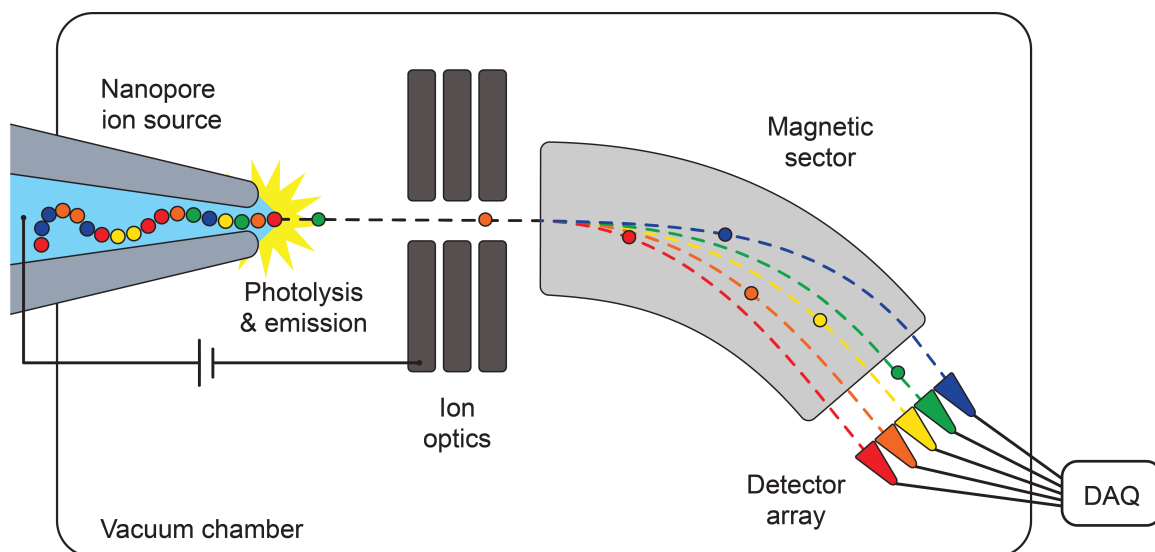


Figure 1.1.4: **Single molecule sequencing by nanopore mass spectrometry.** Our envisioned approach to single molecule protein sequencing. Amino acids are fragmented from a denatured protein within a nanopore ion source and emitted in sequential order. They are separated according to their mass-to-charge ratio by a magnetic sector and detected by an array of single ion detectors. The original sequence can then be reconstructed according to each amino acid ion’s time of arrival.

and limitations of electrospray ionization. This section provides a comprehensive overview of ESI, laying the groundwork for understanding how the nanopore ion source addresses key challenges in ion generation and transmission in mass spectrometry.

1.2.1 A brief history of electrospray

In the year 1590 (± 10), in the bustling market town of Colchester, England, William Gilbert made an observation that would not be fully understood for another three-and-a-half centuries. Gilbert was a physician and natural philosopher, who would go on to be named the personal physician of Queen Elizabeth I, but his primary interest was studying the curious phenomena of electricity and magnetism. At the time, the study of electric and magnetic phenomena had laid practically dormant for nearly 2000 years, since Thales of Miletus first observed the strange way in which little bits of straw were attracted to amber [74]. One day in his laboratory, Gilbert held a piece of charged amber near a droplet of water and observed as the water distorted and stretched upward into a delicate cone, drawn by an invisible force. Gilbert carefully recorded this phenomenon, noting,

“It [the amber] plainly attracts the body itself in the case of a spherical drop of water standing on a dry surface; for a piece of amber held at a suitable distance pulls toward itself the nearest particles and draws them up into a cone.” [75]

This simple observation is likely the earliest recorded example of an electrohydrodynamic phenomenon. In particular, it was the first observation of a class of phenomena we now call electrospray, although it is not clear if any actual charge emission occurred in Gilbert’s early experiments.

The first true electrospray experiments were conducted nearly a century and a half later, in 1747, by the French clergyman Jean-Antoine Nollet. Nollet demonstrated that electrospray could be used as an efficient means to aerosolize a liquid [76, 77, 78]. Nollet’s work is particularly notable for his observations on how the size and geometry of the emitter significantly affect the spray. He documented how narrower capillaries produced a more stable spray and how applying electricity could accelerate the flow of liquids, resulting in a finer and more controlled aerosolization process. Moreover, he recognized that the fluid flow rate could be manipulated to control the spray behavior [78, 77], a crucial insight that is still foundational to the design and operation of ESI systems [79, 80]. Figure 1.2.1 below is an engraving from one of Nollet’s publications on his electrospray experiments [77]. The lower right-hand side of the figure shows a rotating glass globe, which supplied the static electricity for his experiments. On the upper right-hand side, three capillaries of different sizes and geometries are depicted, illustrating the importance of capillary tip geometry even in the earliest recorded electrospray experiments.

By the late 19th century, the once-mysterious phenomena of electricity and magnetism had been largely brought to heel under Maxwell’s comprehensive theory [81]. This theory was soon after made accessible by Heaviside [82], who translated Maxwell’s clumsy theory of 20 equations into the modern four “Maxwell’s equations” still taught today [83]. However, the electrohydrodynamic phenomena underlying electrospray remained largely unexplained. The first major breakthrough came from Lord Rayleigh in 1882, who derived an expression for the stability limit of an electrified droplet [84]. By balancing the electrical repulsive force on the surface of a charged droplet with the inward Laplace pressure from surface tension, Rayleigh derived the following expression,

$$q_R = 8\pi\sqrt{\gamma\epsilon_0 r^3}, \quad (1.2.1)$$

where q_R is the limiting charge on a droplet with radius r and surface tension γ . Equation (1.2.1) highlights the critically important fact that smaller droplets can hold a higher charge density than larger droplets while maintaining stability (this will be important later). Rayleigh observed that if the charge density of a droplet exceeds the limiting value, the droplet will expel a fine jet of liquid. This limit is now known as the Rayleigh limit (not to be confused with the Rayleigh criterion in optics), and is fundamental to the physics of electrospray ionization. It is the crossing of this limit

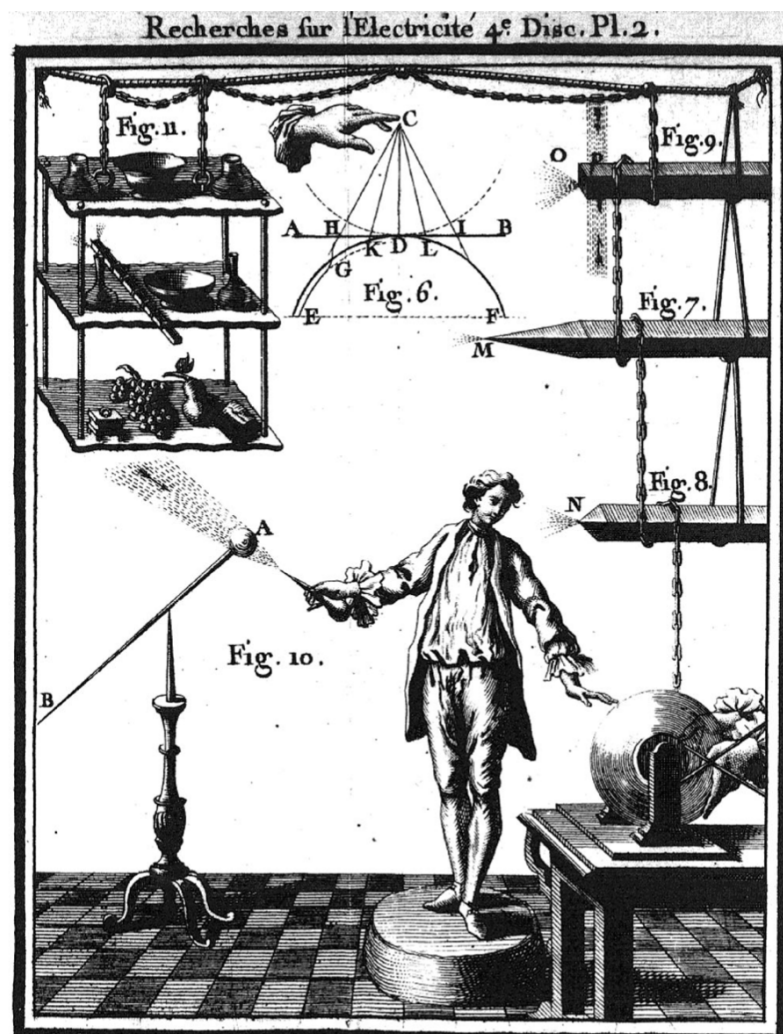


Figure 1.2.1: **18th century electrospray.** Figure showing Nollet's setup for electrospray experiments, with several different emitter geometries displayed in the top right corner of the figure. Reproduced from Nollet 1749.

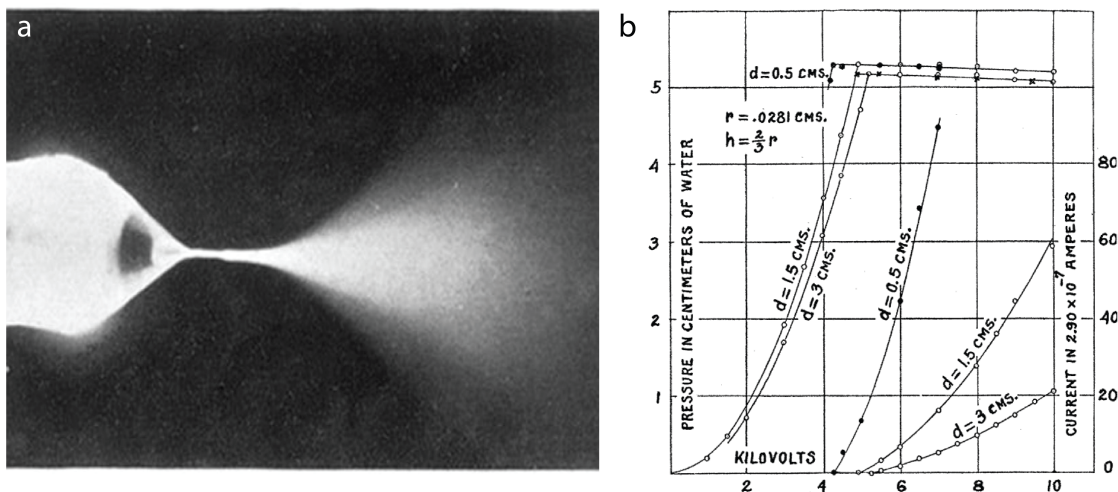


Figure 1.2.2: **Zeleny's experiments.** **a** Photograph of an electrospray plume taken by Zeleny showing the capillary, Taylor cone, jet, and droplet plume. Reproduced from Ref. [85]. **b** An example of Zeleny's measurements of currents and electrostatic pressures generated in electrospray as a function of voltage. Reproduced from [86].

that results in the emission of a charged jet from an electrified meniscus in ESI.

In the 1910s at Yale University, John Zeleny made the first detailed observations of electrospray using high speed photography and careful current and voltage measurements [86, 85]. He applied high voltages to dilute HCl solutions at the end of a glass capillary with a diameter of less than 1 mm, and measured the emitted currents using a telephone receiver. Zeleny noticed that emitted droplets would break up into smaller droplets, and made the connection with Rayleigh's work on the stability of charged droplets. Perhaps Zeleny's greatest contribution was his identification of the different regimes of electrospray operation such as dripping, pulsing, and steady cone-jet modes.

The next major milestone in the development of electrospray ionization comes from the work of fluid dynamicist G.I. Taylor, who developed a theoretical model to explain the behavior of electrified menisci [87, 88]. Taylor's work finally explained Gilbert's observation, made nearly 400 years earlier, that a liquid droplet was distorted into a conical shape by an electric field. By balancing electrical forces and surface tension on an electrified meniscus and assuming that the surface is an equipotential, Taylor demonstrated that the only stable equilibrium shape of the electrified meniscus that could be stable is a cone with half-apex angle of 49.3° . These conical structures are now known as "Taylor cones" in recognition of his work. Photographs of electrospray, such as those taken by Zeleny (Fig. 1.2.2), generally confirmed Taylor's prediction of a conical structure with a 49.3° half-apex angle with small deviations.

The last key developments in this brief historical account of electrospray are the contributions of

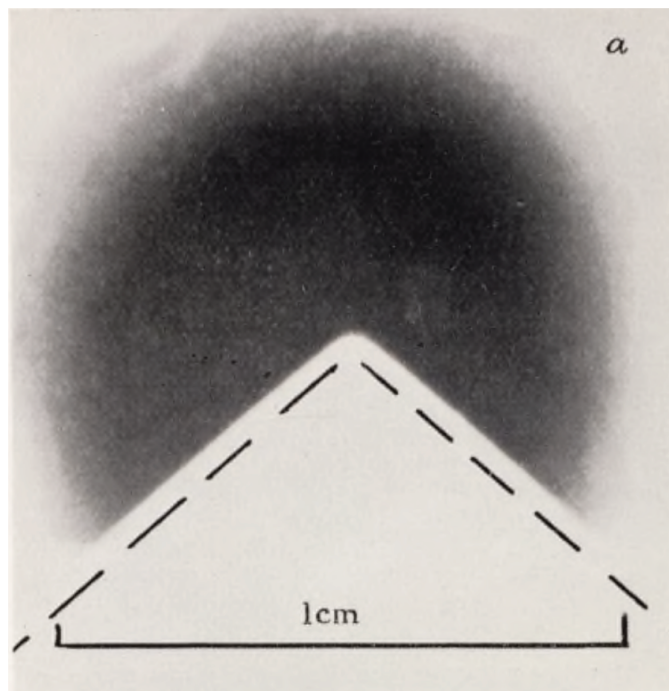


Figure 1.2.3: **The Taylor cone.** Image of a “Taylor cone” formed in an oil/water interface presented in Taylor’s original 1964 publication. Reproduced from Ref. [87].

Malcolm Dole in the 1960s and John Fenn in the 1980s. Dole, a chemist at Northwestern University, was the first to successfully couple electrospray with mass spectrometry (Fig. 1.2.4a), albeit in a rudimentary form [89]. Dole demonstrated that macromolecules like polymers could be ionized by electrospray and subsequently analyzed by mass spectrometry without significant fragmentation. Notably, Dole’s work marked a shift from studying electrospray as a mere physical curiosity to employing it as an innovative analytical tool for ionizing large neutral molecules. This work laid the groundwork for the modern ESI technique but was limited by the technology of the time. It was not until the publication of work by Yamashita and Fenn in 1984 on the design of an “Electrospray ion source” that the true potential of electrospray as a powerful analytical tool began to be widely recognized [90, 34]. They coupled their electrospray ion source to a then state-of-the-art quadrupole mass spectrometer, thoroughly characterized how the behavior of the ion source depended on parameters like the voltage and temperature of the background gas, and demonstrated that nonvolatile organic compounds could be “softly” ionized (transmitted into the gas-phase without unwanted fragmentation) and detected with high mass resolution. A schematic of the ion source design Fenn developed is shown in Fig. 1.2.4b. Fenn was awarded the Nobel Prize in Chemistry in 2002 for this work.

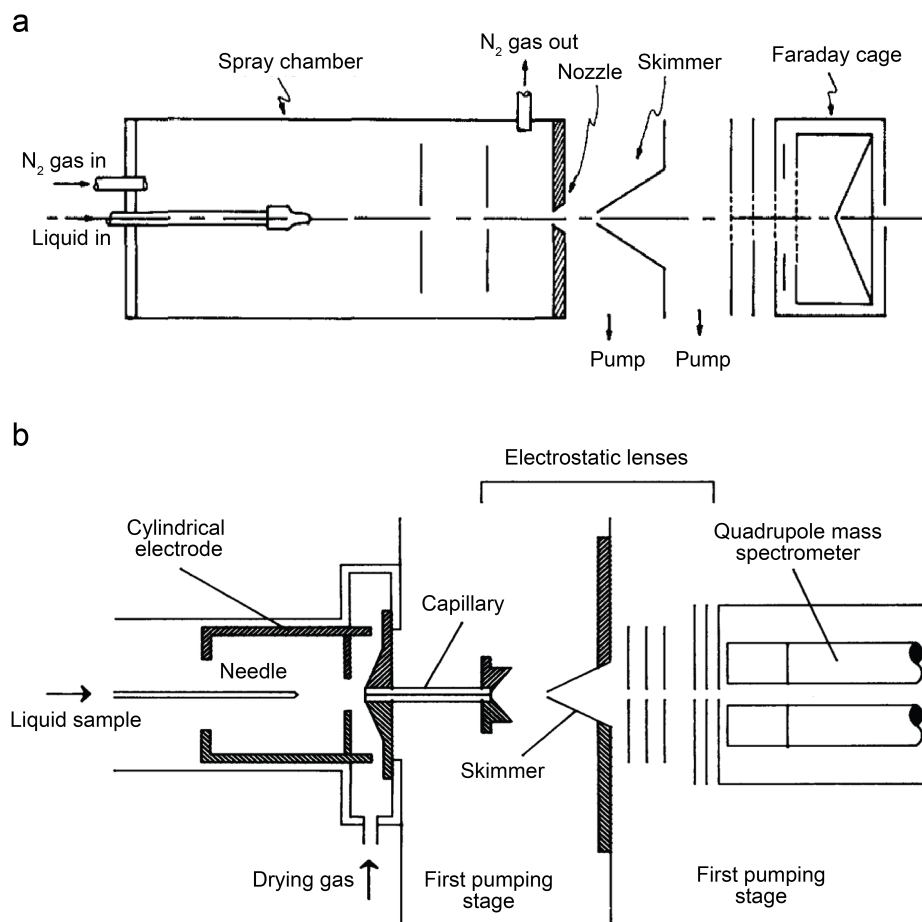


Figure 1.2.4: **Dole and Fenn’s ESI sources.** **a** Schematic of the electrospray ion source used by Dole et al. Adapted from Ref. [89]. **b** Schematic of the electrospray ion source and mass spectrometer design used by Fenn et al. Adapted from Ref. [23].

1.2.2 Electrospray ionization (ESI)

Figure 1.2.5 illustrates the basic process of electrospray ionization (ESI). An electrode supplies a high voltage (V_E) which electrifies the analyte solution contained within a sharpened capillary. Above a critical voltage, the meniscus deforms into a conical shape with a jet emanating from its apex (together this structure is often referred to as a “cone-jet”). The jet breaks up into charged droplets which then collide with a (typically heated) background gas near atmospheric pressure which promotes solvent evaporation. The charge density on the droplets increases as they evaporate and shrink, until eventually electrical repulsion overcomes surface tension and the droplets break apart into smaller, higher charge density droplets. This cycle of evaporation and droplet fission repeats until the droplets reach the nanoscale. At this scale, electric fields at the droplet surface can be sufficiently strong to rip ions directly out of solution and into the gas phase in a process

called ion evaporation [91]. These gas phase ions are then transferred into the high vacuum of the mass spectrometer via a transfer capillary. A detailed description of the physics underlying the electrospray process is presented in § 1.3 below.

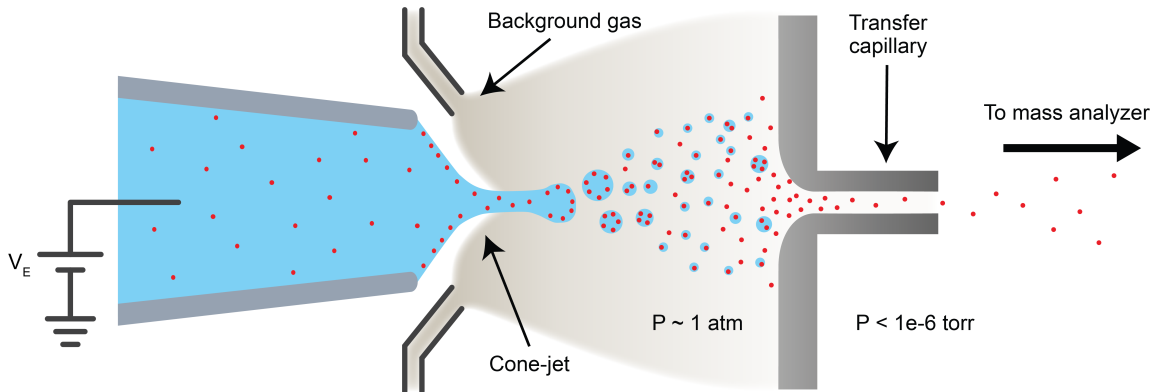


Figure 1.2.5: **Electrospray ionization.** Illustration of electrospray plume highlighting the cone-jet, background/drying gas, plume of charged droplets and gas-phase ions, and the transfer capillary through which ions enter the mass spectrometer.

1.2.3 Limitations of ESI

ESI has limitations that preclude it from achieving the level of sensitivity needed for comprehensive single cell or single molecule analyses. In our view, these limitations are fundamentally linked to the need for a background gas to stimulate the evaporation of droplets and liberate ions. This background gas, along with the plume of charged droplets it creates, widely and chaotically disperses ions. The majority of the ions collide with the mass spectrometer inlet or other hardware components and never reach the mass analyzer [90]. Additionally, many analyte ions are trapped in large droplets that do not fully desolvate before entering the mass spectrometer, reducing the ionization efficiency [92]. Figure 1.2.6a illustrates this process of sample loss [93]; peptide ions are generated in the electrospray plume and are then progressively lost as they run the gauntlet through the front end of the mass spectrometer.

The ion transmission efficiency, which is the fraction of emitted ions that enter the mass analyzer, is a key determinant of the sensitivity of mass spectrometry [56]. In studies of early ESI sources, which used emitter tips hundreds of micrometers in diameter, only one ion in $\sim 10^4$ typically reached the mass analyzer [94]. Nano-electrospray ionization (nanoESI, where the nano refers to the fluid flow rate in nL/min rather than the size of the tip) improved on conventional ESI by using smaller tips ($< 100\ \mu\text{m}$) and lower flow rates ($< 1\ \mu\text{L/min}$) to produce smaller initial electrospray droplets, resulting in higher ionization and transmission efficiencies [95]. Fig. 1.2.6b shows ion transmission

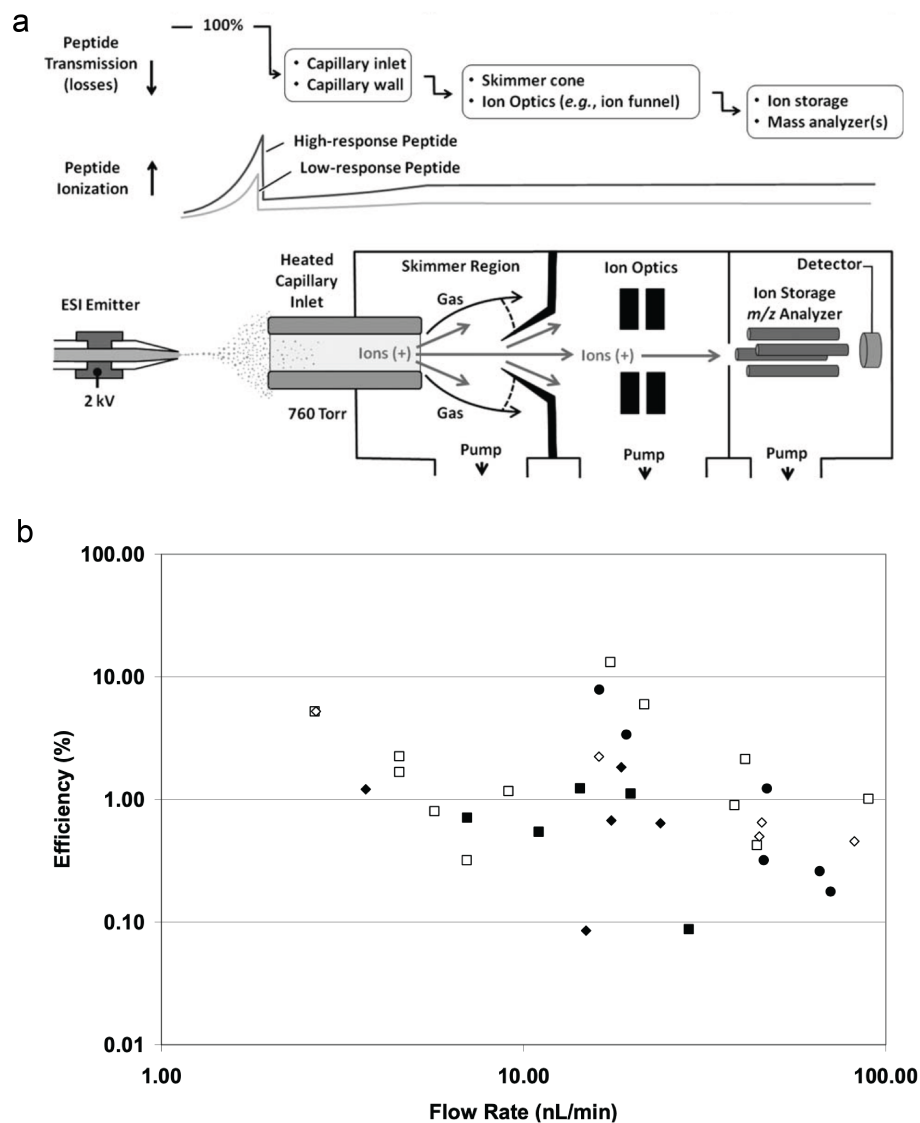


Figure 1.2.6: **ESI's poor transmission efficiency.** **a** Illustration of the main sources of loss in a typical ESI-MS experiment. Reproduced from Ref. [93]. **b** The ion transmission efficiency of 33 different nanoESI sources measured by El-Faramawy et al. The symbols represent different sample solutions. Reproduced from Ref. [54].

efficiency measurements for 33 different nanoESI sources and a variety of solution conditions made by El-Faramawy et al. [54]. NanoESI achieves a transmission efficiency of around 1% in typical measurements and occasionally as high as 12% [95, 54, 93]. A few groups have found clever designs to further improve the ion transmission efficiency in some cases [96]. These include using hydrodynamic simulations to optimize the shape of the MS inlet [97], or operating the ion source at substantially reduced pressures [98]. In particular, Marginean et al. demonstrated that by optimizing the background gas pressure, sheath gas flow rate, and ion funnel voltages, efficiencies as high as 50% could

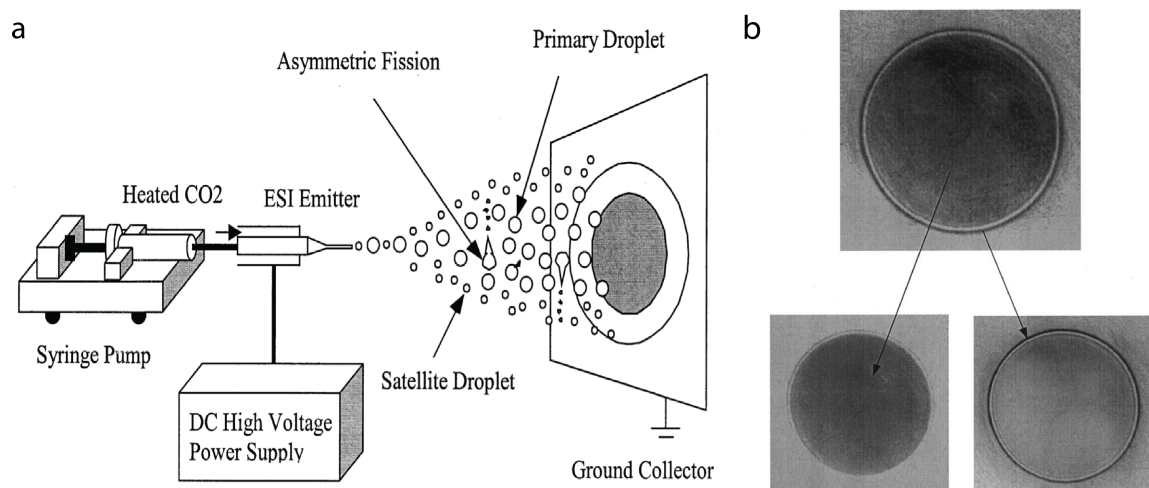


Figure 1.2.7: **Separation of analyte within the electrospray plume.** **a** Experimental setup used by Tang & Smith to demonstrate the physicochemical separation processes that occur in the electrospray plume. **b** Images of the grounded collector showing the separation of methyl green (inner circle), and the brown surfactant (outer ring). The two lower images are taken with color filters to clearly distinguish the two compounds. Reproduced from Ref. [100].

be achieved for a particular small peptide in a sub-ambient pressure nanoESI source [99].

However, co-optimizing the efficiency of multiple analytes remains fundamentally challenging because ESI involves processes that physically separate different ion species within the plume [100]. This was elegantly demonstrated by Tang & Smith with the experiment shown in Fig. 1.2.7a. The authors electrosprayed a solution of hydrophilic methyl green dye along with a brown fluorad carbon surfactant onto a screen to visualize where the compounds ended up in the plume. Fig. 1.2.7b shows the results, the brown surfactant forms a clearly delineated ring around a large spot of methyl green. This separation is the result of multiple physical and chemical processes. Essentially, the more surface active compounds end up concentrated in the smaller satellite and progeny droplets formed in the Rayleigh fissions, which end up on the outside of the plume due to their lower inertia. This separation results in a need to optimize the ESI conditions for each analyte individually, as they will be generated preferentially at different physical locations within the plume [101]. Often ESI emitters are positioned at an angle of up to 90° relative to the MS inlet to better sample the outer portion of the plume [102]. This makes it difficult to achieve high sensitivity for all compounds in a complex mixture, which are likely to vary widely in their surface activities [103]. Consequently, state-of-the-art MS instrumentation for proteomics still requires thousands to millions of copies of proteins for identification [104, 105]. This sensitivity is insufficient for comprehensive single-cell proteomics and single-molecule analyses [53, 50, 106, 58]. Achieving single-molecule sensitivity with MS will require an ion source that circumvents the loss mechanisms intrinsic to spraying charged

droplets into a background gas. To understand how these loss mechanisms may be circumvented, we must first review the fundamental physics underlying ESI.

1.3 The physics of electrospray ionization

This section provides a brief overview of the physics of electrospray ionization, which is essential to appreciate the motivation for developing the nanopore ion source.

1.3.1 The Taylor cone

In his seminal 1964 work, G.I. Taylor provided the theoretical framework that explains the formation and stability of the conical meniscus shape observed in electrosprays (see for example Fig. 1.2.2a), now known as Taylor cones. With two simple postulates, Taylor analytically derived an expression for the electric field, potential, and surface charge density on the surface of a Taylor cone, as well as predicting its apex angle [87, 88]. Those two postulates are: (1) The meniscus is an equipotential surface. (2) The meniscus is in mechanical equilibrium. Postulate 1 is valid when the liquid is highly conductive, such as in an electrolyte solution. Postulate 2 implies that electrical repulsion must be perfectly balanced by surface tension at all points along the meniscus.

By postulate 2, we can write down the pressure balance equation

$$P_L = P_E, \quad (1.3.1)$$

where P_L is the Laplace pressure resulting from the inward force of surface tension and P_E is the electrostatic pressure from the outward force of electrical repulsion. The Laplace pressure is defined as,

$$P_L = \gamma \left(\frac{1}{r_1} + \frac{1}{r_2} \right), \quad (1.3.2)$$

where γ is the surface tension, and r_1 and r_2 are the principle radii of curvature of a point along the surface at a distance d from the apex. From the diagram in Fig. 1.3.1, we deduce that $r_2 = \infty$, and $r_1 = d \tan \alpha_T$. So the Laplace pressure becomes

$$\Delta P_l = \frac{\gamma}{d \tan \alpha_T} \quad (1.3.3)$$

The electrostatic pressure on an element of the meniscus surface can be expressed as $\vec{\sigma}_{nn}$, a component of the Maxwell stress tensor [107], where the subscript nn indicates that the force is

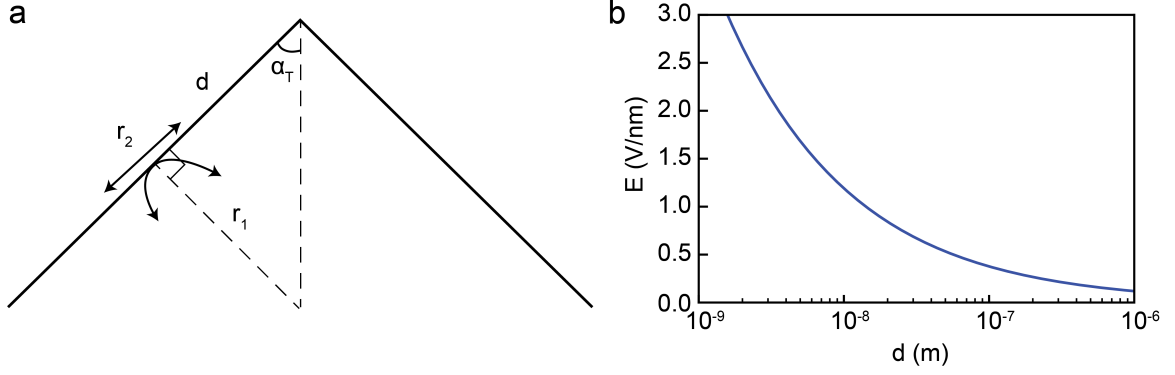


Figure 1.3.1: **Taylor cone structure.** **a** Diagram of a cone showing the coordinate d , the principle radii of curvature, r_1 and r_2 , and the apex half-angle, α_T . **b** Plot of the electric field on the surface of an ideal aqueous Taylor cone as a function of the distance from the apex.

along the $\hat{n}$ direction and applied to a surface which is normal to $\hat{n}$. The Maxwell stress tensor in the absence of magnetic fields is given by

$$\vec{\sigma}_{ij} = \epsilon_0 E_i E_j - \frac{1}{2} \epsilon_0 \delta_{ij} E^2, \quad (1.3.4)$$

where δ_{ij} is the Kronecker delta function. So the electrostatic pressure is

$$P_E = \vec{\sigma}_{nn} = \frac{1}{2} \epsilon_0 E_n^2, \quad (1.3.5)$$

where E_n is the electric field normal to the surface. This is just the classic expression for the energy density of an electric field [107], which has the same units as pressure. Plugging Eqs. (1.3.5) and (1.3.3) into Eq. (1.3.1) yields the pressure balance,

$$\frac{\gamma}{d \tan \alpha_T} = \frac{1}{2} \epsilon_0 E^2, \quad (1.3.6)$$

which we solve to find the electric field strength along the surface,

$$E(d) = \sqrt{\frac{2\gamma}{\epsilon_0 d \tan \alpha_T}}. \quad (1.3.7)$$

Equation (1.3.7) shows that the electric field strength at the surface of a Taylor cone scales as $d^{-1/2}$. Figure 1.3.1b plots $E(d)$ for the 1 μm closest to the tip of an aqueous Taylor cone ($\gamma = 0.072$ N/m). We see that close to the tip of the cone, the electric field becomes very strong, on the order of 10^9 V/m, and diverges at the tip ($d = 0$). An infinite electric field is not physically possible, so one

of the postulates of the model must be violated for small d , this will be explored in the next section.

Equation (1.3.7) above only tells us the electric field on the surface of the cone, and it alone does not reveal the value of the cone-angle, α_T . Taylor deduced the value of this angle by considering the potential near a charged conical conductor [87], which has the form [107],

$$\phi = \phi_0 + Ar^{1/2}P_{1/2}(\cos \theta), \quad (1.3.8)$$

where ϕ_0 and A are constants, r is the radial coordinate with the origin at the tip of the cone, $P_{1/2}$ is the Legendre polynomial of degree $1/2$, and θ is the polar angle. Because the surface is an equipotential, there can be no r -dependence when $\theta = \alpha_T$. So it must be the case that

$$P_{1/2}(\cos \alpha_T) = 0 \quad (1.3.9)$$

The function $P_{1/2}(x)$ has only one zero between $0 < x < 1$ [108], and this zero fixes the value of the apex half-angle of the cone at $\alpha_T = 49.3^\circ$ [87]. This extremely simple derivation leads to the somewhat surprising conclusion that an electrified conical meniscus can only be in equilibrium when its apex half-angle has the value of 49.3° .

In reality, electrified conical menisci have been observed with apex angles that deviate from Taylor's prediction. These departures from Taylor's theory are primarily due to two factors. First, Eq. (1.3.6) does not account for any pressure difference between the liquid inside the meniscus and the atmosphere surrounding it, which can be especially significant when spraying into a vacuum or with large applied pressures [109]. Second, Eq. (1.3.6) ignores the effects of space charge in the electrospray jet and/or plume, which has been shown to decrease the apex angle [110].

To find the full spatial distribution of the potential and electric field, Eq. (1.3.8) can be plugged into Eq. (1.3.6), noting that $E = -\nabla\phi$, to solve for the constant A in terms of γ . Figure 1.3.2a plots the potential around the tip of a Taylor cone ($0 < d < 100$ nm) in polar coordinates. Figure 1.3.2b shows the potential around the tip of a Taylor cone, obtained by numerically calculating the gradient of $\phi(r, \theta)$. This shows clearly how the sharpness of the tip of the Taylor cone enhances local electric fields.

1.3.2 The cone-jet

In the previous section, we saw how the basic theory of Taylor cones predicts a diverging electric field strength at the tip of the cone. Nature resolves this issue through various charge emission processes.

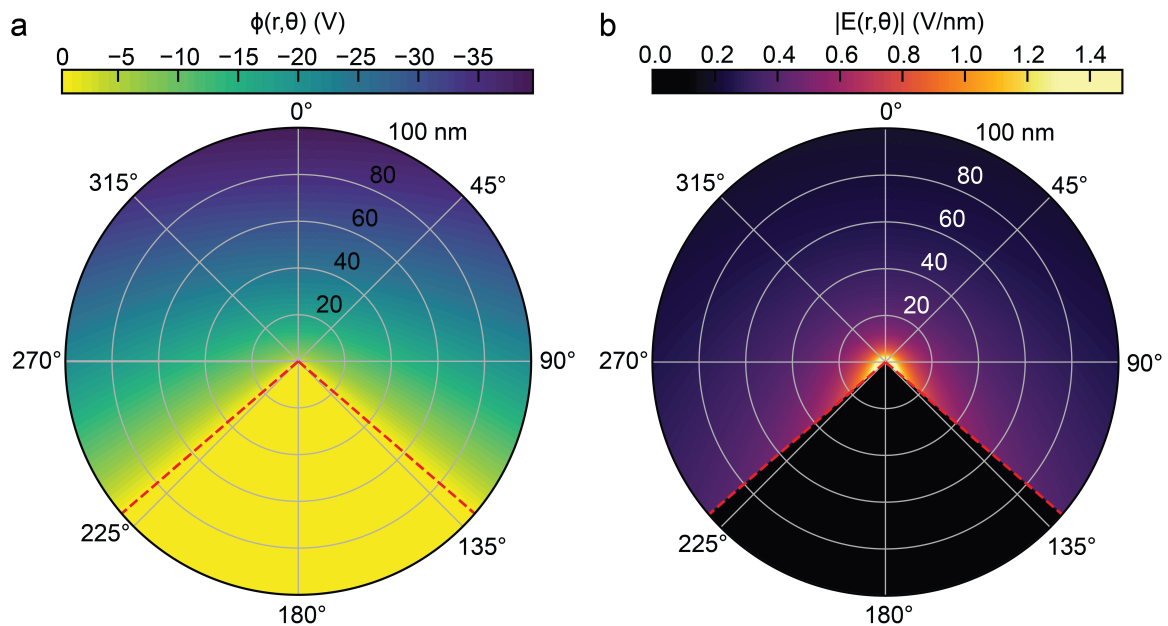


Figure 1.3.2: **Electric field and potential around a Taylor cone.** **a** Plot of the electric potential, $\phi(r, \theta)$, surrounding the tip of a Taylor cone out to $d = 100$ nm. The red dashed lines delineate the surface of the cone. **b** Plot of the electric field strength, $|E(r, \theta)|$, obtained by numerically taking the gradient of ϕ .

Once the electric fields are strong enough, charge can be emitted from the meniscus, preventing the further build up of charge and ever increasing electric fields. The formation of a charged liquid jet at the cone's apex is one of nature's ways of carrying away charge and avoiding a divergence in the electric field strength.

Electrospray has multiple regimes of operation, each with qualitative differences in the way that charge is emitted [111]. In conventional ESI and nanoESI mass spectrometry, electrosprays are most commonly operated in the cone-jet regime, which appears to provide the most stable MS signals [112]. As the name suggests, this regime is characterized by a conical meniscus which emits a jet from its apex as liquid is driven through the emitter. This jet then breaks up into a plume of charged droplets which eventually dry out to yield gas-phase ions for MS analysis. There is a substantial literature describing the physics and scaling laws that govern the behavior of cone-jet electrosprays, much of it from De La Mora and collaborators [110, 113, 109, 114, 115, 116]. The interested reader is directed to those references for a more thorough treatment than what is provided here.

The origin of the cone-jet can be better understood by considering the characteristic time-scales relevant to the motion of charge and fluid near the apex of a Taylor cone. The fluid residence time,

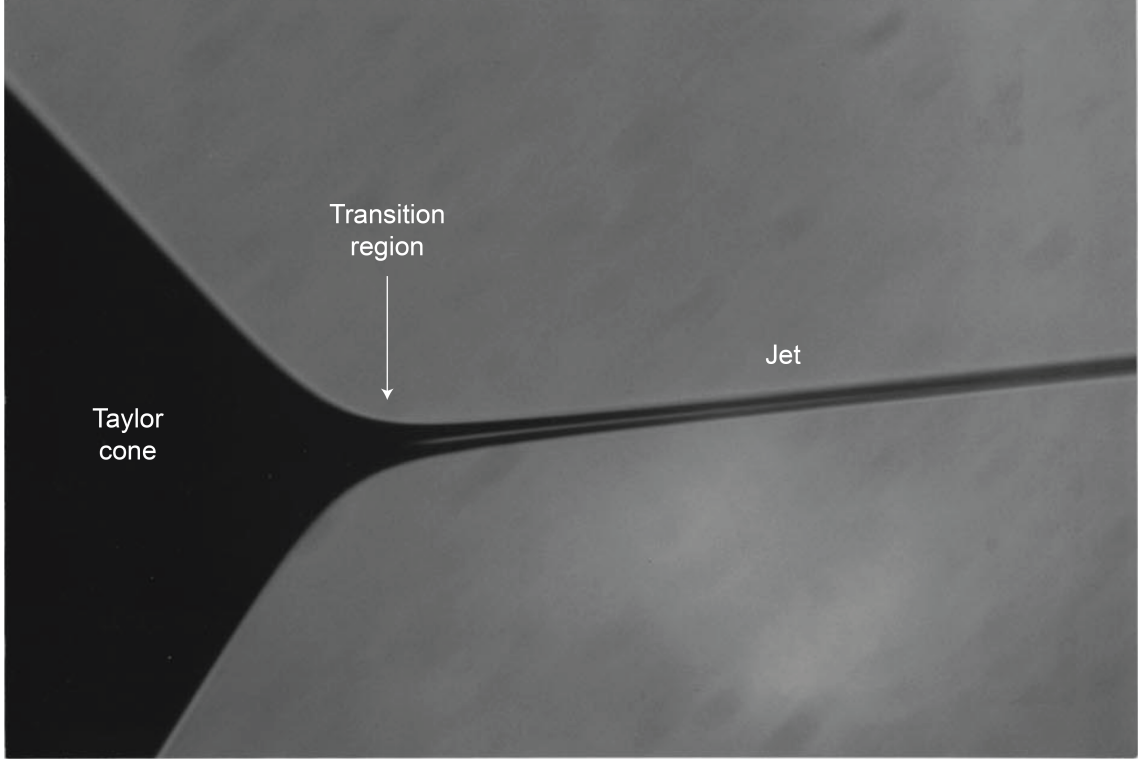


Figure 1.3.3: **The cone-jet.** Image of a cone-jet formed from ethylene glycol. Adapted from Ref. [114].

which describes the timescale over which charge is transferred by advection, is given by

$$t_f = \frac{r^3}{Q}, \quad (1.3.10)$$

where Q is the volumetric flow rate with units of m^3/s , and r is the local radius of the meniscus. The electric relaxation time, which can be thought of as the time needed for built-up charge to equilibrate and is a result of the fluids' finite conductivity, is given by

$$t_e = \frac{\epsilon\epsilon_0}{K}, \quad (1.3.11)$$

where ϵ is the relative permittivity of the fluid, and K is its conductivity. When these two timescales are equal, $t_f \approx t_e$, the motion of fluid can move charges around faster than conduction can relax the charge distribution back to equilibrium [117]. This violates the first postulate from the previous section! As charge builds up, electric stresses can grow larger than the local Laplace pressure can withstand, and the apex of the conical meniscus bursts out into a jet.

Equating Eqs. (1.3.10) and (1.3.11) and solving for r gives the characteristic radius of the tran-

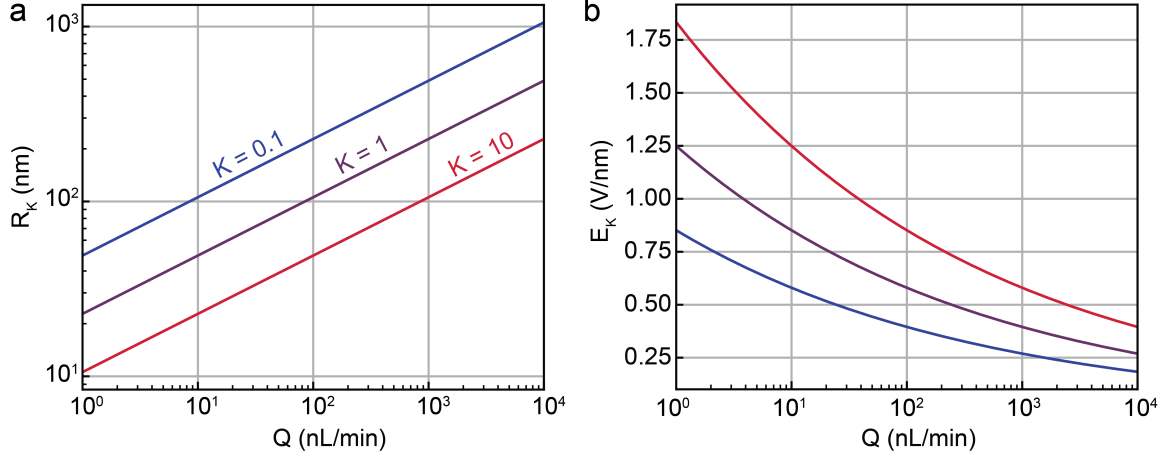


Figure 1.3.4: **Cone-jet transition region scaling relationships.** **a** Plot of the characteristic radius of the cone-jet transition region, R_K , as a function of the flow rate, Q , for three conduction values, $K = 0.1, 1$, and 10 S/m. **b** Plot of the characteristic electric field strength at the surface of the cone-jet transition region.

sition region, where the postulates of the previous section break down and the meniscus transitions from a cone to a jet, which we call R_K in keeping with the convention of Gamero-Castaño et al. [114],

$$R_K \equiv \left(\frac{\epsilon \epsilon_0 Q}{K} \right)^{1/3}. \quad (1.3.12)$$

We now plug this characteristic radius into Eq. (1.3.7) to find the characteristic electric field of the transition region,

$$E_K \equiv \frac{\gamma^{1/2} K^{1/6}}{\epsilon_0^{2/3} Q^{1/6}}, \quad (1.3.13)$$

where we have dropped factors of order 1, again in keeping with the convention of Gamero-Castaño et al. [114] The maximum electric field strength anywhere on the surface of the meniscus is approximately E_K .

Equations (1.3.12) and (1.3.13) are revealing. They describe how the geometry of the cone-jet and the electric field strength depend on the intrinsic properties of the fluid (γ , ϵ , and K) and the flow rate Q . Perhaps most interesting is that they do not depend on anything else, like the size of the emitter or the applied voltage. Figure 1.3.4a and b plot the values of R_K and E_K for an aqueous solution ($\gamma = 0.074$ N/m, $\epsilon = 80$) with $K = 0.1, 1$, and 10 S/m. We see that at lower flow rates, the transition region narrows and the electric field strength rises.

This flow rate dependence was exploited in the development of nanoESI [95, 118]. By using lower flow rates than conventional ESI ($Q < 1$ μ L/min), nanoESI produces a narrower jet which generates smaller initial electrospray droplets, which in turn produce gas phase ions more efficiently and

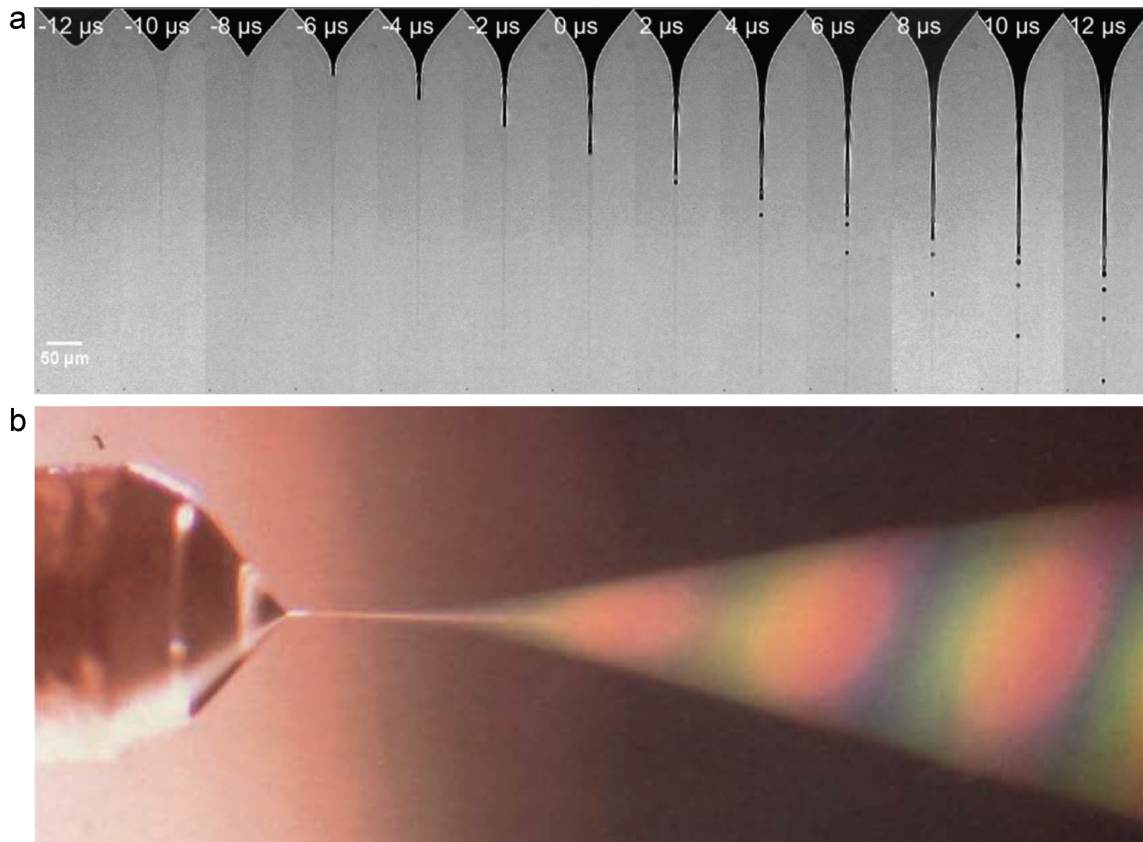


Figure 1.3.5: **The ESI plume.** **a** High speed photographs showing the pinch off of the first droplet following initiation of a cone-jet electro spray. Reproduced from Ref. [121]. **b** Image of an ESI plume from a mixture of methanol and HCl. Adapted from Ref. [122].

enhance sensitivity [95, 119]. Interestingly, when flow rates get down to the order of 1 nL/min, E_K reaches field strengths of approximately 1 V/nm. As we will see in § 1.3.4 below, this is approximately the field strength required for ions to escape the liquid by ion evaporation. In fact, experiments on electrosprays of NaI in formamide have observed ion emission when flow rates dropped below 5 nL/min, which the authors attribute to ion evaporation from the transition region of the cone-jet [114, 120].

1.3.3 The electrospray plume

The jet that emanates from the apex of the Taylor cone extends outwards until it pinches off into droplets due to the Plateau-Rayleigh instability [123]. These droplets contain the excess charge that was carried by the jet. Figure 1.3.5a shows high speed photographs revealing the formation of the cone-jet as well as the generation of the first electrospray droplets. Both theoretical models and direct measurements have shown that the diameter of these initial droplets are close to the diameter

of the jet, which is itself on the order of R_K [113, 124]. The charge density of these first droplets has been measured to be around 50% of the Rayleigh limit [125, 126, 121].

In a typical ESI setup, the emitter tip is placed 2-5 mm in front of the mass spectrometer inlet [96] and bathed in a gas of N_2 [127], which is often heated to temperatures as high as 700 °C [128, 90]. For a 1 μm electrospray droplet, it takes on the order of 1 ms to cross this distance. In that time, countless collisions with background gas molecules transfer thermal energy to the droplet and accelerate the solvent evaporation process, causing the droplet to shrink and increase its charge density. As Rayleigh predicted back in 1882 (Eq. (1.2.1)), there is a limiting charge density above which the droplet will become unstable and undergo what is now called a Rayleigh fission [84, 129].

The Rayleigh fission of a charged droplet (Fig. 1.3.6 inset) is very similar to the formation of a cone-jet on the meniscus at the tip of an ESI emitter [130]. Both processes involve electric stress overcoming surface tension resulting in the ejection of charge to reach a new equilibrium. The Rayleigh fission process has been studied in detail, revealing that it typically occurs when a droplet reaches about 80%-90% of the Rayleigh limit, independent of the nature of the solvent [125, 129, 131, 132]. The droplet forms a conical protrusion on one end and emits a few smaller droplets. These progeny droplets typically contain approximately 20% of the charge originally in the parent droplet, but only about 1% of the mass [131, 133]. Despite having a higher charge density than the original droplet, these smaller progeny droplets are in fact more stable because of the $r^{3/2}$ scaling of the Rayleigh limit. This process repeats in a fractal-like manner, with both the progeny and parent droplets continuing to evaporate and undergo subsequent Rayleigh fission events, producing ever smaller, higher charge density droplets.

Figure 1.3.6 shows the life cycle of a charged droplet in an electrospray plume [131]. In this example, the droplet begins with a radius of 150 nm, and contains 5083 charges, putting it at about 70% of the Rayleigh limit. It evaporates over the next 8 μs , until it has shrunk to a radius of 130 nm, putting it at 86% of the Rayleigh limit. Now a Rayleigh fission occurs, producing five progeny droplets with 20 nm radii and 246 charges, reducing the charge of the parent droplet back to around 70% of the Rayleigh limit. The process repeats until the droplet radius reaches approximately 10 nm, at which point a distinct physical process takes over.

1.3.4 Ion evaporation

When the electric field at the surface of a liquid gets sufficiently high, ions can be emitted directly from the liquid into the gas phase in a process called ion evaporation. This process, which is central

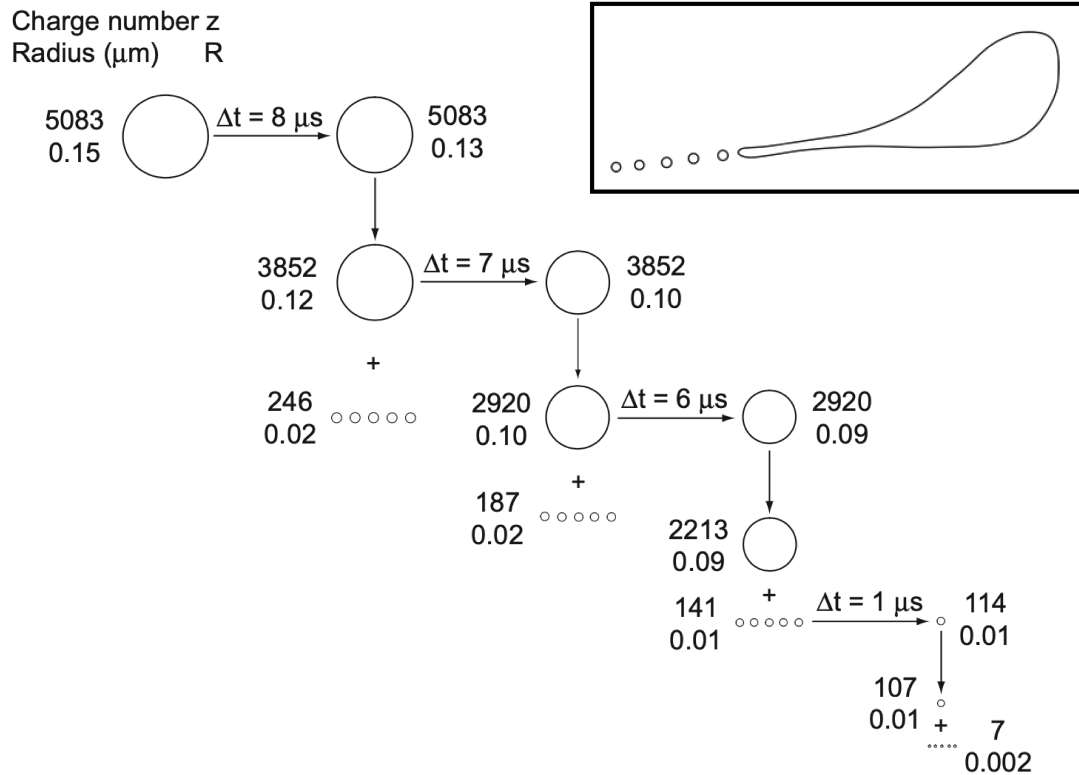


Figure 1.3.6: **ESI droplet lifecycle.** Diagram showing the life-cycle of an electrospray droplet. The droplet in the top left represents an electrospray droplet immediately after splitting from the jet. The numbers next to each droplet show their charge number and radius in μm . The Δt values show the amount of time needed to evaporate and shrink to reach the Rayleigh limit and emit the progeny droplets which appear below the parent droplet. The inset shows the tracing of a photograph of a droplet undergoing a Rayleigh fission, taken by Gomez & Tang [125]. Adapted from Ref. [131]

to the work described in this thesis, was first described by Iribarne and Thompson, who derived the following formula for the current density of evaporating ions [91],

$$j = \sigma \frac{k_B T}{h} \exp \left(- \frac{G_0 - \Delta G(E)}{k_B T} \right), \quad (1.3.14)$$

where σ is the charge density on the liquid surface, k_B is the Boltzmann constant, T is the temperature, h is Planck's constant, G_0 is the ion's solvation energy (essentially the energy barrier to evaporation), and $\Delta G(E)$ is the reduction in the energy barrier due to the presence of the electric field (more on this below). Equation (1.3.14) can be understood quite simply by noticing that the prefactor $\frac{k_B T}{h}$ has units of s^{-1} , so it represents a rate, and the exponential term is just a Boltzmann factor representing a probability. So Eq. (1.3.14) is saying that the ion evaporation current is equal to the surface charge density, times the rate with which each ion “attempts” to evaporate, times the probability that any given attempt results in a successful evaporation. A detailed derivation of Eq. (1.3.14) from first principles can be found in Ref. [134].

In order to use Eq. (1.3.14) to make quantitative predictions, we need an expression for $\Delta G(E)$. Finding this expression is equivalent to finding the work necessary to bring an ion to the droplet surface from infinity. Consider the potential energy landscape of an ion near the surface of a charged droplet, $U(r)$, which is sketched in Fig. 1.3.7. There are two terms that go into $U(r)$, the contribution from the electric field on the surface of the charged droplet, U_E , which repels the ion, and the image charge attraction that the ion feels near the surface, U_{Image} . A few simplifying assumptions are made in this calculation. Most importantly, the droplet surface is treated as a planar conductor, so any effects arising from the curvature of the surface and the liquid's dielectric properties are ignored here. A more detailed model of $\Delta G(E)$ which accounts for the droplets curvature and finite conductivity is described by Magnani et al. in Ref [135].

We derive $\Delta G(E)$ by considering the forces on an ion with charge q at a distance r outside the droplet surface,

$$\vec{F} = \frac{-q^2}{4\pi\epsilon_0(2r)^2} \hat{r} + q\vec{E}. \quad (1.3.15)$$

The first term is the image charge attraction and the second term is the repulsion from the surface electric field. We integrate this from r to ∞ to get $U(r)$, assuming that E vanishes at ∞ ,

$$U(r) = \int_r^\infty \vec{F} \cdot d\vec{r} \quad (1.3.16)$$

$$= -\frac{q^2}{16\pi\epsilon_0 r} - qEr. \quad (1.3.17)$$

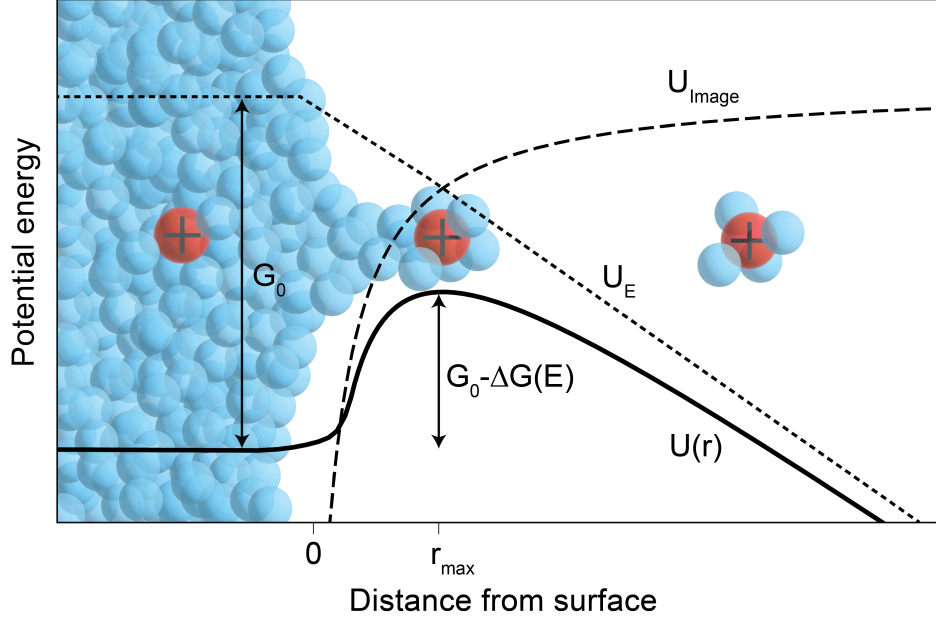


Figure 1.3.7: **Ion evaporation energy landscape.** Illustration of the ion evaporation process and its energy landscape. G_0 is the ions solvation energy, $\Delta G(E)$ is the reduction in the energy barrier due to electrical forces, $U(r)$ is the potential energy of the ion, U_E is the contribution to the potential energy from the electric field of the surface, U_{Image} is the contribution to the potential of the image charge, and r_{max} is the distance from the surface at which the ions potential is maximized.

This function has a global maximum at

$$r_{\text{max}} = \sqrt{\frac{q}{16\pi\epsilon_0 E}}. \quad (1.3.18)$$

Plugging this back into Eq. (1.3.17) yields the expression we seek, the reduction of the energy barrier due to the presence of an electric field and image charge attraction,

$$\Delta G(E) \equiv U(r_{\text{max}}) = \sqrt{\frac{q^3 E}{4\pi\epsilon_0}}. \quad (1.3.19)$$

The full expression for the ion evaporation current now reads,

$$j = \sigma \frac{k_B T}{h} \exp \left(-\frac{G_0 - \sqrt{\frac{q^3 E}{4\pi\epsilon_0}}}{k_B T} \right) \quad (1.3.20)$$

Equation (1.3.20) is perhaps the most important equation presented in this thesis, as it describes the flux of ions evaporating from a liquid surface as a function of the local electric field. Despite its simplifying assumptions, its validity is experimentally well supported for small ions [136, 137, 138].

Crucially, Eq. (1.3.20) provides a way of determining the electric field strength needed to emit ions directly into the gas-phase by ion evaporation. This critical field strength can be estimated by finding the value for which the term in the exponent vanishes. The solvation energy, G_0 , is typically on the order of 1-2 eV for small ions in aqueous solutions [139]. Because water is a polar solvent, it forms strong intermolecular bonds with dissolved ions. In the absence of a strong electric field, Eq. (1.3.14) predicts vanishingly small values for j , since the exponential term is e raised to the power of a large negative number ($G_0/k_B T \approx 50$). It is only when $G_0 \sim \sqrt{\frac{q^3 E}{4\pi\epsilon_0}}$ that the probability of an ion getting over the energy barrier becomes non-negligible. From this condition, we can approximate the critical field necessary for ion evaporation to occur, E^* ,

$$E^* \approx \frac{4\pi\epsilon_0 G_0^2}{q^3}. \quad (1.3.21)$$

For ions with $G_0 \sim 1$ eV, Eq. (1.3.21) predicts $E^* \sim 10^9$ V/m, or 1 V/nm.

At the end of the previous section, we noted that ion evaporation takes over as the dominant charge emission mechanism once electrospray droplets shrink down to the nanoscale. We can now see why this is true. The dashed black curve in Fig. 1.3.8 is a plot of the number of charges on a droplet of radius r charged to the Rayleigh limit (Eq. (1.2.1)). The solid curves plot the number of charges on a drop needed to reach the critical field for ion evaporation, E^* , for three values of G_0 . Each of these curves crosses the Rayleigh limit curve at some critical r value. When r is less than this critical value, a higher charge density is required for the droplet to undergo a Rayleigh fission than for it to emit charge by ion evaporation. For ions with solvation energies $\approx 1 - 2$ eV, this critical radius is in the range of a few nanometers to a few 10s of nanometers.

Ion evaporation explains for how gas-phase ions are produced from charged nanodroplets in an electrospray plume. For sufficiently small droplets, fields on the order of 1 V/nm can be sustained on the droplets surface without hitting the Rayleigh limit. These fields are strong enough to pull ions directly out of solution and into the gas phase by ion evaporation.

It should be noted that there has been a longstanding debate in the field between proponents of the ion evaporation model (IEM) and a distinct mechanism for producing gas phase ions called the charge residue model (CRM) [140, 141, 126]. The CRM proposes that rather than a distinct charge emission process occurring at the nanoscale, Rayleigh fissions and evaporation simply continue until nanodroplets containing just a single analyte ion are produced. The remaining solvent then evaporates away leaving behind a gas-phase ion [89, 142]. While the debate still continues in some corners [143], it is now generally accepted that the IEM accurately describes the process by which

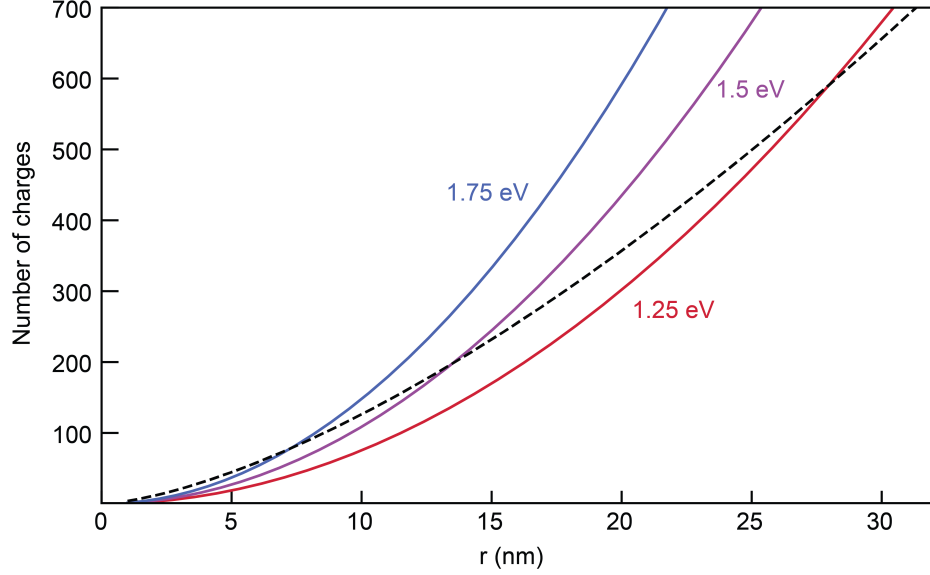


Figure 1.3.8: **Rayleigh fission vs Ion evaporation.** Comparison of the limits for Rayleigh fission and ion evaporation in nanoscale aqueous droplets. The dashed curve shows the number of charges needed to reach the Rayleigh limit as a function of r (from Eq. (1.2.1), and the solid curves show the number of charges needed for the electric field on the droplet surface to reach E^* for three different values of the solvation energy, G_0 .

small ions are generated, such as salt ions and small organic molecular ions like nucleic acids and peptides, while the CRM better describes the formation of very large ions like whole proteins or viruses [144, 145, 131].

In summary, the physics of electrospray encompasses a series of interrelated processes, each governed by the delicate balance between electric forces and surface tension. The process begins with the formation of a Taylor cone at the end of sharp capillary. At the apex of this cone, the balance of electrostatic and hydrostatic forces is broken, and a jet of charged liquid extends outwards, eventually breaking into a plume of charged droplets. As these droplets travel through a background gas and evaporate, they undergo repeated cycles of Rayleigh fission, where the electric stress overcomes surface tension, leading to the ejection of increasingly smaller and higher charge density progeny droplets. Ultimately, when these droplets reach the nanoscale, where surface tension can withstand extreme pressures, the electric field at the surface becomes strong enough to induce ion evaporation, directly emitting ions into the gas phase. The balance of electric forces and surface tension that governs each stage of this process provides a framework for how to think about the physics of the nanopore ion source going forward.

1.3.5 The purely ionic emission regime

The discussion of ESI's limitations in § 1.2.3 above leads naturally to the following question: is there a way to generate ions directly, without the intermediate generation and drying of charged droplets that leads to so much sample loss? The answer is yes, but with some caveats. Consider again the expressions for R_K and E_K (Eqs. (1.3.12) & (1.3.13)). Both depend on the ratio of electrical conductivity and flow rate, K/Q . As this ratio increases, the radius of the transition region shrinks while the electric field gets stronger. There exists a critical threshold for the ratio K/Q where $E_K \approx E^*$, the electric field at the transition region becomes strong enough to induce ion evaporation directly from the liquid meniscus, providing an alternative mechanism for charge release. When this threshold is surpassed, ion evaporation can occur at lower fields than are required for jet formation, effectively extinguishing the jet and eliminating the need for droplet emission to generate gas-phase ions. This is the purely ionic emission regime (PIR) of electrospray (occasionally referred to as the pure ion mode), where the meniscus itself becomes the site of ion production. Figure 1.3.9a shows a comparison of the PIR to the ion-droplet mixed regime, in which there is still a cone-jet but electric fields in the transition region are high enough to evaporate ions [146]. In the PIR, all of the ions are generated from the apex of the conical meniscus.

PIR emission has been observed in high conductivity solutions such as liquid metals [149, 150], ionic liquids [151, 147, 152, 148, 153], as well as electrolyte solutions in low volatility solvents like glycerol [154]. Electrolyte solutions in formamide have generated nearly PIR emission, but the total exclusion of charged droplets has not been conclusively demonstrated [155, 114]. The common features of all these liquids is high conductivity and/or relatively high viscosity, enabling them to reach high K/Q . PIR electrospray describes the operation of the familiar liquid metal ion sources which generate the ion beams used widely in focused ion beam (FIB) applications, although in that application the term field evaporation is typically used in place of ion evaporation [156, 157]. In the field of space propulsion, ionic liquid ion sources (ILIS) operating in the PIR have been the subject of significant research, because the emission of ions provides higher propulsive efficiency than the emission of charged droplets [158, 159, 134]. Figure 1.3.9b shows time-of-flight (TOF) curves for the charge emission from a 20 μm inner diameter emitter filled with the ionic liquid EMI- BF_4 at several flow rates [148]. The sharp step in the TOF curves around 5 μs represent ions, while the wider lower step extending out past 50 μs represents a distribution of charged droplets which take longer to reach the detector. At the lowest flow rates, this distribution of droplets disappears, confirming emission in the PIR.

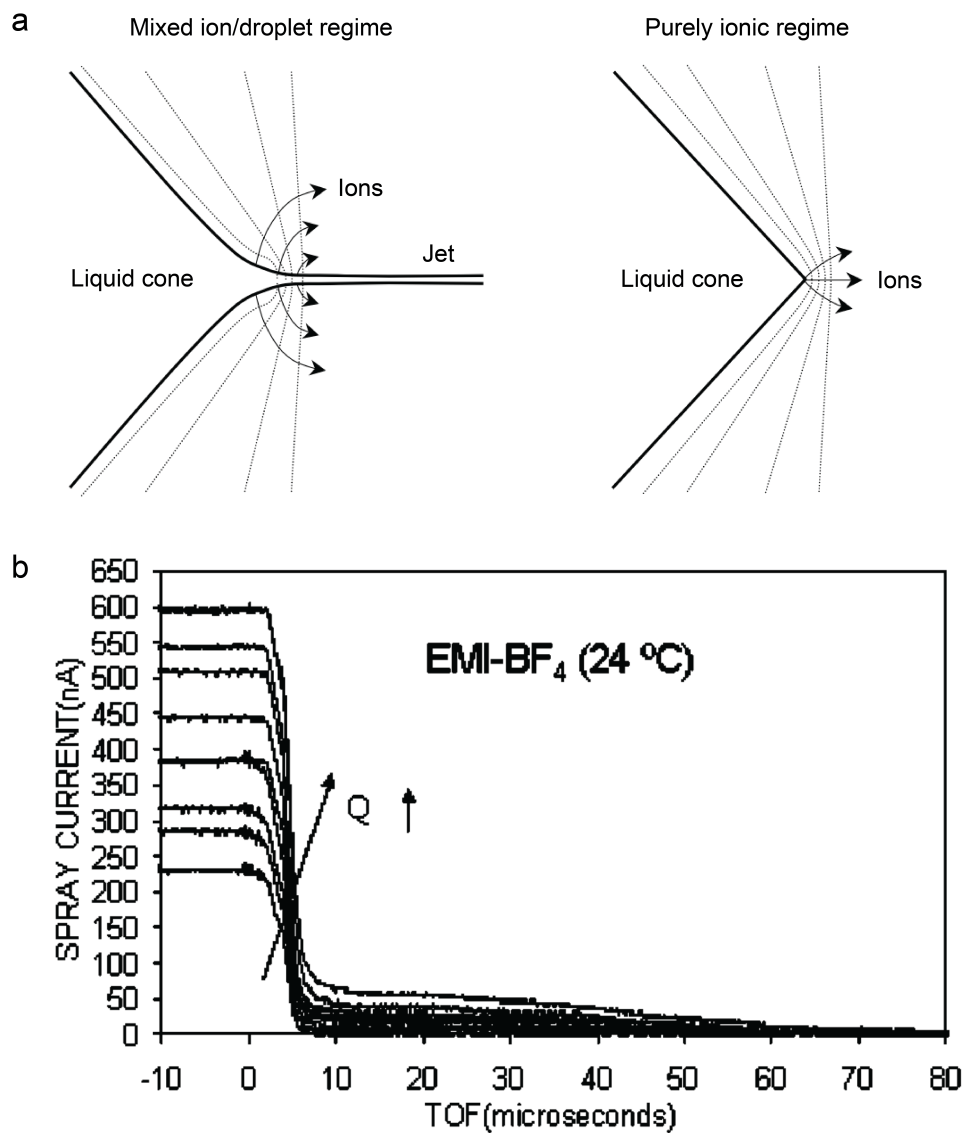


Figure 1.3.9: **Purely ionic emission.** **a** Comparison of the ion-droplet mixed regime, in which ions evaporate from the cone-jet transition region, with the purely ionic regime, in which the jet is extinguished and ions evaporate from the apex of the cone. Dotted lines show the potential distribution. Adapted from Ref [147]. **b** Demonstration of PIR electrospray with the ionic liquid EMI-BF₄. The arrow shows the direction of increasing flow rate. Reproduced from Ref. [148].

The caveat is that PIR electrospray has never been conclusively demonstrated in aqueous solution. There are multiple reasons for this: First, the low viscosity and moderate conductivity of aqueous solutions make it difficult to achieve the K/Q ratio necessary to extinguish the cone-jet. Second, water’s high volatility has prevented it from forming a stable liquid-vacuum interface in past investigations. Historically, efforts to directly emit ions from volatile solvents like water into high vacuum have been hampered by freezing at the emitter tip as well as electrical arcing [160, 154, 161].

1.4 The nanopore ion source

The physics of electrospray reveals a crucial insight: as the radius of curvature of a charged liquid surface decreases, the local electric field strength increases. This principle is evident in the structure of Taylor cones, where higher fields develop closer to the apex. It has also been seen in the electrospray plume, where ever smaller droplets remain stable with ever higher charge densities and surface fields, culminating in ion emission from nanoscale droplets. This recurring theme suggests a tantalizing possibility: what if an ESI emitter were shrunk down to the size of those final ion evaporating droplets that form in an electrospray?

This simple idea forms the basis of the nanopore ion source. By reducing the emitter size to just a few tens of nanometers across, extremely high electric fields (approaching or exceeding 1 V/nm) can be generated at the tip without requiring excessively high voltages. These field strengths are sufficient to induce direct ion emission from the liquid meniscus. Additionally, the extremely high hydraulic impedance of such a narrow emitter would result in very low flow rates, leading to a high K/Q value. This combination of high fields and low flow rates creates conditions highly favorable for emission in the purely ionic regime, eliminating the need for droplet formation and subsequent desolvation processes that are the root cause of sample loss in conventional ESI. Finally, the freezing problems that plagued previous attempts to achieve ion emission from water/vacuum interfaces may be avoided with a nanoscale emitter which limits the power of evaporative cooling. An ion source with these properties could both dramatically improve the sensitivity of MS-based proteomics by achieving near-lossless ion transmission efficiency, and enable single-molecule protein analysis by a combined nanopore/MS approach as described in § 1.1.4.

1.5 UV Photolysis

The nanopore ion source aims to solve one of the main challenges standing in the way of our vision to single molecule protein sequencing laid out in § 1.1.4, the ability to emit amino acid ions directly into vacuum. The other challenge is the ability to fragment the proteins inside of the ion source prior to emission. The final section of this thesis will be related to this challenge.

UVPD, introduced in § 1.1.1.3, is a well-established technique in which UV light is used to fragment peptide and protein ions in the gas phase [43, 162, 45, 46]. In a typical UVPD workflow, ionized proteins or peptides are irradiated by a UV laser pulse while held in an ion trap, producing a diverse array of fragments [43]. Thousands of repeated fragmentation events generate the MS² spectrum from which the protein or peptide can be identified. However, our approach to single molecule sequencing requires a method to cleave peptide bonds while the molecules are still in solution, within the nanopore ion source, rather than in the gas-phase. This approach reverses the order of fragmentation and ionization relative to conventional UVPD.

The process of breaking chemical bonds in solution using light is typically called photolysis. While there is a substantial literature on UVPD (see in particular the thorough review by Brodbelt [43]), less work has been done to study the UV photolysis of peptides in solution, where the chemical environment is more complex. Investigations by Nikogosyan et al. in the 1990s determined the molar absorption coefficients and quantum yields of scission for a variety of amino acids and di-peptides at wavelengths around 200 nm [163, 164, 165, 166, 167]. More recent work by Thøgersen et al. studied the photolysis of the dipeptide gly-gly using UV-pump IR-probe spectroscopy, and found no evidence of peptide bond scission [168]. However, this finding appears to conflict with the earlier reports by Nikogosyan et al. The existing literature on UV photolysis of peptides does not provide sufficient data to evaluate the feasibility of our in-source UV photolysis approach or determine the optimal wavelength to maximize peptide bond cleavage while minimizing damage to amino acid side chains. To that end, more fundamental studies are needed to determine the quantum yield of peptide bond scission for light in the deep UV range.

1.6 Thesis Overview

1.6.1 Chapter 2: Experimental techniques

Chapter 2 details the experimental methods and apparatus designed and used to study the nanopore ion source and UV photolysis of peptides. It first describes how nanopore ion sources are fabricated

and prepared. Next, it describes two custom-built mass spectrometers: a quadrupole mass spectrometer and a magnetic sector mass spectrometer, both designed to operate with the nanopore ion source in high vacuum. Additionally, it outlines methods for measuring ion emission characteristics using sensitive current measurements. Finally, it discusses the methods used to measure the absorbance and quantum yield of bond scission for peptides using deep UV light.

1.6.2 Chapter 3: Nanopore ion sources deliver individual ions of amino acids and peptides directly into high vacuum

Chapter 3 demonstrates the capability of nanopore ion sources to emit individual ions of amino acids and small peptides directly into high vacuum from aqueous solutions. Using the custom quadrupole mass spectrometer described in Ch. 2, this chapter presents mass spectra of various amino acids and small peptides, showing that nanopore sources can deliver many of the same ions generated by conventional ESI directly into high vacuum. The chapter also reports high ion transmission efficiencies (up to 93%) and provides evidence that the nanopore source operates via direct ion evaporation from the electrified meniscus rather than via charged droplet emission like in conventional ESI. These findings suggest that nanopore ion sources could significantly improve the sensitivity of mass spectrometry measurements and are the first step in enabling single-molecule protein sequencing by nanopore mass spectrometry.

1.6.3 Chapter 4: Thermal properties of the nanopore ion source

Chapter 4 focuses on the thermal modeling of nanopore ion sources operating in high vacuum. The chapter begins by analyzing evaporative cooling effects, demonstrating that smaller emitters are more resilient to freezing due to their small size and geometry. It then explores the thermal effects of ion emission, including Joule heating and a novel concept called ion evaporative cooling. The interplay between these heating and cooling mechanisms is examined, revealing critical current values that define distinct thermal regimes. While the ion evaporative cooling effect is predicted to be small in nanopore ion sources, it may be significant in liquid metal ion sources. This thermal analysis provides a framework for understanding and optimizing the performance of nanoscale ion emitters in vacuum environments.

1.6.4 Chapter 5: Emission properties of the nanopore ion source

Chapter 5 details further investigations into the emission properties of nanopore ion sources. A theoretical model is developed to describe the ion source’s IV behavior, predicting a smooth, exponential increase in current with voltage, which is confirmed by experimental measurements with both water and formamide solutions. The chapter demonstrates that the emission mechanism is the same in both air and vacuum environments, and that the emission current is independent of applied pressure, supporting the conclusion of direct ion evaporation from a closed meniscus. Further experiments explore the effects of diffusion and the transition between pure ion emission and mixed ion/droplet emission modes at higher electric fields. These findings provide a more comprehensive understanding of ion emission from nanopore ion sources and support their potential to improve the efficiency of ion transmission in mass spectrometry applications.

1.6.5 Chapter 6: Ultraviolet photofragmentation of peptide bonds

Chapter 6 describes investigations into the use of ultraviolet light to break peptide bonds in aqueous solutions, a crucial step for our proposed single-molecule protein sequencing approach. This chapter presents detailed experiments on the photolysis of glycylglycine (gly-gly) using 193 nm and 222 nm light, including measurements of absorption spectra and quantum yields of peptide bond scission. Results show that 193 nm light is significantly more effective at breaking peptide bonds, with a quantum yield of 1.6% compared to 0.18% for 222 nm light. These findings support the feasibility of UV-based peptide fragmentation for single-molecule sequencing and suggest that 193 nm light is the preferred wavelength. The chapter also discusses broader implications of these results for UV disinfection technologies and origins-of-life studies.

1.6.6 Chapter 7: Conclusions and Outlook

Chapter 7 concludes the thesis with a brief summary and a discussion of the limitations of this work and future directions for further research.

2

Experimental techniques

“The history of science teaches only too plainly the lesson that no single method is absolutely to be relied upon, that sources of error lurk where they are least expected, and that they may escape the notice of the most experienced and conscientious worker.”

– Lord Rayleigh

2.1 The nanopore ion source

The nanopore ion source is a nanoscale glass capillary emitter that emits ions directly from liquid into vacuum. The nanopore ion source takes its name from the nanometer-scale opening at its tip. The emitter is physically identical to the glass nanopipettes used widely in electrochemistry and electrophysiology [169, 170, 171, 172], and differs from conventional electrospray ion sources primarily in its size, mechanism of charge emission, and the vacuum environment in which it operates. The core component of the ion source is a pulled quartz emitter whose tip inner diameter (I.D.) is smaller than 100 nm [173, 174]. This nanoscale tip influences ion emission by enabling a stable liquid-vacuum interface to form, preventing droplet emission through extremely low fluid flow rates, and concentrating electric fields at the sharp, conductive tip. These concentrated electric fields may reach strengths of ~ 1 V/nm, sufficient for ion evaporation [91, 175].

Heat	Velocity	Delay	Pull
660	45	175	180

Table 2.1: Recipe for pulling 30 nm I.D. tips from quartz capillaries using the P-2000 capillary puller.

2.1.1 Preparing nanopore ion sources

2.1.1.1 Fabrication

Nanopore ion source tips are fabricated by pulling standard quartz capillary tubes using a P-2000 pipette puller (Sutter Instruments). We use 7.5 cm long quartz capillaries with 0.7 mm inner diameter, 1 mm outer diameter, and an internal filament to make filling easier (QF100-70-7.5, Sutter Instruments). The tip orifice size and geometry depends sensitively on the pulling recipe used [174]. Table 2.1 shows the single-line recipe that we have found works best for repeatably producing symmetrical, ≈ 30 nm inner diameter, ≈ 100 nm outer diameter tips, with 3-5 mm taper lengths.

The heat and pull values are often adjusted by ± 20 and ± 10 , respectively, to optimize the geometry and smoothness of the tip.

2.1.1.2 Imaging and characterization

Scanning electron microscopy (SEM) is used to determine the inner and outer diameters of the nanopore ion source tips. To prepare nanopore tips for imaging, we first deposit a conductive carbon layer on their outer surface to prevent them from charging up in the SEM. Up to 20 tips at a time are mounted on an SEM sample stage with double sided carbon tape and a strip of copper or aluminum tape on the top to secure them in place. The mounted tips are placed in a thermal evaporator (CED 030, BalTec) which deposits approximately 5 nm of carbon onto their exposed surface by briefly running a high current through double-stranded carbon thread. We then load the tips into the SEM (LEO 1530 VP, Zeiss, Inc.) and rotate the mount such that the tips are tilted up by 45° , halfway between the horizontal plane and the vertical axis of the electron beam. This viewing angle allows us to see the opening of each tip and accurately measure its inner and outer diameter along the perpendicular axis, while also providing a minimally distorted view of the first several micrometers of the taper. Figure 2.1.1 shows an example SEM image of a nanopore tip with arrows to indicate the direction along which the inner and outer diameters are measured. If an SEM is not available but accurate tip size determination is still desired, this can alternatively be done via a conductance measurement with a sensitive electrometer[176].

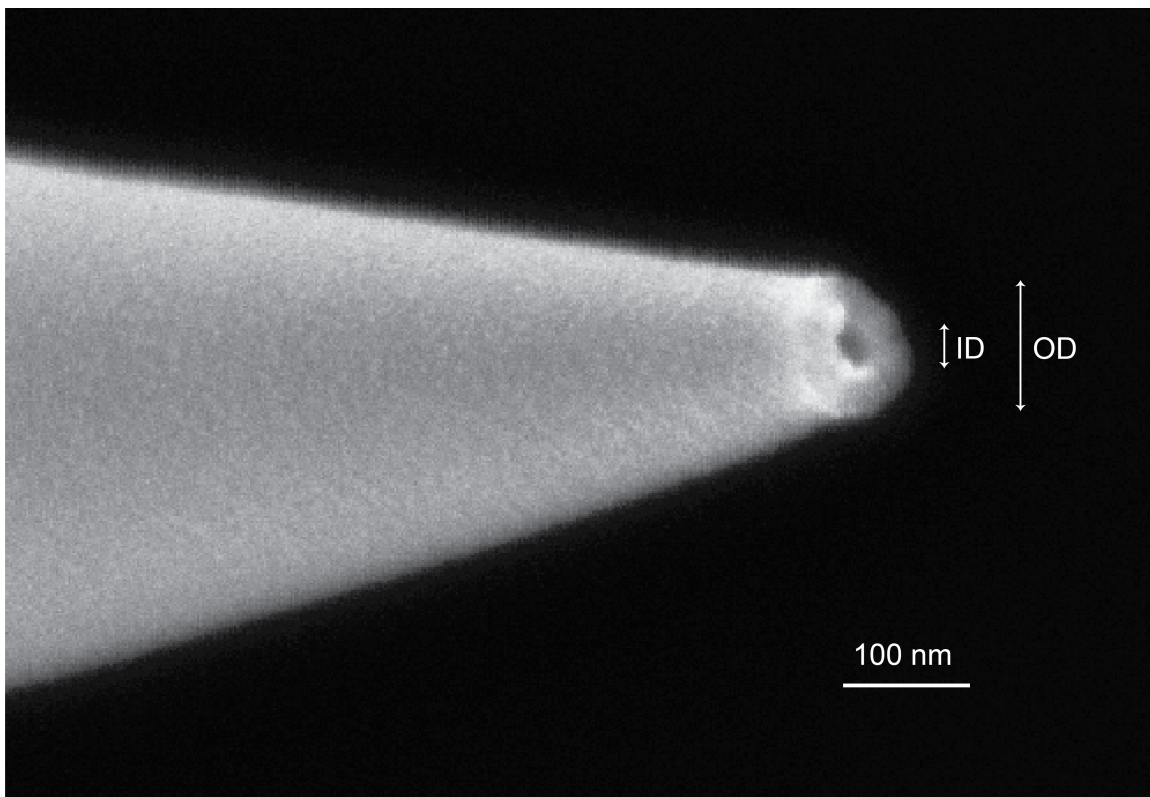


Figure 2.1.1: **SEM image of nanopore tip.** Scanning electron microscope (SEM) image of the tip of a nanopore ion source. Double sided arrows indicate measurements of the inner and outer diameter of the tip.

2.1.1.3 Cleaning and filling

The nanopore ion source emitters need to have a hydrophilic inner surface to be properly filled with aqueous solution. We make them hydrophilic through plasma treatment. A single tip is placed in a barrel reactor (Plasma Preen II-973, Plasmatic Systems), which is purged with pure O_2 and pumped down to 1 torr. The oxygen plasma is ignited with 250 W microwave radiation and allowed to react for 2 minutes. This effectively cleans the surface of organic compounds, including the carbon layer that was deposited for SEM imaging. Plasma treatment makes the surface hydrophilic by breaking surface Si-O-Si bonds, creating dangling silanol (Si-OH) groups which readily form hydrogen bonds with water [177].

Following plasma treatment, the tip is filled with the desired solution using a MicroFil needle (World Precision Instruments). Bubbles can be a major source of frustration and failed experiments throughout micro-and nano-fluidics [178], and the nanopore ion source is no exception [179]. Figure 2.1.2 shows optical microscopy images of the tip after filling successfully (panel a) and with the accidental introduction of a microbubble which may interfere with measurements (panel b). We

have found that gently touching one or two droplets to the rear end of the emitter, allowing capillary action to draw the solution towards the tip, effectively filling the emitter backwards (see Fig 2.1.2c), creates less bubbles than sticking the MicroFil needle all the way into the emitter. Once the liquid meniscus has reached the bottom of the taper, the MicroFil needle can then be inserted past the meniscus to fill the rest of the emitter. The filled tip is then ready for use.

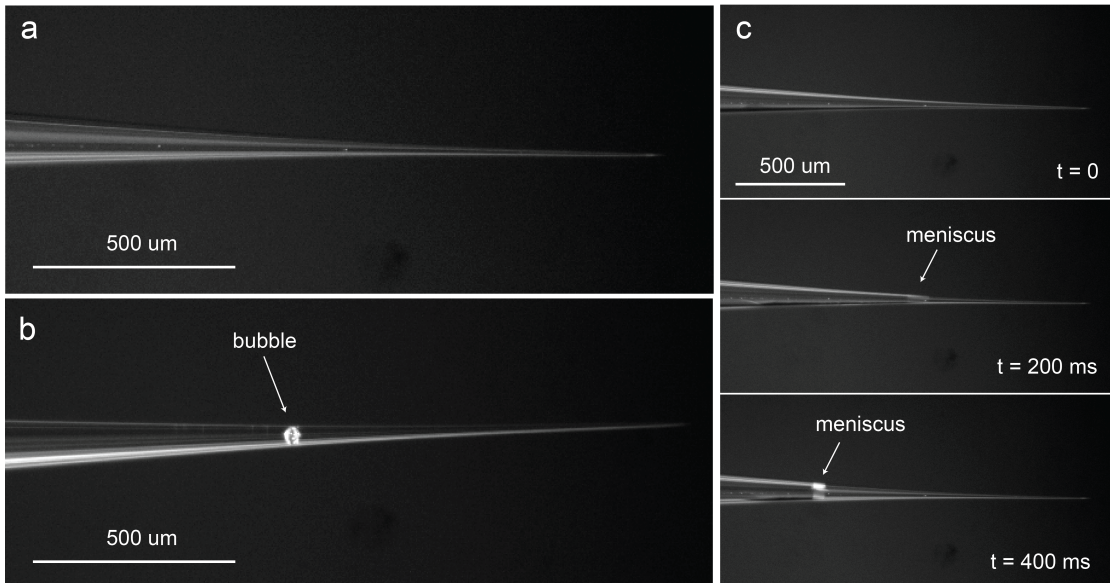


Figure 2.1.2: **Filling a nanopore ion source emitter.** **a** Optical microscope image of tip after being filled with no bubbles. **b** Image of tip after filling in which a small bubble is seen. **c**, Images of a plasma-treated tip at $t = 0$, 200, and 400 ms after initiating filling by touching a droplet to the rear end of the capillary. Capillary action first draws solution all the way to the tip and then fills towards the rear. At $t = 200$ ms and 400 ms, the meniscus (which separates air on the left from liquid on the right) can be seen travelling to the left, away from the tip.

2.1.2 Tip holder and alignment

Operating an ion source in high vacuum presents several unique challenges for the ion source's mounting system. It must supply the tip with high voltage, measure the emission current, carry in fresh sample solution and carry out waste, repeatedly and precisely position the ion source relative to the ion optics and be able to quickly introduce or remove an emitter, all while holding high vacuum. We developed a custom tip holder and mounting system that addresses these challenges by integrating a tube-in-tube design [180] and a kinematic mount [181]. The front-end of the tip holder is illustrated in Fig. 2.1.3. The tube-in-tube system consists of an inner tubing (360 μm O.D. X 150 μm I.D. PEEK) and an Ag/AgCl electrode which are both threaded through an outer PEEK tubing (1/16" O.D. X 0.04" I.D. PEEK). The inner tubing is connected to a syringe mounted on

a syringe pump (New Era Pump Systems NE-300) which supplies fresh solution to the ion source while the outer tubing carries waste solution away. The Ag/AgCl electrode connects to a Keithley 2657a Source-Measure Unit (SMU) which supplies voltage to the tip while measuring the emission current down to ≈ 1 pA. A 1/4" diameter steel tube houses the tube-in-tube system and enters the vacuum chamber through a KF-to-Quick-Connect coupling. This coupling can sustain vacuum down to around 10^{-8} torr, and allows the tip to be inserted into or removed from the vacuum chamber without fully breaking vacuum. The front end of the tip holder has a kinematic mount that ensures proper positioning and alignment of the tip. The mount has three components, a steel "flex" piece that flexibly connects the mount to the steel tubing, a PEEK "chuck" piece which connects the outer PEEK tubing to the back of the nanopore ion source, and a steel "face" piece with three v-grooves which form half of the kinematic mounting system. The other half is a ring shaped "register" piece affixed to the ion optics within the mass spectrometer that has three precision steel balls which make contact with the v-grooves on the face piece, restricting movement along every degree of freedom. Vacuum tight connections are made between the outer tubing and the chuck using a VacuTight fitting (IDEX) and between the nanocapillary and the chuck using a Waters type compression screw and a viton o-ring. The distance and angle between the PEEK chuck piece, which holds the emitter, and the steel face piece can be adjusted by 3 spring-loaded screws. This enables precise control over the position of the ion source's tip to within about 10 microns.

We constructed an alignment system which can ensure that each ion source is placed in the same position relative to the ion optics inside the mass spectrometer. The alignment system comprises a KF flange mounted on an optics table directly in front of a register, which matches the register inside the mass spectrometer. A camera with a microscope objective pointing along the axis of the ion source allows us to measure the tip's precise position. The three adjustment screws are tightened or loosened until the tip is observed to be in the desired alignment, which should be preserved after mounting in the mass spectrometer.

2.2 Quadrupole mass spectrometry

The nanopore ion source is unique in its ability to emit ions directly into high vacuum from aqueous solution. In order to validate this ability, a mass spectrometer in which the ion source is housed in high vacuum was required. This is an unusual requirement for a mass spectrometer, as the ion source typically sits at atmospheric pressure in front of a narrow inlet. We designed and constructed a custom quadrupole mass spectrometer in which the ion source, ion optics, mass analyzer, and

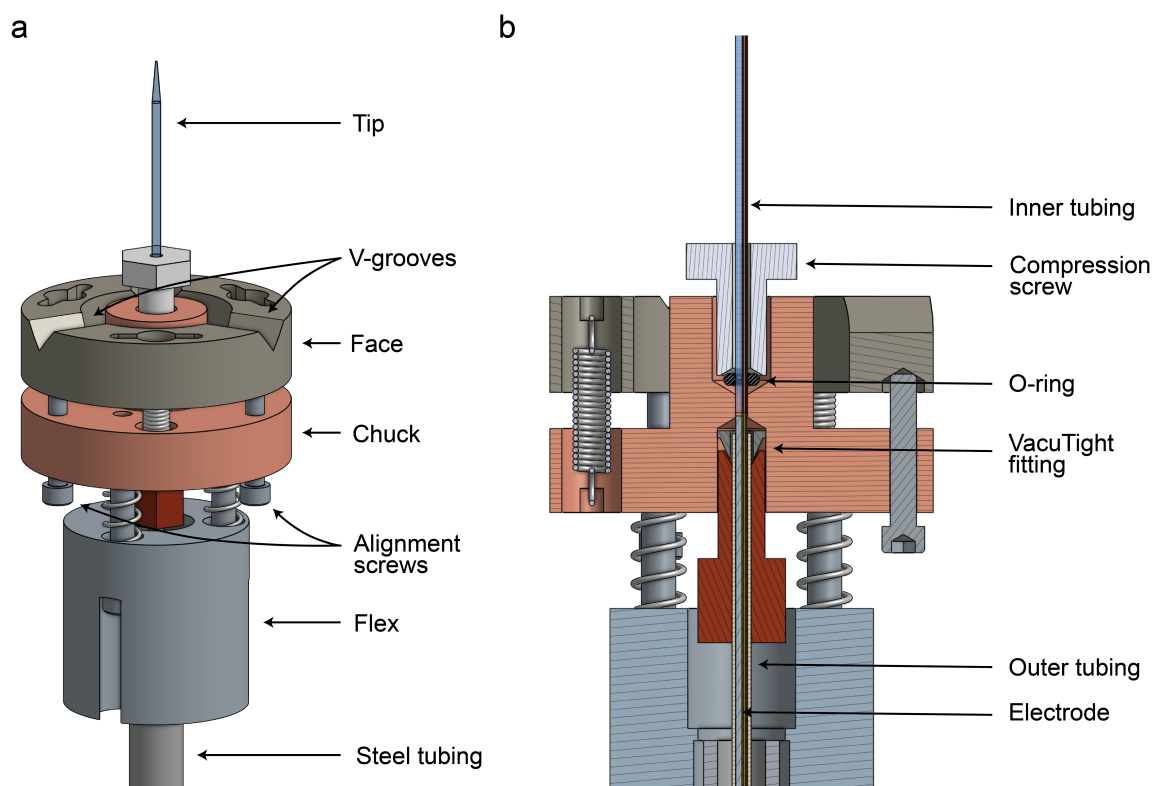


Figure 2.1.3: **Tip holder.** **a**, Perspective view of the front end of the tip holder showing the main components of the mounting system. **b**, Cross-sectional view of the front end of the tip holder showing the tube-in-tube system as well as the O-ring and VacuTight fitting which both make vacuum tight seals.

detector are all housed in a single high vacuum chamber. We decided on a quadrupole mass analyzer as it allows the flexibility and resolution to confidently identify ions across a broad mass range that may be generated by the nanopore ion source. This section provides a brief overview of the theory behind quadrupole mass analyzers before describing the design and features of the quadrupole mass spectrometer we built to test the nanopore ion source.

2.2.1 Quadrupole theory

The quadrupole was first developed to focus charged particle beams in accelerators at Brookhaven National laboratory in 1952 [182], and was independently recognized soon after for its ability to act as a mass filter by W. Paul at the University of Bonn [183] and R.F. Post at the Berkeley Radiation Laboratory [184]. Paul et al. developed the theory of quadrupole mass filters throughout the 1950s [185, 186], and it has remained largely unchanged up to today [187, 29]. I provide a brief overview of this theory below, deriving the equations of motion for an ion in a quadrupole field and determining the stability conditions of its trajectory as a function of its mass-to-charge-ratio.

The general expression for the electric potential, Φ , within a quadrupole in Cartesian coordinates is given by

$$\Phi(x, y, t) = \Phi_0(t)(\lambda x^2 + \sigma y^2 + \gamma z^2), \quad (2.2.1)$$

where Φ_0 is the potential applied to each pair of electrodes, and λ , σ , and γ are constants. Taking the quadrupole to be parallel to the z-axis, we find $\gamma = 0$ and the problem reduces to 2 dimensions. Laplace's equation, $\nabla^2 \Phi = 0$, gives $\lambda = -\sigma$. Additionally, the boundary condition at any electrode surface (take $x = r_0$ and $y = 0$ for example) gives $\lambda = 1/r_0^2$, where r_0 is the distance between the central axis and the edge of any of the four electrodes. Equation (2.2.1) now reads

$$\Phi(x, y, t) = \Phi_0(t) \frac{x^2 - y^2}{r_0^2}. \quad (2.2.2)$$

The applied potential has DC and AC components, so it is typically written as $\Phi_0(t) = U - V \cos \omega t$. The electric fields are obtained from (2.2.2),

$$E_u = -\frac{d\Phi}{du} = \mp (U - V \cos \omega t) \frac{2u}{r_0^2}, \quad (2.2.3)$$

where $u = x, y$, and the upper sign always applies to x while the lower sign applies to y . Now we can apply Newton's 2nd law, $F_u = zq_e E_u$ where z is the number of charges on the ion and q_e is the

elementary charge, to get the equations of motion,

$$\frac{d^2u}{dt^2} \pm \frac{2zq_e}{mr_0^2}(U + V \cos \omega t)u = 0. \quad (2.2.4)$$

Equation 2.2.4 has the form of a Mathieu equation, a linear second order homogeneous differential equation that appears widely throughout physics and engineering [188, 189]. The equation of motion can be rewritten in the canonical form of the Mathieu equation using the following change of variables,

$$\xi \equiv \frac{m\omega}{2}, \quad q_u \equiv \pm \frac{8zq_e U}{m\omega^2 r_0^2}, \quad \text{and} \quad a_u \equiv \pm \frac{4zq_e V}{m\omega^2 r_0^2}. \quad (2.2.5)$$

Equation 2.2.4 then becomes

$$\frac{d^2u}{d\xi^2} + (a_u - 2q_u \cos 2\xi)u = 0. \quad (2.2.6)$$

The Mathieu equation has solutions of the form

$$u(\xi) = A \sum_{\infty} C_{2n} \cos(2n + \beta) + B \sum_{\infty} C_{2n} \sin(2n + \beta), \quad (2.2.7)$$

where A and B are functions of the initial conditions, and C_{2n} and β are functions of a_u and q_u [189, 187]. These solutions can be stable or unstable depending on the values of a_u and q_u , which are themselves functions of m/z . It is the difference between these two families of solutions that allows a quadrupole mass analyzer to distinguish ions by their mass-to-charge ratio. Figure 2.2.1 shows examples of each of these classes of solutions found by numerically integrating Eq. (2.2.6). Figure 2.2.1a shows the x-component of an example stable trajectory through a 440 kHz quadrupole with parameters $a_x = 0.15$ and $q_x = 0.67$. Figure 2.2.1b shows the x-component of the phase space trajectory for the same ion, showing a stable orbit. Figure 2.2.1c shows an unstable ion trajectory with parameters $a_x = 0.15$ and $q_x = 0.67$. Figure 2.2.1d shows the x-component of the phase space trajectory for the same ion, showing an unstable orbit. By mapping out the stable and unstable solutions in the a - q parameter space, one finds a region of stability near the origin. There are other stability regions at higher a and q values, but these are not often used [29]. The main region of stable solutions is shown in Fig. 2.2.1e, along with points marking the locations of the example trajectories shown in panels a-d.

A quadrupole mass spectrometer scans across the mass range by maintaining a fixed ratio of DC to RF voltage (U/V) and increasing both voltages simultaneously. Notice from Eq. (2.2.5) that $q_u/a_u = 2U/V$, so keeping $U/V = \text{const.}$ is equivalent to moving through a - q space along

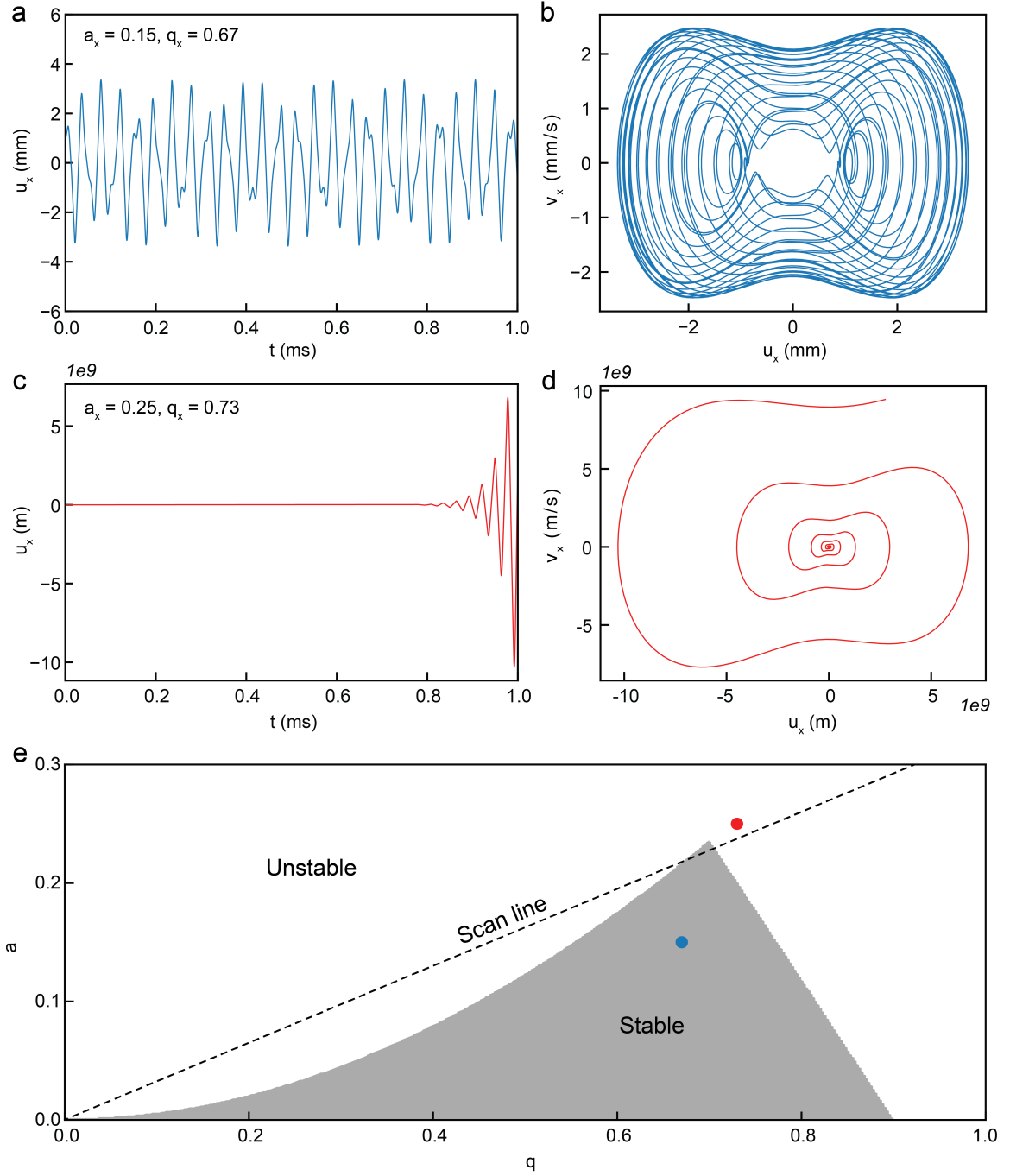


Figure 2.2.1: **Ion trajectories in a quadrupole.** **a**, x-component of a simulated stable trajectory of an ion through a quadrupole with Mathieu parameters $a_x = 0.15$ and $q_x = 0.67$. **b**, Phase space plot of the x-component of the same stable trajectory. **c**, x-component of a simulated unstable trajectory of an ion through a quadrupole with Mathieu parameters $a_x = 0.25$ and $q_x = 0.73$. **d**, Phase space plot of the x-component of the same unstable trajectory. **e**, Mathieu stability diagram with the stable and unstable trajectories of panels a,b and c,d labelled in blue and red, respectively. The dashed line represents a mass scan-line at fixed U/V .

a straight line, called the mass scan-line and illustrated in Fig. 2.2.1e. As the voltages increase, the scan-line clips the tip of the stable regions of successively higher mass ions. The width of the intersection between the scan-line and the stable region determines the resolution of the peak in the mass spectrum. For a more thorough treatment of quadrupole theory, see the following references [190, 187, 191].

2.2.2 The quadrupole mass spectrometer

We designed and built a quadrupole mass spectrometer to validate the nanopore ion source and identify the species it emits. The mass spectrometer, shown in Fig. 2.2.2, comprises a custom ion lens, a quadrupole mass filter, an ion bender, and a detector sensitive to single ions, all housed in a vacuum chamber. The ion optics were home-built and consist of four electrodes. The first is the extraction electrode which applies a negative voltage relative to the nanopore tip to draw ions out, and the other three electrodes act as an electrostatic lens to focus the ion beam [192, 193]. Typically, the tip is held at +100 V while the extraction electrode is held between approximately -50 and -300 V. The quadrupole (QC MAX-500, Extrel inc.) consists of four parallel conducting rods that generate oscillating electric fields through applied DC and RF voltages, allowing only ions with specific mass-to-charge ratios to maintain stable trajectories and pass through [194]. A mass spectrum is generated by scanning the quadrupole across the desired m/z range while counting the number of ions detected at each m/z value. The quadrupole in our instrument is driven by a 440 kHz RF oscillator and scans across an m/z range of 50 to 1000 in about 0.6 s. The ion bender (Extrel inc.) is itself another quadrupole, but it applies only a DC voltage between its inner and outer poles to deflect the ions, it does not discriminate by m/z . The ion detector (Extrel inc., 810132) has two parts, a conversion dynode which generates a burst of electrons when an ion strikes it, and a channel electron multiplier (often called a Channeltron), which receives and multiplies the burst of electrons to produce a measurable voltage pulse at its output [195, 193]. The dynode is typically held at around -3000 V while the electron multiplier is held at approximately +1500 - 2000 V.

The vacuum chamber housing the instrument consists of an 8", 6-way cross attached to an 8", 6-way cube. Each major component is mounted on one of the 8" flanges with steel rods. The chamber is evacuated by a single turbo pump (Osaka vacuum TG450F), backed by a dry screw pump (Osaka vacuum DSP251). A thermocouple gauge (Televac 2A) and cold cathode gauge (Televac 7B) monitor the pressure in the main chamber. This system typically achieves base pressures around 10^{-8} torr. Two valves in between the turbo pump and dry pump can be switched to seal the main chamber and

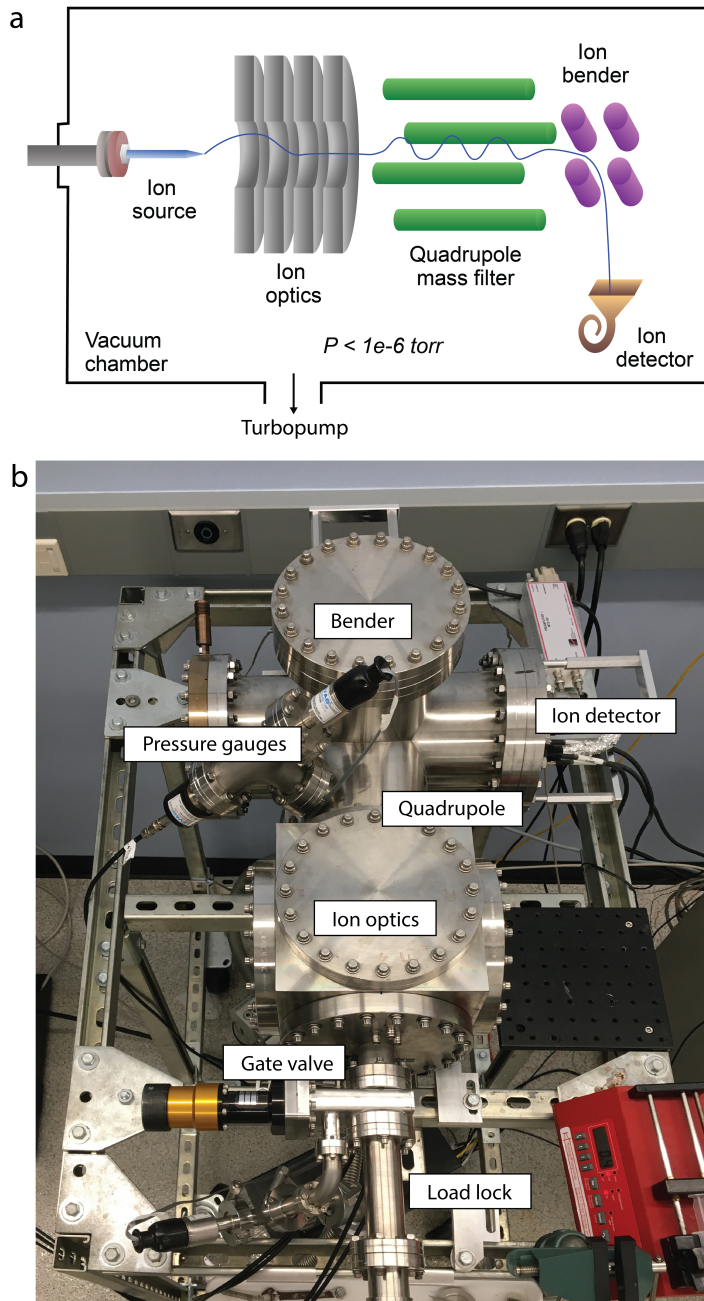


Figure 2.2.2: **The quadrupole mass spectrometer.** **a** Diagram of the quadrupole mass spectrometer showing the ion source, ion optics, quadrupole, ion bender, and ion detector. **b** Overhead image of the quadrupole mass spectrometer with labels on the locations of critical components.

route the dry pump inlet to the load-lock at the mass spectrometer's inlet. We can easily insert and remove ion sources without breaking vacuum in the main chamber by the following process: First, we insert the ion source into the load-lock, which is sealed from the main chamber by a gate valve. We then close the valve connecting the two pumps and open the valve connecting the dry pump to the load-lock. Once the pressure in the load-lock drops below ≈ 10 mTorr, we swap the valve positions

to reconnect the two pumps and open the gate valve. The ion source can then be pushed all the way into the main chamber by pushing the steel tubing through the slightly loosened KF-to-Quick Connect adapter, and mounted to the register in front of the ion optics. A more detailed description of our quadrupole mass spectrometer can be found in Ref. [180].

2.2.3 Example data from quadrupole MS

Figure 2.2.3 shows an example of the kind of data we obtain from the quadrupole mass spectrometer using a nanopore ion source. Figure 2.2.3a shows the mass spectrum measured for a 1 M solution of NaI in formamide which was emitted from a nanopore ion source with I.D. = 28 nm and O.D. = 72 nm. The spectrum shows a series of peaks corresponding to a Na^+ ion solvated by n formamide (FA) molecules, where $n \geq 3$. The majority of ions are detected with a solvation state of $3 < n < 6$, with the $n = 4$ state being the most abundant. There is a broad distribution of higher solvation states extending beyond the end of the mass range with a local maximum at $n = 14$. Figure 2.2.3b shows the MS signal intensity, emission current, and extraction voltage over about 10 minutes of emission. The onset voltage, the voltage at which emitted ions are first detected, was 205 V, with an initial emission current of about 3 nA. The emission current and MS signal both declined exponentially until emission stopped altogether after 10 minutes (this phenomenon is discussed in more detail in § 5.6). The voltage was increased by about 2 V at $t \approx 100$ s (not visible in figure due to noise), causing a jump in the emission current by about 30% and a smaller jump in the MS signal. The difference in response is likely due to improved focusing of the ion beam following the change in the extractor voltage. Figure 2.2.3c shows a scatterplot of the Emission current and MS signal, showing a highly linear relationship between the two. The upper distribution of points corresponds to the period before the extraction voltage was raised, and the lower distribution of points corresponds to the period after.

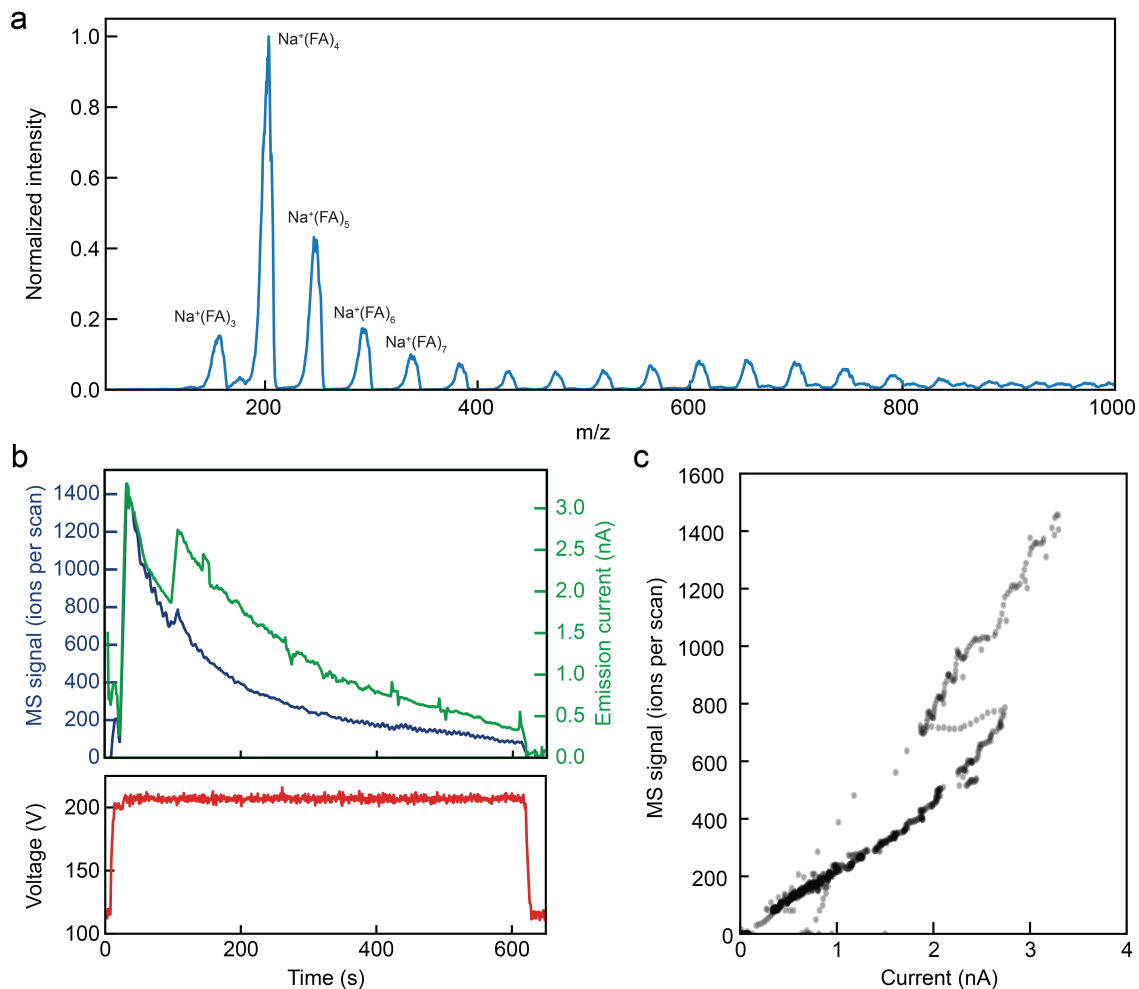


Figure 2.2.3: **Quadrupole MS example data.** **a**, Mass spectrum of 1 M NaI in formamide. **b**, Plot of emission current and MS signal vs time during emission showing a characteristic decay. **c**, Scatter plot of emission current and MS signal showing a linear relationship between the two. The upper distribution corresponds to the first ≈ 100 s of emission, and the lower distribution corresponds to the subsequent ≈ 500 s, after the voltage was slightly increased.

2.3 Magnetic Sector Mass Spectrometry

We built a second mass spectrometer with a removable magnetic sector mass analyzer to carry out measurements that would not have been possible with the quadrupole instrument. The quadrupole MS is fundamentally limited by the quadrupole mass filter’s principle of operation. The quadrupole throws away any ion that is outside of its narrow m/z acceptance window as it scans across the full m/z range, meaning that the vast majority of emitted ions are never measured. Such an instrument is incompatible with our eventual goal of sequencing single molecules, as it cannot simultaneously detect multiple fragments of a single molecule with different m/z values. Additionally, the ion losses incurred by the quadrupole complicate measurements of the ion transmission efficiency, as it is difficult to separate losses incurred by the source from those incurred by the mass filter.

2.3.1 Theory of magnetic sector MS

A magnetic sector is simply a region of magnetic field that the ions must traverse, causing them to spatially separate by their mass-to-charge ratio according to the Lorentz force law. With the magnetic field perpendicular to the ion beam axis,

$$\frac{mv^2}{R} = zq_e v B, \quad (2.3.1)$$

where v is the ion’s velocity, B is the magnetic field strength, and R is the radius of curvature of the ions trajectory. If the ions have been accelerated by a voltage V , then they will have a kinetic energy given by

$$\frac{1}{2}mv^2 = zq_e V, \quad (2.3.2)$$

Combining Eqs. (2.3.1) and (2.3.2) and solving for R yields

$$R = \sqrt{\frac{2Vm}{q_e B^2 z}} \quad (2.3.3)$$

Given an ion beam composed of multiple species with distinct m/z , accelerated by a voltage V , and traversing a magnetic field of strength B , Eq. (2.3.3) reveals how the ions will take circular trajectories of radius R which depend on their mass-to-charge ratio.

In combination with an array of detectors or an imaging detector, a magnetic sector MS can detect ions with wide ranging m/z values simultaneously, unlike a quadrupole MS. For our approach to single molecule sequencing (Fig. 1.1.4), we envision using a detector array where the detection

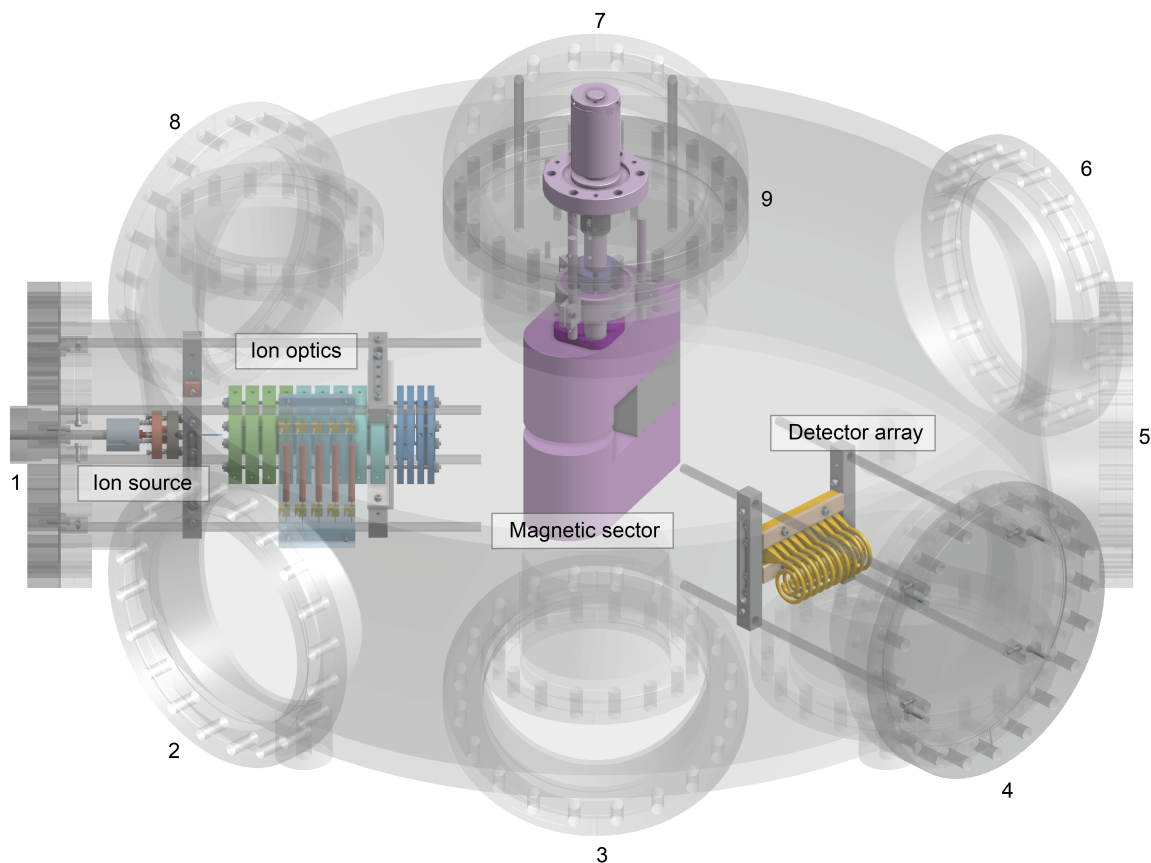


Figure 2.3.1: **The magnetic sector mass spectrometer.** Schematic of the magnetic sector mass spectrometer showing the cylindrical vacuum chamber, ion source, ion optics, magnetic sector, and detector array. Flanges are numbered for easy referencing throughout the text.

location identifies the amino acid, while the *time* of each detection contains the sequence information. In a quadrupole MS the temporal information is used to identify the ion; the m/z value is known based on when the ion strikes the detector during a scan over the m/z range. In a magnetic sector instrument, spatial information is used to identify the ion, leaving the temporal information available for sequencing.

2.3.2 The magnetic sector mass spectrometer

2.3.2.1 System overview

Our magnetic sector mass spectrometer, illustrated in Fig. 2.3.1, was designed to maximize adaptability for a variety of different measurements. Primarily, the instrument was designed with measurements of ion transmission efficiency and the ion vs. charged droplet content of the emitted beam in mind. It is housed in a 24" diameter, 8" tall cylindrical vacuum chamber with eight lateral 8"

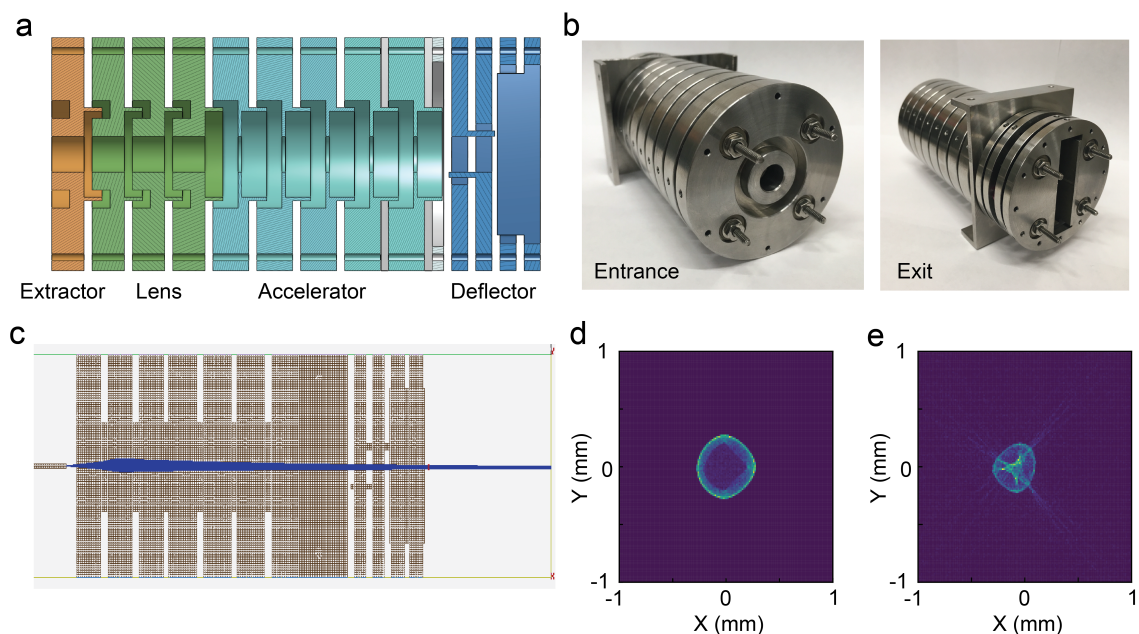


Figure 2.3.2: **Ion optics.** **a** Diagram showing the cross section of the designed ion optics with the extractor, lens, accelerator, and deflector labeled and displayed in different colors. **b** Photographs showing both sides of the assembled ion optics. **c** A simulated image of an ion beam focused by the ion optics taken in SIMION. **d** An image of the cross section of a simulated ion beam just as it is exiting the ion optics. **e** An image of the cross section of the same simulated ion beam, with the trajectories of all the ions extrapolated out 50 cm beyond the ion optics, where the detector would generally be located.

flanges, and two pairs of 8" and 6" flanges on the top and bottom sides respectively. This adaptable design allows for modular arrangement of detectors and other components to alter the configurations as needed. The primary components of the instrument are the ion optics to extract and focus the beam, the magnetic sector which separates emitted ions based on m/z , and detectors (of which there are multiple options). Flanges can also be configured as viewports when not mounting any of these components. The instrument uses the same ion source holder and mounting/alignment system as the quadrupole mass spectrometer described above. This allows us to easily move an ion source from one instrument to the other without dismounting it from its holder. The subsections below describe in detail the design and simulation/testing of each component.

The vacuum system is very similar to that of the quadrupole MS (§ 2.2.2). It features a turbo-molecular pump (Osaka Vacuum TG450F) backed by a rotary vane pump (Pfeiffer DUO 20M), and an identical load-lock and valve system to the quadrupole MS described in § 2.2.2. When pumping down after being vented to atmosphere, it reaches a base pressure of about 10^{-8} torr in a few days.

2.3.2.2 Ion Optics

The ion optics comprise a stack of steel electrodes with a 1 cm bore through their center through which ions can pass, illustrated in figure 2.3.2a and b. There are 4 sections of the ion optics with distinct purposes. The first electrode is the extractor, which is biased relative to the tip to generate the electric field necessary to extract ions. The ion lens comprises the next three electrodes, which focus the extracted ion beam [193]. The next five electrodes make up the “accelerator”, which maintains a linearly decreasing potential down to 0 V, ensuring that the ion beam exits the ion optics at ground potential while minimizing defocusing. The fourth and final section of the ion optics is the deflector, comprising two pairs of parallel plate electrodes which can deflect the focused ion beam in the x and y directions, respectively, by applying a voltage between the pair of electrodes. These deflectors provide an additional level of control over the ion trajectory, which can be crucial for fine-tuning the beam path, compensating for any misalignments in the system, and ensuring optimal ion transmission to the detector. This redundancy in beam control allows for real-time adjustments during experiments, and improves the overall flexibility of the instrument. All of the electrodes are machined from stainless steel and mounted together on four threaded steel rods with insulating Vespel washers keeping them electrically insulated. Insulating ruby balls lodged between the electrodes maintain a uniform separation of 2.5 mm between each one. To prevent ions from striking any insulating components leading to the buildup of charge and unwanted electric fields, each electrode is designed with a protrusion that nests inside a recess in the neighboring element. This prevents the ion beam from having any line-of-sight to an insulating component.

The voltage on each electrode in the first three sections is controlled using an 8-channel high voltage power supply (CAEN DT8033) and a 3-channel low voltage power supply (Siglent SPD3303X-E) for the deflectors. The voltage on the extractor and lens electrodes are each set independently. The accelerator electrodes, however, are arranged in a voltage-ladder configuration. The voltage on the first electrode is specified, and the subsequent four electrodes are connected in series with 100 M Ω hermetically sealed resistors between them, creating a uniform voltage drop between each electrode. Applying a voltage V_A to the first accelerator electrode sets the voltages for the other four electrodes as $3V_A/4$, $V_A/2$, $V_A/4$, and 0 V, respectively.

The design of the ion optics was guided by numerical simulations carried out using SIMION [196]. The simulations yield ion trajectories, as illustrated in Fig. 2.3.2c, which are analyzed to determine the focused beam width at the position of the detector. Figure 2.3.2d shows the beam cross section as the ions exit the optics, and Fig. 2.3.2e shows the cross section of the same beam

50 cm downstream from the optics, at the position of the detector. We find from the simulations that a focused beam width < 1 mm should be easily achieved. The simulations also show that the beam does not have perfect rotational symmetry, and some caustic-like features appear when the beam is extrapolated out past the optics. This is caused by the fields generated by the deflector electrodes, which break the rotational symmetry. For our purposes this should not be too troubling, but future instrument designs seeking higher resolution may want to address this issue.

2.3.2.3 Magnetic Sector

Figure 2.3.3a shows the assembled magnetic sector mass analyzer. The magnetic sector comprises a custom rectangular neodymium magnet (KJ magnetics) sandwiched between two pieces of low-carbon magnetic iron (ASTM A848, Scientific Alloys inc.) that make up the magnet's yoke. The yoke directs the magnetic field to a circular region 6 cm in diameter and 1 cm in height, this is the region that ions pass through. The low carbon magnetic iron is an ideal material for this purpose due to its high magnetic permeability and magnetization saturation [197]. We measured the magnetic field in this circular region using a gaussmeter (Lake Shore Cryonics, 450) and found a fairly homogeneous field of $0.54 \text{ T} \pm 0.02 \text{ T}$. A 1 cm resolution map of the field is shown in Fig. 2.3.3b.

The magnetic sector is mounted such that it can be rotated to modulate the width of the magnetic field region the ions have to traverse. This is achieved by mounting the magnetic sector to a rotary vacuum feedthrough (MD40N, Kurt J. Lesker) via a steel shaft along an axis that is offset from the center of the circular magnetic field region. Figure 2.3.3c shows numerical simulations of ion trajectories ($m/z = 60, 140, 220, 300,$ and 380) for two different rotation angles of the magnetic sector (0° and 90°). At 90° , the ions experience a shorter path through the magnetic field, and are therefore less strongly deflected. This illustrates how rotation of the magnetic sector about the steel shaft provides fine control over the deflection of the ions. Because of the substantial weight of the magnet and yoke, a trapeze system with a thrust bearing was designed for additional support. This system allows the rotary feedthrough to rotate the magnetic sector without having to support its entire weight.

2.3.2.4 Detectors

As mentioned above, we have several detectors that can be swapped in and out as desired depending on the needs of the particular experiment, including a Faraday plate detector, a Faraday cup detector, a microchannel plate detector, and an array of channeltron detectors.

In cases where we want to measure the current over a wide area, for example to measure the

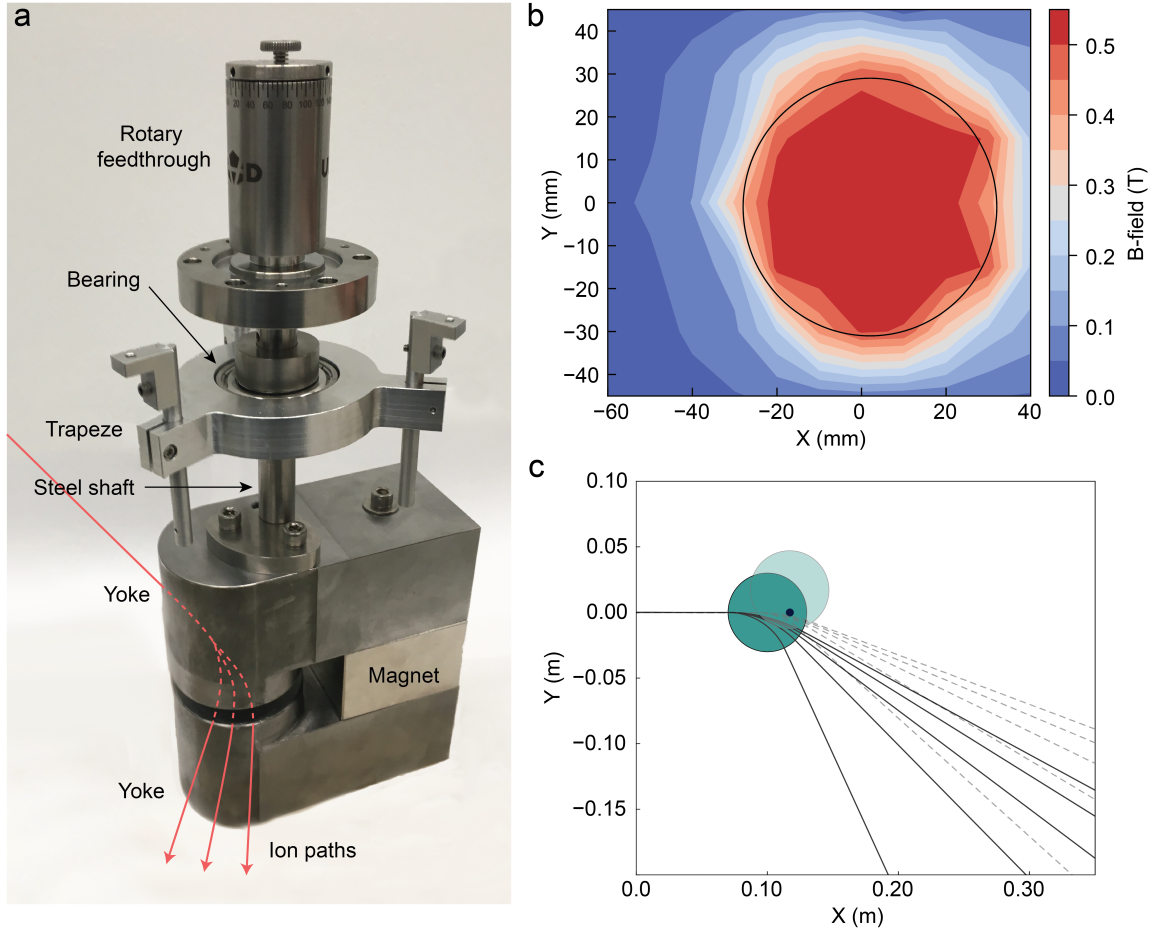


Figure 2.3.3: **Magnetic sector.** **a** Picture of the magnetic sector and its mounting system. The flange has been removed so all the other components could be visible in a single image. **b** A map of the measured magnetic field in the circular region of the magnetic sector. **c** Simulations of ion trajectories after passing through the magnetic sector. The turquoise circle is the circular magnetic region, and the small black spot shows the axis of rotation of the magnetic sector. The black solid lines show the trajectories for ions passing through the magnetic sector at 0° , and the lighter dashed lines show the trajectories for ions passing through the magnetic sector which has been rotated 90° about its axis.

total ion current transmitted by the magnetic sector, we use a simple Faraday plate detector. This detector comprises a 5 inch diameter steel disk spot welded to a 0.05 inch diameter steel wire which connects to a BNC vacuum feedthrough via a set screw contact (FTASSC094, Kurt J. Lesker). The steel wire is strong enough to support the weight of the Faraday plate while also acting as an electrical connection. The current striking the Faraday plate is measured by a current to voltage converter (SR570, Stanford Research Systems).

While the Faraday plate functions as a basic ion detector, it lacks any spatial specificity and has no mechanism to prevent biased current measurements that may result from secondary electron emission. When ions strike a metal surface with high energy, secondary electrons may be released from the surface, leading to an overestimation of the detected current. A Faraday cup with a narrow aperture and a secondary electron suppression electrode solves these problems. Figure 2.3.4a shows the design of the Faraday cup. The central component of the design is a graphite cup 3.8 cm in diameter and 3.5 cm deep (Almath Crucibles). Graphite's rough texture and electronic properties helps to reduce the number of secondary electrons that escape the cup [198]. A steel suppression electrode at the entrance to the Faraday cup also helps prevent the escape of secondary electrons when a negative voltage is applied to it. The graphite cup and suppression electrode are housed in a grounded aluminum cylinder with a 2.4 cm diameter aperture at one end, and electrically insulated by thin PEEK spacers. A kapton-insulated wire connects to a steel screw at the back of the graphite cup to carry the current to a BNC vacuum feedthrough. The entire Faraday cup assembly is mounted on an 8" flange with four steel rods and a shaft collar. Figure 2.3.4 shows the fully assembled and mounted Faraday cup detector.

When we carry out experiments in which we primarily care about spatial specificity of ion detection, but do not require precise current measurements, we use a microchannel plate (MCP) detector mounted on an 8" flange (Photonis APD 2 PS 40/32/25/8 I 60:1 NR 8"FM P43). The MCP acts as an imaging detector; it does not directly measure a current, but visually displays where ions are striking it. The MCP consists of two stacked arrays of thousands of microchannels in a "Chevron" configuration, with each microchannel acting as an electron multiplier when a voltage of a few kV is applied across it [199]. The output of the stacked microchannel arrays faces a fluorescent phosphor screen, so that the burst of electrons generated when an ion strikes one of the microchannels causes a small blip of light to appear on the screen. The MCP detector is especially useful for checking the focusing properties of the ion optics and visualizing mass spectra when using the magnetic sector. The MCP has a circular detector area 4 cm in diameter and was typically operated at voltages of 2.25 kV on the phosphor screen and 2.0 kV across the microchannel array.

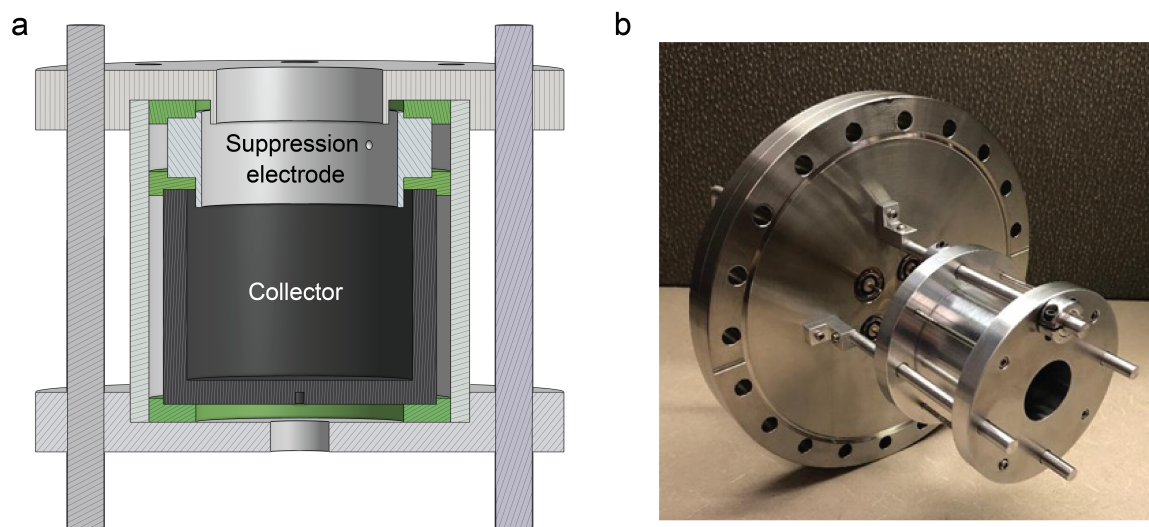


Figure 2.3.4: **Faraday cup.** **a** Cross section view of the Faraday cup detector. The graphite collector and suppression electrode are insulated from each other and from the grounded housing by PEEK dividers which are illustrated in green. **b** Image of the assembled Faraday cup detector mounted on an 8" flange.

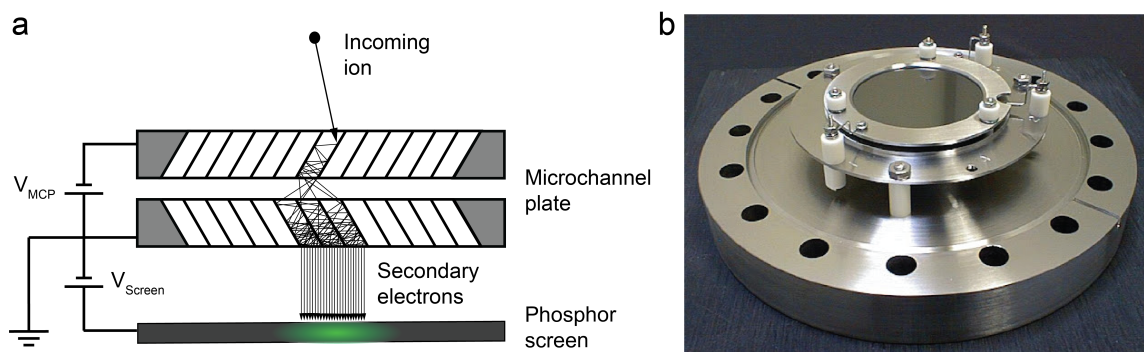


Figure 2.3.5: **Microchannel plate detector.** **a** Schematic showing the principle of operation of a microchannel plate detector coupled to a phosphor screen, configured in positive ion detection mode. **b** Photograph of an MCP detector mounted to a vacuum flange. Credit: Jordan TOF Products, inc.

In experiments where information on the timing of single ion detections is desired, such as in proof-of-concept sequencing experiments, an array of channeltron detectors can be used. This is the detector array shown in Fig. 2.3.1. Channeltrons have quantum efficiencies of $> 95\%$, and produce output pulses just 10-20 ns wide, allowing for high temporal resolution [26]. Up to ten individual channeltrons can be mounted side-by-side on a PEEK holder that we designed. The channeltrons are secured using a PEEK clamp lined with nitrile rubber, which provides gentle pressure to avoid damaging the delicate detectors. The output of each channeltron connects to an independent BNC feedthrough, and then to the timing electronics, which typically consists of a series of preamplifiers connected to a digitizer or oscilloscope.

2.3.3 Validation of the mass spectrometer design

We carried out several validation experiments to test out the ion optics and magnetic sector. In these experiments we typically used nanopore ion sources filled with 1 M NaI in formamide, which has previously been demonstrated to support ion evaporation directly from the meniscus into vacuum [114, 155]. In each of these experiments, we use the MCP detector to visualize the ion beam.

2.3.3.1 Focusing

In order to test the ion optics' ability to focus the ion beam, we removed the magnetic sector and mounted the MCP detector on flange 5, directly opposite the ion source. We initiated emission from a nanopore ion source filled with 1 M NaI in formamide by setting the tip voltage to +405 V and the extractor voltage to +250 V. We then varied the voltages on the three lens electrodes while monitoring the appearance of the beam on the MCP. Optimal focusing in this experiment was found when the lens voltages were set to 400 V, 100 V, and 300 V, respectively. Figure 2.3.6 shows a photograph of the resulting beam spot as seen on the fluorescent screen of the MCP. The spot's width is approximately 1 mm, and it displays some of the caustic-like features that were predicted in the simulations (Fig. 2.3.2e). The optimal focusing voltages depend on the specific experimental conditions, particularly the tip and extractor voltages. The tip voltage is determined by the desired ion energy (which controls the magnetic deflection via Eq. (2.3.3)), while the total extraction voltage must be kept close to the onset voltage of the emitter, which is not known *a priori*, to maintain stable ion emission. Consequently, the focusing voltages may need adjustment between experiments to accommodate these source-specific parameters and achieve optimal beam focusing.



Figure 2.3.6: **MCP image of focused ion beam.** Photograph of the MCP screen showing a well-focused ion beam.

2.3.3.2 Tip alignment

We conducted a series of experiments and simulations to evaluate our alignment system and quantify the relationship between tip position and beam trajectory. For these tests, we removed the magnetic sector and mounted the MCP detector on the rear flange of the instrument. Using a nanopore ion source filled with 1M NaI in formamide, we aligned the tip in seven different radial positions and recorded the resulting beam spot locations on the MCP screen. Concurrently, we performed SIMION simulations to model the ion trajectories for various emitter positions relative to the ion optics entrance. The results of these experiments and simulations are presented in Figure 2.3.7. The alignment system’s view of the emitter tip at three representative positions is shown in Fig. 2.3.7a. Figure 2.3.7b and c offer a side-by-side comparison of the simulated and experimentally observed beam spots for these same tip positions, respectively. This visual comparison demonstrates the close agreement between our simulations and actual beam behavior.

To quantify this relationship, we plotted the beam spot positions from all seven measurements along with the simulation results for both the x- and y-axes in Figure 2.3.7d and e. The strong correlation between experimental data and simulations validates our alignment system’s accuracy and our understanding of the beam’s behavior. This analysis reveals a consistent relationship: a ≈ 1 mm radial deflection of the ion source corresponds to ≈ 1 cm of deflection at the detector. These findings also demonstrate the importance of precise control over the tips position for optimal

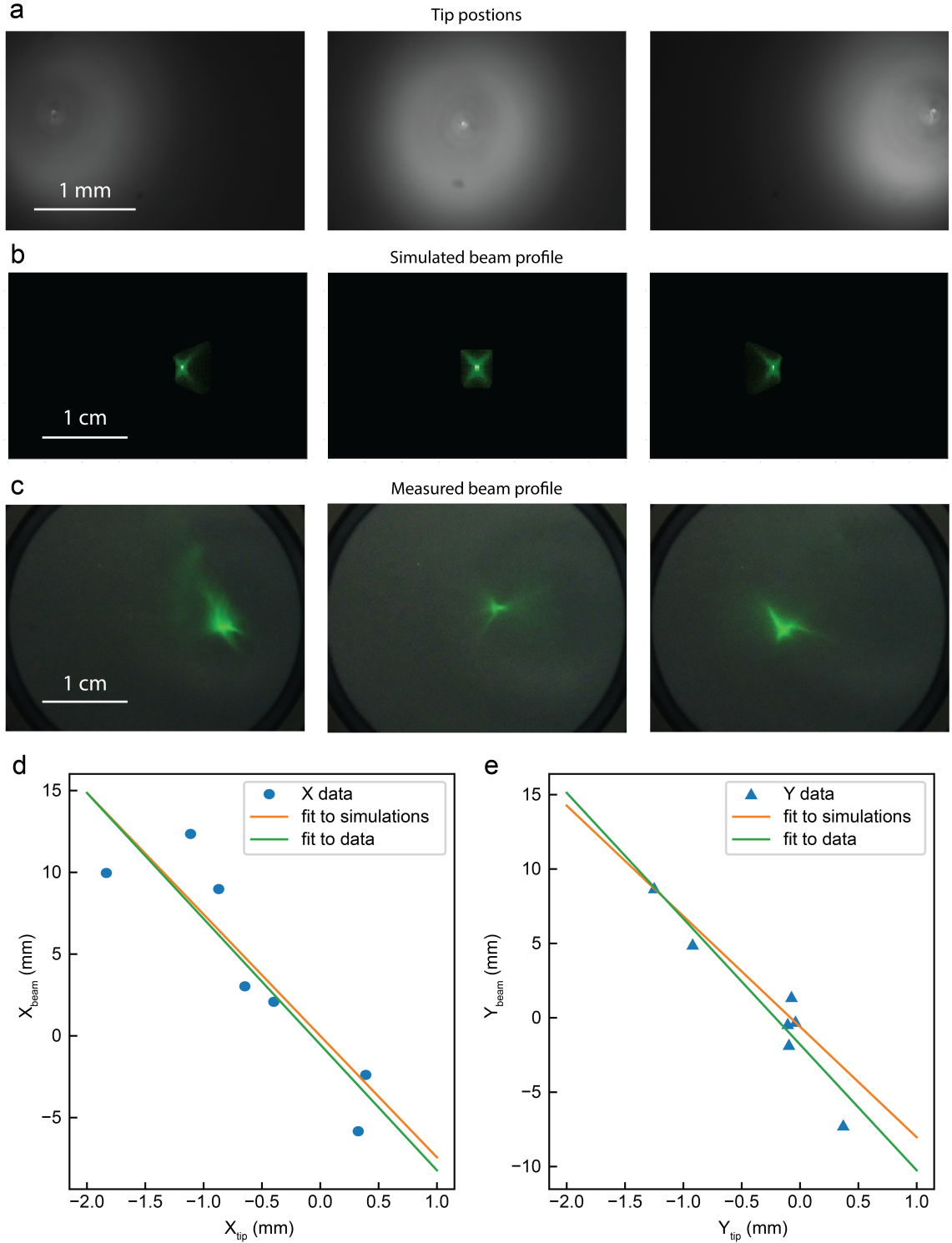


Figure 2.3.7: **Effect of tip displacement.** **a** Images of tip taken in the alignment system at three example positions separated by about 1 mm each. **b** Simulated beam position at the detector for $x_{\text{tip}} = -1, 0$, and $+1$ mm. **c** Beam images in MCP screen for the tip positions shown in panel a. **d** and **e** Scatterplots of the measured beam position vs tip position in the x- and y-directions, respectively. The best fit line (green) to the measured data is compared to the simulation results (orange) and found to be in good agreement.

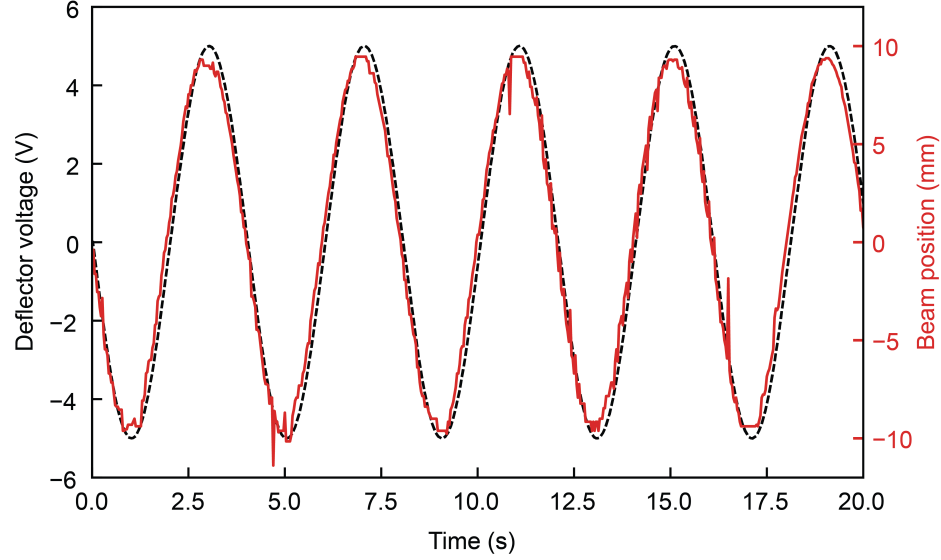


Figure 2.3.8: **Ion beam deflection.** Plot of the measured beam position on the x-axis over time alongside the voltage applied to the x-deflector electrodes.

performance.

2.3.3.3 Deflection of the ion beam

To validate the deflector electrodes' ability to adjust the ion beam trajectory, we conducted deflection experiments using time-varying voltages applied to the deflector electrodes and measured the resulting changes in the beam position. Ion emission from the source was initiated with a tip voltage of +300 V and an extractor voltage of +90 V. The deflector voltage on both pairs of deflectors was varied sinusoidally with a peak-to-peak amplitude of 10 V and a frequency of 0.25 Hz. We simultaneously recorded the beam position on the MCP with a video camera, and used ImageJ to extract the position of the beam [200]. Figure 2.3.8 presents the results of these experiments for deflection in the x-direction, showing excellent correlation between the applied deflector voltage and the measured beam position over multiple cycles. Similarly good results were obtained for deflection in the y-direction (not shown). The beam position closely follows the sinusoidal pattern of the deflector voltage, with a peak-to-peak deflection of about 20 mm on the detector. Note, however, that the amount of beam deflection depends on the ions' kinetic energy. So the required voltages to achieve a desired amount of beam deflection will depend on the tip voltage.

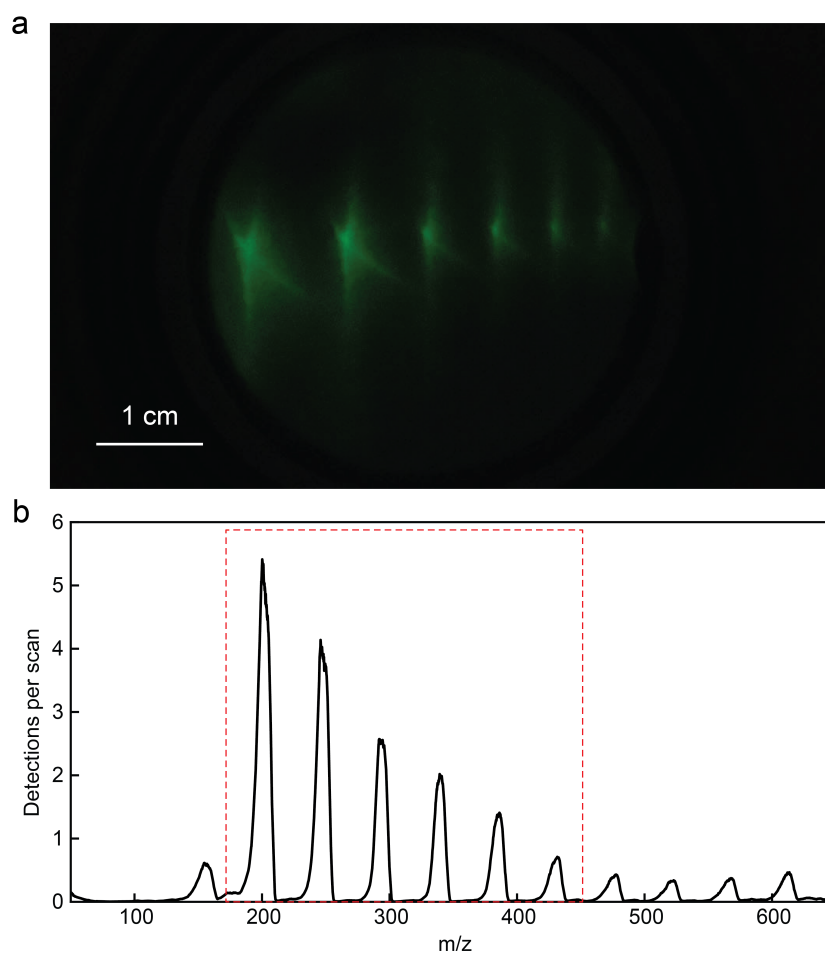


Figure 2.3.9: **Magnetic sector mass spectrum example.** **a** Mass spectrum of 1 M NaI in formamide as seen through the MCP detector. **b** Mass spectrum of the same solution obtained in the Quadrupole MS using the same nanopore ion source.

2.3.3.4 Mass separation

In order to test the magnetic sector's ability to separate ions by their m/z value, we mounted the magnetic sector on flange 9, in the center of the MS, and moved the MCP to flange 4, which is offset at 45° . Ion emission was initiated at a tip voltage of 300 V and extractor voltage of 200 V. The lens voltages were set to 200 V, 210 V, and 205 V, respectively. Fig. 2.3.9a shows a photograph of the MCP screen taken during the experiment. Six distinct beam spots of incrementally decreasing intensity are observed. By comparing this image with the mass spectrum measured in the quadrupole MS using the same tip and solution (Fig. 2.3.9b), we can identify that the spots correspond to sodium ions clustered with $n = 4 - 9$ formamide molecules. The position of the beam spots was observed to shift left or right on the MCP screen in response to rotations of the magnetic sector, as expected. Crucially, the beam maintains its good focusing properties after passing through the magnetic sector. This experiment validates the design of our magnetic sector mass spectrometer and demonstrates that it can measure the mass spectrum of ions emitted directly into vacuum from a nanopore ion source.

2.4 IV characterization of ion emission

The current-voltage (IV) characteristic of an ion source provides fundamental insight into its electrical properties and can shed light on the ion emission mechanism. The IV characteristic can in principle be measured with either of our mass spectrometer systems, as they both continuously measure the emission current and extraction voltage applied to the ion source during experiments. In practice however, the MS experiments are time-consuming to set up, and the source-measure units employed have limited bandwidth and relatively high noise, making quick and precise measurements of the IV characteristic impractical. We constructed an electrical test system in which we can rapidly measure the IV characteristics of a nanopore ion source within a Faraday cage using sensitive ammeters, separate from our MS instruments.

Figure 2.4.1a shows a schematic of the experimental setup. Essentially, a negative voltage is applied to a counterelectrode to initiate emission from the grounded tip, while a sensitive ammeter measures the emitted current. The tip is mounted on a PEEK tee connector (IDEX P-712) with a tubing sleeve (IDEX F-252, 1/16 OD, 0.042" ID) to make a water-tight fit. A 0.5 mm diameter Ag/AgCl electrode is threaded through the tee connector and held in place with another tubing sleeve to make electrical contact with the sample solution. The bottom port of the tee connector

can be used to apply extra pressure to the liquid inside the tip if desired. The Tee connector is mounted to a standard optical table via a custom made adapter and an optical post. The 1" diameter copper counterelectrode is mounted on a linear stage (Thorlabs MT1B) to allow precise control of the distance between the counterelectrode and the tip. Electrical isolation is between the counterelectrode and the linear optical post using an insulating washer and nylon screw. Figure 2.4.1b shows the Faraday cage which encloses the setup, as well as the BNC and SHV feedthroughs which connect to the tip electrode and counterelectrode, respectively.

The electronic hardware and data recording device are located outside of the Faraday cage. The extraction voltage, V , is generated by a high voltage power supply (Stanford Research Systems HV350). The emission current, which is typically in the range of 10^{-12} to 10^{-9} A, is converted to a voltage by a sensitive current preamplifier with adjustable gain up to 10^{12} A/V (Stanford Research Systems SR570). This voltage is then read by Labview via a DAQ (National Instruments USB6251). A custom Labview program automates the measurement of the IV characteristic by sweeping over a range of extraction voltages in discrete steps while recording the emission current. Once the emission current reaches a user-defined threshold value, the sweep is reversed until the initial voltage is reached.

2.5 Measuring UV Photolysis of Peptides

In Chapter 6, we turn our attention to the UV photolysis of peptides, with the eventual aim of breaking proteins apart into their constituent amino acids within a nanopore ion source. To that end, we carried out a series of fundamental studies on the absorbance of small peptides in the deep UV range and determined the quantum yield of peptide bond scission for 193 nm and 222 nm irradiation.

2.5.1 UV-vis spectroscopy

Determining quantum yields of bond scission after irradiation requires knowledge of the molar absorption coefficients of the relevant compounds at the specific wavelengths of interest. We used a commercial UV-vis spectrophotometer, the NanoDrop ONE, to measure the absorption coefficients of model peptides and amino acids [201]. The NanoDrop One is a microvolume UV-vis spectrophotometer that employs a xenon flash lamp as its light source and operates over a wavelength range of 190-850 nm. It features a small sample pedestal that allows for measurements using only 1-2 μ L of sample, pipetted directly onto the pedestal. The Nanodrop One automatically adjusts the path-

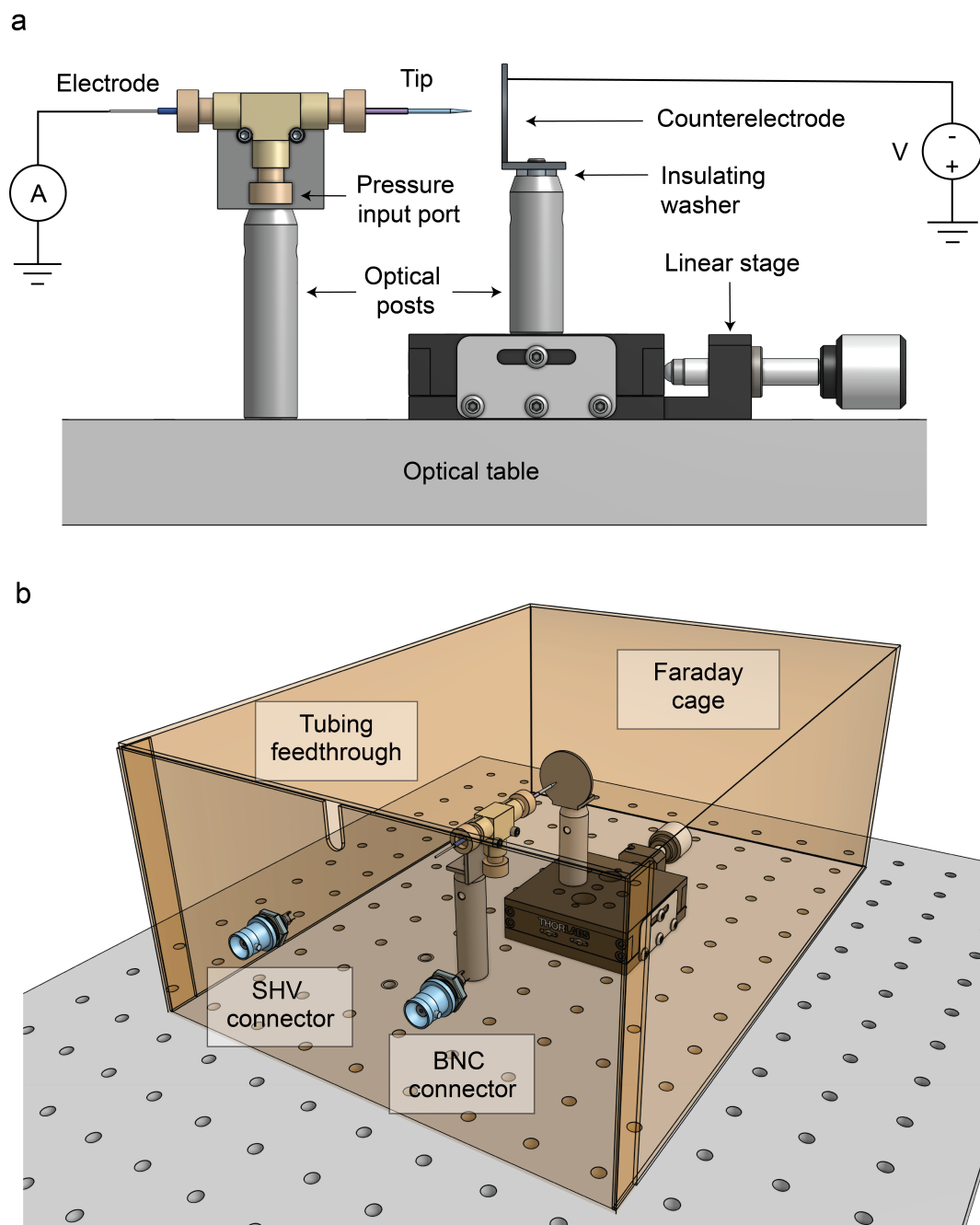


Figure 2.4.1: **Faraday cage for IV characterization.** **a** Side view of IV measurement setup showing the how the tip and counterelectrode are mounted and electrically connected. **b** Perspective view of IV measurement setup showing the Faraday cage and feedthroughs.

length through the sample based on the concentration, allowing for a higher dynamic range than traditional spectrophotometers with fixed 10 mm pathlength. In order to track the changing absorption spectrum of samples over the course of UV irradiation experiments (described below), we periodically take small aliquots of the sample using a micropipette for offline UV-vis measurement, allowing us to monitor photolysis progress while minimizing sample consumption.

2.5.2 *in situ* 222 nm irradiation and absorbance measurements

I constructed a device to irradiate aqueous solutions with 222 nm light while measuring the 222 nm absorbance of the sample *in situ*, illustrated in Fig. 2.5.1a. The device consists of a 222 nm excimer lamp (Larson Electronics, FRL-EMY-5-5W-FUVC), a quartz cuvette with 10 mm path length (Spectrocell, R-3010-S), a far UV photodiode optimized for 222 nm (Electro Optical Components, GFUV-T10GD-L), and a custom amplifier circuit. These components are all housed in a steel box which acts as a Faraday cage and has two BNC feedthrough for power in and signal out. There is also a steel plate with a small window cut out that separates the lamp from the other components in the box, this ensures that any light hitting the photodiode has to travel through the sample cuvette. The small current produced by the photodiode is amplified by the amplifier circuit, then recorded by a DAQ card (National Instruments, NI PCIe-6251) using a custom LabView script. Fig. 2.5.1b shows image of the box with its lid removed and its components labelled.

Figure 2.5.2a shows the amplifier circuit used to amplify the photodiode current. The circuit is a simple trans-impedance amplifier with a low pass filter at the output. The filter acts to smooth out the rapid signal oscillations that result from the high frequency discharge rate of the excimer lamp. We use an AD822 op-amp (Analog Devices) which has a very low input bias current, making it ideal for a photodiode amplifier. The circuit also supplies power to two cooling fans (visible in the lower left and right in fig. 2.5.1b). Figure 2.5.2b compares the filtered and unfiltered signal from the photodiode after being amplified as measured by an oscilloscope (Tektronix TD2024B). The unfiltered signal captures the rapid pattern of discharges from the lamp which makes the signal appear extremely noisy on longer time-scales. Using resistor and capacitor values of 10 k Ω and 1 μ F gives an RC time-constant of 10 ms for the low pass filter, more than long enough to smooth out the discharge pattern, and fast enough not to affect the measurement over second-to-minute timescales.

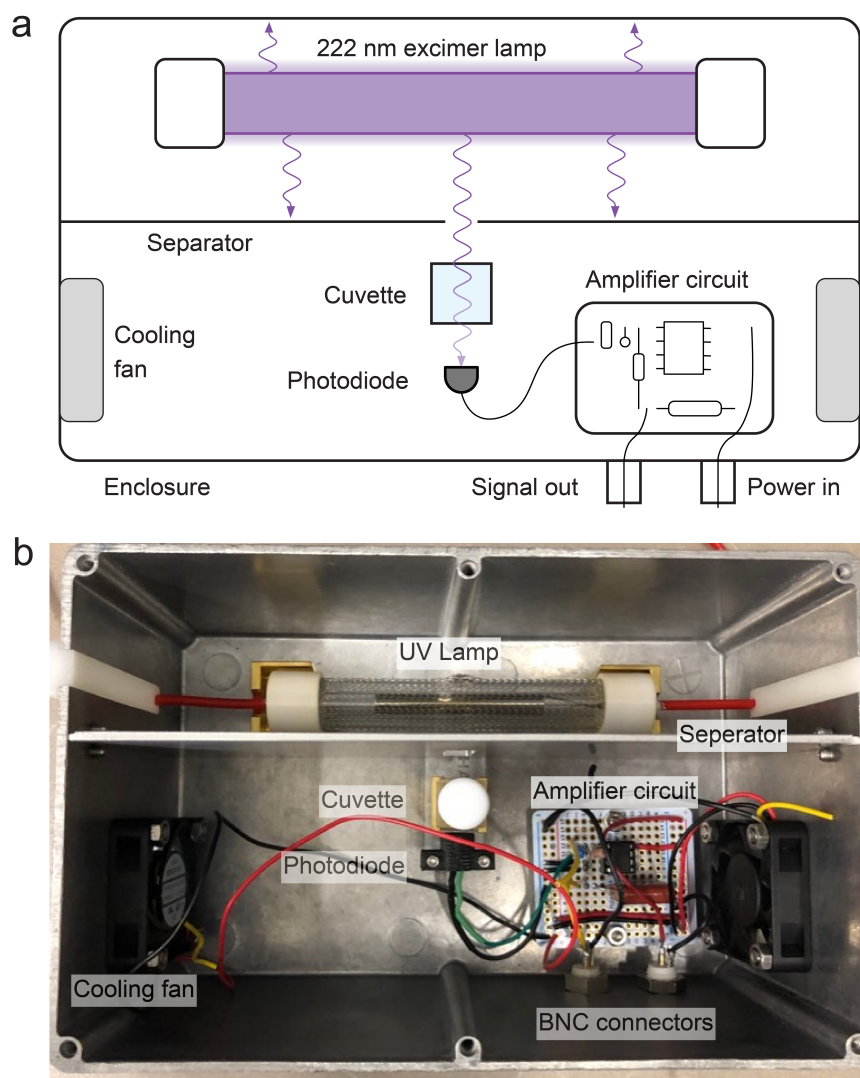


Figure 2.5.1: **222 nm irradiation experiment.** **a** Schematic of the device to irradiate samples with 222 nm light while measuring the samples absorbance *in situ*. **b** Photograph of the 222 nm irradiation device with components labelled.

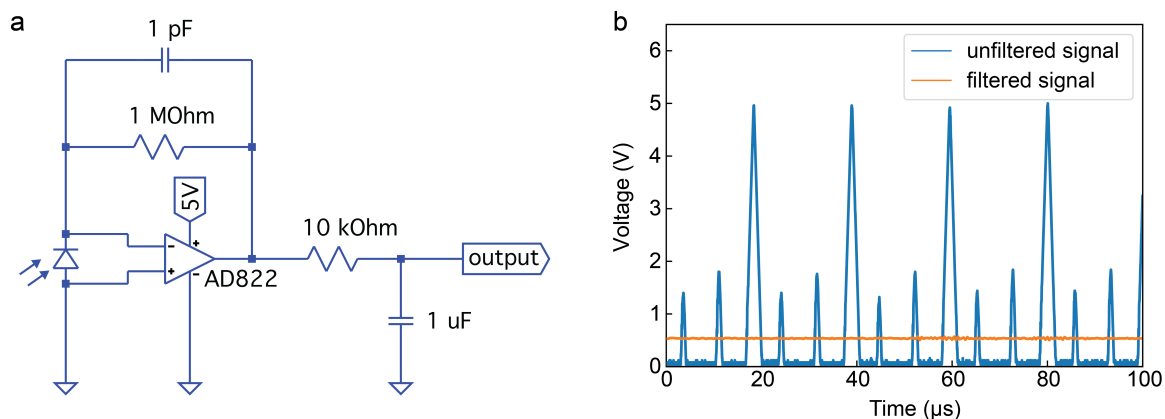


Figure 2.5.2: **Photodiode amplifier circuit.** **a** Circuit used to amplify and filter the signal from the photodiode. The 1 MΩ feedback resistor produces a gain of 10^6 . The 10 kΩ resistor and 1 μF capacitor form a low pass filter with a cutoff frequency of about 16 Hz. **b** Plot of the amplified signal from the photodiode under irradiation from the 222 nm excimer lamp both with and without filtering using the low-pass filter shown in panel a.

2.5.3 *in situ* 193 nm irradiation and absorbance measurements

Figure 2.5.3 shows our setup for irradiating and monitoring the scission of peptide bonds by 193 nm laser light *in situ*. A Ti:Sapphire laser (Chameleon Ultra II, Coherent) generated tunable IR light in 140 fs pulses at an 80 MHz repetition rate with a total output power of around 3.5 W. The IR beam is directed through a Faraday isolator to prevent back reflections and then into a harmonics module (APE Harmonixx FHG 3+1) which generates the fourth harmonic of the fundamental frequency. We tuned the IR laser to 772 nm so that the harmonics module would output a 193 nm beam. The power is significantly reduced with each harmonic generated, resulting in a typical output power of around 0.5 mW at 193 nm. The UV beam is directed into an acrylic enclosure that is continuously purged with N₂ to remove oxygen, which absorbs strongly at 193 nm. The beam passes through an aperture and is then directed towards a beam splitter using two UV mirrors. The splitter directs about 20% of the beam towards a reference power meter to track the laser power, which may drift over the duration of an experiment. The remainder of the beam passes through a 1 cm path length quartz cuvette (Spectrocell R-3010-S) which contains the sample solution. The sample is continuously stirred with a magnetic stir bar which is kept near the edge of the cuvette to avoid interfering with the beam. A second power meter measures the transmission of 193 nm light through the sample.

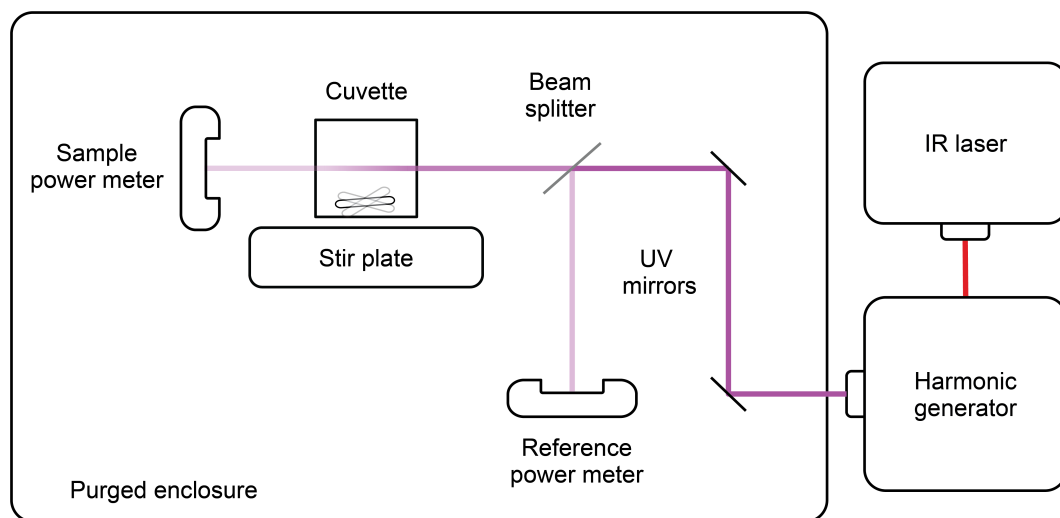


Figure 2.5.3: **193 nm irradiation experiment.** Diagram of the experimental setup for irradiating samples with 193 nm light while monitoring absorbance *in situ*.

3

Nanopore ion sources deliver individual ions of amino acids and peptides directly into high vacuum

“New directions in science are launched by new tools much more often than by new concepts.”

– Freeman Dyson

3.1 Introduction

Mass spectrometry (MS) is the workhorse of proteomics research thanks to its ability to distinguish amino acids and small peptides by their mass to charge ratio (m/z) [202]. Its utility also significantly derives from the availability of soft ionization techniques for transferring peptide ions into the gas phase intact [90]. In particular, electrospray ionization (ESI) transfers analyte into a mass spectrometer via a plume of charged droplets that emerge from a liquid cone-jet at the end of a voltage-biased emitter, as illustrated in fig. 3.1.1a [23, 88]. The droplets pass through a background gas that induces a series of evaporation and Rayleigh fission cycles that ultimately release analyte ions into the gas phase [131]. However, the background gas needed to liberate ions from droplets is also a source of significant sample loss that limits the sensitivity of MS.

The background gas and the plume of charged droplets it creates widely disperse ions, the ma-

jority of which collide with the transfer capillary, which bridges the ambient-pressure ion source and the first pumping stage of the mass spectrometer, and other hardware components upstream of the detector [90]. The ion transmission efficiency of an ion source, which refers to the fraction of emitted ions that enters the mass analyzer, is a key determinant of the sensitivity of mass spectrometry [56]. Early ESI sources had emitter tips with diameters of hundreds of micrometers, and only one ion in $\sim 10^4$ reached the mass analyzer [94]. Nano-electrospray ionization (nano-ESI) increased the ion transmission efficiency to $\sim 1\%$ in typical measurements (occasionally reaching as high as 12%) by using emitters with micrometer-scale tips that reduced the flow rates to the range of several nL per min [95, 54]. Marginean et al. demonstrated that by optimizing the background gas pressure, the sheath gas flow rate, and the ion funnel voltages of a subambient pressure nano-ESI source, they could achieve an ion utilization efficiency (the fraction of analyte originally in solution reaching the detector) as high as 50% for a particular small peptide [99]. However, co-optimizing the efficiency of multiple analytes is fundamentally challenging because ESI involves processes that physically separate different ion species within the plume [100]. State-of-the-art MS instrumentation still requires thousands to millions of copies of proteins for their identification [104, 105]. This sensitivity falls short of what is desired for single-cell proteomics and single-molecule analyses [53, 50, 106, 58]. Achieving single-molecule sensitivity requires an ion source that circumvents the loss mechanisms intrinsic to spraying charged droplets into a background gas.

We have developed a nanopore ion source that emits amino acid and small peptide ions directly into high vacuum from aqueous solution at its tip (fig. 3.1.1b). The heart of the ion source is a pulled quartz pipette with a tip whose inner diameter (I.D.) is smaller than 100 nm [173, 174]. We reason that the smallness of the tip will influence ion emission in three important ways: First, the surface tension of water can maintain a stable liquid-vacuum interface that supports many atmospheres of pressure when stretched across a nanoscale opening [203]. Second, the fluid flow rate, which scales as the inverse cube of the tip diameter, is too low for a stable electrospray cone-jet to form [204, 79], and this may prevent charged droplets from being emitted altogether. Third, electric fields will concentrate at a sharp, conductive tip like an electrolyte-filled nanopipette, and may reach ≈ 1 V per nm at the meniscus, the approximate field needed to draw out ions at high rates by the process of ion evaporation [205, 175].

Here we characterize the emission of amino acid and small peptide ions from aqueous solutions directly into high vacuum using a nanopore ion source. We obtain mass spectra using a custom quadrupole mass spectrometer in which the nanopore ion source operates at pressures below 10^{-6} torr (fig. 3.2.1a) [180]. Separately, we measure the transmission efficiency between the ion source and a

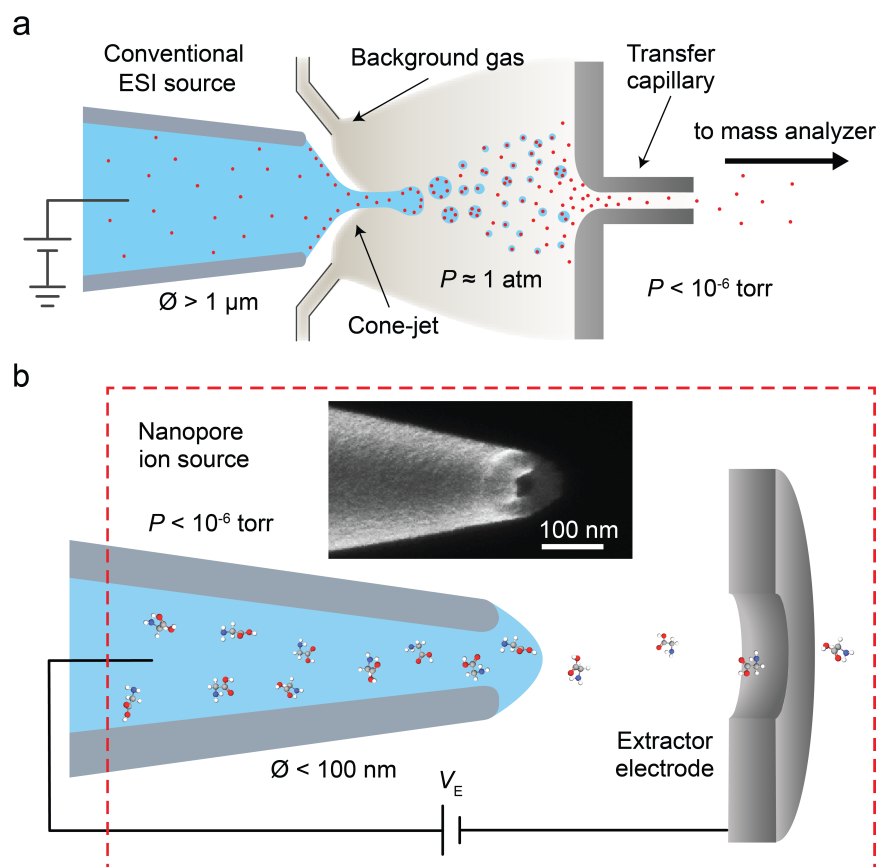


Figure 3.1.1: **Electrospray ion source vs nanopore ion source.** **a**, Schematic of conventional electrospray ionization highlighting the background gas that stimulates evaporation of solvent from droplets, as well as the transfer capillary where significant ion loss occurs. P represents pressure. **b**, Schematic of a nanopore ion source showing the liquid-filled nanopipette tip, the extractor electrode, and the extraction voltage V_E applied between them. Inset shows an scanning electron microscopy image of the tip of a pulled quartz nanopipette with a tip I.D. of 30 nm.

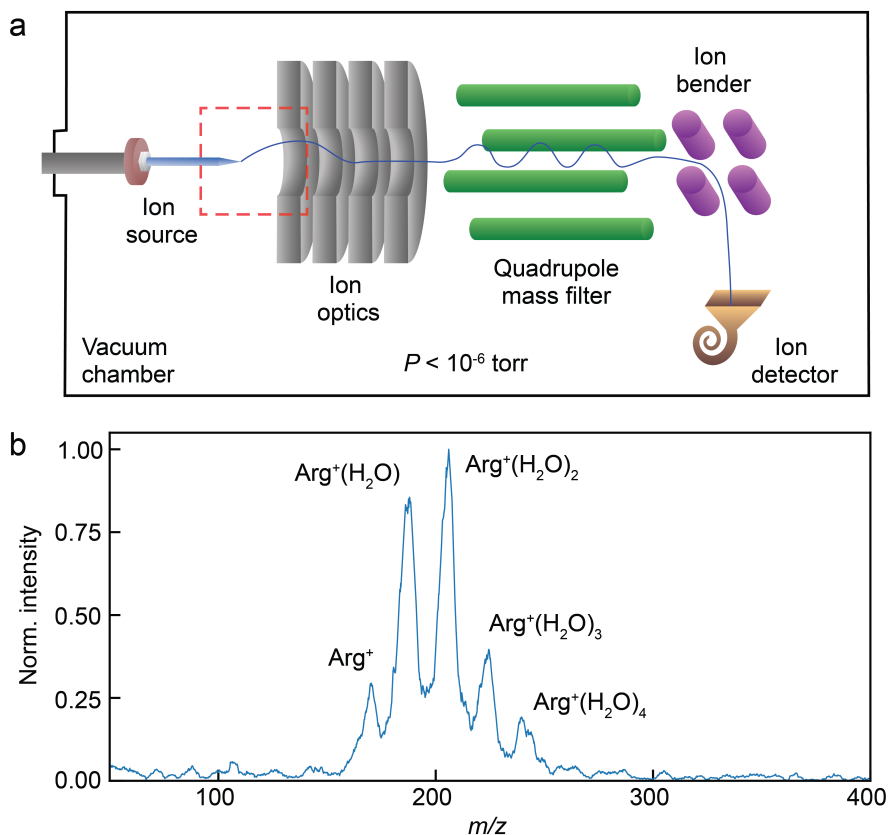


Figure 3.2.1: **Quadrupole mass spectrometer and example arginine spectrum.** **a**, Schematic of the mass spectrometer used in this study. Ion optics comprising an extractor electrode and an Einzel lens extract ions from the liquid meniscus at the ion source and focus them through a quadrupole mass filter and an electrostatic ion bender. The transmitted ions strike a channel electron multiplier detector which is sensitive to single ions. **b**, Mass spectrum of 100 mM arginine in aqueous solution obtained with a 41 nm I.D. nanopore ion source in our quadrupole mass spectrometer. Peaks are labelled as $\text{Arg}^+(\text{H}_2\text{O})_n$, where Arg^+ represents protonated arginine $[\text{Arg} + \text{H}]^+$, and n denotes the number of attached water molecules.

downstream Faraday cup for both aqueous salt solutions and aqueous amino acid solutions and find it to exceed that of ESI by two orders of magnitude. We further use a magnetic sector to separate charged droplets from small ions and thereby demonstrate that the nanopore source can be made to emit only ions. We also measured the pH dependence of amino acid ion emission rates to probe the emission mechanism.

3.2 Results

3.2.1 Emitting amino acid ions from a nanopore ion source

We characterize the emission of amino acids from aqueous solution in a custom quadrupole mass spectrometer shown in fig. 3.2.1a [180]. Amino acid solutions comprised the amino acid of interest ($\geq 98\%$, Sigma-Aldrich, Merck) dissolved in deionized water ($18\text{ M}\Omega\text{ cm}$, Millipore) at a concentration of 100 mM , with the exception of tryptophan, whose concentration was 50 mM . To ensure that the amino acids were positively charged in solution, we reduced the pH of each solution below the amino acid's isoelectric point by adding $0.1\%-0.5\%$ v/v glacial acetic acid ($\geq 99\%$, Sigma-Aldrich, Merck). In a typical experiment, ion emission from a nanopore ion source is initiated by applying an extraction voltage, V_E , in the range $+190$ to $+380\text{ V}$ between the tip and the extraction electrode. The emission current, I_E , is typically in the range of $2\text{--}20\text{ pA}$. Significantly higher currents, ranging from hundreds of pA to a few nA are often measured when working with aqueous salt solutions. The onset of current is abrupt and accompanied by the measurement of ions striking the instrument's detector. We collect easily interpretable mass spectra within seconds to minutes at these low emission currents. We observed that an individual nanopore ion source can be kept in operation for several weeks without clogging.

fig. 3.2.1b shows a typical mass spectrum obtained from a 100 mM solution of arginine in water. This spectrum is obtained in positive ion mode using a nanopore ion source with $\text{I.D.} = 41\text{ nm}$. Five peaks are clearly visible. The m/z peak at 175 corresponds to the singly protonated arginine ion (labelled as Arg^+ , shorthand for the protonated form $[\text{Arg} + \text{H}^+]$). The higher m/z peaks are all separated by 18, the shift induced by an additional water molecule. Thus, the other peaks correspond to solvated arginine clusters (labelled as $\text{Arg}^+(\text{H}_2\text{O})_n$, where the integer n denotes the number of attached water molecules).

Figure 3.2.2 illustrates how the tip diameter can influence the mass spectrum obtained from an aqueous amino acid solution, again using 100 mM arginine. The spectra shown are obtained using tips with I.D.s of 20 , 125 , and 300 nm . The largest tip produces a broad spectrum of peaks that includes the bare arginine ion, eight incrementally hydrated arginine ion clusters, and an m/z peak at 349 that corresponds to the arginine dimer ion $((\text{Arg})_2^+)$. The intermediate-sized tip produces a narrower spectrum that includes the bare arginine ion, six incrementally hydrated arginine ion clusters, and a relatively diminished arginine dimer ion peak. The smallest tip primarily produces the bare arginine ion, but attenuated peaks corresponding to the singly and doubly hydrated arginine ion clusters are also visible in the spectrum. Smaller tips tend to produce relatively stronger signals

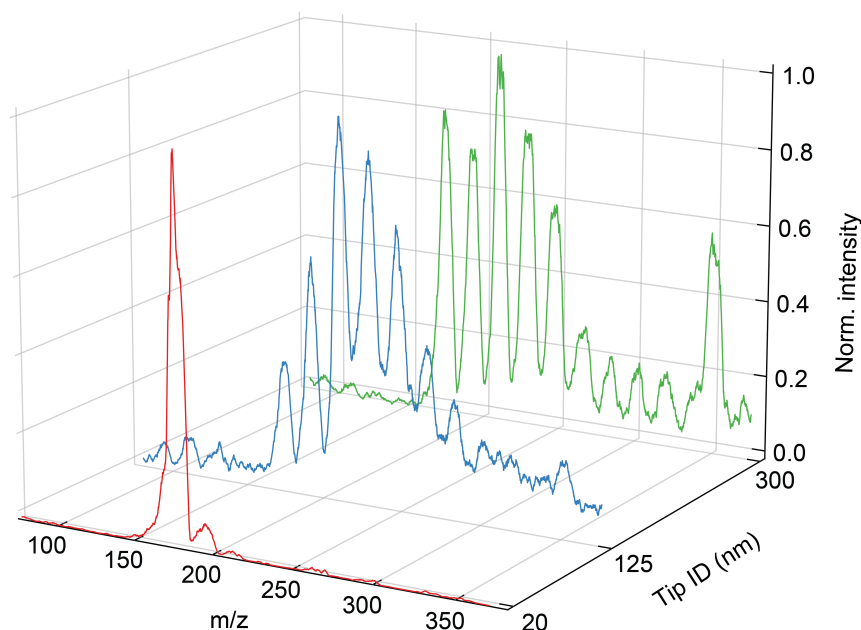


Figure 3.2.2: **Mass spectra with different emitter sizes.** Mass spectrum of 100 mM arginine solution in H_2O using nanopore ion sources with 3 different inner tip diameters (I.D. = 20 nm, 125 nm, and 300 nm).

and less noisy spectra than larger tips, as can be seen by comparing the baselines of the three spectra in Fig. 3.2.2. We observe some variance in the distribution of solvation states between nanopore ion sources with similar tip sizes, and even in repeated measurements with the same tip. This is illustrated, for example, by the different distributions of arginine solvation states obtained with a 20 nm I.D. tip (Fig. 3.2.2) and obtained with a 24 nm I.D. tip (Fig. 3.2.13). However, only tips with I.D.s smaller than about 65 nm produced spectra where most of the amino acid ions were in the desolvated state.

The tube-in-tube system described in § 2.1.2 allows different samples to be swapped within the ion source on the fly. Figure 3.2.3 shows how, when replacing the sample solution being pumped through the ion source, the old solution (in this case cysteine) is gradually displaced by the new solution (phenylalanine) over a period of about 30 minutes.

Figure 3.2.4 shows mass spectra obtained from 16 different aqueous amino acid solutions, all at 100 mM concentration with the exception of tryptophan which was 50 mM. Four different tips with I.D.s of 20, 25, 57 and 58 nm were used for these measurements. The conditions of each amino acid measurement are summarized in Table 3.1. The most prominent amino acid peak in every spectrum shown in Fig. 3.2.4 corresponds to a singly charged and unsolvated ion. The spectra for glycine, alanine, proline, valine, cysteine, glutamine, and phenylalanine show no additional peaks

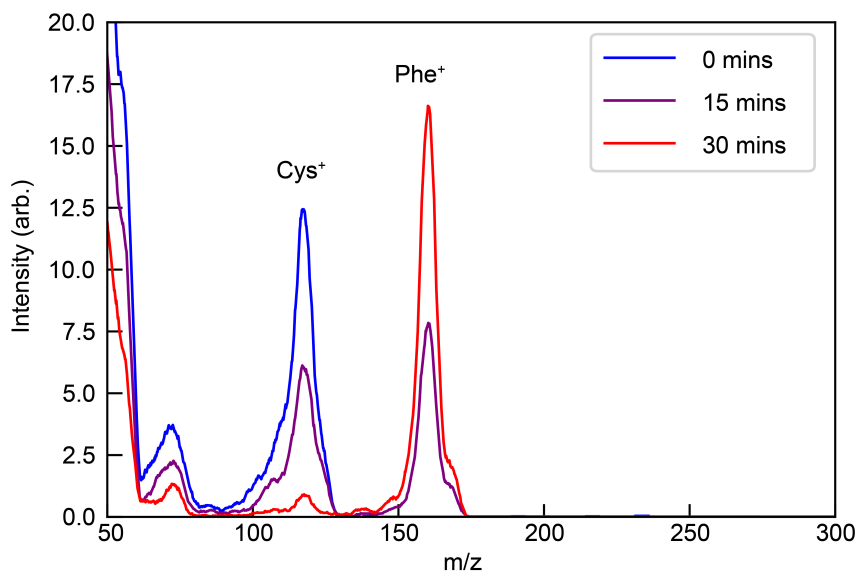


Figure 3.2.3: **Swapping amino acid solutions.** Mass spectra obtained 0 min (blue line), 15 min (purple line), and 30 min (red line) following the introduction of 100 mM Phenylalanine into an ion source tip initially filled with 100 mM Cysteine. As the new solution gradually displaces the old solution at the tip of the ion source, peaks corresponding to both amino acid types are visible in the spectrum.

which could correspond to solvated amino acid ions. The spectra for serine, threonine, asparagine, lysine, methionine, histidine, arginine, and tryptophan show a secondary m/z peak 18 to the right of the unsolvated peak, corresponding to the singly hydrated amino acid ion. Leucine shows a third and fourth peak corresponding to higher solvation states. The spectra for leucine, methionine, and histidine show additional small peaks corresponding to bare, singly charged amino acid dimers. A number of spectra also show peaks below 200 corresponding to singly protonated water clusters ($\text{H}_3\text{O}^+(\text{H}_2\text{O})_n$). Four proteinogenic amino acids are absent from Fig. 3.2.4: we do not attempt to measure aspartic acid and glutamic acid in positive ion mode because of their low isoelectric points; we also left out isoleucine because it is indistinguishable from leucine based on m/z ; and tyrosine gave poor emission characteristics, likely related to its low solubility in water. We worked with high (50 - 100 mM) analyte concentrations to obtain interpretable spectra quickly. Reliable identification of amino acids in these measurements required the release of only attomoles of analyte from the ion source (see § 3.2.7 below). The minimum concentration of analyte at which identification is possible is an alternative measure of sensitivity that we did not explore in this work.

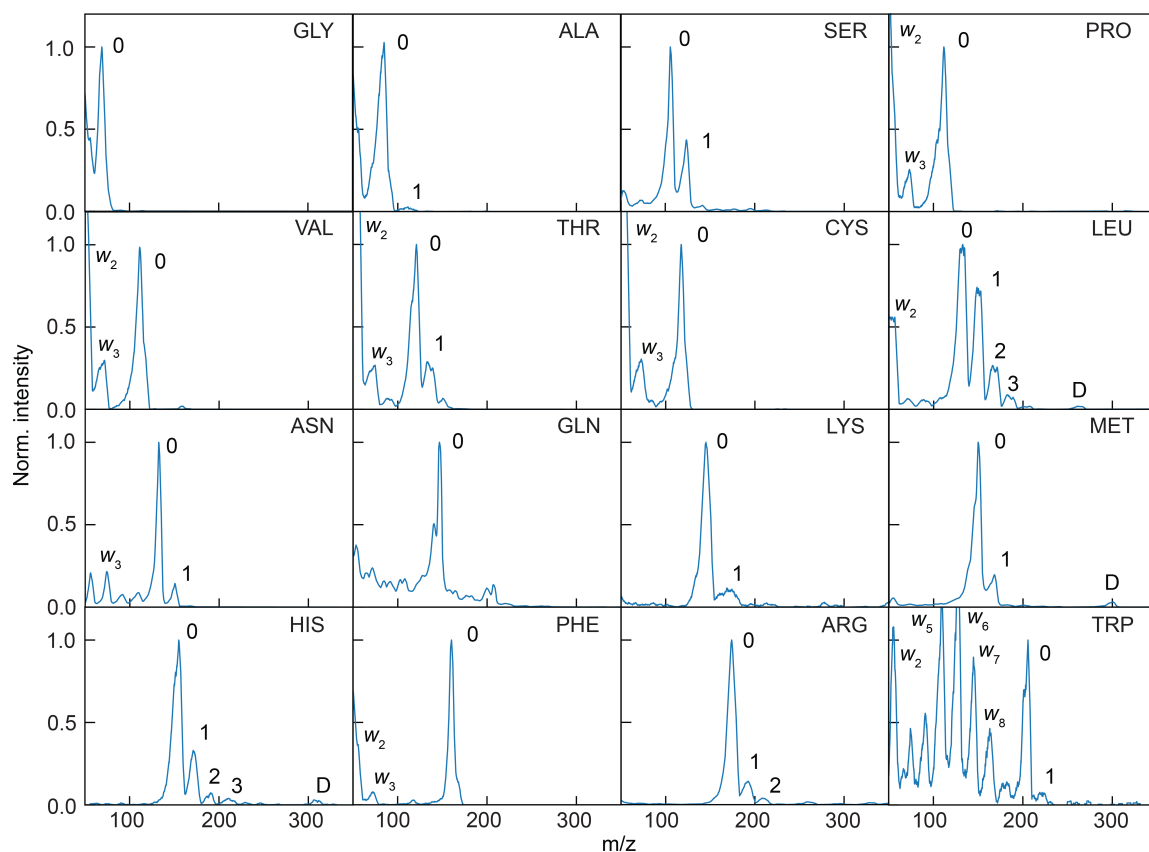


Figure 3.2.4: **Gallery of amino acid spectra.** Gallery of 16 amino acid mass spectra, ordered from top left to bottom right by mass. Peaks labelled w_n correspond to water clusters ($\text{H}_3\text{O}^+(\text{H}_2\text{O})_n$), peaks labelled 0 correspond to the desolvated amino acid ion, 1 corresponds to the singly solvated amino acid ion, etc, and peaks labelled D correspond to the desolvated amino acid dimer ion. All experiments were carried out using nanopore ion sources with 20-60 nm inner tip diameters (see Table 3.1). Each spectrum comprises thousands of individual detection events.

Table 3.1: Experimental conditions of amino acid measurements presented in Fig. 2b of the main text. The table lists the solution properties (amino acid identity and mass, concentration, pH, and conductivity), the ion source properties (tip inner diameter (I.D.), tip outer diameter (O.D.), and tip number), as well as the time-averaged chamber pressure, extraction voltage, and emission current.

Amino acid	Molecular weight (g mol ⁻¹)	Conc. (mM)	pH	K (S m ⁻¹)	Tip I.D. (nm)	Tip O.D. (nm)	P (torr)	V_E (V)	I_E (pA)	Tip number
Arginine	174.20	100	8.06	0.205	20	64	8×10^{-8}	225	2	1
Lysine	146.19	100	5.75	0.486	20	64	5×10^{-8}	225	1	1
Histidine	155.16	100	6.22	0.247	20	64	1.3×10^{-7}	280	2	1
Glycine	75.07	100	4.96	1.96×10^{-2}	25	58	6×10^{-8}	190	4	2
Alanine	89.09	100	4.03	1.94×10^{-2}	25	58	6×10^{-8}	190	4	2
Proline	115.13	100	3.96	1.25×10^{-2}	25	58	6×10^{-8}	190	4	2
Valine	117.15	100	3.95	1.75×10^{-2}	25	58	6×10^{-8}	190	4	2
Threonine	119.12	100	3.88	1.82×10^{-2}	25	58	6×10^{-8}	190	4	2
Cysteine	121.15	100	3.78	1.53×10^{-2}	25	58	6×10^{-8}	190	4	2
Leucine	131.18	100	3.86	2.10×10^{-2}	25	58	6×10^{-8}	190	5	2
Phenylalanine	165.19	100	4.01	1.08×10^{-2}	25	58	6×10^{-8}	190	4	2
Serine	105.09	100	4.08	1.56×10^{-2}	58	111	6×10^{-8}	311	5	3
Asparagine	132.12	100	4.50	1.74×10^{-2}	58	111	1.4×10^{-7}	380	25	3
Methionine	149.21	100	3.90	1.35×10^{-2}	58	111	6×10^{-8}	311	5	3
Tryptophan	204.23	50	4.20	0.50×10^{-2}	58	111	6×10^{-8}	311	5	3
Glutamine	146.15	100	3.39	5.58×10^{-2}	57	115	3×10^{-8}	360	30	4

3.2.2 Measuring post-translationally modified peptides

Figure 3.2.5 shows mass spectra of glutathione and two chemically modified variants, *S*-nitrosogluthione, and *S*-acetylglutathione. Glutathione is a tripeptide (amino acid sequence: GSH) found in high concentrations in most cells [206], and the variants we studied result from common post-translational modifications [207, 208]. Peptide solutions comprised the peptide of interest dissolved in deionized water at a concentration of 100 mM with pH between 3.1 and 3.9, adjusted by the addition of acetic acid. Glutathione and *S*-acetylglutathione were purchased in powder form ($\geq 98\%$, Sigma-Aldrich, Merck), and *S*-nitrosogluthione was synthesized from glutathione according to a protocol from T.W. Hart [209]. Ion sources with 20 nm I.D. tips were used to generate the peptide ions. The glutathione spectrum shows a single m/z peak at 308, which corresponds to the singly protonated, unsolvated glutathione ion. The spectra of *S*-acetylglutathione and *S*-nitrosogluthione show dominant peaks at 350 and 337, respectively, corresponding to the singly protonated, unsolvated peptide ions; each spectrum also shows two progressively smaller peaks 18 and 36 to the right of the dominant peak, corresponding to singly and doubly solvated peptide ions, respectively.

3.2.3 Measuring a multiply charged peptide

Figure 3.2.6 shows mass spectra obtained from an experiment where an aqueous glutathione solution was replaced with an angiotensin II solution (amino acid sequence: DRVYIHPF) inside a 29 nm I.D. tip. The mass spectrum of the 100 mM glutathione solution (pH 3.0) initially inside the emitter tip shows a large peak at 308 that we assign to protonated glutathione, along with additional smaller peaks at 326, 344, and 615 that we assign, respectively, to singly protonated, singly hydrated glutathione, singly protonated, doubly hydrated glutathione, and singly protonated glutathione

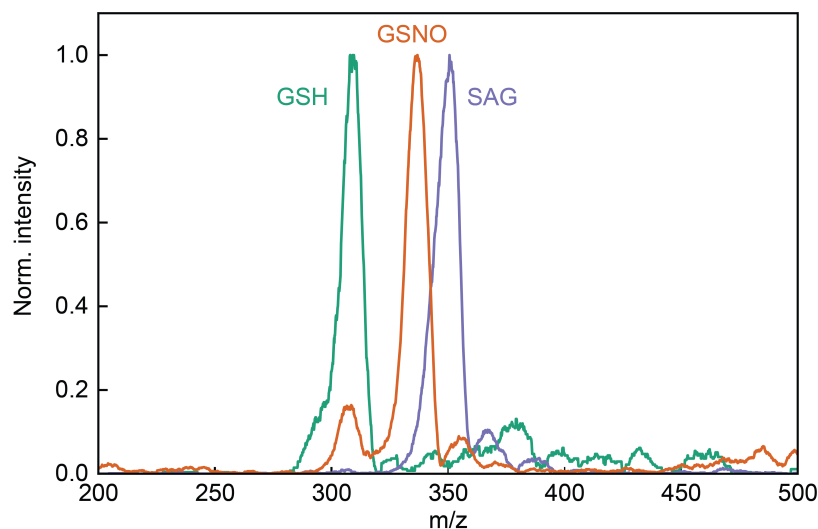


Figure 3.2.5: **Glutathione and PTMs.** Overlaid mass spectra of glutathione (GSH, blue) and two of its PTM variants, *S*-nitrosoglutathione (GSNO, red) and *S*-acetylglutathione (SAG, green).

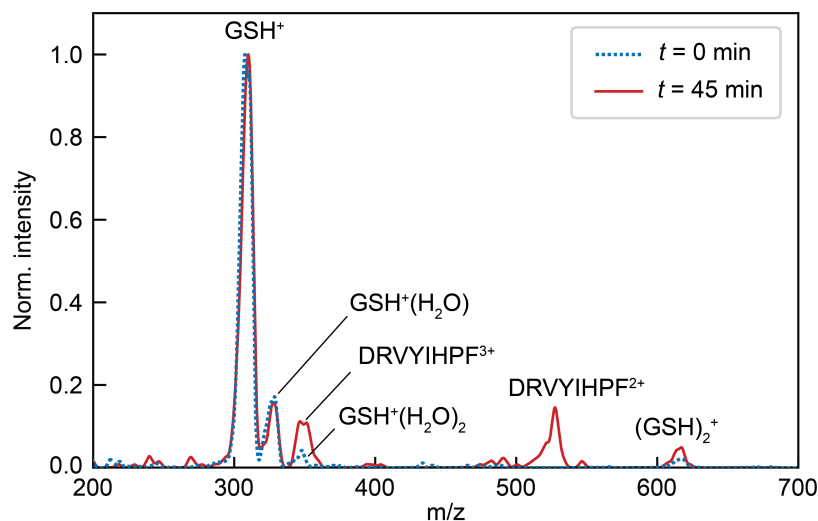


Figure 3.2.6: **Detection of angiotensin II.** Mass spectra of 100 mM glutathione (dashed blue line) and a mixture of glutathione and angiotensin II obtained about 45 min after introducing a 1 mM angiotensin II solution into the tip of the ion source (solid red line). Peaks assigned to glutathione (GSH^+), singly hydrated glutathione ($\text{GSH}^+(\text{H}_2\text{O})$), glutathione dimer ($(\text{GSH})_2^+$), and the doubly and triply protonated states angiotensin II (DRVYIHPF^{m+} , $m = 2, 3$) are indicated.

dimer. We gradually introduced a 1 mM angiotensin II solution into the tip using a previously described tube-in-tube fluid handling system [180]. Additional peaks appeared in the mass spectrum at 524 and 350, which we assign to doubly and triply protonated angiotensin II, respectively. The emission current throughout the measurement was 10 ± 3 pA with an extraction voltage of 400 V.

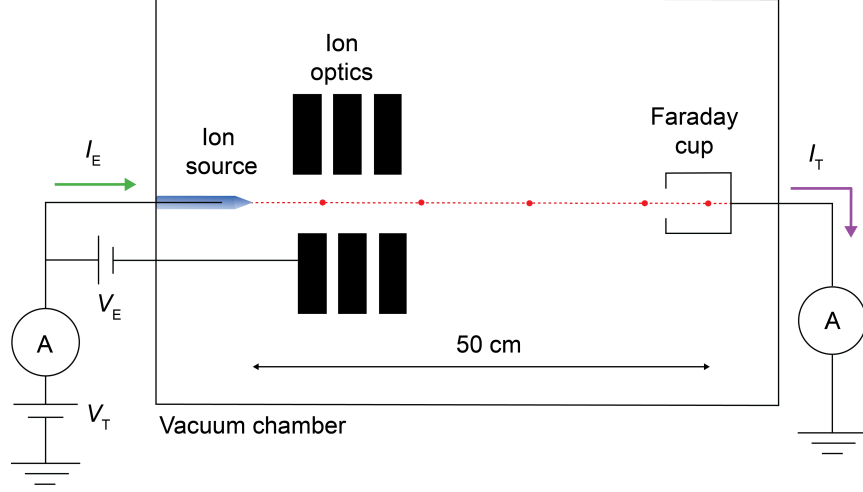


Figure 3.2.7: **Ion transmission efficiency measurement setup.** Diagram of the setup used to measure the ion transmission efficiency of a nanopore ion source. I_E : emission current, I_T : transmitted current, V_T : tip voltage, V_E , extraction voltage (difference between V_T and voltage on first element of the ion optics)

3.2.4 Ion transmission efficiency

We measure the efficiency with which ions pass from the nanopore source to a distant detector in a high-vacuum environment as illustrated in Fig. 3.2.7. Ions emitted by the source are focused into the 2.4 cm diameter opening of a Faraday cup located ≈ 50 cm away. A high voltage source measure unit (Keithley 2410 for the NaI data, Keithley 2657a for the arginine data) measured the current emitted from the nanopore tip, I_E , while also applying a voltage, V_T , to it. A current-preamplifier (SR570, Stanford Research Systems) converted the transmitted current, I_T , striking the Faraday cup into a voltage that was recorded by a DAQ (NI PCIe-6251, National instruments). The ion transmission efficiency is the ratio of the current transmitted to the Faraday cup, I_T , to the current emitted from the nanopore, I_E .

Figure 3.2.8a shows I_E , I_T , and the ion transmission efficiency measured over several minutes using a 39 nm I.D. tip filled with a 100 mM aqueous solution of sodium iodide. The mean ion transmission efficiency in this measurement is $93.4\% \pm 1.7\%$. Figure 3.2.8b shows the results of a similar measurement made with a 49 nm I.D. tip filled with 100 mM arginine in aqueous solution at pH 8.95. The mean ion transmission efficiency is $65.3\% \pm 8.1\%$. In both measurements, small changes in I_E are mirrored by I_T . We had more difficulty optimizing the focusing voltages in the amino acid measurement because of the very low emission currents, which were at the single pA level.

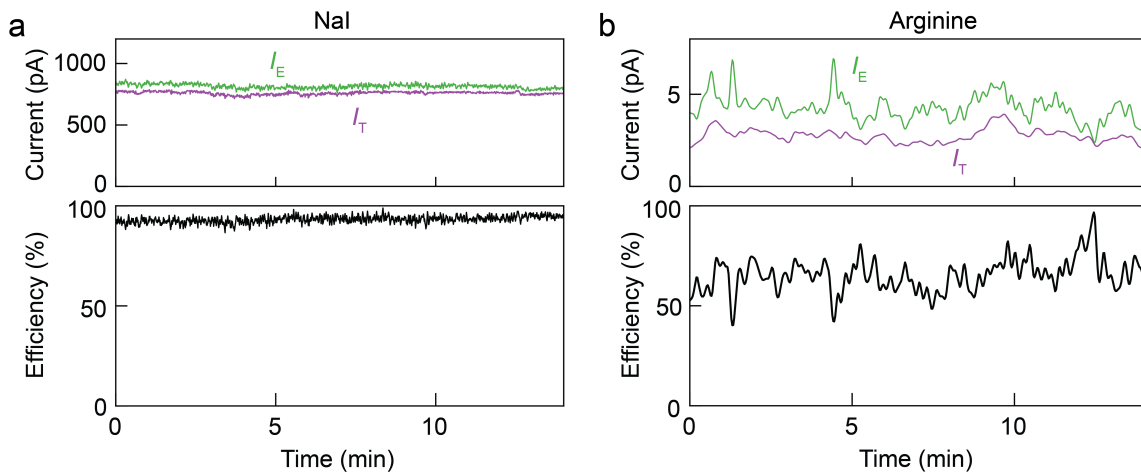


Figure 3.2.8: **Ion transmission efficiency results.** **a**, I_E and I_T and the resulting transmission efficiency of a 39 nm I.D. nanopore ion source containing a 100 mM aqueous solution of NaI. **b**, I_E and I_T and the resulting transmission efficiency of a 49 nm I.D. nanopore ion source containing a 100 mM aqueous solution of arginine at pH 8.95.

3.2.5 Separating ions and charged droplets

We investigate the possibility that the nanopore ion source emits charged droplets in addition to ions by adding a magnetic sector to the flight path as shown in Fig. 3.2.9. The 6 cm diameter, 0.54 T magnetic sector deflects charged species based on their mass-to-charge ratio. Droplets with an $m/z > 33,000$ deflect by less than 3.1° and enter the Faraday cup where their current, I_{Drop} , is measured. We do not expect the droplet size to change appreciably between emission and detection because nanoscale aqueous droplets freeze rapidly in vacuum [210, 211]. Meanwhile, ions with m/z in the range of about 70 to 325 deflect onto a separate Faraday plate where the ion current, I_{Ion} , is measured § 3.2.5.1 below describes how the above m/z ranges were determined. Figure 3.2.10a shows I_{Ion} , I_{Drop} , and the ion fraction, defined as $(\frac{I_{\text{Ion}}}{I_{\text{Ion}} + I_{\text{Drop}}})$, for a measurement performed using a 28 nm I.D. tip filled with a 100 mM aqueous solution of NaI. I_{Ion} varies between about 60 pA and 80 pA while no I_{Drop} is observed. Figure 3.2.10b shows the results of a similar measurement made using a 41 nm I.D. tip filled with a 100 mM aqueous solution of arginine at pH 8.75. I_{Ion} varies between about 1 pA and 2 pA while there is no detectable I_{Drop} . In both measurements, the nanopore source was only observed to emit ions.

3.2.5.1 Magnetic sector simulations

We performed simulations to determine the m/z range of particles expected to strike the Faraday cup and Faraday plate detectors used in the magnetic sector experiments. The trajectories of

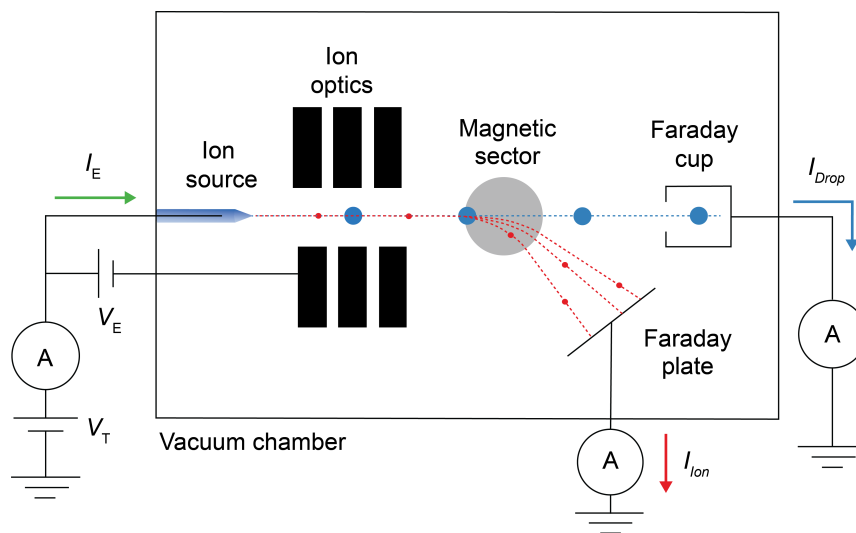


Figure 3.2.9: **Ion fraction measurement setup.** Setup used to separately measure the ion current, I_{ion} , and the droplet current, I_{drop} .

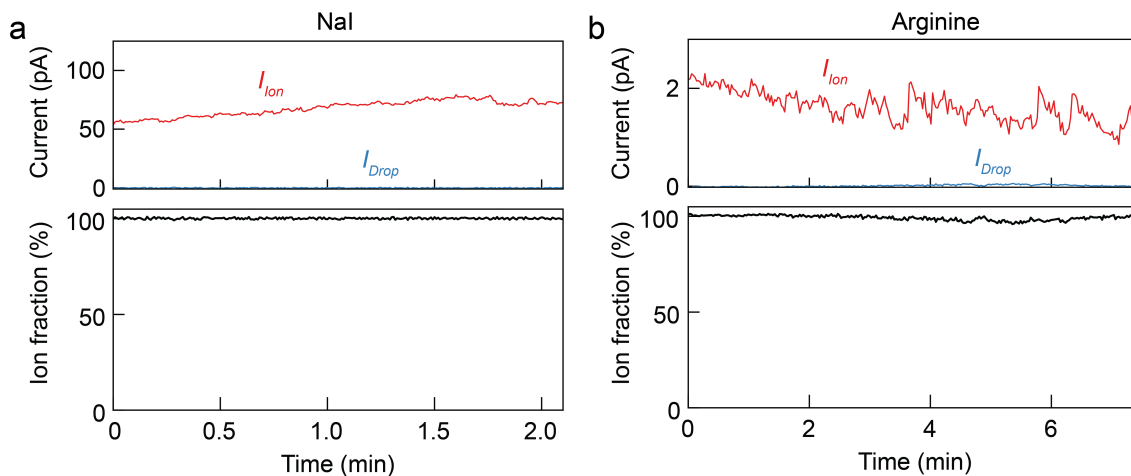


Figure 3.2.10: **Ion fraction results.** **a** I_{ion} and I_{drop} and the resulting ion fraction emitted by a 39 nm I.D. nanopore ion source containing a 100 mM aqueous solution of NaI. **b** I_{ion} and I_{drop} and the resulting ion current fraction emitted by a 41 nm I.D. nanopore ion source containing a 100 mM aqueous solution of Arginine at pH 8.95.

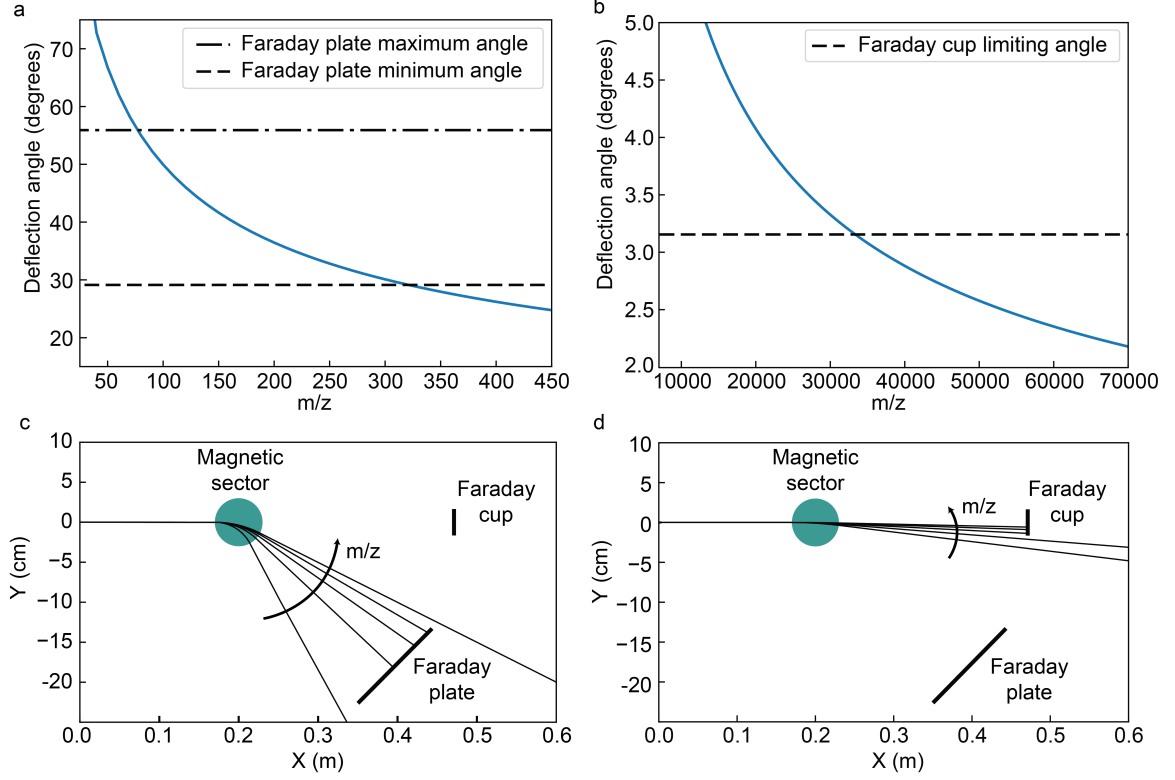


Figure 3.2.11: **Magnetic sector simulations.** **a** Simulation results of deflection angle vs m/z for small ions. The region between the dashed lines represents the position and extent of the Faraday plate detector, so ions with $70 < m/z < 325$ should strike the Faraday plate. **b** Simulation results of deflection angle vs m/z for nanoscale charged droplets. The region below the dashed line represents the position and extent of the Faraday cup aperture, so species with $m/z > 33000$ be detected. **c** Selection of simulated trajectories for five singly charged ions of mass between 60 and 460 amu, the arrow points in the direction of increasing m/z , and the blue circle represents the magnetic sector. **d** Selection of simulated trajectories for five nanodroplets charged to the Rayleigh limit with radius between 5 and 25 nm, the arrow points in direction of increasing m/z .

particles passing through the magnetic sector, which are subject to the Lorentz force, are calculated numerically by solving Newton's second law using a fourth-order Runge-Kutta scheme with a custom Python script. Particles are given a kinetic energy of qV , where $V = 500$ is the tip voltage used in the experiments presented in Fig 3.2.10, and q is the charge on the particle. The magnetic field has a magnitude of 0.54 T, is oriented in the $+z$ -direction within the bounds of the magnetic sector (a circle with a diameter of 6 cm), and vanishes outside these bounds. Given the geometry of the instrument, particles deflected less than 3.2° will strike the Faraday cup and contribute to I_{Drop} , while particles deflected by between 31° and 59° will strike the Faraday plate and contribute to (I_{Ion}). We find that particles with $75 < m/z < 315$ strike the Faraday plate, while particles with $m/z > 33,000$ strike the Faraday cup. The latter m/z value corresponds to a 14 nm radius water droplet charged to the Rayleigh limit [84] as well as a 7 nm radius droplet sufficiently charged to

readily evaporate ions [205].

3.2.5.2 Freezing of charged droplets

One may challenge the conclusion that the source emits only ions on the grounds that the detected ion current could be generated by ion evaporation from charged droplets in flight between the source and magnetic sector. However, this explanation is implausible given the high vacuum environment, as an aqueous nanodroplet would rapidly freeze before emitting significant charge.

We used the model developed by Shin et al. to work out the rate of evaporative cooling for nanoscale aqueous droplets emitted into a vacuum [212]. The rate of mass loss is given by

$$\frac{dm}{dt} = -\frac{4\pi r D_v M}{R} \left(\frac{P_a}{T_a} - \frac{P_\infty}{T_\infty} \right), \quad (3.2.1)$$

where r is the droplet radius, D_v is the vapor diffusion coefficient, M is the molar mass, R is the ideal gas constant, P_a and T_a are the vapor pressure and temperature at the droplet surface, and P_∞ and T_∞ are the background pressure and temperature in the vacuum. The rate of evaporative cooling at the droplet surface is given by

$$\frac{dT_a}{dt} = -\frac{3}{\rho c_P r^2} \left[\frac{h_v D_v M}{R} \left(\frac{P_a}{T_a} - \frac{P_\infty}{T_\infty} \right) - k_v (T_\infty - T_a) \right], \quad (3.2.2)$$

where c_P is the specific heat capacity, h_v is the heat of vaporization, and k_v is thermal conductivity of vapor. The vapor pressure at the surface, P_a , is calculated using the Kelvin equation to account for the effect of the droplet curvature [213]. Figure 3.2.12 plots the temperature vs time curves for aqueous nanodroplets with $r = 10, 25, 50, \& 100$ nm, calculated from eq. (3.2.2), using the following parameters: $c_P = 4.18 \times 10^3$ J/kgK, $h_v = 2.26 \times 10^6$ J/kg, $D_v = 2.5 \times 10^{-5}$ m²/s, $k_v = 2.3$ W/mK, $P_\infty = 0$, & $T_\infty = 273$ K. Nanodroplets with $r < 100$ nm reach freezing temperature within nanoseconds after shedding only a very small fraction of their mass. Such rapid freezing makes Coulomb explosions unlikely. Additionally, measurements by Evan Williams' group have shown that aqueous nanodroplets charged near the Rayleigh limit in high vacuum evaporate ions no faster than about 1 per second [214]. Taken together, these results demonstrate the implausibility of the ion currents shown in Fig. 3.2.10 originating from charged droplets downstream of the nanopore tip.

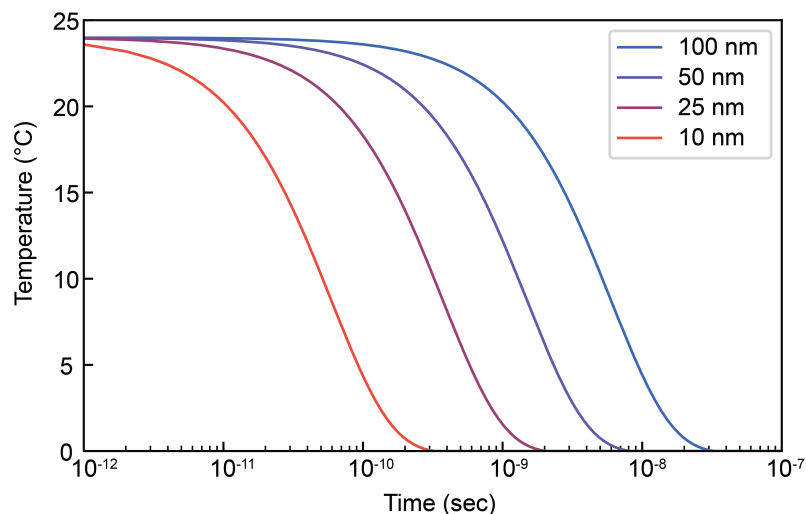


Figure 3.2.12: **Droplet freezing.** Plots of temperature vs time for aqueous nanodroplets of different radii (10 nm, 25 nm, 50 nm, & 100 nm) undergoing evaporative cooling in a vacuum. Calculated from Eq. (3.2.2).

3.2.6 pH dependence of amino acid emission

To further investigate the emission mechanism, we measured the pH dependence of the emission rates of histidine and arginine. For each amino acid, we prepared solutions at two different pH values but with a fixed concentration of Na^+ to serve as a reference ion. We made all measurements with a single, 24 nm I.D. tip. Figure 3.2.13a shows the mass spectra of 100 mM aqueous histidine solutions with pH values of 5.5 and pH 8.5; the concentration of Na^+ is 24 mM. At pH 5.5, peaks corresponding to the bare histidine ion and several solvated states appear at m/z between approximately 150 and 275; the histidine peaks are more intense than the solvated sodium peaks which appear between approximately 50 and 150. At pH 8.97, the histidine peaks are still visible, but their intensity is significantly diminished relative to the sodium peaks. Figure 3.2.13b shows the mass spectra of 100 mM arginine solutions with pH values of 9.1 and 10.8; the solutions also contain 50 mM of Na^+ . Similarly to histidine, bare and solvated arginine peaks, which appear in the m/z range between 170 and 350, have a higher intensity than the sodium peaks at the lower pH of 9.1. The relative intensity of the arginine peaks decreases dramatically at the higher pH of 10.8. The results shown in Fig. 3.2.13 are representative of repeated measurements. To quantify the pH dependence of the amino acid emission rates, we normalized them by the sodium emission rates. The histidine emission rate increased by an average factor of 8.14 ± 1.65 as the pH decreased from 8.5 to 5.5. The arginine emission rate increased by an average factor of 22.6 ± 10.5 as the pH decreased from 10.8 to 9.1.

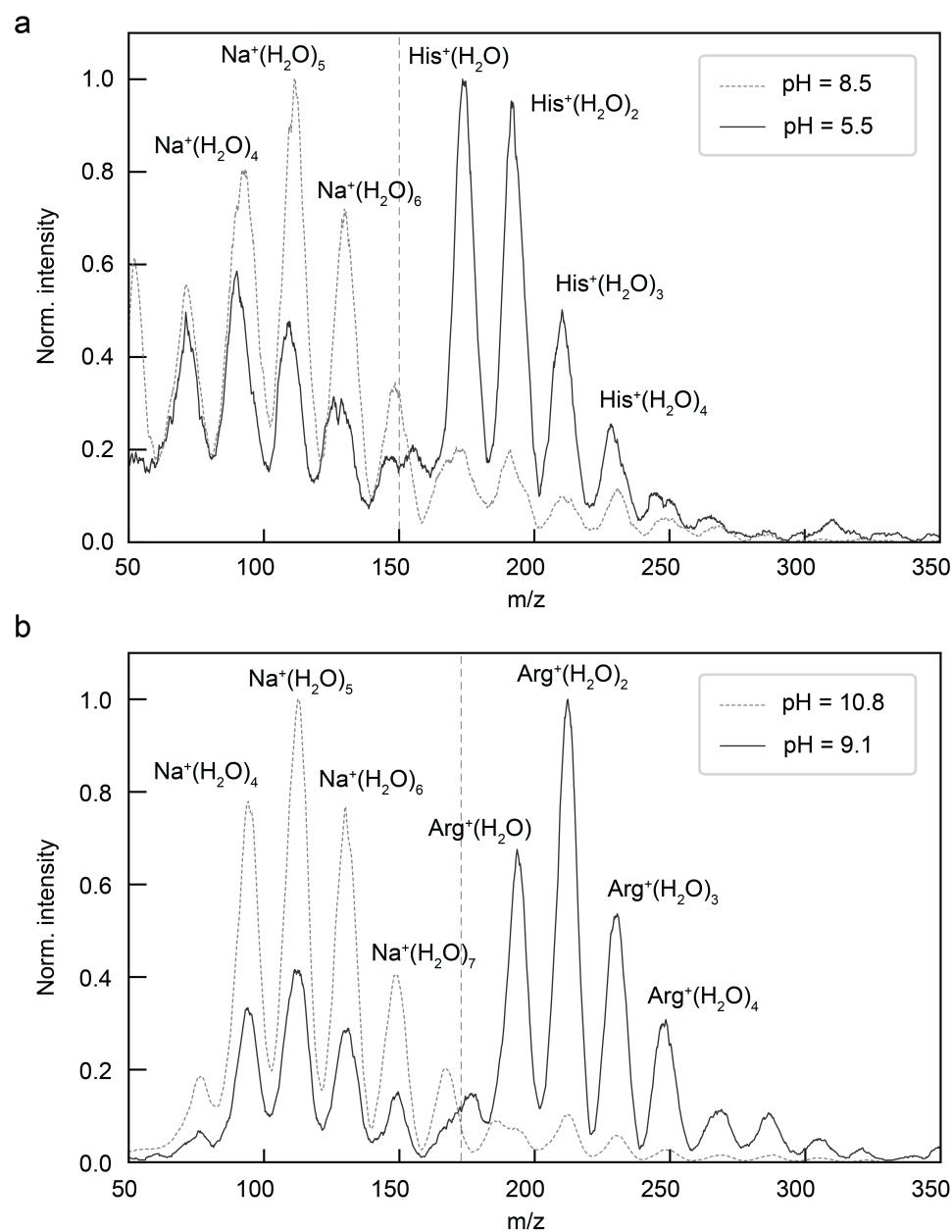


Figure 3.2.13: **Amino acid spectra above and below the isoelectric point.** **a** Normalized mass spectra of 100 mM Histidine and 24 mM NaI/NaOH in aqueous solution at pH 5.5 (darker solid curve) and 8.5 (lighter dashed curve) respectively. **b** Normalized mass spectra of 100 mM Arginine and 50 mM NaI at pH 9.1 (darker curve) and 10.8 (lighter curve) respectively. In both panels, the vertical dashed line shows the m/z value used in the analysis to separate the amino acid signal from sodium signal.

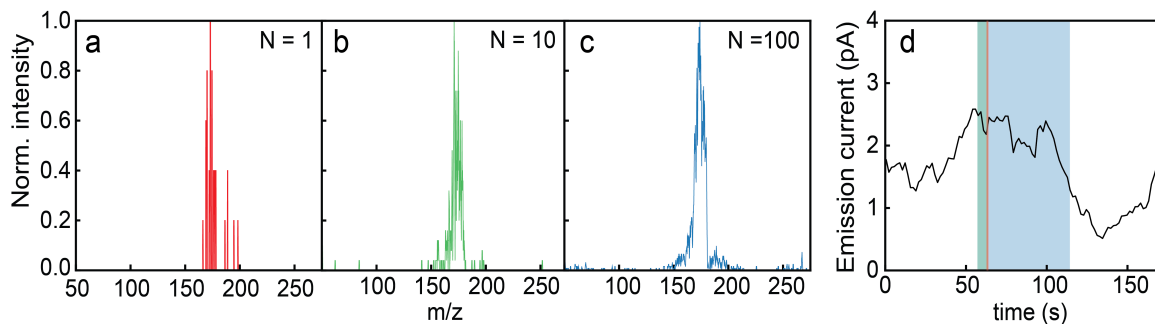


Figure 3.2.14: **MS sensitivity estimate.** Mass spectra of 100 mM Arginine recorded with (a) 1 scan (≈ 0.6 seconds), (b) 10 scans (≈ 6 seconds), and (c) 100 scans (≈ 60 seconds). d Emission current as a function of time over the period in which the detections in a, b and c were measured. The red, green, and blue regions correspond to the 0.6 s, 6 s, and 60 s of data shown in a, b and c, respectively.

3.2.7 Sensitivity of quadrupole MS measurements

In this section we present an estimate of the minimum number of emitted analyte molecules required for a reliable detection in our MS experiments, which is an important measure of the sensitivity [215]. An alternative measure of sensitivity is the minimum detectable concentration of analyte, which we did not explore in this work. The quantity of ions emitted per quadrupole scan is given by I_E , integrated over the duration of a scan, Δt , and divided by the charge on each ion, zq_e ,

$$N_{\text{emitted}} = \frac{I \Delta t}{z q_e}. \quad (3.2.3)$$

Figure 3.2.14a-c shows mass spectra of a 100 mM aqueous arginine solution obtained with 1, 10, and 100 scans. The median scan duration is $\Delta t = 0.64$ s. A single scan (Fig. 3.2.14a) produces a spectrum composed of 55 individual ion detection events with a clear m/z peak at 175. Thus, a single scan enables the identification of arginine. Fig. 3.2.14d plots I_E during the measurement. Using Eq. (3.2.3) with $I_E = 2.2$ pA gives $N_{\text{emitted}} = 8.8 \times 10^6$ analyte ions, or 15 attomoles. Note that N_{emitted} provides an upper bound on the number of analyte molecules emitted because analyte only escapes in the form of ions, and non-analyte ions can carry some portion of the emission current. Using N_{emitted} as we have done here thus gives a worst-case estimate of the amount of analyte consumed. The 15 attomole sensitivity of our measurement is similar to the typical sensitivity of a proteomics MS measurement, which is in the range of an attomole (~ 1 million copies) to a femtomole (~ 1 billion copies) [8]. However, it is worth noting that we are comparing measurements performed on small amino acid ions with large proteins, which may differ in their emission characteristics.

Sensitivity depends on every step of the measurement leading up to detection, and any step

could be the limiting one. ESI is often the limiting step, losing 99% or more of the analyte. In contrast, many ion detectors and mass analyzers (e.g. orbitraps) waste very few ions. However, in our instrument, the quadrupole mass filter is the limiting step, because its narrow mass acceptance window rejects the vast majority of ions over the course of a scan [194]. For most of a scan, the acceptance window is far from the m/z of the analyte, and even when the two align, the transmission is typically about 10%-20% or less [94, 216]. Additionally, a misalignment of the tip with respect to the central axis of the ion optics and the quadrupole of about 1 mm can cause significant deflection of the ion beam, reducing transmission through all subsequent parts of the instrument. We reason that significant improvements in sensitivity can be achieved by combining the nanopore ion source, which wastes very few ions, with a highly efficient ion trapping mass analyzer.

3.2.8 Calculating the probability of ions scattering

Calculations indicate that most ions trace collisionless trajectories from the ion source to the detector. In conventional ESI, collisions with gas molecules are the primary mechanism that removes solvent molecules from ions. However, our instrument operates under high vacuum conditions where collisions with gas molecules are rare. We applied the kinetic theory of gases to calculate the probability that an emitted ion cluster would collide with at least one gas molecule. This addresses the question of whether ions emerge from the solution unsolvated, or whether they emerge with a solvation shell which is knocked off by collisions with gas molecules on their way to the detector.

We take the distribution of gas particles within the vacuum chamber to be the sum of two components, a uniform background of number density n_b and a distribution of water molecules n_w that evaporate from the meniscus at the nanopore tip [217]. The background gas pressure in our experiments was typically around 7×10^{-8} torr (see table 3.1). The mean free path of a water molecule in nitrogen gas at this pressure is greater than 1 km, so we model the evaporating water molecules as travelling radially outward from a hemispherical meniscus, as illustrated in Fig. 3.2.15. The density of water molecules decays as $n_w \sim r^{-2}$ near the ion source, where r is the distance from the center of the hemisphere. The rate at which water evaporates from the nanoscale liquid meniscus into vacuum is difficult to establish precisely [218]. However, the flux of water molecules evaporating from the liquid surface at a given temperature cannot exceed the flux of incoming molecules at equilibrium. Thus, if we subtract the inward-moving molecules, the highest possible density that the (outward-moving) evaporating water molecules could achieve on the vacuum side of the meniscus is half the water vapor density at equilibrium. We therefore estimate the upper

limit of the probability that an emitted ion collides with a gas molecule by assuming the density of water vapor on the vacuum side of the meniscus is $\frac{p_0}{2k_B T}$, where $p_0 = 17.5$ torr is the approximate equilibrium vapor pressure of water at 20 °C [219], and $k_B T$ is the thermal energy.

We are interested in the cumulative probability that an ion cluster leaving the source undergoes at least one collision before reaching r , $C_P(r)$. To compute $C_P(r)$, it is easier to first consider the probability that an ion survives to a distance r without undergoing a collision, $P_s(r) = 1 - C_P(r)$, which can be defined recursively as

$$P_s(r) = P_s(r - dr) (1 - P_c(r - dr \rightarrow r)), \quad (3.2.4)$$

where $P_c(r - dr \rightarrow r)$ is the probability that a collision occurs between $r - dr$ and r . In the limit of dilute gasses and infinitesimal displacements,

$$P_c(r - dr \rightarrow r) = \sigma_w n_{w,0} \left(\frac{r_0}{r}\right)^2 dr + \sigma_b n_b dr, \quad (3.2.5)$$

where σ_w is the cross section for a collision between an ion cluster and a water molecule, σ_b is the cross section for an ion collision with a background gas molecule, $n_{w,0}$ is the number density of water on the vacuum side of the meniscus, and r_0 is the radius of the meniscus. Combining Eqs. (3.2.4) and (3.2.5) gives a differential equation for P_s

$$\frac{P_s(r) - P_s(r - dr)}{P_s(r - dr)} = \frac{dP_s(r)}{P_s(r)} = -\sigma_w n_{w,0} \left(\frac{r_0}{r}\right)^2 dr - \sigma_b n_b dr. \quad (3.2.6)$$

Integrating Eq. (3.2.6) from r_0 to r , and applying the boundary condition $P_s(r_0) = 1$ gives

$$P_s(r) = \exp \left[-\sigma_w n_0 \left(\frac{1}{r_0} - \frac{1}{r} \right) - \sigma_b n_b (r - r_0) \right]. \quad (3.2.7)$$

Finally, the cumulative probability that an ion experiences at least one collision before reaching r is given by $1 - P_s(r)$,

$$C_P(r) = 1 - \exp \left[-\sigma_w n_0 \left(\frac{1}{r_0} - \frac{1}{r} \right) - \sigma_b n_b (r - r_0) \right]. \quad (3.2.8)$$

Figure 3.2.15 plots $C_P(r)$ using parameters corresponding to the conditions of our MS measurements. The cross sections σ_w and σ_b are given respectively by $\pi(a_i + a_w)^2$ and $\pi(a_i + a_b)^2$, where $a_w = 1.3$ Å is the kinetic radius of water, $a_b = 1.8$ Å is the kinetic radius of the background gas

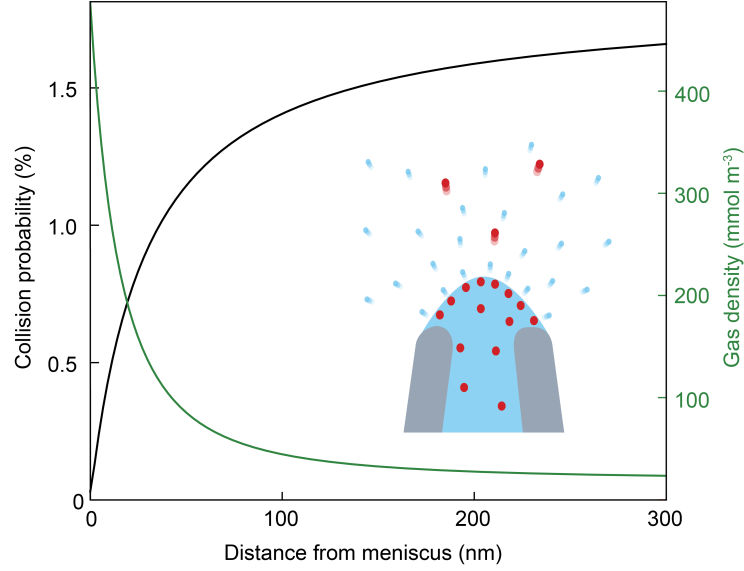


Figure 3.2.15: **Ion collision probability.** Plot of the cumulative probability that an emitted amino acid with a small hydration shell (radius = 7 Å) will collide with an evaporated water molecule or background gas molecule as a function of distance from the meniscus, r . The green curve shows the calculated maximum water vapor density as a function of the distance from the meniscus

molecules [220], and $a_i = 7$ Å is the approximate radius of an amino acid with a full hydration shell, which we estimated using the Stokes-Einstein relation and reported diffusion coefficients of amino acids in water [221]. $n_b = 2.25 \times 10^{15} \text{ m}^{-3}$ and $n_{w,0} = 6.44 \times 10^{23}$ are the number densities of background gas and water molecules on the vacuum side of the meniscus, respectively, at a background pressure of 7×10^{-8} torr and equilibrium water vapor pressure of 17.5 torr. We use a meniscus radius of $r_0 = 30$ nm. Figure 3.2.15 shows $C_P(r)$ rise rapidly with r over the first 100 nm before beginning to saturate near 1.8 %. $C_P(r)$ continues to increase slowly over centimeter-scale distances due to the non-zero density of background gas molecules. The cumulative probability of an ion colliding with a gas molecule over the entire 50 cm trajectory from the source to the detector is just 2.1%, meaning the vast majority of ions do not experience any collisions.

These results show that, in contrast to conventional electrospray, collisions between gas particles and ion clusters emitted from the nanopore ion source are rare. The majority of ions follow collisionless trajectories from the source to the detector because the instrument operates under high vacuum conditions. The lack of collisions implies that ions are emitted in the same state they are detected in, leading us to conclude that the nanopore ion source is capable of emitting predominantly desolvated amino acid and small peptide ions.

3.3 Discussion

There are two distinct mechanisms by which the nanopore source could generate the ions that we measure. The first is conventional electrospray ionization, a multi-step mechanism in which an ion source first produces charged droplets which go on to evaporate ions into the vapor phase [90]. The second is a single-step mechanism in which ions evaporate directly from the electrified meniscus of the ion source [148]. We highlight the important physical distinctions between these two mechanisms before presenting an analysis that rules out the former.

ESI begins when a voltage applied to the liquid sample generates electrical forces on the meniscus that are strong enough to overcome the surface tension and draw out charged droplets (fig. 3.2.1a). The droplets typically measure hundreds of nanometers to tens of micrometers in radius and contain fully solvated analyte molecules [126]. A background gas is used to evaporate solvent from the droplets [126]. As a droplet shrinks, its charge density increases, and the resulting internal Coulomb forces can once again overcome surface tension when the electric field strength at the surface reaches the Rayleigh limit, $E_R = 2\sqrt{\frac{\gamma}{\epsilon_0 r}}$, where γ is the surface tension, ϵ_0 is the permittivity of free space, and r is the droplet radius [84, 23]. The unstable droplet undergoes a Coulomb explosion and expels smaller charged droplets [23, 131]. The cycle repeats, producing ever smaller droplets requiring ever stronger electric fields to reach the Rayleigh limit. When the field strength at the surface reaches about 10^9 V m^{-1} , it becomes possible for ions to quickly escape the droplet by a different process: Ion evaporation is a thermal process in which ions overcome an energy barrier associated with their solvation energy, ΔG_0^S , to enter the vapor phase [205]. Coulomb explosions cease and ion evaporation becomes the main charge release mechanism for small molecules when droplets reach around 10 nm in radius [205, 222, 141].

Alternatively, the nanopore source might deliver ions and ion clusters into the vapor phase directly from the meniscus at the tip by an ion evaporation process. This alternative mechanism circumvents the emission of droplets and their interactions with a background gas that are integral to ESI. Figure 3.3.1a illustrates the direct evaporation of a solvated ion cluster from an electrified meniscus and a schematic of the energy landscape [205, 135]. An ion inside the nanopore source is fully solvated by water molecules, which reduce the free energy by an amount ΔG_0^S , called the solvation energy. The free energy increases as the ion protrudes from the meniscus, distorting it against surface tension. At this point, the ion is also attracted by its own image charge as it begins to break free of the solvent. Far from the meniscus, Coulomb forces drive the ion (or cluster) away from the tip and its free energy decreases. Modelling the height of the energy barrier as $\Delta G_0^S - G(E)$,

where $G(E)$ is the field-dependent reduction in the barrier height [205, 135, 23], the current density of ions escaping the meniscus is given by

$$j = \sigma \frac{k_B T}{h} \exp \left(-\frac{G_0 - \Delta G(E)}{k_B T} \right), \quad (3.3.1)$$

where σ is the surface charge density, $k_B T$ is the thermal energy, and h is the Planck constant. The ion evaporation current density only becomes appreciable when the electric field at the meniscus approaches $\approx 10^9 \text{ V m}^{-1}$, at which point $G_0 - \Delta G(E) \sim k_B T$ [114]. The ion evaporation mechanism applies equally well to bare ions and small ion clusters, as each is a charged species that can escape the liquid over a solvation energy barrier [205, 155, 223]. On the other hand, charged nano-droplets comprising hundreds of solvent molecules or more are too large to evaporate.

Multiple lines of evidence lead us to conclude that the nanopore source produces ions directly by ion evaporation rather than by the conventional ESI mechanism in which ions emerge from charged droplets. First, the chain of Coulomb explosions that characterizes ESI cannot occur under the high vacuum conditions of our experiments. Droplets quickly freeze before shedding a significant fraction of their mass when there is no background gas to provide the thermal energy that normally sustains solvent evaporation [210]. A previous study showed that nanoscale charged aqueous droplets survive under high vacuum for more than a second without undergoing Coulomb explosions [214].

Secondly, we searched for droplets and measured none among the charged species delivered by the source (Fig. 3.2.10). Our measurements, which are sensitive to species with $m/z > 33,000$, exclude droplets with a radius larger than 14 nm and a charge as high as the Rayleigh limit. For comparison, nano-ESI begins with a spray of droplets that are in the detectable range of tens to hundreds of nanometers in diameter [90, 224]. If even smaller charged droplets were produced [225, 226], they would evaporate ions readily, lose a significant fraction of their charge, and move into the detectable range of m/z ; when the electric field at the surface falls to 0.75 V per nm, the approximate threshold for ion evaporation, droplets as small as 7 nm in radius are detectable.

Thirdly, the nanopore ion source is sufficiently small that as the electric field at the liquid meniscus increases, it reaches the threshold for ion evaporation well before the Rayleigh limit. Figure 3.3.1b, which compares the critical electric field for ion evaporation with the Rayleigh limit, illustrates why ions evaporate from the meniscus while the meniscus remains stable. If we consider a nanopore source with a radius of 15 nm, we expect ions to evaporate from the meniscus when the electric field reaches about 0.75 V per nm, whereas droplets cannot emerge unless the electric field grows about twice as strong. On the other hand, a conventional ESI source with a radius of 1 μm

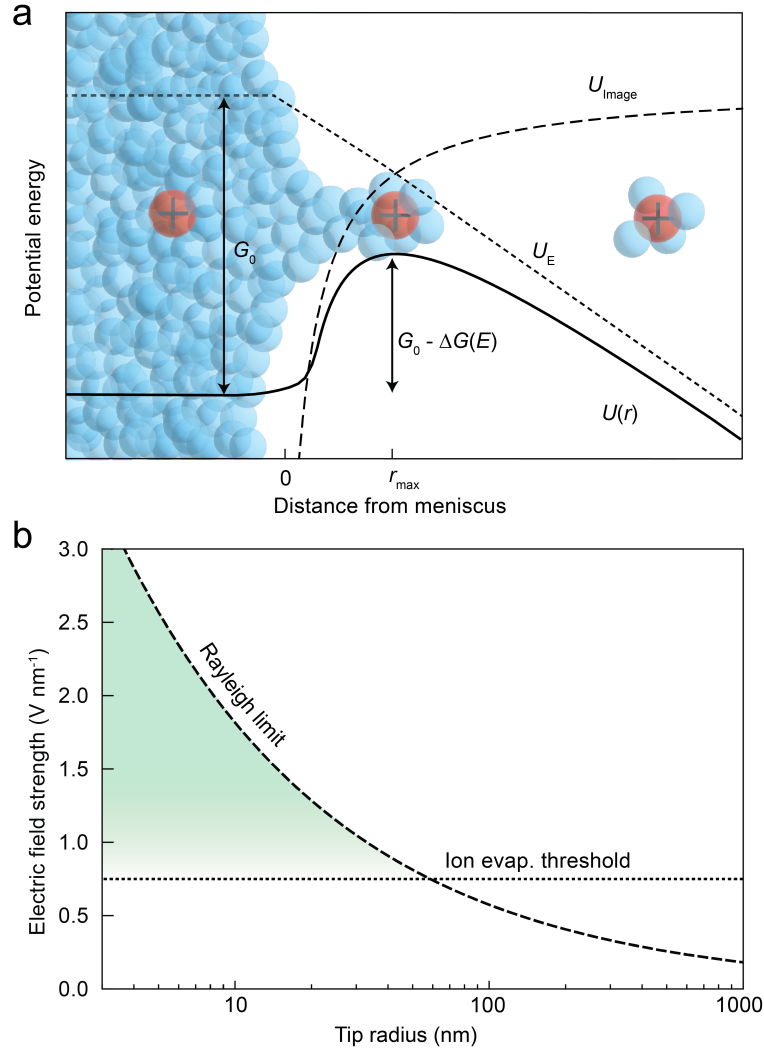
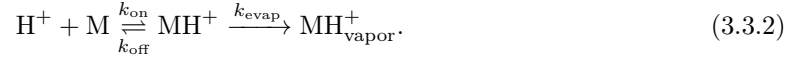


Figure 3.3.1: **Ion emission mechanism.** **a** Diagram of the potential energy, $U(r)$, showing the contributions to the energy barrier for ion evaporation: the solvation energy, G_0 , image charge attraction, U_{image} , and electrostatic repulsion, U_E . r_{max} represents the distance from the meniscus where the potential energy is highest. $\Delta G(E)$ is the reduction in the energy barrier height due to the presence of an electric field of magnitude E . **b** Plot of the approximate limiting fields for ion evaporation and droplet generation (Rayleigh limit). The region where ion evaporation should dominate is highlighted in green.

produces charged droplets when the electric field at the meniscus reaches about 0.2 V per nm, which is well below the threshold for ion evaporation. The crossover from a conventional droplet-emitting source to a nanopore source that can evaporate ions directly is predicted to occur at a radius of about 60 nm.

Finally, the pH dependence of the emission rates of arginine and histidine we measured (Fig. 3.3.2) is consistent with a simple kinetic model of ion evaporation from the meniscus. We consider a two-step process in which an analyte molecule M can undergo reversible protonation/deprotonation with rates $k_{\text{on}}[\text{H}^+]$ and k_{off} , and non-reversible evaporation from the protonated state with rate k_{evap} ,



This process is illustrated in Fig. 3.3.2a. For the purposes of this calculation, assume that the total concentration of analyte remains fixed at c_0 , so that $c_0 = [\text{M}] + [\text{MH}^+]$. This is equivalent to assuming diffusion is fast enough to replace any emitted ion with one from the bulk. The rate of ion emission is given by $v = [\text{MH}^+]k_{\text{evap}}$, where $[\text{MH}^+]$ is the concentration of protonated ions near the surface. In order to find an expression for $[\text{MH}^+]$, we write down its kinetic equation

$$\frac{d[\text{MH}^+]}{dt} = k_{\text{on}}[\text{H}^+][\text{M}] - k_{\text{off}}[\text{MH}^+] - k_{\text{evap}}[\text{MH}^+]. \quad (3.3.3)$$

The system can be considered to be in a quasi steady-state when $\frac{d[\text{MH}^+]}{dt} = 0$. With this assumption we can derive an expression for $[\text{MH}^+]$,

$$[\text{MH}^+] = \frac{k_{\text{on}}c_0[\text{H}^+]}{k_{\text{off}} + k_{\text{evap}} + k_{\text{on}}[\text{H}^+]}. \quad (3.3.4)$$

Using the fact that $[\text{H}^+] = 10^{-\text{pH}}$, we find that the quasi-steady-state ion emission rate scales as,

$$v \propto \frac{10^{-\text{pH}}}{10^{-\text{pK}_a} + 10^{-\text{pH}}}. \quad (3.3.5)$$

Figure 3.3.2b plots the relative emission rates predicted by this model for both histidine and arginine and compares them to the measured values. For histidine, two protonation reactions occur within the experimental pH range (with $\text{pK}_{a,1} = 6.04$ and $\text{pK}_{a,2} = 9.09$); a single dissociation constant corresponding to the isoelectric point ($\text{pK}_a = 7.59$) models the combined effect [219]. For arginine, only the protonation of the amino group ($\text{pK}_a = 9.00$) is relevant [219, 227]. The kinetic model predicts that the emission rates should increase by factors of 9.2 and 27.3 over the pH

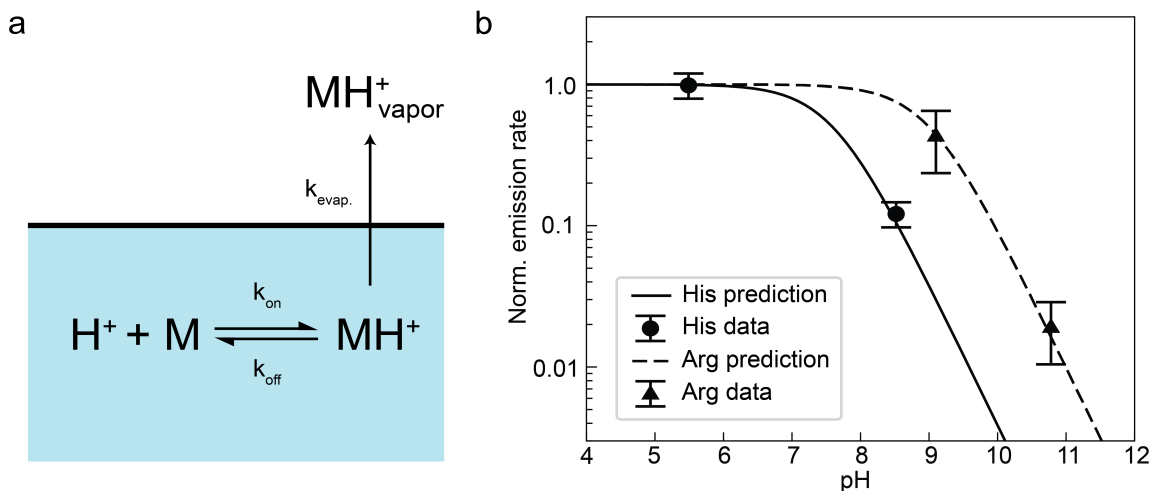


Figure 3.3.2: **pH dependence of amino acid emission, comparison to model.** **a** Diagram illustrating simple kinetic model of evaporation. **b** Summary of repeated measurements of the relative emission rates of histidine (circles, $N = 8$) and arginine (triangles, $N = 3$) compared to theoretical predictions (solid curve for arginine, dashed curve for histidine). The emission rate at the lower pH, v_{low} , is calculated using Eq. (3.3.5), and the emission rate at the higher pH is calculated using v_{low}/F , where F is the ratio of measured signal between low and high pH. The error bars represent standard error of the mean.

ranges investigated for histidine and arginine, respectively, in agreement with our measured values of 8.14 ± 1.65 and 22.5 ± 10.5 . By contrast, previous measurements have shown that ESI emits arginine and histidine at rates that depend much more weakly on pH (by a factor less than three over the same pH range), and this observation was attributed to the acidification of droplets that accompanies the ESI process [228, 229].

The nanopore source appears to operate in a pure ion mode where no droplets are emitted. Pure ion emission was previously observed from highly conductive liquids including liquid metals [230], ionic liquids [148, 147], and concentrated electrolyte solutions in formamide [114, 155], but not aqueous solutions. Previous attempts to evaporate ions directly into vacuum from a fixed water meniscus encountered significant challenges, as the evaporation of water led to electrical arcing and freezing [160]. The nanopore source overcomes those challenges because the evaporation rate from the nanoscale meniscus is minuscule (less than 1 pL of water evaporating per min); this low gas load also eases the pumping requirements of the instrument [180]. Previously, even the smallest ion emitters were used in combination with a background gas and the emission mechanism was presumed to be conventional ESI [231].

It is striking that we measure mostly desolvated amino acid and small peptide ions in many experiments (Fig. 3.2.4, Fig. 3.2.5, Fig. 3.2.6). This property simplifies the interpretation of mass spectra without requiring a drying gas. Were the ions emitted in an already desolvated state, or

clustered with one or more water molecules which were shed on the way to the detector? Given that just 2% of emitted ions collide with a gas molecule even once (see Fig. 3.3.1a), post-emission collisions can be dismissed as a primary desolvation mechanism. Furthermore, the tip size appears to influence the solvation state, as only sub-100 nm nanopores produced bare ions (Fig. 3.2.2). These observations suggest that it is the local environment at the source that controls the solvation state rather than processes occurring in flight. The conditions that give rise to bare ion emission are not entirely clear. The electric fields that develop near the smallest tips are strong enough to reorient and weaken hydration bonds, which may play a role [232, 233]. Joule heating may also play a role by raising the local temperature and further weakening hydration bonds [234, 235]. In addition to single amino acid ions, we observed desolvated amino acid dimer ions in some spectra (e.g., Fig. 3.2.4, His). This is likely due to the aggregation of amino acids in solution, which is known to occur at concentrations of 1 mM and above [236]. We also observed glutathione and its post-translationally modified variants as bare, singly charged ions. Subsequent work should investigate how the charge and hydration states depend on the peptide length, composition, and other properties.

3.4 Conclusions

This chapter makes three significant contributions: First, our measurements and analyses of the nanopore source provide strong evidence for the direct ion evaporation mechanism. Second, the nanopore source achieves near unit ion transmission efficiency. This holds the promise of significant improvements in the sensitivity of MS measurements, which are universally desirable and could be achieved by combining a nanopore source with a sensitive mass filter (e.g., a trap-based mass filter). Third, the nanopore source is compatible with biologically relevant aqueous samples.

The findings reported here present multiple opportunities for further research. We envision sequencing a single protein molecule by fragmenting it and delivering its constituent amino acids into a mass spectrometer in sequential order [180, 237, 58]. The nanopore ion source provides the crucial means of transmitting virtually every amino acid to the detector, and because they avoid collisions in flight, the timing of detection events can be used to accurately reconstruct the order in which they left the source. However, significant challenges persist. A means of reliably fragmenting polypeptides into free monomers is required, as is a means of preserving the order of monomers prior to emission, perhaps using force gradients [237] or a narrow nanotube emitter [238] to prevent amino acids from diffusing past one another. These findings could also impact various fields outside of single-molecule sequencing or proteomics. Electrospray propulsion, a method for generating thrust

through ion emission, is currently constrained by a limited selection of non-volatile ionic liquids that can be used as propellants [151, 239], but the nanopore ion source highlights the possibility of using volatile liquids. Atom probe tomography, a technique for generating compositional maps of materials using field-emitted ions, is entering a new frontier with studies of liquid samples [240, 241, 242], which may be simplified by using a nanopore ion source. Finally, the nanopore source may shed new experimental light on the ionization of water, its orientation, or its evaporation at highly electrified surfaces [243, 244].

4

Thermal properties of the nanopore ion source

“The universe began as an enormous breath being held. Who knows why, but whatever the reason, I’m glad it did, because I owe my existence to that fact. All my desires and ruminations are no more and no less than eddy currents generated by the gradual exhalation of our universe. And until this great exhalation is finished, my thoughts live on.”

– Ted Chiang

4.1 Introduction

Ion sources are crucial components in various analytical and materials processing applications, including mass spectrometry and focused ion beam (FIB) systems. These devices generate beams of charged particles by extracting ions from a liquid or solid material and transferring them into vacuum [90, 245]. Historically, efforts to directly extract ions from volatile solvents like water into a vacuum have been hampered by freezing at the emitter tip [160, 154, 161]. This phenomenon is attributed to evaporative cooling, which becomes significant in the absence of a surrounding gas to replenish heat. Conventional ESI sources generate gas-phase ions for MS from volatile solvents, but operate in a background gas which is often heated to aid in the desolvation of charged droplets; this gas also prevents freezing. However, operation in a high-pressure environment has its own drawbacks, such as significant reductions in ion transmission efficiency, which ultimately limits the sensitivity of mass spectrometry analyses [54, 93, 246].

In chapter 3, we presented a new type of ion source comprising a capillary with a pulled conical tip that ends in a ≈ 30 nm diameter orifice [246]. These nanopore ion sources generate beams of ions from a nanoscale water/vacuum interface. Strong evidence supports the ion evaporation mechanism being responsible for the emitted ion current [91, 114]. A surprising aspect of this work is that the aqueous solution at the tip appears to remain a liquid even when exposed to the high vacuum environment ($P < 10^{-6}$ torr) inside a mass spectrometer. We hypothesize that the ability of the nanopore ion source to maintain a stable liquid water/vacuum interface without freezing is due to the ion source's small tip size and conical geometry, which limits the extent of evaporative cooling and allows for sufficient thermal conduction to maintain the liquid state at the tip.

With a high enough current and resistance, it is conceivable that enough heat could be generated within an ion source through Joule heating to boil the liquid, altering the charge emission mechanism and possibly causing damage to the emitter. This effect was first proposed by Joseph Crowley in two papers from 1977, which suggested that the emission of charged droplets rather than small ions may be due to explosive boiling at the tip caused by Joule heating [247, 248]. This idea was generally dismissed at the time in favor of the hydrodynamic cone-jet model [154, 249], but the possibility of emitter "self-heating" has begun receiving renewed interest in recent years [250, 251, 252]. We expect Joule heating to have a stronger effect in small emitters like the nanopore ion source due to their larger electrical resistance.

This chapter explores the thermal effects of nanoscale ion sources operating in high vacuum. We begin by analyzing how evaporative cooling affects the tip of the emitter, showing that the cooling effect is sufficient to freeze water for emitters with micrometer-scale or larger orifices. As the emitter orifice size decreases to nanoscale dimensions, the reduced cooling power and enhanced thermal conduction prevent the tip from freezing. We then consider the thermal effects of ion emission, showing that for sufficiently small emitters and high emission currents, boiling at the emitter tip may be possible. Additionally, we will introduce the concept of ion evaporative cooling, in which each evaporating ion carries away some thermal energy and cools the tip. This effect, which has not been thoroughly explored in previous studies, can generate a net cooling effect in certain current regimes, counteracting the effect of Joule heating.

4.2 Evaporative cooling and freezing

Past attempts to emit ions from volatile solutions directly into a vacuum have been impeded by freezing of the emitter tip due to evaporative cooling [160, 154]. However, we recently demonstrated

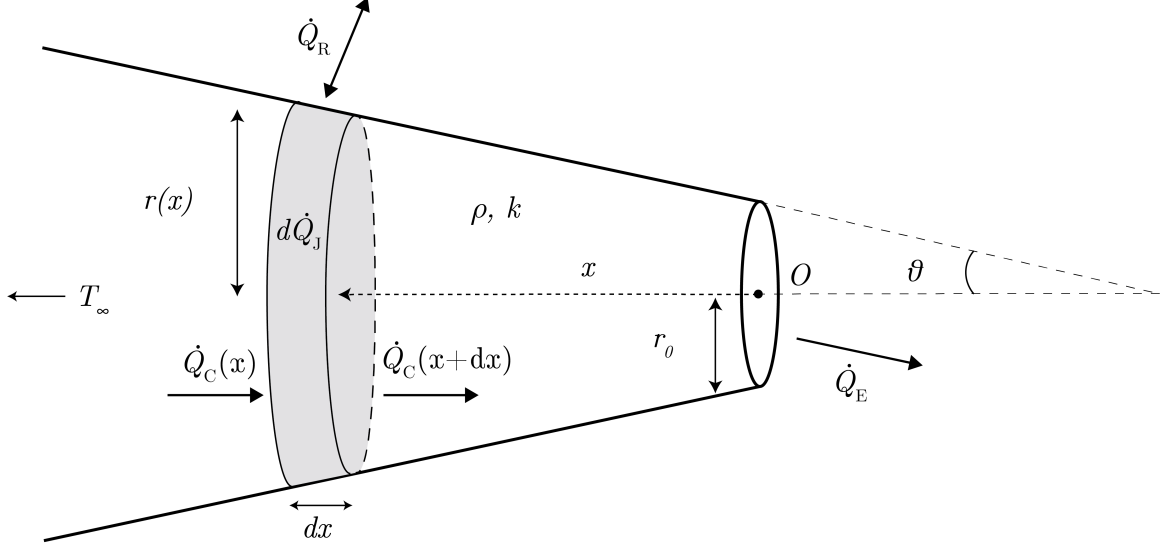


Figure 4.2.1: **Thermal model diagram.** Truncated cone model of the emitter tip. The x-direction points to the left, with the origin at the point labelled O. The cone has an apex-half angle of θ and a tip radius of r_0 . The fluid within the cone has a resistivity of ρ and thermal conductivity k .

that emission of organic ions from aqueous solutions into vacuum can be maintained with stable emission currents over several hours from sub-100 nm glass emitters [246]. We infer that these nanopore ion sources are able to sustain stable liquid-vacuum interfaces without freezing, and attribute the prevention of freezing in these emitters to their size and conical geometry. The nanopore at the emitter tip restricts the area over which evaporation occurs, limiting cooling power, while the conical geometry of the emitter allows sufficient thermal conduction to keep the liquid above its freezing point.

Here we develop a simple thermal model of a conical ion emitter and show that the problem of volatile solutions freezing can be avoided altogether using sufficiently small emitter tips. Figure 4.2.1 shows a diagram of the truncated cone model we use with all of the relevant heat fluxes and dimensions noted. To model the solvent freezing process, we consider just conduction and evaporative cooling. In the steady-state, conservation of energy demands that the total heat flux, $\dot{Q}$, into a differential slice must equal the total heat flux out of the slice, $\dot{Q}_C(x) = \dot{Q}_C(x + dx)$. The heat flux is related to the local temperature gradient by Fourier's law, $\dot{Q} = -kA(x)\frac{dT}{dx}$, where k is the thermal conductivity in $\text{Wm}^{-1}\text{K}^{-1}$, and $A(x) = \pi(r_0 + x \tan \theta)^2$ is the cross-sectional area of the cone at x . Combining Fourier's law with the conservation of energy yields the governing ODE for 1-dimensional heat conduction through a truncated cone,

$$\frac{d^2T}{dx^2} + \frac{2 \tan \theta}{r_0 + x \tan \theta} \frac{dT}{dx} = 0, \quad (4.2.1)$$

which has solutions of the form

$$T(x) = \frac{C_1}{r_0 + x \tan \theta} + C_2. \quad (4.2.2)$$

C_1 and C_2 are integration constants that depend on the chosen boundary conditions.

Evaporation only occurs at the liquid meniscus at the tip of the ion source. So we incorporate evaporative cooling in the thermal model as a heat flux boundary condition at the tip,

$$\left. \frac{dT}{dx} \right|_{x=0} = \frac{\dot{Q}_E}{k\pi r_0^2}. \quad (4.2.3)$$

Using $T(\infty) = T_\infty$ as the second boundary condition, we find

$$T(x) = T_\infty - \frac{\dot{Q}_E}{k\pi \tan \theta (r_0 + x \tan \theta)}. \quad (4.2.4)$$

The change in temperature at the tip of the ion source is then given by

$$\Delta T_{\text{tip}} = T(0) - T_\infty \quad (4.2.5)$$

$$= -\frac{\dot{Q}_E}{k\pi r_0 \tan \theta}. \quad (4.2.6)$$

There may be several valid approaches for determining the value of $\dot{Q}_E$, but it should scale with the area of the evaporating surface ($\dot{Q}_E \propto r_0^2$) in any reasonable model. This fact and Eq. (4.2.6) alone are sufficient to show that the temperature change at the tip depends linearly on r_0 . This explains why smaller emitters like the nanopore ion source are more resilient to freezing when exposed to high vacuum.

In order to make quantitative predictions of the temperature change at the ion source tip, we assume that $\dot{Q}_E \approx \alpha \pi r_0^2 \dot{n} H_V$, where α is a geometric factor that depends on the meniscus shape, $\dot{n}$ is the flux of evaporating solvent molecules (units of $\text{m}^{-2}\text{s}^{-1}$), and H_V is the latent heat of vaporization of the solvent. This expression is based on the simple assumptions that evaporation takes place over the entire area of the meniscus, $\alpha \pi r_0^2$, and that each evaporating molecule carries away some thermal energy which is given by H_V . We use the Hertz-Knudsen equation to approximate $\dot{n}$ [253, 217], which predicts an evaporative flux of about 10^8 water molecules per nm^2 per second at $T = 300$ K, in agreement with experimentally measured evaporation rates of water from nanopores by Li et al. [218]. Combining the Hertz-Knudsen equation with the expression for $\dot{Q}_E$ above, we find,

$$\dot{Q}_E = \frac{\alpha \pi r_0^2 H_V P_0(T)}{\sqrt{2\pi m k_B T}}, \quad (4.2.7)$$

where $P_0(T)$ is the solvent vapor pressure as a function of temperature [254], m is the mass of a solvent molecule, and k_B is the Boltzmann constant.

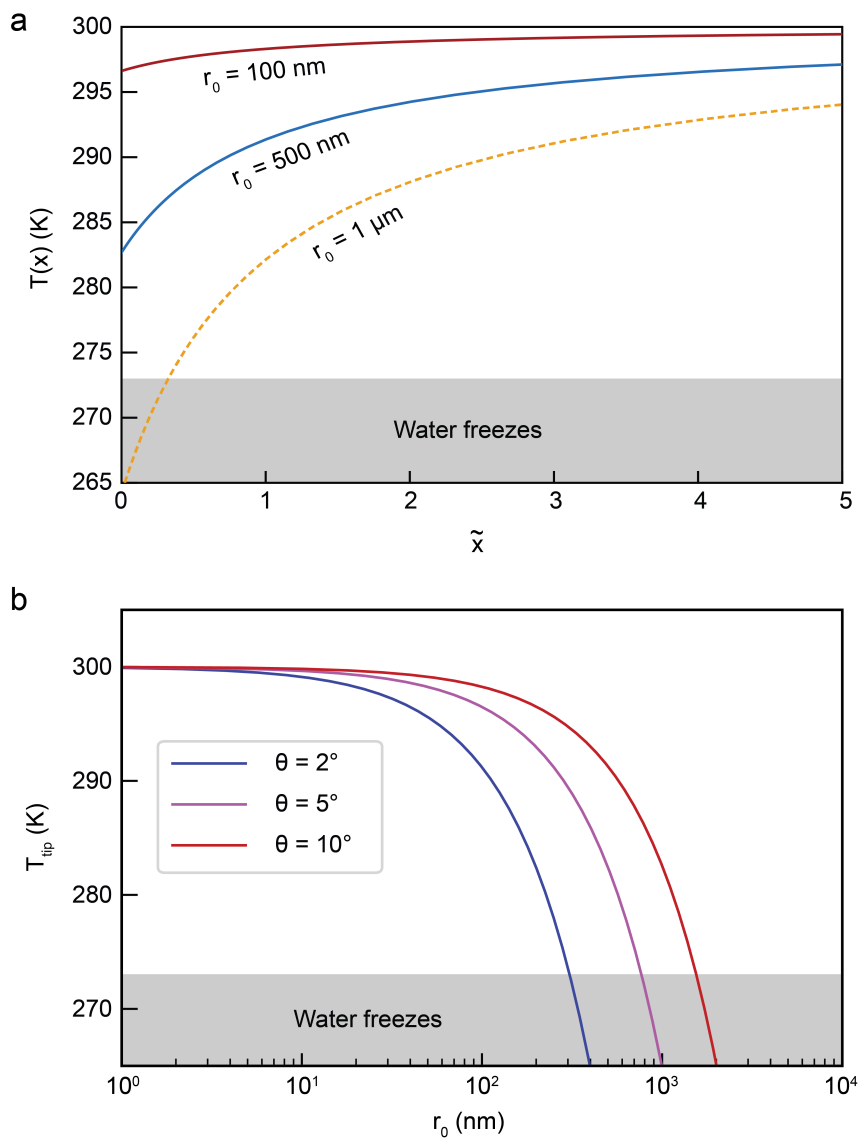


Figure 4.2.2: **Evaporative cooling.** **a** Temperature profiles for a water filled ion source under the influence of evaporative cooling plotted as a function of $\tilde{x} = x \tan \theta / r_0$ for $r_0 = 100$ nm, 500 nm, & 1 μm and $\theta = 5^\circ$. Other parameters used were $k = 0.75 \text{ W m}^{-1}\text{K}^{-1}$ and $\alpha = 1.5$. The curve for $r_0 = 1 \mu\text{m}$ is dashed as the model becomes unphysical when $T(x)$ falls below the freezing point. **b** Temperature at the tip of a water filled ion source as a function of the tip radius, r_0 .

Figure 4.2.2a plots the temperature profile for water filled ion emitters as a function of $\tilde{x} = x \tan \theta / r_0$. Examples with $r_0 = 100$ nm, 500 nm, and 1 μm and $\theta = 5^\circ$ are shown. All of the temperature profiles scale with $1/x$. The drop in temperature at each emitter tip is proportional to r_0 , as expected given the linear dependence of ΔT_{tip} on r_0 discussed above. Figure 4.2.2b plots the

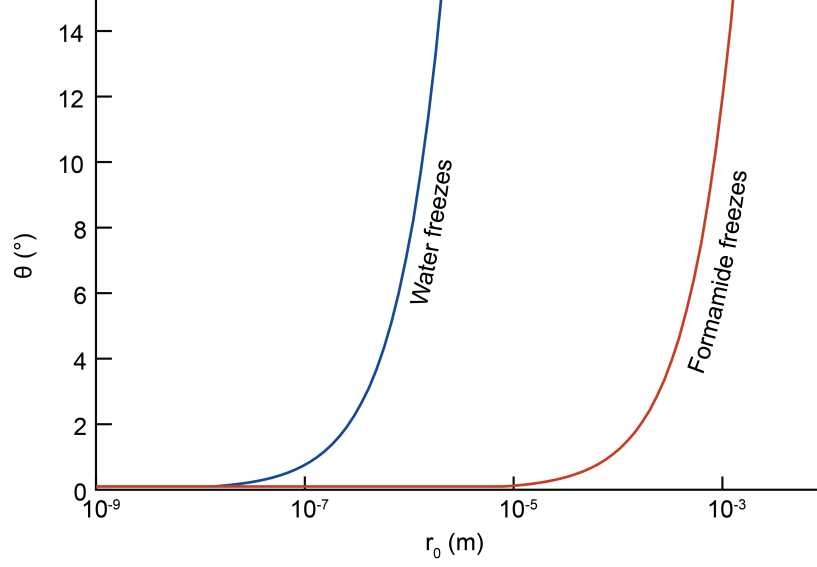


Figure 4.2.3: **Water and formamide freezing conditions.** Plot of the threshold r_0 and θ values which can hold liquid samples in contact with vacuum without freezing. Tip geometries that fall to the right of the boundary curve will be predicted to freeze.

temperature at the tip of a water filled ion source, $T(x=0)$, as a function of r_0 for $\theta = 2^\circ$, 5° , and 10° . The plot shows how evaporative cooling causes larger drops in temperatures for tips with larger r_0 and smaller θ . For values of θ near 5° , water filled ion sources are predicted to freeze if they have a tip radius larger than about $1 \mu\text{m}$. For tips with radii smaller than about 500 nm , conduction through the liquid in is sufficient to prevent evaporative cooling from causing freezing. This explains why the nanopore ion source with $r_0 < 50 \text{ nm}$ is able to maintain a stable water-vacuum interface at its tip without freezing.

Equation (4.2.6) in combination with Eq. (4.2.7) reveals the importance of volatility in determining the conditions that lead to freezing. The change in temperature at the tip depends linearly on the vapor pressure of the solvent. Direct ion evaporation from a stable liquid meniscus into vacuum has previously been reported for non-volatile solvents like formamide [114, 155], whose vapor pressure is $\approx 10^3$ times lower than that of water at their respective freezing points. Figure 4.2.3 delineates the conditions at which an emitter filled with water or formamide is expected to freeze. This clearly illustrates why direct ion evaporation from solutions like formamide has been previously observed but water hasn't; formamide will remain in a liquid state even with $r_0 > 100 \mu\text{m}$ emitters, whereas water will only remain liquid in emitters where $r_0 < 1 \mu\text{m}$.

This analysis leaves out the effect of thermal radiation, which tends to equilibrate the temperature of the ion source tip with the walls of the vacuum chamber. For an ion source with $r_0 = 1 \mu\text{m}$ and

$\theta = 5^\circ$, the total heat transferred by radiation is about 2 orders of magnitude smaller than the heat transferred by evaporative cooling. Including this small additional heat transfer would slightly increase the predicted temperature at the tip, but the effect is small enough that it can be safely left out of this analysis.

4.3 Thermal effects of ion emission

The previous section left out any consideration of the thermal effects of charge emission. When the ion source is in operation and emitting an ion current, there are two additional sources of heat transfer to consider. The first is Joule heating, which results from the dissipation of heat as current flows through the solution with finite conductivity. The second is cooling from ion evaporation, where the thermal energy needed to evaporate an ion is lost, a mechanism akin to evaporative cooling. We will first analyze the effect of Joule heating alone, and later incorporate the ion evaporative cooling effect.

4.3.1 Joule Heating

We incorporate Joule heating into the thermal model by adding a forcing term to Eq. (4.2.1), explicitly treating the heat generation as being distributed throughout the entire cone. The balance of heat entering and exiting a differential slice in the steady state is now given by,

$$\dot{Q}_C(x) + d\dot{Q}_J(x) = \dot{Q}_C(x + dx). \quad (4.3.1)$$

The heat generated in a differential slice is given by $d\dot{Q}_J = \frac{I^2 \rho}{\pi r(x)^2} dx$, where I is the current and ρ is the resistivity of the liquid. Plugging this and Fourier's law into Eq. 4.3.1 yields the ODE for heat conduction through a truncated cone with Joule heating,

$$\frac{d^2 T}{dx^2} + \frac{2 \tan \theta}{r_0 + x \tan \theta} \frac{dT}{dx} = - \frac{I^2 \rho}{k \pi^2 r(x)^4}. \quad (4.3.2)$$

We solve Eq. (4.3.2) with the same evaporative boundary conditions we used above to find the temperature profile,

$$T(x) = T_\infty + \frac{I^2 \rho}{k \pi^2 \tan^2 \theta} \left(\frac{1}{r_0 r(x)} - \frac{1}{2r(x)^2} \right) - \frac{\dot{Q}_E}{k \pi \tan \theta r(x)} \quad (4.3.3)$$

as well as the temperature change at the tip,

$$\Delta T_{\text{tip}} = \frac{I^2 \rho}{2k\pi^2 \tan^2 \theta r_0^2} - \frac{\dot{Q}_{\text{E}}}{k\pi \tan \theta r_0}. \quad (4.3.4)$$

Equation 4.3.4 shows that the increase in temperature at the tip due to Joule scales with r_0^{-2} , in contrast to the evaporative cooling term which scales with r_0 as described in the previous section. Joule heating should therefore dominate when r_0 is small. Note that not all current emitted by an ion source necessarily contributes to Joule heating, only the ohmic currents. Any currents carried by advection, such as streaming currents near a charged surface [255] or the advected current in an electrospray cone-jet [113], do not contribute directly to Joule heating.

Figure 4.3.1a shows example temperature profiles predicted for ion sources of $r_0 = 5$ nm, 100 nm, and 1 μm , respectively, all with $\theta = 5^\circ$, and $I = 10$ nA. The other parameters ($k = 0.75$ W m $^{-1}$ K $^{-1}$, and $\rho = 1$ Ωm) were chosen to approximate 100 mM NaI in water. The figure shows the increased effect of Joule heating on tips with smaller r_0 . Joule heating is the dominant effect for an ion source with $r_0 = 5$ nm, whose tip temperature increases by about 35 K. Joule heating and evaporative cooling are nearly balanced for the the ion source with $r_0 = 100$ nm. Evaporative cooling dominates for the ion source with $r_0 = 1$ μm , whose tip temperature is predicted to fall below the freezing point.

Figure 4.3.1b shows the temperature at the tip of an ion source under the influence of Joule heating and evaporative cooling as a function of r_0 and with a range of current values from 1 nA to 100 nA. The other parameters were again $\theta = 5^\circ$, $\rho = 1$ Ωm , and $k = 0.75$ W m $^{-1}$ K $^{-1}$. This plot demonstrates the two different scales at which each effect is significant. The inclusion of evaporative cooling has a negligible effect on the conditions at which a tip may reach the boiling point, while Joule heating has a negligible effect on the freezing conditions. For sub-micron tips like our nanopore ion sources, Joule heating may be significant if $I \gtrsim 10$ nA, while evaporative cooling can be largely ignored. For ion sources with $r_0 \gtrsim 400$ nm, Joule heating may be ignored while evaporative cooling be significant. The effect of thermal radiation was again left out of this analysis as its effect is more than 2 orders of magnitude smaller than the effect of either joule heating or evaporative cooling in the relevant parameter range.

4.3.2 Cooling from the evaporation of ions

Ions are emitted by the nanopore ion source through ion evaporation, a thermally activated process in which ions overcome their solvation energy and enter the gas phase with the help of a strong

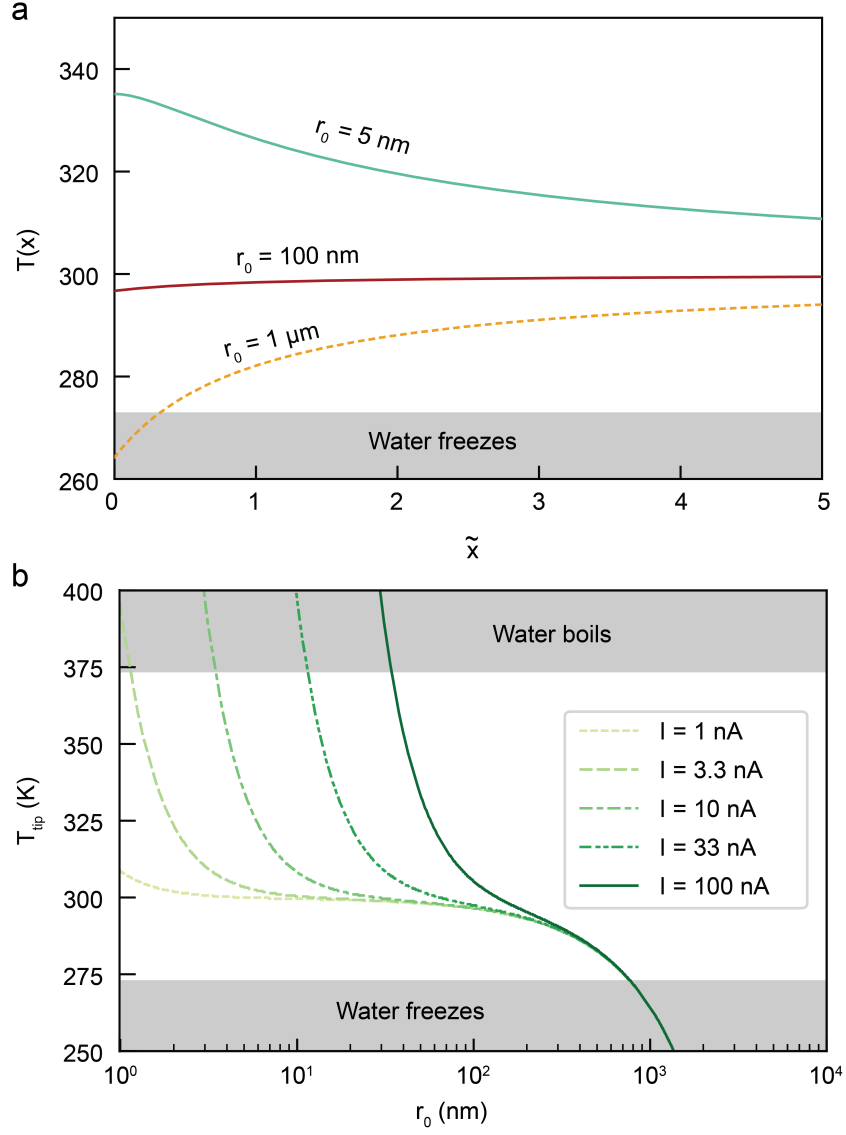


Figure 4.3.1: **Joule heating.** **a** Temperature profiles as a function of $\tilde{x}$ for ion sources under the influence of Joule heating and evaporative cooling. Profiles for three different r_0 values (5 nm, 100 nm, and 1 μm) and $\theta = 5^\circ$ are shown. Other parameters used were $\rho = 1 \Omega\text{m}$, $k = 0.75 \text{ W m}^{-1} \text{ K}^{-1}$, and $I = 10 \text{ nA}$. The curve for $r_0 = 1 \mu\text{m}$ is dashed as the model becomes unphysical when $T(x)$ falls below the freezing point. **b** Predicted temperature at the tip of an ion source as a function of r_0 for different I values. Solid curves show only the effect of Joule heating and dotted curves show the combined effect of Joule heating and evaporative cooling include it.

electric field [91]. Much like evaporating solvent molecules, evaporating ions require a thermal kick to overcome an energy barrier, and therefore cool the liquid as they evaporate. This ion evaporative cooling effect has not been previously discussed in the ion evaporation literature to our knowledge. We model the cooling power due to ion evaporation by $\dot{Q}_{\text{E,ion}} = (I/q_e)G^\ddagger$, where q_e is the electron charge and $G^\ddagger = G_0 - \Delta G(E)$ is the height of the energy barrier for ion evaporation. This effect can be handled identically to evaporative cooling of solvent, as a Neumann boundary condition applied at the tip when solving Eq. (4.3.2). However, unlike the cooling power from solvent evaporation, which is proportional to r_0 , the ion evaporative cooling power is independent of r_0 .

A current of evaporating ions therefore generates two competing thermal effects in the source: Joule heating and ion evaporative cooling. Joule heating is distributed throughout the source, while the cooling from ion evaporation occurs only at the tip. Combining these effects, we find that the net heating power is given by

$$\dot{Q}_{\text{net}} = \frac{I^2 \rho}{\pi r_0 \tan \theta} - \frac{IG^\ddagger}{q_e}, \quad (4.3.5)$$

where the first term is the integral of $d\dot{Q}_J$ from $x = 0$ to ∞ . Joule heating acts to increase the temperature at the tip proportionally to I^2 , while ion evaporative cooling counteracts this by removing heat proportionally to I . The temperature change at the tip, ΔT_{tip} , is similarly determined by the competition between these two effects:

$$\Delta T_{\text{tip}} = \frac{I^2 \rho}{2k\pi^2 \tan^2 \theta r_0^2} - \frac{IG^\ddagger}{q_e k \pi \tan \theta r_0}. \quad (4.3.6)$$

If, in our analysis, we had assumed that all of the joule heating power was deposited at the tip rather than being distributed throughout, Eq. (4.3.6) would change only by losing the factor of 1/2 in the first term.

To better understand the interplay between these effects, we introduce characteristic scales for the current, temperature change, and heating power, respectively,

$$\alpha = \frac{G^\ddagger \pi r_0 \tan \theta}{q_e \rho}, \quad \beta = \frac{G^{\ddagger 2}}{q_e^2 k \rho}, \quad \gamma = \frac{G^{\ddagger 2} \pi r_0 \tan \theta}{q_e^2 \rho}. \quad (4.3.7)$$

Using these scales, we can non-dimensionalize the net heat flux and temperature change equations, resulting in

$$\tilde{\dot{Q}}_{\text{net}} = \tilde{I}^2 - \tilde{I} \quad (4.3.8)$$

$$\Delta\tilde{T}_{\text{tip}} = \frac{\tilde{I}^2}{2} - \tilde{I}, \quad (4.3.9)$$

where $\tilde{I} = I/\alpha$, $\Delta\tilde{T}_{\text{tip}} = \Delta T_{\text{tip}}/\beta$, and $\tilde{Q}_{\text{net}} = \dot{Q}_{\text{net}}/\gamma$ are the dimensionless current, temperature change at the tip, and heat flux, respectively. The zeros and minima of Eqs. (4.3.8) and (4.3.9) reveal universal critical points that delineate the different regimes of heating and cooling caused by ion evaporation.

Figure 4.3.2a plots the non-dimensionalized heating power (red curve) and the temperature change at the tip (green curve) as a function of the current and illustrates the critical points on both curves. Increasing the current from zero initially has a net cooling effect. At $\tilde{I} = \frac{1}{2}$ (i.e., $I = \frac{\alpha}{2}$), $\tilde{Q}_{\text{net}}$ reaches its minimum value, $-1/4$, indicating the point of maximum total cooling power. At this current the tip temperature changes by $\Delta\tilde{T}_{\text{tip}} = -3/8$. However, $\Delta\tilde{T}_{\text{tip}}$ only reaches its minimum value of $-1/2$ when the current doubles to $\tilde{I} = 1$. At this current, the Joule heating power perfectly balances the ion evaporative cooling power and $\tilde{Q}_{\text{net}} = 0$. Beyond $\tilde{I} = 1$, $\Delta\tilde{T}_{\text{tip}}$ begins increasing, eventually reaching zero when $\tilde{I} = 2$ and $\tilde{Q}_{\text{net}} = 2$.

Figure 4.3.2b plots the non-dimensionalized temperature profile of an ion source for the three critical current values given above. The temperature profiles shed light on the counter-intuitive results that the maximum cooling power does not result in the lowest tip temperature, and that $\Delta\tilde{T}_{\text{tip}} > 0$ only if $\tilde{Q}_{\text{net}} > 2$. Focusing only on the temperature at the tip ignores the full effect of Joule heating, which is distributed throughout the tip. We see that raising the the current from 0 to α causes increased cooling of the tip. The profile for $\tilde{I} = 1$ indeed shows the lowest tip temperature $\Delta\tilde{T}_{\text{tip}}$, but far from the tip there is a stronger cooling effect at $\tilde{I} = 1/2$, which is expected in light of the fact that this is the current that generates the most overall cooling power. At $\tilde{I} = 2$, the maximum value of $\Delta\tilde{T}(x)$ is $1/2$ and occurs at $\tilde{x} = 1$, while $\Delta\tilde{T}(x) = 0$ as expected. Further increases of the current raise the temperature everywhere along the tip, with the location of the maximum moving closer to $x = 0$.

A quantitative estimate of the ion evaporative heat flux requires knowledge of $G^\ddagger$, which is difficult to measure directly. We estimate $G^\ddagger$ to be in the range of approximately 0.4 - 1.2eV over a wide range of emission currents (1pA - 1 μ A) and tip radii (10nm - 1 μ m) using the Iribarne and Thomson model of ion evaporation [91]. We use the mean value $G^\ddagger = 0.8$ eV in our calculations. Since the energy lost is the height of the barrier after being reduced by the electric field, $G^\ddagger$ depends on the field strength but is independent of the particular ion's solvation energy.

For the nanopore ion source, the effect of ion evaporative cooling is small. Using the values

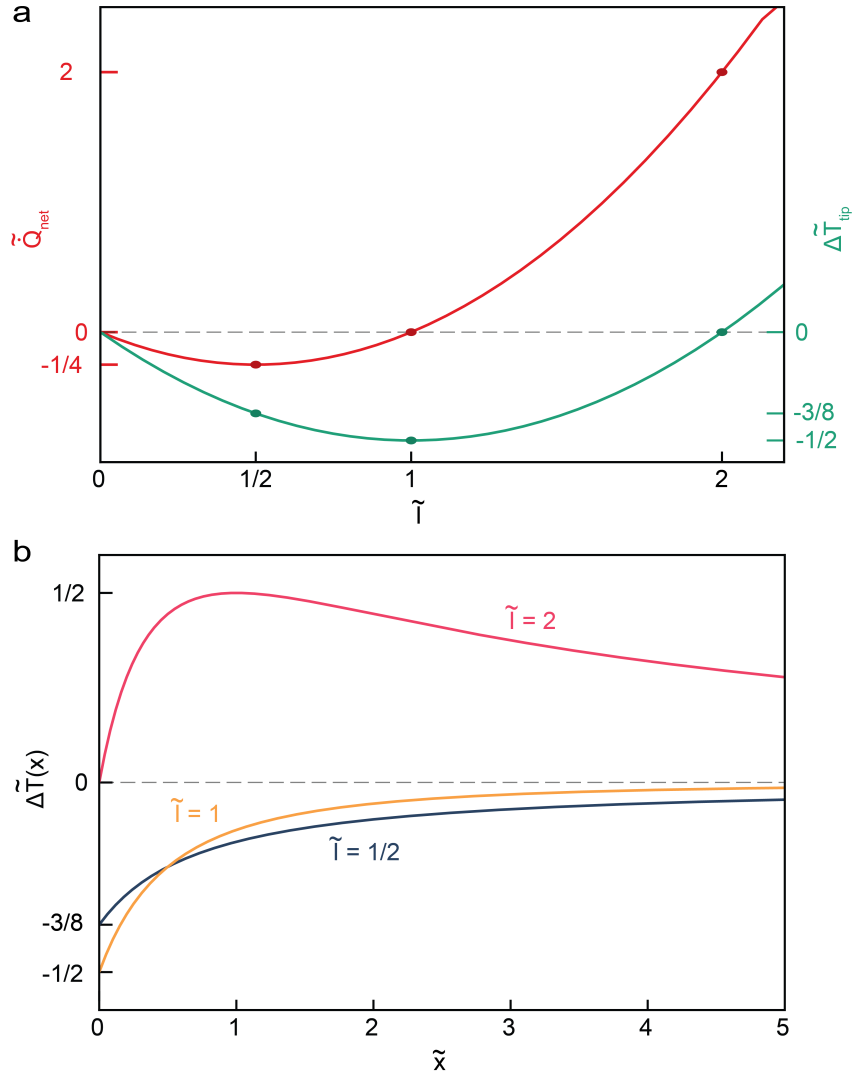


Figure 4.3.2: **Ion evaporative cooling.** **a** Plot of the net heating power (red curve) and the change in temperature at the tip of an ion source (green curve) generated by an ion evaporation current $\tilde{I}$. Darker dots label the points on each curve which correspond to the critical currents $\tilde{I} = 1/2, 1$, and 2 . **b** Profiles for the change in temperature along an ion source plotted for the three critical currents listed above.

$r_0 = 15$ nm, $\theta = 5^\circ$, $k = 0.75$ W/mK, and $\rho = 1$ Ω m, we find a characteristic current of $\alpha = 2.75$ nA at which the tip will reach its minimum temperature, just 0.17 K below T_∞ . Using values for an ionic liquid ion source, which generates an ion current via ion evaporation and are typically used for satellite propulsion [159, 151], we find a slightly larger effect, with a maximum cooling effect of around 1 K at a characteristic current of around 15 nA.

However, in the case of liquid metal ion sources (LMIS) [230], the very low resistivity may make the ion evaporative cooling effect significant. For example, liquid gallium has a resistivity of just 2.6×10^{-7} Ω m and a thermal conductivity of about 30 W/mK. TEM studies have shown that LMIS form a Taylor cone with an apex radius and angle of about 3 nm and 49° respectively [256, 257, 87]. Using these values we find a characteristic current of $\alpha = 20$ mA. This is far beyond the standard operating current from a liquid metal ion source, which is typically in the range of microamps [257]. Therefore, ion emission from liquid metal ion sources should cause cooling rather than heating in virtually all cases. At these currents, our model predicts a cooling effect at the tip ranging from 1.5 K to 150 K.

To our knowledge, the cooling effect of ion evaporation has been previously discussed in the LMIS literature only once in passing [258]. However, measurements of the energy distributions of ions emitted from liquid metal ion sources have revealed an apparent cooling effect, the presence of a secondary energy peak at lower energies than expected [259, 260]. This observation was attributed to inertial effects due to oscillations of the emitter apex, and other possible explanations were not considered [260]. While speculative, our model suggests the alternative explanation that this apparent cooling effect may in fact be a result of ion evaporative cooling.

4.4 Conclusions

This study modelled the thermal properties of nanopore ion sources in vacuum, emphasizing the interaction between evaporative cooling, Joule heating, and a previously overlooked effect we call ion evaporative cooling. The temperature change at the tip due to evaporative cooling is shown to scale linearly with the tip radius, explaining the increased resilience of smaller emitters like the nanopore ion source against freezing. This linear scaling gives nanopore ion sources their unique ability to maintain a stable liquid water/vacuum interface. Joule heating, whose effect on the tip temperature scales with the square of the tip radius, can cause significant heating in nanoscale emitters when emission currents are high. We introduced the concept of ion evaporative cooling, where each emitted ion carries away thermal energy, creating a cooling effect that scales linearly with current. There

are critical currents which depend on the fluid characteristics (k and ρ) and the tip geometry (r_0 and θ) that define distinct regimes where the net effect of ion emission is heating or cooling. While this ion evaporation effect should be small in the nanopore ion source, it may be significant in liquid metal ion sources, and possibly account for previously reported observations of an apparent cooling effect in the measured energy distributions of emitted ions. These findings provide a framework for predicting the thermal behavior of conical ion emitters in vacuum and guides the design and operation of nanopore ion sources.

Emission properties of the nanopore ion source

“Never get so attached to a poem you forget truth that lacks lyricism.”

– Joanna Newsom

5.1 Introduction

In this chapter, our goal is to understand how the emission the emission current depends on the applied voltage, local electric field, fluid flow rate, and the tip size. We are also interested in the structure of the meniscus itself, whether it has an open or closed geometry during emission [109], and what the local curvature is at the fields required for ion emission.

A few groups, led primarily by F.J. Higuera and P. Lozano, have made significant contributions to the theoretical understanding of ion emission from small menisci, mostly focusing on developing computational models of ionic liquid ion sources for spacecraft propulsion. Higuera provided a foundational model of purely ionic emission from the ionic liquid ion sources, and found a predicted family of equilibrium meniscus profiles in which a constant current of evaporating ions can be emitted from a stable meniscus over a range of applied electric fields [175]. This work indicated that the meniscus may only be stable within a limited range of electric fields, and that upstream flow conditions have a significant impact on the meniscus profile and emission behavior [175]. Coffman et al. extended this understanding by identifying two families of stable meniscus profiles, a rounded profile at low field which emits a negligible ion current, and a stable “cusp”-like profile at high fields

which emits a significant ion current [159, 261]. Importantly, this work noted that both families of stable meniscus profiles can deviate significantly from the ideal Taylor cone. Additionally, Coffman et al. identify maximum flow rates beyond which the closed meniscus structure characteristic of purely ionic emission becomes unstable, noting the importance of high hydraulic impedance of the emitter and upstream fluid path [261]. Most recently, Gallud and Lozano expanded on Coffman's model by incorporating thermal effects, suggesting that ohmic heating may expand the region of stability for purely ionic emission by enhancing conductivity near the meniscus (the authors do not consider the ion evaporative cooling effect discussed in the previous chapter) [234]. These studies all focused on menisci with diameters in the range of 0.5 - 5 μm , where the curvature of the "low-field" family of rounded meniscus profiles is insufficient to sustain the fields required for ion emission. In the case of the nanopore ion source, however, this may not be the case.

Here, we present a simple model of ion emission from the meniscus at the tip of the nanopore ion source based on the Iribarne and Thomson model of ion evaporation [91], and derive an expression for the emitted current as a function of the applied voltage. We measure the IV curves for several nanopore ion sources with water and formamide solutions using the experimental setup described in § 2.4, and show that the emission behavior is well described by our model. Measurements of emission from the same ion source both in atmosphere and in vacuum suggest that the emission mechanism is identical in either environment. Additionally, we report measurements showing that the emission current from a nanopore ion source is independent of applied pressure to its feed line, providing further evidence of operation in the purely ionic emission regime. Finally, we note the variability in the observed emission characteristics from nanopore ion sources, and discuss how it may be related to the wetting conditions at the tip.

5.2 The IV characteristic of nanopore ion sources

5.2.1 Theoretical model of the IV characteristic

Our goal in this section is to derive an expression for the emission current as a function of voltage, $I(V)$, for the nanopore ion source operating in the purely ionic emission regime. The total emission current is given by the integral of the ion evaporation current density (Eq. (1.3.20)) over the area of the meniscus,

$$I = \int j \cdot dA. \quad (5.2.1)$$

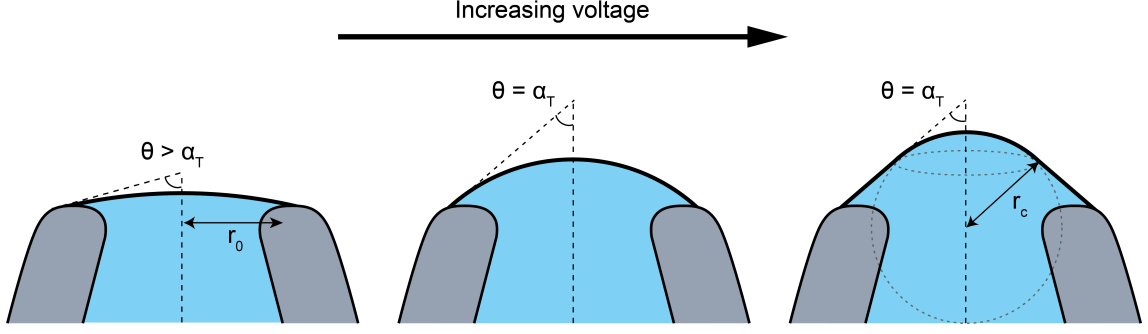


Figure 5.2.1: **Meniscus shape.** Diagram showing the assumed evolution of the meniscus shape in response to increasing voltage.

To evaluate this integral, we assume that the meniscus evolves in response to an applied voltage as illustrated in Fig. 5.2.1. At low voltages, the meniscus forms a spherical cap with base radius r_0 and radius of curvature $r_c > r_0$, much like the low-field family of hydrostatic meniscus profiles identified by Coffman and Higuera [159, 175]. As the voltage increases and electrostatic stress on the meniscus increases, its radius of curvature decreases (it becomes sharper). This can also be characterized by the angle θ , which is complementary to the meniscus' contact angle. Once θ reaches the Taylor angle, $\alpha_T = 49.3^\circ$, further increases in voltage reduce r_c while maintaining $\theta = \alpha_T$, forming a spherical cap atop a Taylor cone. For the sake of simplicity, we will assume that the ion evaporation current density is uniform over the spherical cap, so that $I = j(V)A(V)$. These assumptions reduce the problem to finding separate expressions for $j(V)$ and $A(V)$.

Beginning with $j(V)$, inspection of Eq. (1.3.20) reveals that the voltage-dependence must appear via the electric field strength E and the surface charged density $\sigma = \epsilon_0 E$. There are several expressions in the literature relating the electric field strength on the surface of a sharp emitter to the applied voltage [88, 249, 262, 263, 264]. They all take the form

$$E = \frac{V}{f(r_0, d)}, \quad (5.2.2)$$

where $f(r_0, d)$ is a geometrical function of the emitter tip radius, r_0 , and the distance between the counterelectrode and the tip, d [249]. We will use the expression derived by Maulbetsch, using a semi-infinite line charge model [204],

$$E(V) = \frac{\sqrt{2}V}{r_0 \log\left(\frac{4d}{r_0}\right)}. \quad (5.2.3)$$

With Eq. (5.2.3) in hand, we can write down the expression for $j(V)$,

$$j(V) = \epsilon_0 E(V) \frac{k_B T}{h} \exp \left\{ - \left(\frac{G_0 - \sqrt{\frac{q^3 E(V)}{4\pi\epsilon_0}}}{k_B T} \right) \right\}. \quad (5.2.4)$$

We now turn to finding an expression for $A(V)$. The area of a spherical cap in terms of the radius of curvature, r_c and the angle θ is given by,

$$A = 2\pi r_c^2 (1 - \sin \theta). \quad (5.2.5)$$

To determine how r_c and θ depend on V , we will follow the example set by Rayleigh and Taylor and exploit the balance between electrostatic stress and surface tension [84, 87],

$$\frac{\epsilon_0 E^2}{2} = \frac{2\gamma}{r_c}. \quad (5.2.6)$$

Combining Eqs. 5.2.6 & (5.2.3) and solving for r_c yields

$$r_c(V) = \frac{2\gamma r_0^2 \ln \left(\frac{4d}{r_0} \right)^2}{\epsilon_0 V^2} \quad (5.2.7)$$

To find $\theta(V)$, note that when the entire meniscus is a spherical cap, i.e. before the Taylor angle is reached, $\cos \theta = r_0/r_c$. So using Eq. (5.2.7), we find that

$$\theta(V) = \begin{cases} \arccos \left(\frac{\epsilon_0 V^2}{2\gamma r_0 \ln \left(\frac{4d}{r_0} \right)^2} \right) & \text{for } V < V_T \\ \alpha_T & \text{for } V \geq V_T, \end{cases} \quad (5.2.8)$$

where V_T is the threshold voltage at which θ reaches α_T . Equipped with Eqs. (5.2.4), (5.2.5), (5.2.7), & (5.2.8), we can now write down the complete expression for $I(V)$,

$$I(V) = \epsilon_0 E(V) \frac{k_B T}{h} \exp \left\{ - \left(\frac{G_0 - \sqrt{\frac{q^3 E(V)}{4\pi\epsilon_0}}}{k_B T} \right) \right\} 2\pi r_c(V)^2 (1 - \sin(\theta(V))). \quad (5.2.9)$$

Equation (5.2.9) predicts a relationship between I and V that is markedly different from that of a cone-jet electrospray [265]. Figure 5.2.2 shows example IV curves for emitters with $r_0 = 20, 40$, & 60 nm, and $G_0 = 1.3$ & 1.5 eV. While conventional cone-jet models describe a discrete onset voltage where the emission current abruptly jumps from zero to a finite value [90, 109], our ion evaporation-

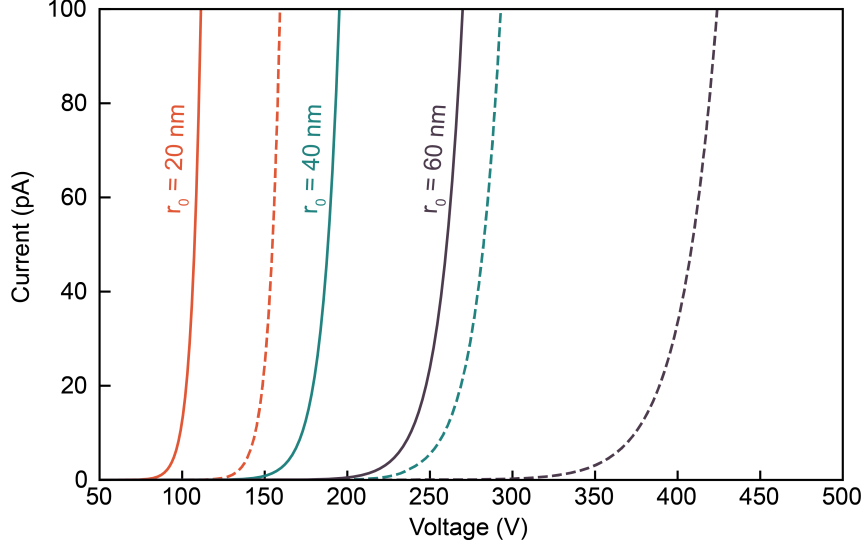


Figure 5.2.2: **Predicted IV characteristics.** Example IV curves predicted by Eq. (5.2.9) for three values of r_0 (20, 40, & 60 nm) and two values of G_0 (1.3 & 1.5 eV). Solid curves correspond to $G_0 = 1.3$ eV and dashed curves correspond to $G_0 = 1.5$ eV. Other parameters used were $\gamma = 0.072$ N/m, $d = 1$ mm, $T = 300$ K.

based model suggests a smooth, exponential increase in current with voltage. This continuous behavior reflects the gradual enhancement of ion evaporation as the electric field intensifies and the meniscus sharpens, rather than the sudden transition of a closed meniscus with low curvature to a cone-jet. Additionally, the emission current at a given voltage depends strongly on the solvation energy of the emitted ion, G_0 , with higher G_0 values requiring significantly higher extraction voltages to reach the same emission current.

The onset voltage, or the voltage at which emission currents begin to be generated, is a common metric used to evaluate the behavior of various electrospray ion sources [266]. Without a distinct onset voltage, one could reasonably define the “threshold voltage” for emission in a few different ways. One natural definition is the voltage at which the electric field reaches the critical field for ion evaporation, V^* , i.e. the voltage which satisfies $E(V^*) = E^*$, where E^* is given by Eq. (1.3.21). Another natural definition for a threshold voltage is the minimum voltage at which $\theta = \alpha_T$ (illustrated in the middle diagram of Fig. 5.2.1), which we will call V_{Taylor} . From Eq. (5.2.8) we can see that this is given by,

$$V_{\text{Taylor}} = \sqrt{\frac{2\gamma r_0 \cos \alpha_T}{\epsilon_0}} \ln \left(\frac{4d}{r_0} \right). \quad (5.2.10)$$

This is identical to the theoretical prediction for the onset voltage of a Taylor cone in electrospray [249]. Lastly, we may define a threshold voltage, V_I , at which the predicted emission current surpasses some threshold value, I_{thresh} .

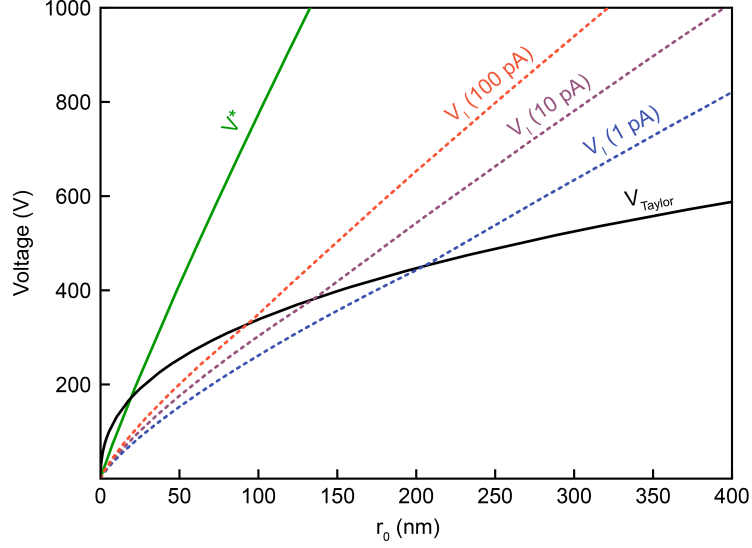


Figure 5.2.3: **Threshold voltages** Plots of the three threshold voltages as functions of r_0 , V^* (solid green curve, obtained from Eq. (5.2.3)), V_{Taylor} (solid black curve, from Eq. (5.2.10)), and V_I where $I_{\text{thresh}} = 1, 10, \& 100$ pA (dashed curves, from Eq. (5.2.9)).

Figure 5.2.3 plots these three distinct threshold voltages, V^* , V_{Taylor} , and V_I , as a function of r_0 for $G_0 = 1.25$ eV. V_I is plotted for three different values of I_{thresh} , 1, 10, & 100 pA. This figure illustrates two important predictions about nanoscale emitters. First, significant and easily measurable currents can be emitted at voltages well below V^* . Recall that V^* is the voltage at which the so-called critical field for ion evaporation is reached, i.e. the energy barrier for ion evaporation ($G_0 - \Delta G$) goes to 0. However this condition is far more restrictive than what is actually needed to generate a measurable emission current. For example, Eq. (5.2.9) predicts an emission current of 10 pA for an emitter with $r_0 = 20$ nm when $G_0 - \Delta G \approx 20k_B T$, i.e. when the energy barrier for ion evaporation is still approximately 0.5 eV, in agreement with recently reported simulations of ion evaporation from small droplets [223]. At $V = V^*$, where the height of energy barrier is 0, Eq. (5.2.9) predicts emission currents of nearly 100 μA , much higher than the picoampere currents we typically measure from nanopore ion sources.

Second, for emitters with $r_0 < 100$ nm, measurable currents are emitted at voltages well below V_{Taylor} . It is typically taken for granted that the Taylor cone approximately describes the basic structure of any charge-emitting electrified meniscus. For nanoscale emitters, however, Eq. (5.2.9) predicts that the formation of a Taylor cone may be unnecessary for emission. At radii below 100 nm, significant emission currents occur at voltages well below V_{Taylor} , suggesting that the classical Taylor cone model may not apply. Instead, ion emission can proceed directly from a rounded meniscus, driven by high electric fields generated at modest voltages due to the sharpness of the

emitter tip. This is similar to the predictions of Higuera and Coffman et al. for the equilibrium shape of the meniscus at low fields, but in the case of a nanoscale emitter significant ion emission can be sustained with this meniscus profile. To put it another way, charge emission from any ion source requires *sharpness*, and the formation of a Taylor cone is typically required to reach that level of sharpness. For nanopore ion sources, the sharpness is already baked into the geometry of the emitter itself.

5.2.2 Measured IV characteristics

We measured the IV characteristic of several nanopore ion sources using a wide variety of different solutions using the experimental setup described in § 2.4. Unless otherwise noted, all experiments were carried out at atmospheric pressure and room temperature.

5.2.2.1 NaI in water

Figure 5.2.4 shows the measured IV characteristic for a nanopore ion source with I.D. = 31 nm and O.D. = 57 nm filled with 100 mM NaI in water. Fig. 5.2.4a shows an SEM image of the tip. As shown in Fig. 5.2.4b, the extraction voltage was swept upward from 45 V until an emission current greater than 15 pA was measured, at about 150 V, at which point the voltage sweep was reversed. Fig. 5.2.4c plots the measured current and voltage at each discrete voltage step, and the errorbars represent the standard deviation of the current and voltage at each step. This IV characteristic qualitatively matches the predictions of our model; the emission current increases exponentially at modest voltages rather than experiencing the sudden onset characteristic of cone-jet electrosprays. The solid curve in Fig. 5.2.4c is a fit of Eq. (5.2.9) to the measured IV data with r_0 and G_0 as the only free parameters. The fit returns values of $r_0 = 30$ nm and $G_0 = 1.29$ eV. The r_0 value is in excellent agreement with the measured outer radius of the emitter tip, 28 nm, while the G_0 value appears low relative to previously reported solvation energies of Na^+ in water, which are typically around 2.4 eV [140, 267, 223]. The model predicts that the maximum electric field at the meniscus surface reached $E \approx 0.6$ V/nm at 150 V in this experiment.

5.2.2.2 NaI in Formamide

The IV characteristic for electrolyte solutions in formamide have the same basic form as electrolyte solutions in water. This is notable since electrolyte solutions in formamide have previously been confirmed to emit ions directly from an electrified meniscus by ion evaporation [114, 155]. Figure 5.2.5

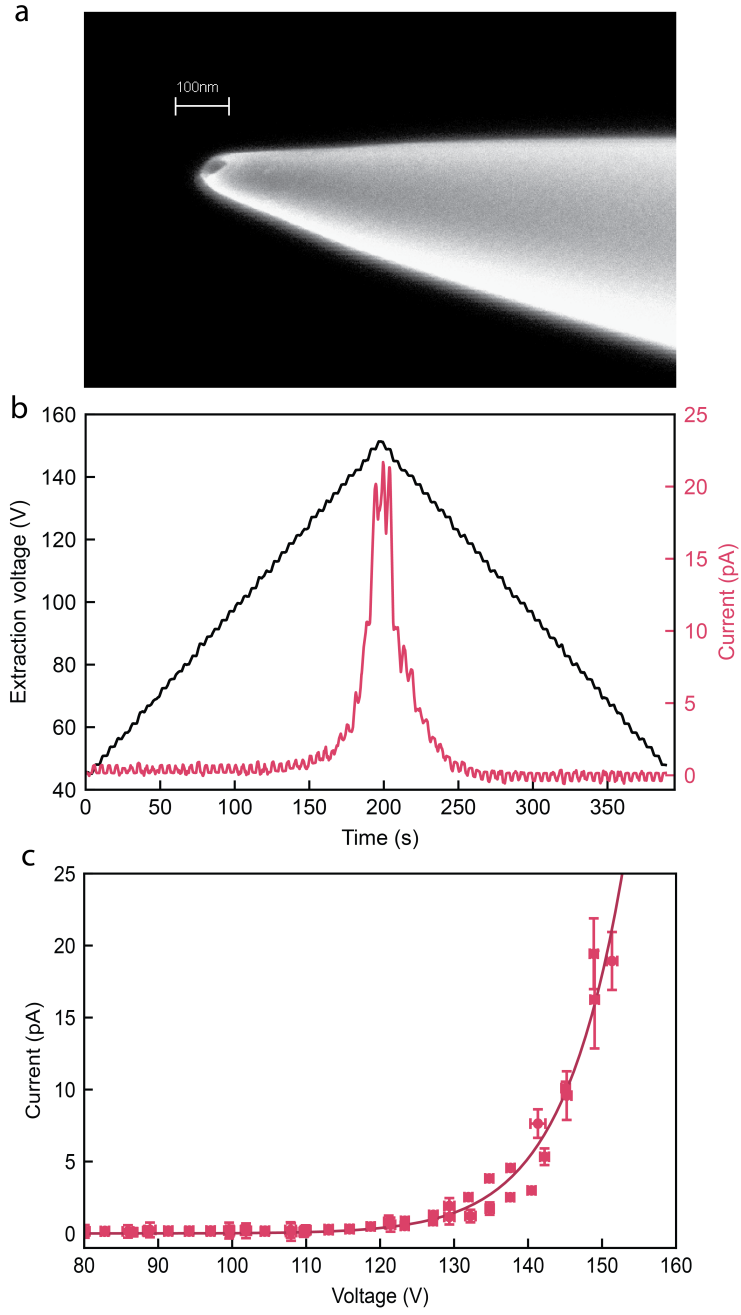


Figure 5.2.4: **Measuring the IV characteristic.** **a** SEM image of the nanopore ion source used in these measurements. The tip has an I.D. and O.D. of 31 & 57 nm, respectively. **b** Plot of the measured extraction voltage and emission current vs time during an automated voltage sweep. **c** Plot of the IV characteristic extracted from the data in panel b. The solid curve is a fit of Eq. (5.2.9) to the data, which yielded best fit values of $r_0 = 30$ nm and $G_0 = 1.29$ eV.

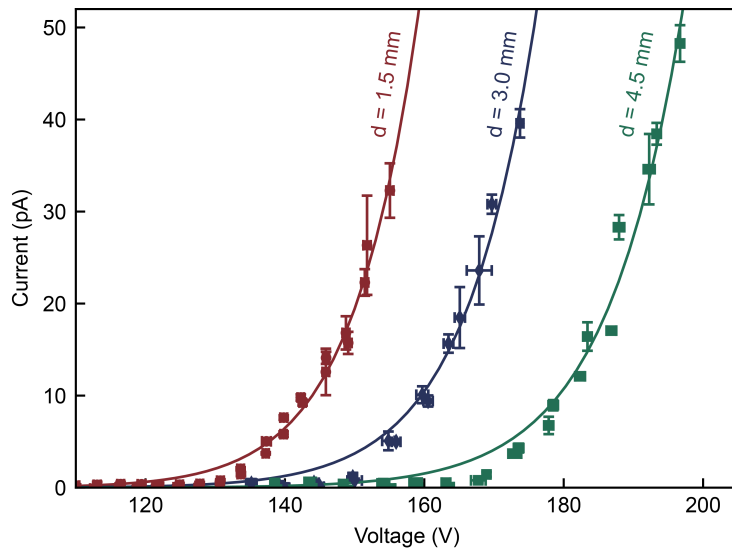


Figure 5.2.5: **IV characteristic of NaI in formamide.** Plot of the measured IV characteristic for 1 M NaI in formamide measured at three different values of the tip-electrode spacing, d .

shows the IV characteristics measured for the emission of 1 M NaI in formamide from a nanopore ion source with I.D. = 25 nm and O.D. = 86 nm at three different counterelectrode positions ($d = 1.5$, 3.0, & 4.5 mm). The curves again show an exponential increase in emission current rather than a sudden onset, and larger d values necessitate higher voltages to achieve equivalent current levels. For this data, Eq. (5.2.9) was fit to the measured IV curves with G_0 as the only fitting parameter while r_0 is taken to be half of the tip OD, or 43 nm for this tip. The fits return $G_0 = 1.22 \pm 0.02$ eV, where the uncertainty represents the standard deviation of the best fit G_0 values between the three measurements. This is slightly lower than the solvation energy of Na^+ for water reported above, but in closer agreement with previously reported solvation energies for Na^+ in formamide of about 1.7 eV [114, 152].

We also carried out IV measurements in a single tip with different concentrations of NaI in formamide (1 mM, 10 mM, 100 mM, 1000 mM) to determine the effect of conductivity on the IV characteristic. The tip had an O.D. of 55 nm, so we used an r_0 value of 28 nm when fitting Eq. (5.2.9) to the data. Table 5.1 summarizes the results of $N = 10$ repeated measurements at each concentration. The best fit G_0 values at each concentration are in agreement within experimental uncertainty despite varying in conductivity by over an order of magnitude. They also agree within the uncertainty with the measured G_0 value for Na^+ in formamide reported above from a different emitter. This further validates our model which is independent of the solute concentration or solution conductivity.

conc. (mM)	G_0 (eV)
1	1.18 ± 0.03
10	1.19 ± 0.03
100	1.15 ± 0.03
1000	1.20 ± 0.03

Table 5.1: Best fit G_0 values for various concentrations of NaI in formamide

5.2.2.3 Arginine in water

The IV characteristic obtained from the emission of larger molecular ions from aqueous solution are similar to those obtained from aqueous salt solutions. Figure 5.2.6a shows the measured IV characteristic for a 100 mM aqueous solution of arginine (pH 8.9, $K = 3.2$ mS/cm) from a nanopore ion source with I.D. = 32 nm & O.D. = 99 nm. Eq. (5.2.9) with G_0 as the only fitting parameter again nicely fits the data, returning a value of $G_0 = 1.63$ eV for arginine. The higher solvation energy relative to that obtained for Na^+ is expected as arginine’s charge is distributed over two positively charged groups (amino and side-chain guanidino) and one negatively charged group (carboxyl), so it should interact more strongly with the surrounding water molecules.

For comparison, Fig. 5.2.6b shows the measured IV characteristic of a much larger emitter (I.D. = $6.7 \mu\text{m}$, O.D. = $7.0 \mu\text{m}$), similar in size to conventional nanoESI emitters. The emitter contained the same 100 mM arginine solution as the nanopore ion source emitter shown in panel a. The IV characteristic looks markedly different, rather than a gradual exponential increase in current, there is a sudden onset in emission current at 1175 V of about 10 nA. This is within 15% of the predicted onset voltage for a tip of this size (Eq. (5.2.10)). After onset, the emission current increases linearly with voltage. As the voltage is decreased from its maximum value, the tip continues emitting at voltages below its onset voltage, consistent with other groups’ observations that a higher voltage is required to initiate Taylor cone formation than to maintain it [80].

5.2.3 Emission mechanism is the same in air and vacuum

All of the data presented in this chapter thus far was measured with an ion source in air at ambient temperature and pressure. However, all of the data presented in Ch. 3, which was used to demonstrate that the nanopore ion source operates by emitting charge directly from the electrified meniscus into high vacuum, was measured with the ion source under high vacuum. In order to use the IV measurements presented in this chapter to make any inferences about the behavior of ion source under vacuum conditions, we must first verify that the IV characteristic is the same under both ambient and vacuum conditions.

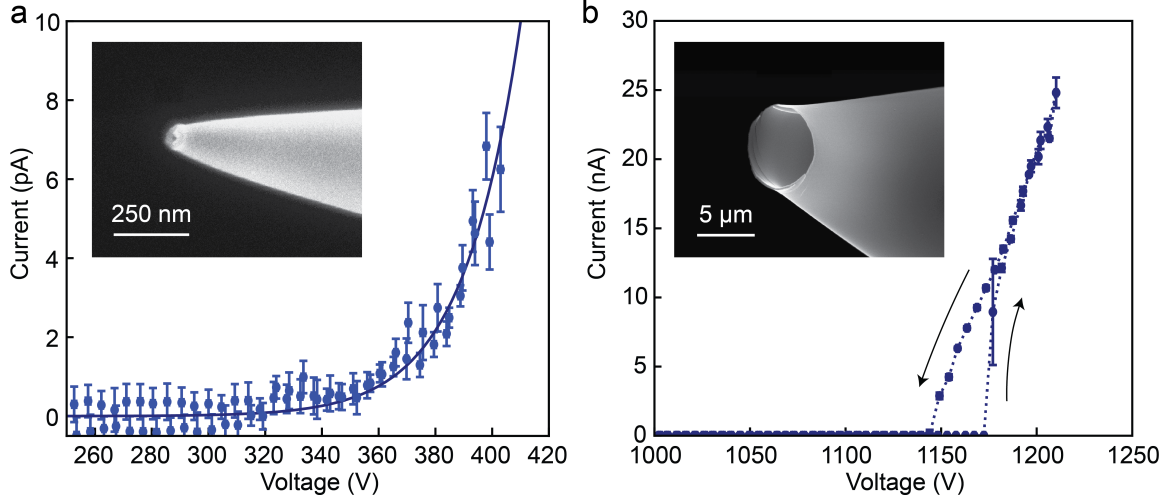


Figure 5.2.6: **IV curves for small and large emitter.** **a** Measured IV curve for a nanopore ion source (I.D. = 32 nm, O.D. = 99 nm, shown in inset) filled with 100 mM arginine in water. The solid curve shows the fit to Eq. (5.2.9). **b** Measured IV curve for a much larger emitter (I.D. = 6.7 μm , O.D. = 7.0 μm , shown in inset) filled with the same 100 mM arginine solution. The arrows indicate the direction of the IV curve as the voltage was increased and decreased.

We measured the IV characteristic of a nanopore ion source in both vacuum and atmosphere. We used the same ion source for these measurements that was used to obtain the data presented in Figs. 3.2.13 & 3.3.2, with I.D. = 24 nm and O.D. = 77 nm. The tip was filled with 100 mM Arginine & 50 mM NaI in water at pH 9.1, and then mounted in the Faraday cage to measure the IV characteristic in air as described above. Following this measurement, the same ion source filled with the same solution was mounted in the quadrupole mass spectrometer described in § 2.2.2 to measure the IV characteristic in vacuum. The extraction voltage was manually swept from approximately 265 V to 395 V while recording the emission current using the source-measure unit (Keithley 2657a) as well as the detector current using a counting pre-amplifier (Advanced Research Instruments Corporation F-100T) hooked up to the channeltron detector in the MS.

Figure 5.2.7 plots the IV curves measured both in air at atmospheric pressure (red diamonds) and under high vacuum ($P \approx 10^{-7}$ torr) within the MS (blue circles). The emission current increases exponentially with extraction voltage in both environments, and the IV curves match in the overlapping voltage range of 265-365 V within the uncertainty margins. The detector current (black squares) also follows the same trend as the emission current, although it is a noisier measurement. Both sets of emission current data are fit to Eq. (5.2.9) with G_0 as the only free parameter, the best fit curves in vacuum and air are shown as solid blue and red curves, respectively. The fit returns values of $G_0 = 1.71$ eV for the atmospheric pressure IV measurement and $G_0 = 1.72$ eV for

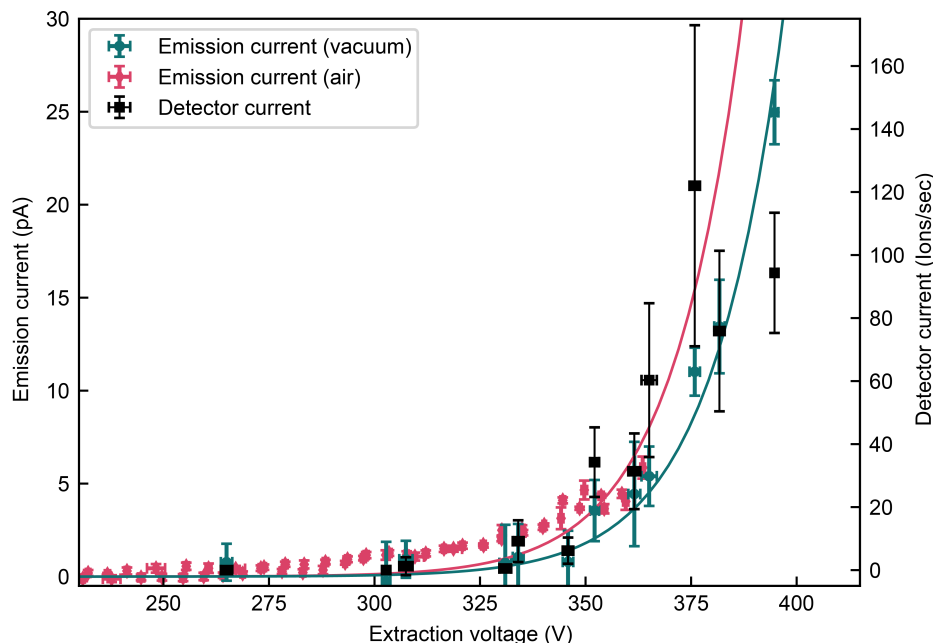


Figure 5.2.7: **IV characteristic in air and in vacuum.** Plot of the measured IV characteristic for a nanopore ion source filled with 100 mM arginine & 50 mM NaI in water (pH 9.1) in both high vacuum (blue circles) and ambient air (red diamonds) conditions. The MS detector current corresponding to the vacuum emission current measurement is plotted along the right-hand axis (black squares).

the vacuum IV measurement. Since the solution is a mixture of arginine and NaI, these G_0 values represent an effective solvation energy for the entire solution rather than for any specific ion. The close agreement of the IV curves in both measurements supports the conclusion that the emission process is the same in both air and vacuum.

There are theoretical reasons to expect the nanopore ion source's emission process to be largely unchanged between ambient and vacuum environments. Two additional physical effects arise when considering emission in the vacuum environment: increased solvent evaporation and the extra atmosphere of pressure difference across the liquid column that will shift the pressure balance at the meniscus. Regarding evaporation, as demonstrated in Chapter 4, evaporative cooling is negligible for tips with radii smaller than 100 nm, even for volatile solvents like water. This mitigates concerns about potential freezing or significant temperature changes in vacuum. As for the pressure difference, this will manifest as an additional $\Delta P = 1$ atm term on the LHS of Eq. (5.2.6). But for $r_c < 100$ nm, the laplace pressure term will be > 14 atm for water, so the additional atmosphere of pressure will only have a minor effect on the structure of the meniscus. Therefore, both experimental evidence and theoretical considerations strongly support the conclusion that the emission mechanism of the

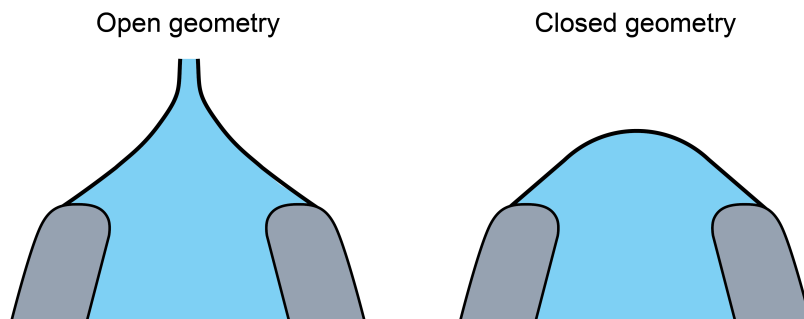


Figure 5.3.1: **Diagram of open and closed meniscus geometries.**

nanopore ion source is ion evaporation directly from the electrified meniscus, regardless of whether it operates in ambient conditions or high vacuum.

5.3 The emission current from a nanopore ion source is independent of applied pressure

An important difference between the nanopore ion source's emission mechanism and that of conventional ESI sources can be revealed by examining the dependence of the emitted current on the applied pressure. In a cone-jet electrospray, there are well established relationships between the emission current carried by the jet and the fluid flow rate, Q . Since the jet emanating from the tip of a Taylor cone contains a net charge, a change in the flow rate necessarily changes the current. de la Mora and Loscertales established a fundamental scaling law for highly conducting liquids, where the emission current is proportional to the square root of the flow rate, $I \sim Q^{1/2}$ [113]. This scaling was confirmed for a wide range of solutions [268, 269, 270, 271]. Higuera later showed that the scaling could deviate from that predicted by de la Mora, and may be closer to $I \sim Q^{4/5}$ for a very small meniscus [272].

At very low flow rates, these scaling relationships break down as ions begin to evaporate directly from the meniscus [114, 120]. As described in §1.3.5, lower flow rates result in a shrinking jet and higher electric fields in the transition region (see Figs. 1.3.4 & 1.3.3), eventually reaching the threshold field for ion evaporation. The ion evaporation current grows with decreasing flow rate, violating the scaling relationships described above. If the ratio K/Q is high enough, as is the case in liquid metals and ionic liquids, the jet can be completely extinguished and the meniscus takes on a closed geometry [109, 175]. In this scenario, the flow rate is solely attributed to the volume flux of evaporating ions and solvent molecules.

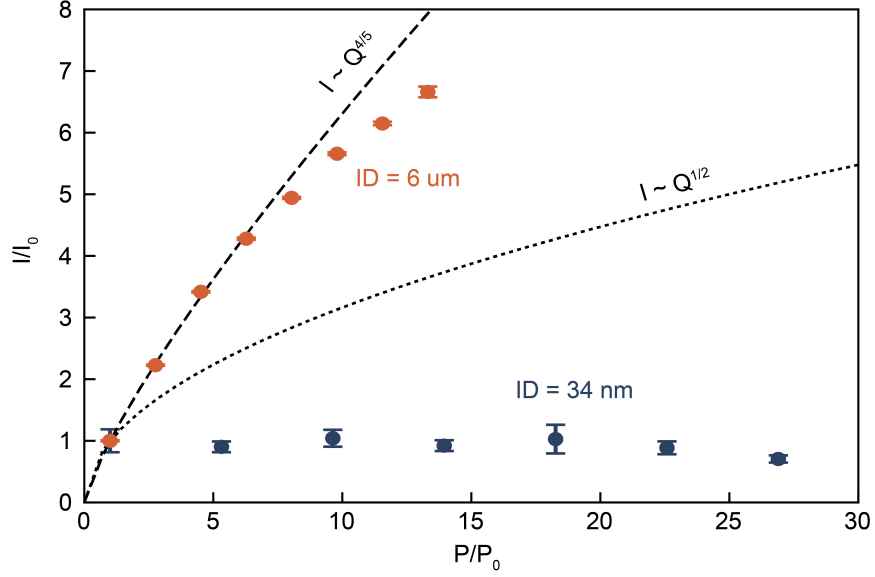


Figure 5.3.2: **Emission current is independent of flow rate.** Plot of the normalized emission current for two ion sources vs. the non-dimensional flow rate at fixed voltage. One ion source has I.D. = 34 nm (dark blue) and the other has I.D. = 6 μm (orange), in the size range of a conventional nanoESI source. Both ion sources contained a 100 mM solution of glycine in water ($K = 0.22 \text{ S/m}$). The dashed curves show the $I \sim Q^{1/2}$ scaling predicted by de la Mora [113] and the $I \sim Q^{4/5}$ scaling predicted by Higuera for a small meniscus [272].

We devised a simple experiment to test whether the meniscus at the tip of a nanopore ion source has an open or closed geometry during emission (Fig. 5.3.1). A nitrogen cylinder with a regulator connected to the pressure input port on the tee connector holding the ion source (see Fig. 2.4.1) enables direct control over the applied pressure on the liquid within the ion source. The flow rate through the emitter should be directly proportional to this applied pressure [204]. A nanopore ion source with I.D. = 34 nm and O.D. = 76 nm was compared with a much larger source with I.D. = 6.0 μm and O.D. = 6.7 μm , closer to the dimensions of typical nanoESI sources [273]. Both ion sources were filled with 100 mM glycine in water with 0.5% acetic acid ($K = 0.22 \text{ S/m}$) before being mounted in the Faraday cage. While applying a pressure of $P_0 = 4 \text{ kPa}$, the extraction voltage was raised until stable emission was observed from each emitter, then held fixed for the duration of the experiment. The nanopore ion source generated a current of $I_0 = 20 \pm 3 \text{ pA}$ at 310 V, while the nanoESI source generated a current of $I_0 = 13.7 \pm 0.2 \text{ nA}$ at 950 V. We recorded the emission current as the pressure was swept in discrete steps up to 105 kPa in the case of the nanopore ion source, and up to 60 kPa in the case of the nanoESI source, since the emission current became unstable at higher pressures for this emitter. At each pressure, we recorded the emission current over a 30 second period.

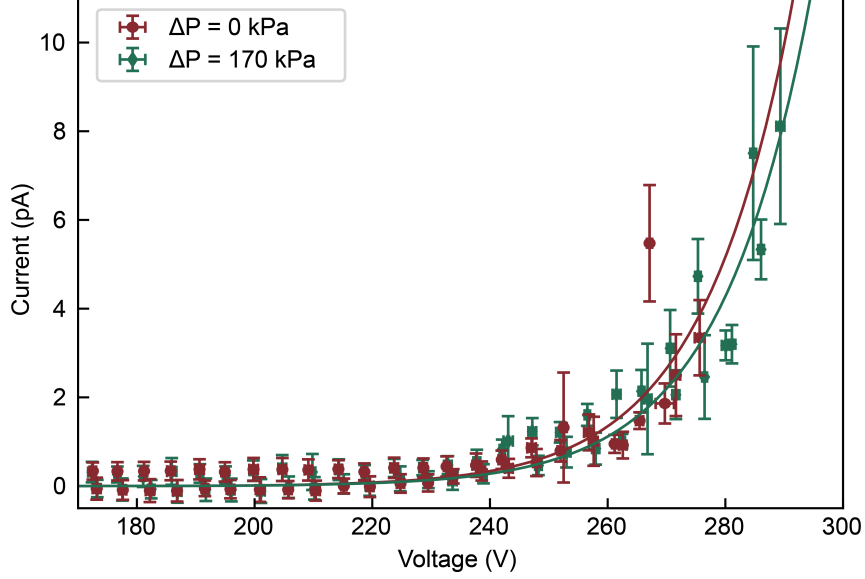


Figure 5.3.3: **IV curves are unaffected by applied pressure.** Plot of the measured IV characteristic for an ion source with I.D. = 29 nm and O.D. = 80 nm filled with 100 mM LiCl in water, with an applied back pressure of $\Delta P = 0$ & 25 psi.

Figure 5.3.2 plots the normalized current I/I_0 vs. the normalized applied pressure P/P_0 for both ion sources. The nanoESI emitter with I.D. = 6 μm shows a clear dependence on the pressure, as expected for a cone-jet electrospray. The scaling laws proposed by de la Mora and Higuera are also plotted [113, 272], and Higuera's $Q^{4/5}$ scaling shows much better agreement with the data from the larger emitter. In either case, the strong dependence on pressure illustrates that the meniscus at the tip of the larger emitter has an open geometry, where higher pressure pushes more charge out of the meniscus via a charged jet or charged droplets. The emission current from the nanopore ion source, by contrast, does not appear to show any dependence on the applied pressure. This is consistent with a closed meniscus geometry and purely ionic emission.

In a separate set of experiments, we measured the IV characteristic of a single nanopore ion source (I.D. = 29 nm, O.D. = 80 nm) filled with 100 mM LiCl in water ($K = 1.08 \text{ S/m}$) with applied pressures of 0 and 170 kPa respectively. Figure 5.3.3 plots the measured IV characteristics, showing no significant differences between them. Each set of IV data was fit to Eq. (5.2.9) with G_0 as the only free parameter. Repeated measurements ($N = 10$) at each pressure returned $G_0 = 1.56 \pm 0.02$ at $P = 0$ kPa and $G_0 = 1.56 \pm 0.01$ at $P = 170$ kPa. The excellent agreement between the IV characteristics despite a difference of almost 2 atmospheres of applied pressure supports the conclusion that the meniscus at the tip of a nanopore ion source has a closed geometry during emission, and that one atmosphere of applied pressure should not significantly effect the emission

process.

5.4 Over-/under-wetting may account for variation between ion sources.

Not all of the nanopore ion source emitters we test produce IV characteristics which match the predictions of Eq. (5.2.9) as well as those shown in the figures above. Occasionally, nanopore ion sources exhibit sudden onset behavior with high currents (> 1 nA), similar to that shown in Fig. 5.2.6b. Some ion sources we test under vacuum don't emit any detectable current even up to extraction voltages as high as 1 kV. Other times, they may exhibit an exponential onset with picoampere currents as expected, but with significant instability or hysteresis (tracing different paths in I-V space depending on the direction of the voltage sweep). Figure 5.4.1 displays an example of this behavior in an ion source (I.D. = 27 nm, O.D. = 92 nm) filled with 100 mM arginine in water. As the extraction voltage rises above 290 V, the current begins increasing exponentially as expected. Past 320 V, however, the emission current starts following a shallower trajectory, until at 375 V the voltage sweep reverses direction and the emission current decreases exponentially with voltage along a higher voltage path. There is no reason to expect G_0 for an ion to vary over the course of an experiment, so according to the model developed in § 5.2.1, the most reasonable explanation appears to be a change in the effective tip radius r_0 . Figure 5.4.1b shows the measured IV curve, along with fits of Eq. (5.2.9) to the exponential rise and fall sections, where r_0 is the only fitting parameter and G_0 was fixed at 1.65 eV. The fits return r_0 values of 39 nm and 45 nm respectively.

This analysis suggests a potential explanation for the observed hysteresis effect, the wetting of the meniscus at the tip of the emitter may be changing over the course of the measurement. Consider the illustration in Fig. 5.4.2. Our IV model assumes that the meniscus looks like the “optimal wetting” condition. But given the extremely small size of the emitter tip, this is not easy to confirm. It is entirely possible that the actual meniscus at the tip of the ion source looks more like the under-wetting or over-wetting condition, or that it moves between these states depending on the applied voltage due to electrowetting effects [274]. As the voltage increases, electrowetting can reduce the contact angle, causing the meniscus to advance and change shape. However, contact angle hysteresis may prevent the meniscus from immediately returning to its original state as the voltage decreases [275]. In the case of the IV curve shown in Fig. 5.4.1, one possibility is that the meniscus shifted from an under-wetted state to a more optimally wetted state as the voltage changed, increasing the

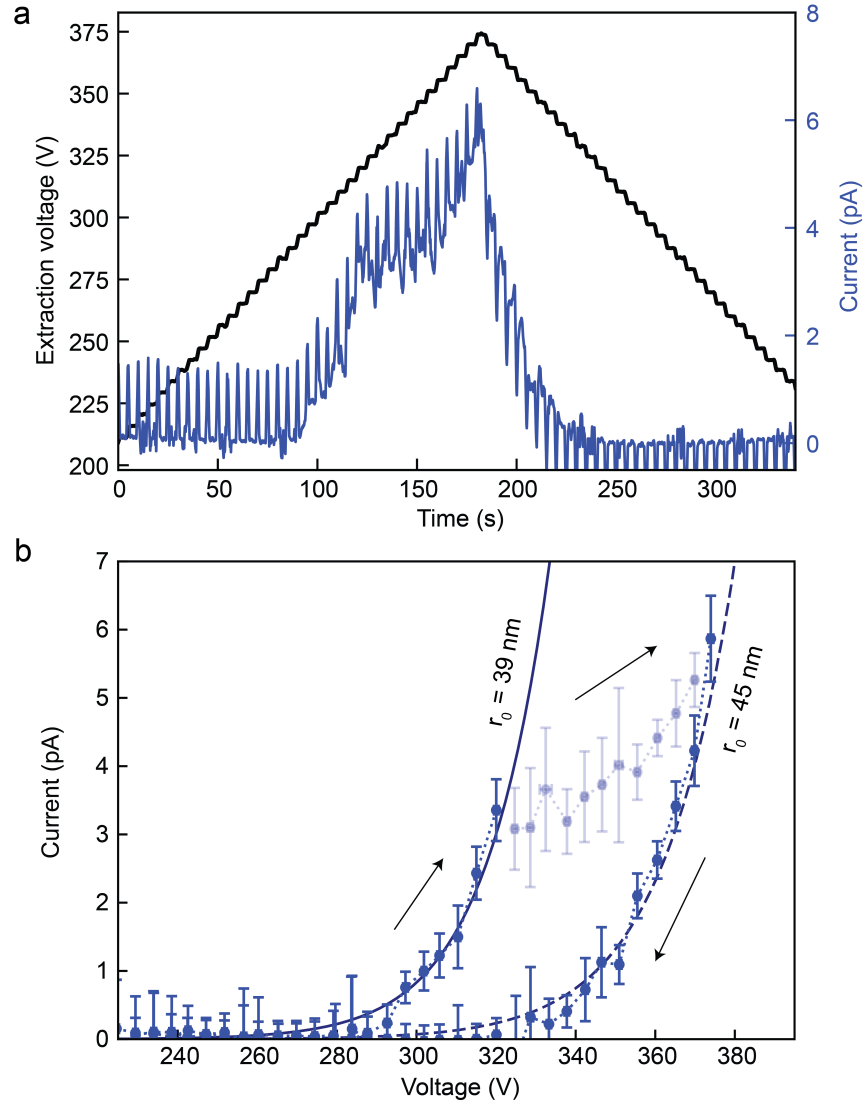


Figure 5.4.1: **IV characteristic suggesting moving meniscus.** **a** Plot of the measured extraction voltage and emission current vs time during an automated voltage sweep. **b** Plot of the IV characteristic extracted from the data in panel a. The solid curve is a fit of Eq. (5.2.9) to the first exponential section, yielding fit value of $r_0 = 39 \text{ nm}$, and the dashed curve is a fit to the second exponential section, yielding $r_0 = 45 \text{ nm}$.

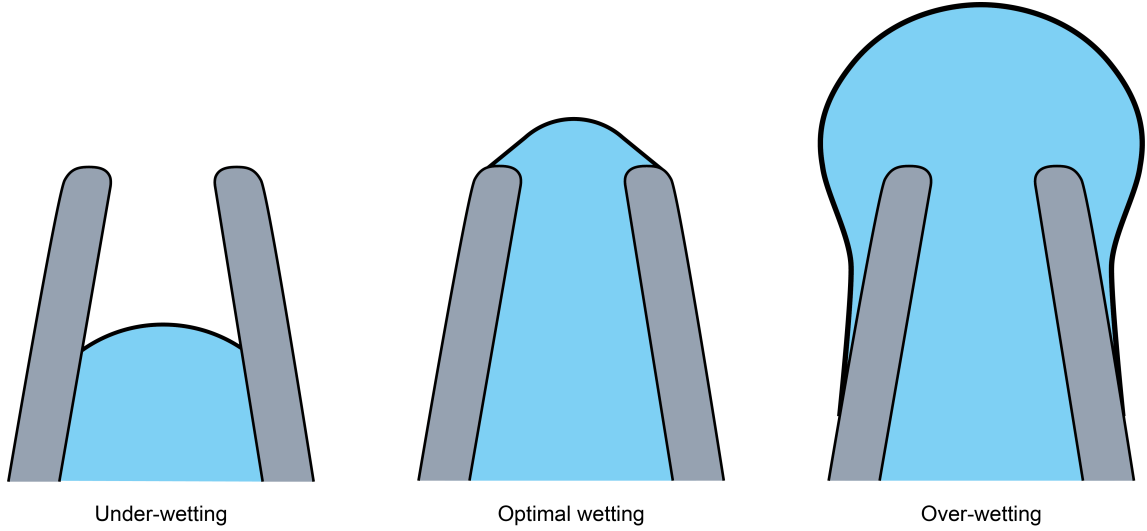


Figure 5.4.2: **Under- and over-wetting.** Illustrations of possible wetting conditions at the tip of the emitter.

effective tip radius r_0 and leading to the observed hysteresis effect. In the cases where a nanopore ion source exhibits a sudden onset with high current, it may be that the tip is “over-wetted”, with a large droplet on the outside of the emitter providing the conditions necessary for the emission of charged droplets or the formation of a cone-jet. Such over-wetting conditions would complicate the thermal analysis of the previous chapter, as the surface area over which evaporative cooling occurs would be substantially larger, potentially leading to freezing of the emitter. This may provide an explanation for why some fraction of nanopore ion sources we test do not appear to emit charge at all when filled with aqueous solutions and placed in vacuum.

Additional evidence for this over-wetting phenomenon is provided by SEM images taken before and after filling a nanopore ion source with aqueous solution. An emitter (I.D. = 25 nm and O.D. = 75 nm) was treated by oxygen plasma for 2 minutes, then filled with 10 mM NaI in water and left out in ambient conditions for 30 minutes. The emitter was then soaked in DI water at 80° C for 30 minutes and dried at 80 C for another 30 minutes. Before the second round of imaging, the emitter was coated with a thin layer of carbon, since the carbon coating from the first round of imaging would have been removed by the plasma treatment. Fig. 5.4.3 shows SEM images of the emitter taken before and after the filling and soaking process. In the latter image, there is clearly a residue deposited on the exterior surface of the emitter. It seems likely that these deposits are composed of sodium iodide crystals strongly bound to the glass which formed when the tip was left out in ambient conditions after filling with NaI solution. The fact that the residue formed on the tip exterior indicates that the exterior surface was wetted by the NaI solution, supporting our

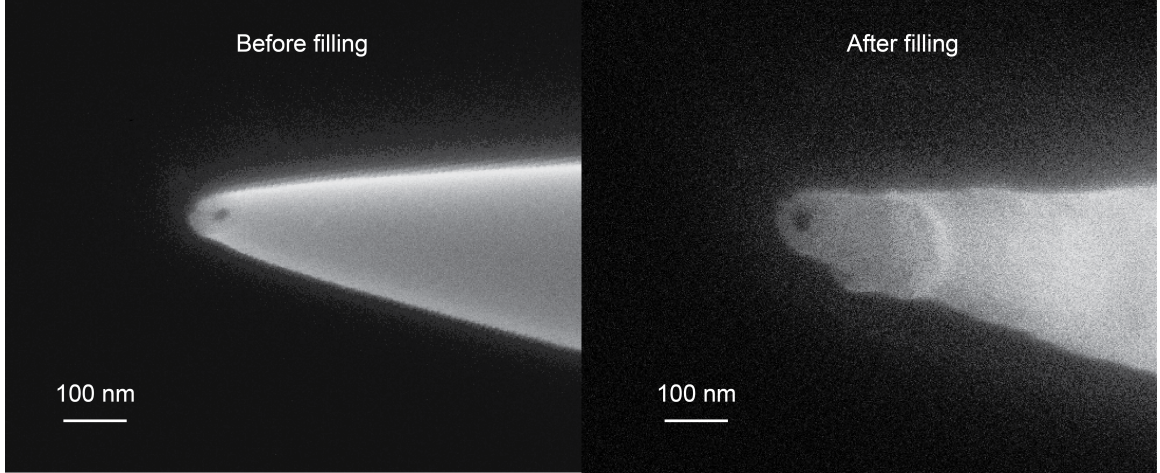


Figure 5.4.3: **SEM images showing evidence of tip over-wetting.**

hypothesis that over-wetting conditions may be responsible for variations in emission behavior.

5.5 Mixed ion/droplet regime at higher currents

The nanopore ion source is most likely to operate in the pure ion emission mode when the extraction voltage is kept as close as possible to V_{onset} and the emission current is kept as low as possible. This was the case in both measurements presented in Fig. 3.2.10. When the voltage is increased, a transition to a mixed ion/droplet emission mode with higher emission current (> 1 nA) is typically observed. By varying the extraction voltage, we can map out the transition between modes.

Figure 5.5.1 shows an example of an experiment using a nanopore ion source (I.D. = 65 nm, O.D. = 119 nm) filled with 100 mM NaI in water where this transition was observed. This measurement was carried out with the magnetic sector mass spectrometer configured to measure the emitted ion fraction as illustrated in Fig. 3.2.9. Figure 5.5.1a shows the extraction voltage and measured emission, ion, and droplet currents. Initially, the source was emitting around 200 pA with an extraction voltage of 134 V and only an ion current was detected downstream, indicating pure ion emission. The extraction voltage was then slightly increased up to 136 V, causing a discrete jump in the emission current up to 10 nA along with the sudden appearance of a droplet current. As the extraction voltage and emission current were further increased, the droplet current increased relative to the ion current (ie. the ion fraction fell). Figure 5.5.1b shows a scatter plot of the calculated ion fraction vs the emission current. A distinct pure ion emission mode is clearly visible at low current and near 100% ion fraction, while the mixed ion/droplet emission mode appears as a continuous distribution of points at higher current. The precise shape of these ion fraction vs

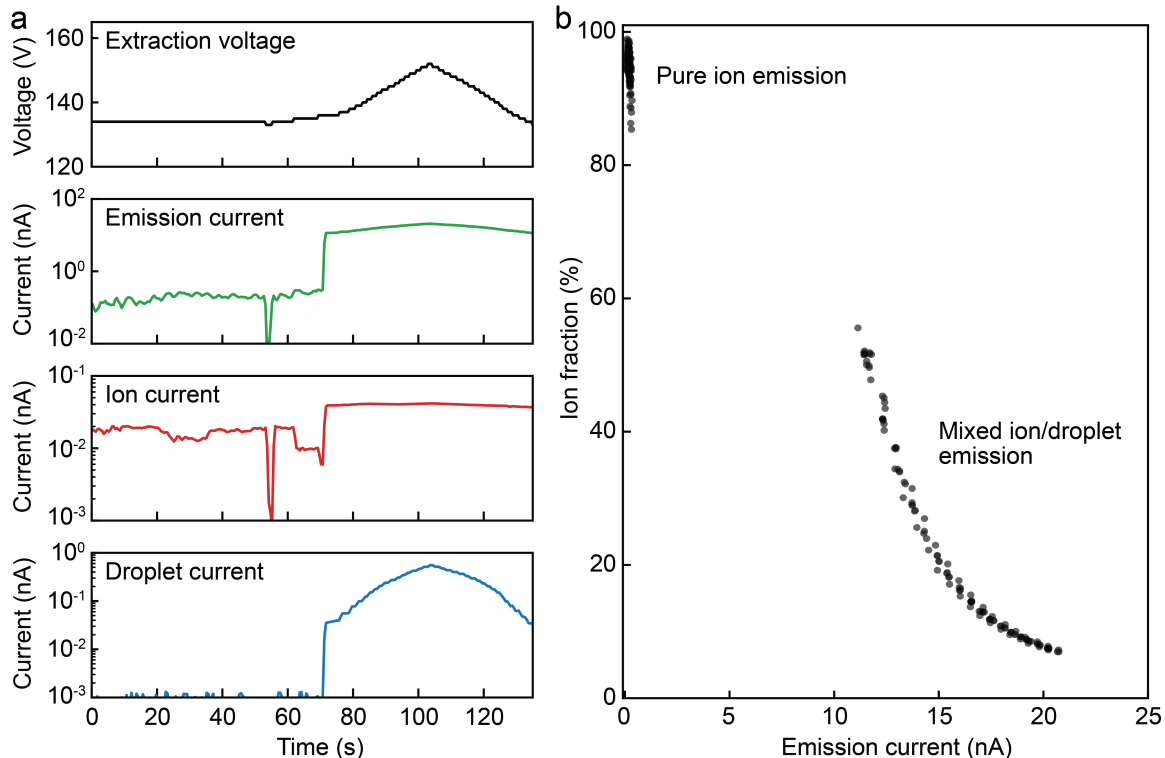


Figure 5.5.1: **Mixed ion/droplet emission.** **a** Plots of the extraction voltage, emission current, transmitted ion current, and transmitted, droplet current. **b** Scatter plot of the measured ion fraction vs emission current showing two distinct regimes.

emission current scatter plots varied for different tips and occasionally for repeated experiments with the same tip, but the overall structure was conserved. There was typically a discontinuous jump in emission current between pure ion emission and mixed ion / droplet emission. Whenever a pure ion mode was observed, it appeared at low currents (< 1 nA), while a mixed ion/droplet emission mode was always observed for currents $\gg 1$ nA. These results are consistent with our observations in the quadrupole mass spectrometer, where emission currents > 1 nA are typically associated with a significant reduction of ion signal at the detector.

The existence of a transition between a purely ionic regime and a mixed ion/droplet regime is consistent with the theoretical models developed to describe the behavior of ionic liquid ion sources discussed in § 5.1 [175, 159, 234]. These models predict a narrow range of applied electric field strengths in which a stable hydrostatic meniscus is emitting charge in the purely ionic regime. The transition to a mixed emission regime likely occurs when the electric field at the tip exceeds a critical value, causing the meniscus to become unstable and emit charged droplets.

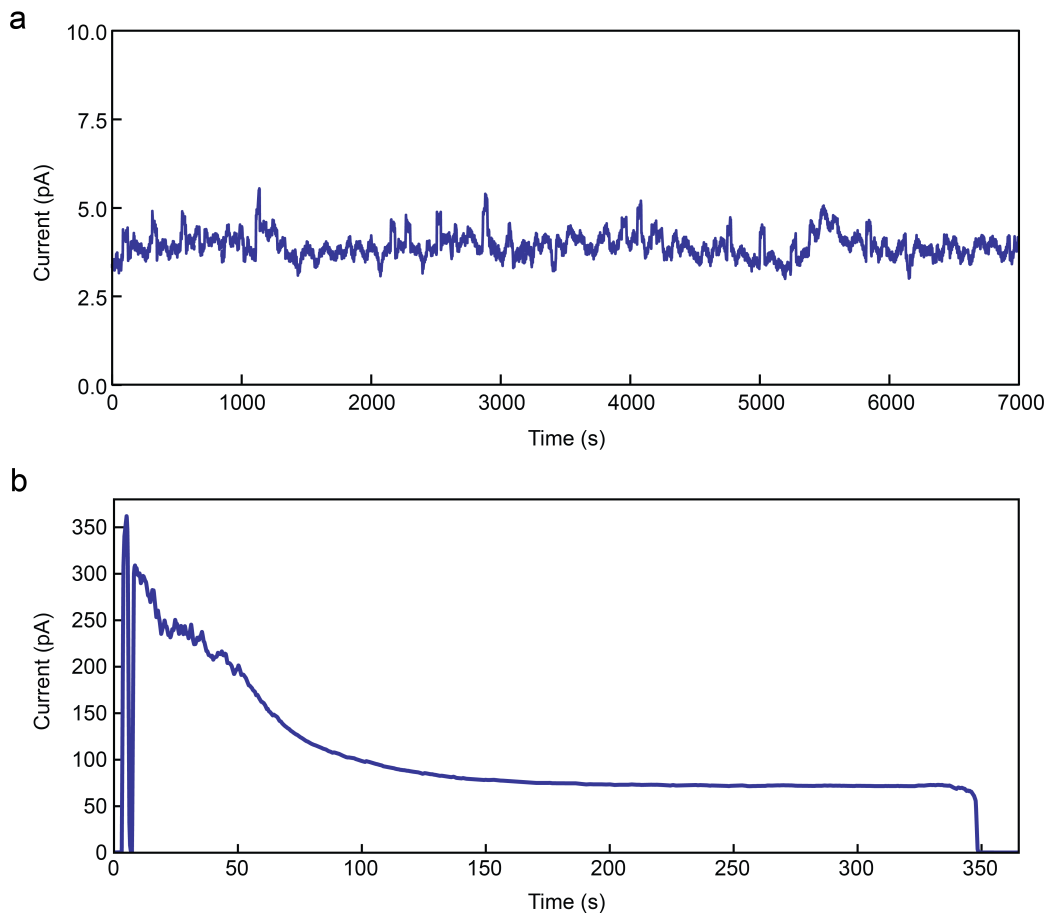


Figure 5.6.1: **Stable and unstable emission currents.** **a** Plot of the emission current from a nanopore ion source during the emission of two 100 mM aqueous amino acid solutions (cysteine and phenylalanine), showing stability for two hours. **b** Plot of the unstable emission current from a nanopore ion source containing 10 mM NaI in water, showing instability and decay.

5.6 Emission current stability

The nanopore ion source can emit a stable current of ions for hours at a time. Figure 5.6.1a plots the emission current from a nanopore ion source (I.D. = 57 nm, O.D. = 115 nm) over about two hours. During that time, the sample solution was swapped between a 100 mM aqueous solution of cysteine, to a 100 mM aqueous solution of phenylalanine. This data comes from the same measurement that produced the data shown in Fig. 3.2.4. The emission current remains stable throughout the entire measurement at $I = 4 \pm 1$ pA, with an extraction voltage of $V_E = 190$ V. We have observed that currents in the low picoampere range, like that shown in Fig. 5.6.1a, can be emitted stably from aqueous solutions of modest conductivity ($K \sim 0.01 - 1$ S/m).

Emission currents in the range of 100s of pA or higher, however, tend to be unstable and quickly decay to a more stable value or shut off altogether. Figure 5.6.1b shows an example from a nanopore

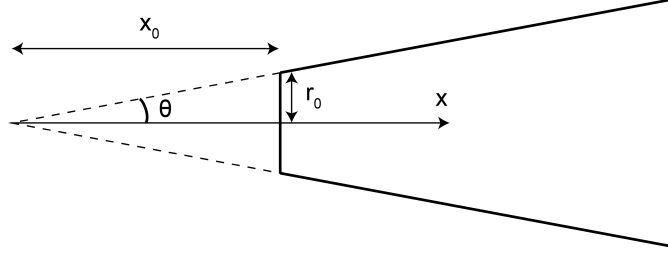


Figure 5.6.2: **Diffusion model coordinate system.**

ion source (I.D. = 24 nm, O.D. = 74 nm) emitting 10 mM solution of NaI in water. The emission current turns on with about 350 pA at $V_E = 140$ V and appears unstable. It then decays over about 100 s to a more stable emission current of $I = 72 \pm 2$ pA. At $t = 350$ s the emission current abruptly falls to zero, despite a constant extraction voltage since onset.

5.6.1 Diffusion limited ion transport

The instability of high emission currents can be explained by considering a very simple model of ion transport through the nanopore ion source. The source emits positive ions while receiving no negative ions from the vacuum. In this way, it behaves much like a perfectly selective ion exchange membrane [276]. We can therefore apply models developed for ion exchange membranes to gain insight into the behavior of the nanopore ion source. The simple question we want to answer here is, what is the limiting current if we consider diffusion as the only form of ion transport? In other words, beyond what emission current is diffusion alone insufficient to supply ions to the meniscus?

We derive an expression for the limiting current by balancing the diffusive ion flux with the emitted ion flux. This latter flux, J_E , is given by

$$J_E(x) = \frac{I}{F\pi r_0^2} \left(\frac{x_0^2}{x^2} \right), \quad (5.6.1)$$

where F is Faraday's constant, x_0 is the distance from the tip to the apex of the hypothetical *un*-truncated cone as illustrated in Fig. 5.6.2. The diffusive flux, J_{Diff} , is given by

$$J_{\text{Diff}} = -D \frac{dc}{dx}. \quad (5.6.2)$$

Equating Eqs. 5.6.1 and 5.6.2 and integrating both sides from x_0 to ∞ we find,

$$\frac{Ix_0}{F\pi r_0^2} = D(c_0 - c(x_0)). \quad (5.6.3)$$

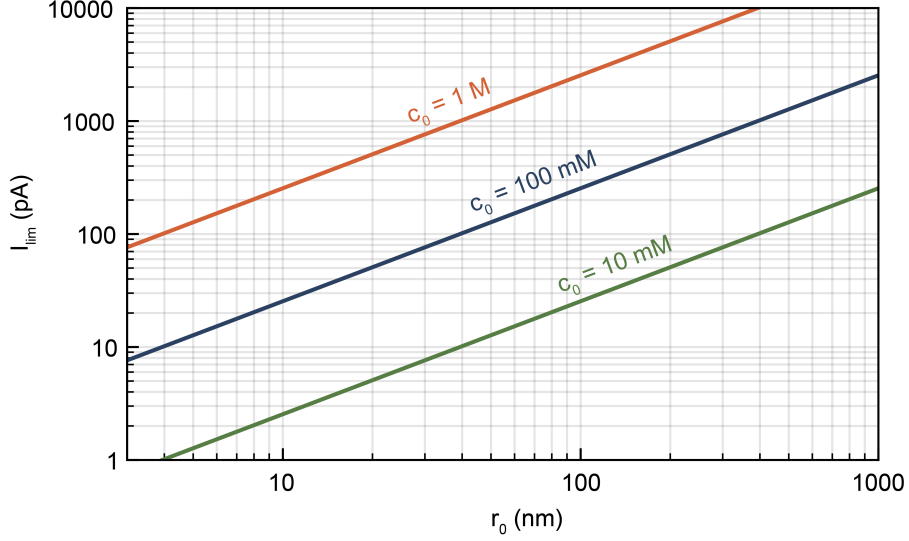


Figure 5.6.3: **Diffusion-limited current predictions.** Predictions of I_{lim} as a function of r_0 for $c_0 = 10, 100$, & 1000 mM, $D = 10^9$ m²/s, and $\theta = 3^\circ$ from Eq. (5.6.4).

To find the limiting current, we solve for I in limit where $c(x_0) \rightarrow 0$. This yields

$$I_{\text{lim}} = F D \pi c_0 r_0 \tan \theta, \quad (5.6.4)$$

where we have used the fact that $x_0 = r_0 / \tan \theta$. Note that c_0 in this expression is in units of mol/m³, an additional factor of 10^3 is required to convert to the more commonly used units of mol/L.

Equation (5.6.4) is very straightforward. The amount of current that diffusion can “keep up with” is directly proportional to each parameter that would be naturally expected to play a role, the diffusion coefficient, the ion concentration, the tip radius, and the apex half-angle. Figure 5.6.3 shows the limiting current predicted by Eq. (5.6.4) for $c_0 = 10$ mM, 100 mM, & 1 M as a function of r_0 . The other parameters used in the simulations were $D = 10^9$ m²/s, and $\theta = 3^\circ$. For the two example datasets shown in Fig. 5.6.1, Eq. (5.6.4) predicts limiting currents on the order of 100 pA & 10 pA respectively. These predictions are largely consistent with our observations, higher currents tend to be less stable, while higher concentration solutions tend to generate higher currents.

While this model provides valuable insights into the general behavior of the nanopore ion source, it does not account for the complex phenomenon of concentration polarization and its associated effects [277]. In reality, as the emission current approaches the diffusion-limited current, an effect akin to concentration polarization should occur, in which cations are depleted near the tip of the emitter. I say “akin to concentration polarization”, because in the case of the nanopore ion source, the other side of the meniscus is just vacuum, so counter-ions do not accumulate on the other

side as they do in conventional concentration polarization. This polarization induces an electric field within the solution, which in turn drives electromigration of ions. The electromigration effect can partially counteract the depletion caused by the limited speed of diffusion, potentially allowing for over-limiting currents that exceed the simple diffusion-limited prediction [276, 278]. Similar concentration polarization effects have been observed in sub-micron nanoESI emitters [279]. Future work should aim to incorporate these more complex transport phenomena into a more comprehensive model of the nanopore ion source.

5.7 Conclusions

This chapter revealed several key emission characteristics that distinguish the nanopore ion source from conventional ESI sources. The IV characteristics of nanopore ion sources exhibit a smooth, exponential increase in current with voltage, rather than the abrupt onset typical of cone-jet electrosprays. This behavior is consistent with emission in the purely ionic regime, where ions evaporate directly from the electrified meniscus. We developed a theoretical model based on ion evaporation that accurately describes the observed IV characteristics, allowing us to extract important parameters such as the effective tip radius and ion solvation energy from experimental data. Additionally, the emission current from nanopore ion sources is independent of applied pressure, in contrast to the behavior of conventional ESI sources. This suggests a closed meniscus geometry, providing further support for purely ionic emission. We demonstrated that the emission mechanism is the same in both air and vacuum environments, an important finding that connects our atmospheric IV measurements with mass spectrometry measurements performed in vacuum. By mapping the transition between pure ion emission and mixed ion/droplet emission regimes, we showed that pure ion emission is favored at lower voltages and currents, consistent with predictions made by other groups for ionic liquid ion sources. These findings collectively support the conclusion that nanopore ion sources operate primarily through direct ion evaporation from a nanoscale electrified meniscus, but that droplet emission may occur with sufficiently high voltages or over-wetting conditions at the emitter tip.

Despite these insights, many aspects of the nanopore ion source’s behavior require further study. The model we developed agrees nicely with individual IV curve measurements, but it fails to accurately model the relative contributions of different ion species in a mixture, such as those presented for mixtures of NaI and arginine/histidine in Fig. 3.2.13. Due to the differences in G_0 , Eq. 5.2.9 predicts that the current from Na^+ ions should be orders of magnitude larger than the current

from Arg^+ or His^+ ions, but that is not observed. A more sophisticated model that deals with solutions containing multiple different solutes, perhaps accounting for the finite occupancy of ions at the meniscus, may be necessary to capture this behavior. Additionally, the role of electrowetting phenomena in determining the meniscus shape requires further investigation, as many of our observations suggest that the wetting conditions at the tip significantly impact emission behavior. Future work should explore ion sources with hydrophobic exteriors and hydrophilic interiors, as this design may ensure more optimal wetting conditions. Finally, a far more detailed model of ion transport within the emitter should be developed to understand the dynamic behavior of the emitted current as well as the effect of concentration polarization on the stability of emission.

6

Ultraviolet photofragmentation of peptides

"All the fifty years of conscious brooding have brought me no closer to answer the question, "What are light quanta?" Of course today every rascal thinks he knows the answer, but he is deluding himself."

– Albert Einstein

6.1 Introduction

Light is widely used to fragment peptides in mass spectrometry-based proteomics [162, 280, 43, 281, 282, 283, 284, 285]. Ultraviolet photodissociation (UVPD) is a technique that stands out for its ability to achieve extensive fragmentation and high sequence coverage, making it a powerful tool for top-down proteomics [43, 45, 46]. In a typical UVPD workflow, ionized proteins or peptides are irradiated by a UV laser pulse while held in an ion trap [43]. To maximize signal and sequence coverage, each ion should undergo just one fragmentation event [286, 43], generating an informative pair of N- and C-terminal fragments. Multiple fragmentations of a single precursor generate internal fragments which complicate the analysis and are typically ignored, reducing the total usable signal [286, 287, 288]. Thousands or more copies of the protein or peptide ion of interest typically need to be fragmented to generate a sufficiently detailed fragmentation spectrum to identify the ion [289]. The need for large ensembles of precursor ions makes these UVPD methods fundamentally unsuitable for single-molecule analyses. The aim of analyzing and sequencing single protein molecules has received

growing interest, driven by the need to provide ultimate sensitivity for detecting low-abundance proteins and enabling comprehensive single-cell proteomics [57, 58, 290, 60], but UVPD methods have not yet been applied to the study of single molecules.

We envision analyzing a single peptide by fragmenting it with light in the liquid phase before every fragment is ionized and delivered into a mass spectrometer [58, 180, 246]. Single molecule protein sequencing may be possible if one can preserve the ordering of fragments [237] and faithfully measure every ion. One important element of this strategy is an ion source that delivers ions with minimal loss. We recently reported such an ion source, which uses a nanopore to deliver amino acid and small peptide ions directly into the high vacuum region of a mass spectrometer and transmit them to the detector with near unit efficiency [246]. We also theoretically analyzed the conditions required to preserve the ordering of fragments as they emerge from such a source [237]. A second element of our strategy is a method for using light to cleave peptides into separate amino acids within the ion source. This concept reverses the order of fragmentation and ionization relative to conventional UVPD. Our first step is to identify wavelengths that efficiently induce peptide bond scission in water and quantify the quantum yields of these processes. Ultraviolet (UV) light with a wavelength near 200 nm appears to be the ideal candidate because it is strongly absorbed by peptide bonds [43], it carries enough energy in a single photon to break the peptide bond [44], and it is weakly absorbed by water and quartz [291, 292].

UVPD of peptides in the gas phase has been extensively studied, particularly at 157 nm [293, 294, 295], 193 nm [296, 297, 298], 213 nm [299, 300], and 266 nm [301, 302]. In this wavelength range, UVPD proceeds via a combination of direct dissociation pathways and internal conversion [286], producing a diverse array of fragments. The longest of those wavelengths produces the highest fraction of b- and y-type fragments, which result from cleavage of the peptide bond. With decreasing wavelength (and hence increasing photon energy), one obtains more a- and x- type fragments, which result from cleavage of the backbone C-C $_{\alpha}$ bond, as well as more v- and w-type fragments from side chain cleavages [295, 43, 40]. This is consistent with results from collision induced dissociation (CID) experiments, where b- and y-type fragments are generated at lower energies than other fragments. Near-UV photons with wavelengths of 351 nm and 355 nm carry less energy than the dissociation energy of the peptide bond [44], but these wavelengths have still found use in identifying post-translational modifications (PTMs) like phosphorylation and nitration [303, 304], as well as for fragmenting specific peptides tagged with near-UV chromophores [305, 281].

UV fragmentation of peptides in water (termed photolysis) occurs in a complex chemical environment, making the analysis of photoproducts more challenging compared to gas-phase studies where

mass spectrometry can directly measure the fragmentation products. Investigations by Nikogosyan et al. in the 1990s determined the molar absorption coefficients and quantum yields of scission and decomposition for a wide range of amino acids and di- and tri-peptides at several wavelengths around 200 nm [166, 165, 163, 167, 164]. Around the same time, Hill et al. carried out detailed analyses of the photolysis products of a few dipeptides induced by irradiation with 254 nm light and determined that deamination and decarboxylation were the primary fragmentation pathways [306, 307]. More recent work by Thøgersen et al. used UV pump - IR probe transient absorption spectroscopy to observe the photolysis of gly-gly over 200 ps following absorption of 200 nm photons, and observed CO₂ as the only major photoproduct [168]. Thøgersen et al. concluded that the peptide bonds were “unharmed by deep UV excitation”, however, that finding appears to be in disagreement with the earlier reports by Nikogosyan et al.

Here, we report measurements of the UV photolysis of gly-gly in aqueous solution upon irradiation by broad spectrum UV light, as well as monochromatic 193 nm and 222 nm light. This work seeks to clarify the question of whether single UV photons near 200 nm can break peptide bonds in aqueous solution, as illustrated in Fig. 6.1.1, and to establish whether this is a viable approach for our single molecule peptide analysis scheme. Our approach combines *in situ* UV absorption measurements at the irradiation wavelength with UV-vis spectra sampled following varying irradiation doses. Gly-gly was chosen as a model peptide for these measurements as it is the simplest dipeptide, and the peptide bond is the only major absorber of deep UV light in the molecule. Light with a wavelength of 193 nm was chosen because it is located near the peptide bond absorption peak and is the most common wavelength used in UVPD [308, 43]. Light with a wavelength of 222 nm was also investigated because it has become widely and cheaply available in the form of KrCl excimer lamps following the COVID19 pandemic due to its use in disinfection [309, 310, 311], however the quantum yield of peptide bond scission at 222 nm has not been previously reported. A simple model of absorption and bond scission allows us to extract the quantum yield of peptide bond scission at 193 nm and 222 nm from the *in situ* transmission and offline UV-vis measurements. Additionally we discuss the relevance of these results for our single-molecule protein sequencing scheme, as well as to other fields such as origins of life and UV disinfection.

6.2 Theoretical photodissociation model

We developed a simple physical model that describes the rate of peptide bond scission as a function of the irradiance, peptide concentration, molar absorption coefficient, and the geometry of the

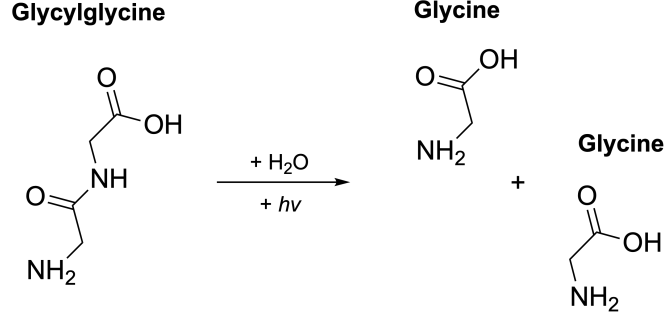


Figure 6.1.1: **UV photolysis scheme.** Reaction scheme for the UV photolysis of gly-gly causing peptide bond scission.

irradiation setup. We start by defining the absorbed light intensity, $I_{\text{abs}} = I_0 - I$, where I_0 is the incident intensity and I is the transmitted intensity, both having units of W/m^2 . Using Beer's law, we find that

$$I_{\text{abs}} = I_0 (1 - 10^{-\epsilon c l}), \quad (6.2.1)$$

where c is the peptide concentration, ϵ is the molar absorption coefficient at the wavelength of the incident light, and l is the path length through the sample. The absorbed intensity is related to the number of absorbed photons, N_{abs} by

$$dN_{\text{abs}} = I_{\text{abs}} A dt / E_\nu, \quad (6.2.2)$$

where Δt is the total irradiation time, A is the illuminated cross sectional area, and E_ν is the photon energy. We then find the change in peptide concentration resulting from peptide bond scission, Δc , by multiplying N_{abs} by the quantum yield of peptide bond scission, Φ_{sc} , and applying the necessary unit conversions,

$$dc = - \frac{dN_{\text{abs}} \Phi_{\text{sc}}}{10^3 V N_A} \quad (6.2.3)$$

$$= - \frac{A \Phi_{\text{sc}} I_0}{10^3 V N_A E_\nu} (1 - 10^{-\epsilon c l}) dt, \quad (6.2.4)$$

Avogadro's number, N_A , and the volume, V , appear in Eq. (6.2.4) to convert from total number to concentration, while the 10^3 converts the units from mol/m^3 to mol/L . This can then be rewritten as an ODE which can be solved numerically,

$$\frac{dc}{dt} = - \frac{A \Phi_{\text{sc}} I_0}{10^3 V N_A E_\nu} (1 - 10^{-\epsilon c(t) l}). \quad (6.2.5)$$

Compound	ϵ_{193} ($\text{M}^{-1}\text{cm}^{-1}$)	σ_{193} (10^{-18} cm^2)	ϵ_{222} ($\text{M}^{-1}\text{cm}^{-1}$)	σ_{222} (10^{-18} cm^2)
Glycine	264 ± 29	1.01 ± 0.11	15 ± 11	0.057 ± 0.042
Gly-gly	7210 ± 830	27.6 ± 3.2	268 ± 31	1.02 ± 0.12

Table 6.1: Molar absorption coefficients (ϵ) and absorption cross sections (σ) for glycine and gly-gly at 193 nm and 222 nm.

The solution to Eq. (6.2.5) is related to the absorbance at any wavelength λ by

$$A_\lambda(t) = \epsilon_\lambda c(t)l. \quad (6.2.6)$$

6.3 UV Absorption of the peptide bond

6.3.1 Mixtures of gly-gly and glycine

I first measured the UV absorption spectra of aqueous mixtures of gly-gly and glycine with concentrations ranging from 1 mM gly-gly and 0 mM glycine to 0 mM gly-gly and 2 mM glycine. This serves two purposes, (1) it simulates the results of an “ideal” gly-gly photolysis experiment, where 100% of the peptide bonds are broken but all other bonds remain intact, (2) it allows us to measure the molar absorption coefficient of the peptide bond itself.

I made aqueous mixtures of gly-gly and glycine in ratios of 100:0, 75:25, 50:50, 25:75, and 0:100 by combining the appropriate volumes of 1 mM gly-gly solution and 2 mM glycine solution. Figure 6.3.1a shows the UV absorption spectrum of each mixture measured using a Nanodrop One UV/vis spectrophotometer. Each curve represents the mean of three repeated measurements, and there is a narrow shaded region which represents the standard deviation of the measurements (the measurements are highly reproducible so this shaded region may be too small to see). The absorption near 200 nm decreases approximately linearly with decreasing gly-gly concentration, which is expected given that the peptide bond is the dominant absorber in this wavelength range.

The molar absorption coefficients of gly-gly and glycine can be obtained from the 1 mM gly-gly and the 2 mM glycine spectra, respectively, using the Beer-Lambert law (Eq. (6.2.6)). The molar absorption coefficient (in $\text{M}^{-1}\text{cm}^{-1}$) can be converted to an absorption cross section (in units of cm^2) using

$$\sigma = \frac{10^3 \ln(10)}{N_A} \epsilon. \quad (6.3.1)$$

Table 6.1 shows the measured ϵ and σ values for glycine and gly-gly at both 193 and 222 nm.

Figure 6.3.1b shows the molar absorption coefficient of the peptide bond, ϵ_{pb} , as a function of

$\epsilon_{pb,193} \text{ (M}^{-1}\text{cm}^{-1}\text{)}$	$\sigma_{pb,193} \text{ (10}^{-18} \text{ cm}^2\text{)}$	$\epsilon_{pb,222} \text{ (M}^{-1}\text{cm}^{-1}\text{)}$	$\sigma_{pb,222} \text{ (10}^{-18} \text{ cm}^2\text{)}$
6680 ± 830	25.6 ± 3.2	238 ± 33	0.91 ± 0.13

Table 6.2: Molar absorption coefficients (ϵ_{pb}) and absorption cross sections (σ_{pb}) for the peptide bond at 193 nm and 222 nm.

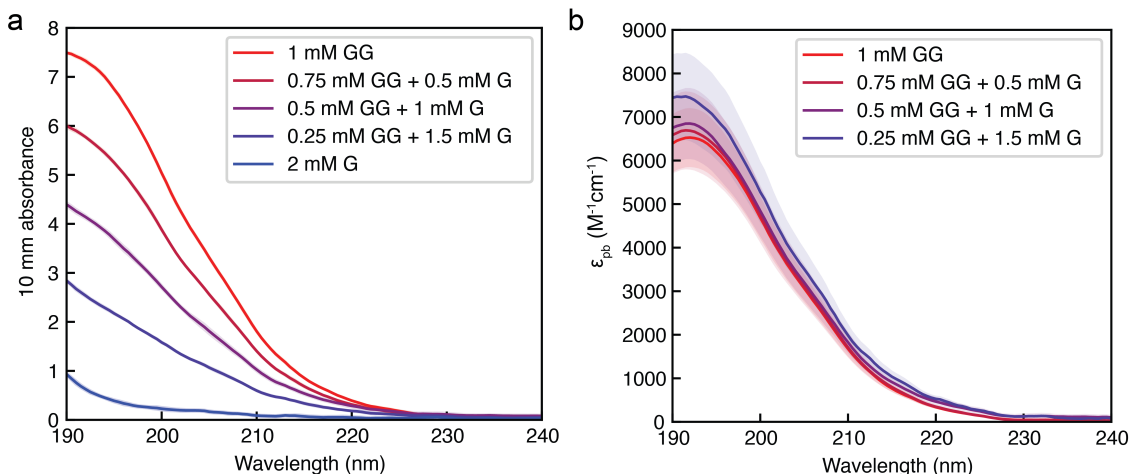


Figure 6.3.1: **Absorption of glycine and gly-gly mixtures.** **a** Absorption spectra of mixtures of gly-gly (GG) and glycine (G). **b** The molar absorption coefficient of the peptide bond, ϵ_{pb} as a function of wavelength for each mixture of gly-gly and glycine.

wavelength. This is calculated using Eq. (6.2.6) by first subtracting the 2 mM glycine absorbance spectrum from the absorbance of the other mixtures to isolate the contribution of the peptide bond, then dividing by the concentration of peptide bonds (concentration of gly-gly) and the path length. Each of the four mixtures constitutes an independent measurement of ϵ_{pb} , and they all agree within the experimental uncertainty. There appears to be a slight bias towards higher ϵ_{pb} values for the lower concentrations of gly-gly, which may be explained by a slight underestimation in the concentration of the gly-gly stock solution. The large uncertainty is due primarily to uncertainty in the concentration of the stock solutions and diluted mixtures, as these were prepared with a 10 μ L micropipette which has close to 10% uncertainty. Table 6.2 summarizes the ϵ_{pb} and σ_{pb} values at 193 nm and 222 nm. At 193 nm, we measure $\epsilon_{pb} = 6680 \pm 830$, in agreement with a previous measurement by Nikogosyan *et al* [166]. The fact that ϵ_{pb} appears highest around 193 nm indicates that this is the optimal wavelength with which to *monitor* the photolysis of gly-gly, as it affords the highest sensitivity to peptide bond scission. However, this does not necessarily imply that 193 nm is an optimal wavelength for *specifically fragmenting* the peptide bond.

6.3.2 Glycine homopolymers

I measured the absorption spectrum of several short $N \times$ gly homopeptide solutions at multiple concentrations, where $N = 1, 2, 3, 4$, & 6 (G, GG, GGG, GGGG, and GGGGGG). This experiment provides another measurement of the absorption coefficient of the peptide bond, as each solution should absorb deep UV light in proportion to the concentration of peptide bonds. Table 6.3 shows the concentration of each solution used in these experiments (c_{actual}) and the uncertainty in each concentration (Δc_{actual}). The uncertainty is calculated from the uncertainties associated with the scale, glassware, and micropipettes used to make the stock solutions and dilutions.

Compound	Molar mass (g/mol)	c_{nominal} (mM)	c_{actual} (mM)	Δc_{actual} (mM)
G	75.07	10	10.0	0.764
G		5	5.00	0.520
G		2	2.00	0.207
GG	132.12	2	2.05	0.214
GG		1	1.03	0.106
GG		0.33	0.342	0.0353
GGG	189.17	1	1.05	0.0951
GGG		0.5	0.526	0.0603
GGG		0.2	0.210	0.0240
GGGG	246.22	1	1.08	0.102
GGGG		0.5	0.540	0.0574
GGGG		0.2	0.216	0.0276
GGGGGGG	360.32	0.05	0.054	0.00296

Table 6.3: Concentrations of $N \times$ gly solutions used in absorption measurements. Uncertainty in concentration is calculated from the uncertainty in concentration of original stock solution and the uncertainty associated with the micropipettes used to make the dilutions.

Figure 6.3.2 shows the measured UV absorbance spectrum of each solution in the left column. Each spectrum shown is the mean of 3 repeated measurements, and the standard deviation of these measurements was typically < 0.05 absorbance units, too small to display clearly on these axes. The right column of Fig. 6.3.2 shows the molar absorption coefficient as a function of wavelength, which was calculated by dividing the absorbance by the concentration and path length. Molar absorption coefficient is an intrinsic property of the solute, so it should be independent of concentration. The measurements at different concentrations are generally in agreement within the experimental uncertainty, with the exception of the highest concentration solutions of GG, GGG, and GGGG below 200 nm, which were close to the detection limit of the spectrophotometer.

Figure 6.3.3a plots molar absorption coefficient spectrum of each $N \times$ gly compound on a single axis. Each spectrum shown is the mean of the spectra presented in Fig. 6.3.2, excluding the highest concentration spectrum of GG, GGG, & GGGG. Table 6.4 summarizes the measured mean molar

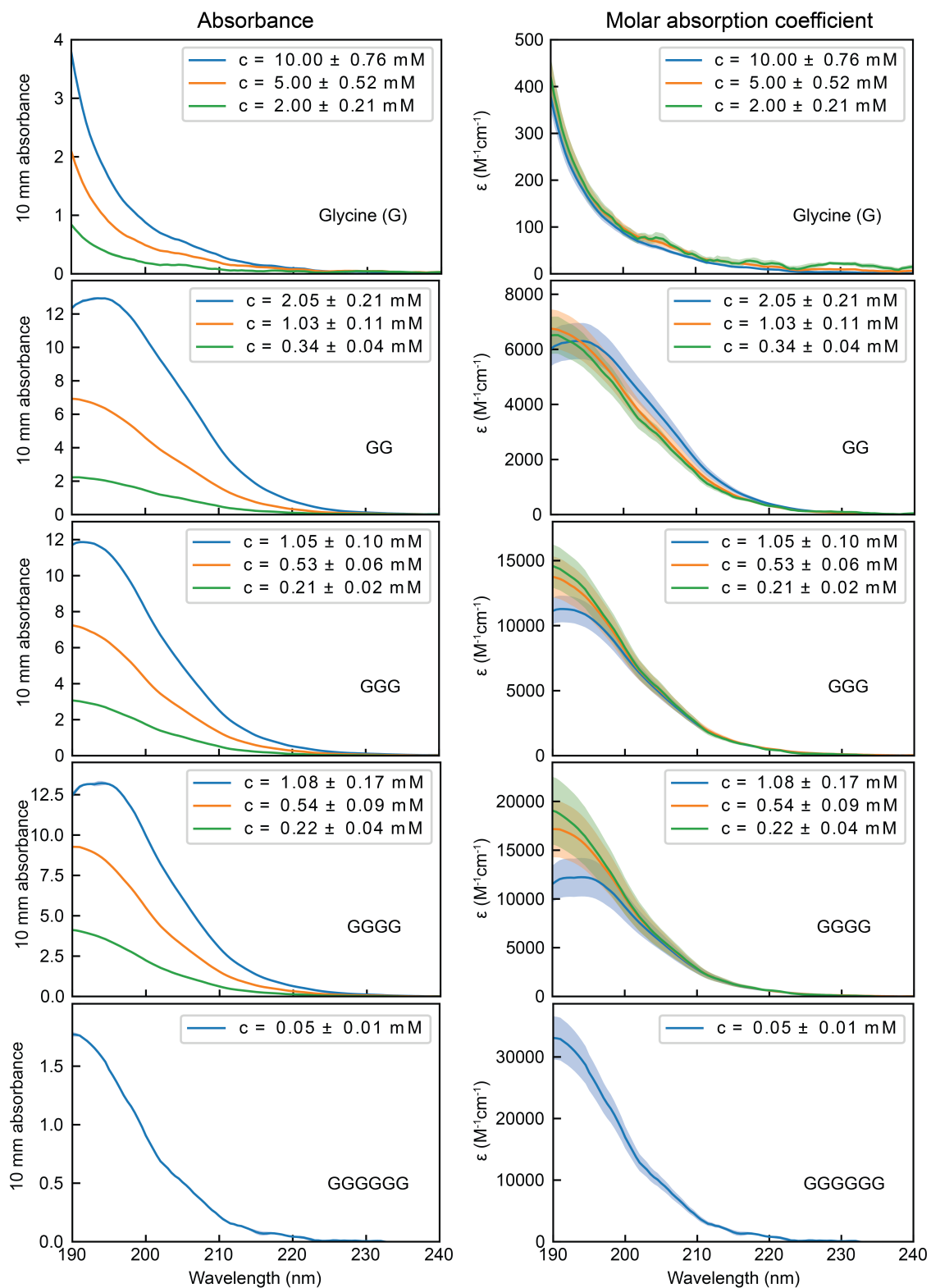


Figure 6.3.2: **N-gly absorbance results.** UV absorbance spectra and calculated molar absorption coefficients for glycine homopeptides.

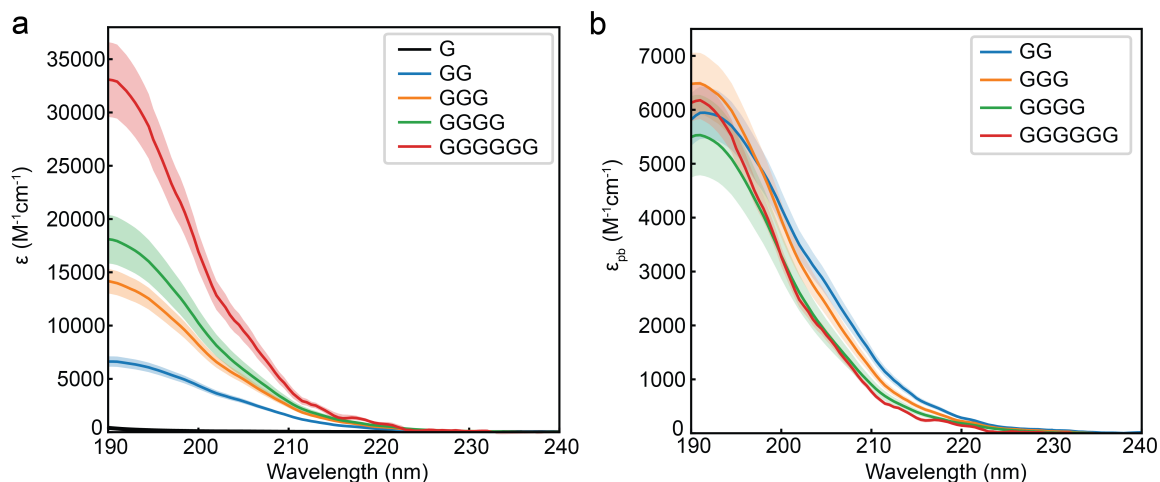


Figure 6.3.3: **Peptide bond absorption from N-gly measurements.** (a) Molar absorption coefficient spectrum of $N \times$ gly homopeptides, where $N = 1, 2, 3, 4,$ & 6 . (b) Molar absorption coefficient of the peptide bond as a function of wavelength.

absorption coefficients and absorption cross sections for glycine and each homopeptide at 193 nm and 222 nm. Figure 6.3.3b shows the molar absorption coefficient of the peptide bond, calculated by subtracting the glycine spectrum off of each N-gly spectrum N times, then dividing by $N-1$, the number of peptide bonds [312, 313]. The results are summarized in Table 6.5, which give the measured molar absorption coefficient and absorption cross section of the peptide bond, ϵ_{pb} & σ_{pb} , at both 193 nm and 222 nm for each glycine homopeptide measured. The values obtained from the four homopeptides are all in agreement with each other within the experimental uncertainty for wavelengths below around 200 nm, and begin to diverge at higher wavelengths. In fact, the measured values for $\epsilon_{pb,222}$ appear to decrease with increasing peptide length. A similar effect has been reported in the literature, but this was attributed to the background electrolyte composition [313], which is not present in our solutions. I suspect that this may be a systematic bias from the UV-vis spectrophotometer, which is not particularly sensitive at the absorbance level measured at 222 nm in these experiments. Regardless of the source of this effect, it further highlights the point that to measure peptide bond concentration, using a measurement wavelength close to 193 nm is far more reliable than one close to 222 nm.

The resulting peptide bond molar absorption coefficient at 193 nm is a bit lower than that found in the experiments with mixtures of gly-gly and glycine from the previous section ($5860 \pm 360 M^{-1}cm^{-1}$ vs. $6700 \pm 600 M^{-1}cm^{-1}$), but still agrees within the uncertainty. These measurements are a bit lower than that of Nikogosyan et al., who reported a value of $\epsilon_{pb,193} = 6980 \pm 220$, although the first measurement agrees within uncertainty. Taken together, these experiments show beyond reasonable

Compound	ϵ_{193} ($\text{M}^{-1}\text{cm}^{-1}$)	σ_{193} (10^{-18} cm^2)	ϵ_{222} ($\text{M}^{-1}\text{cm}^{-1}$)	σ_{222} (10^{-18} cm^2)
Glycine	236 ± 13	0.90 ± 0.05	13.0 ± 1.4	0.050 ± 0.005
GG	6360 ± 460	24.3 ± 1.8	233 ± 22	0.89 ± 0.08
GGG	13300 ± 1100	50.7 ± 4.1	363 ± 30	1.39 ± 0.11
GGGG	17000 ± 2100	65 ± 8	444 ± 56	1.70 ± 0.21
GGGGGG	30900 ± 3300	118 ± 13	500 ± 240	1.9 ± 0.9

Table 6.4: Molar absorption coefficients (ϵ) and absorption cross sections (σ) for glycine, gly-gly (GG), GGG, GGGG, and GGGGGG at 193 nm and 222 nm.

Compound	$\epsilon_{pb,193}$ ($\text{M}^{-1}\text{cm}^{-1}$)	$\sigma_{pb,193}$ (10^{-18} cm^2)	$\epsilon_{pb,222}$ ($\text{M}^{-1}\text{cm}^{-1}$)	$\sigma_{pb,222}$ (10^{-18} cm^2)
GG	5890 ± 465	22.5 ± 1.8	207 ± 24	0.79 ± 0.09
GGG	6280 ± 540	24.0 ± 2.1	162 ± 16	0.62 ± 0.06
GGGG	5360 ± 706	20.5 ± 2.7	130 ± 19	0.50 ± 0.07
GGGGGG	5900 ± 325	22.5 ± 1.2	85 ± 24	0.32 ± 0.09
Mean	5860 ± 360	22.4 ± 1.4	146 ± 45	0.56 ± 0.17

Table 6.5: Molar absorption coefficient (ϵ) and absorption cross section (σ) of the peptide bond at 193nm and 222nm, obtained from UV-vis measurements of glycine homopeptides.

doubt that deep UV absorption of a glycine homopolymer solution is a reliable measure of the concentration of peptide bonds.

6.4 Broad spectrum irradiation

As a first test of feasibility, we irradiated a 5 mM aqueous gly-gly solution with a broad-spectrum light source (Energetiq EQ-99) to see if any fragmentation would occur and produce a measurable change in the absorption spectrum. The light source uses a focused laser to ignite a Xenon plasma, producing broad spectrum radiation from 170 nm to 2500 nm with a radiance of about $10 \text{ mW mm}^{-2} \text{ nm}^{-1} \text{ sr}^{-1}$ over its full wavelength range. A refocusing system consisting of two off-axis parabolic mirrors focused the output of the light source into $0.5 \text{ cm} \times 1.0 \text{ cm}$ spot with a focal distance of about 23 cm. The light source irradiated 0.25 mL 5 mM solution of gly-gly in DI water for 90 hours. We measured the UV-vis absorption spectrum before and after irradiation and each measurement was repeated 5 times to estimate the uncertainty. The absorption spectrum of the irradiated gly-gly solution was corrected for the evaporation of solvent by measuring the volume before and after irradiation and scaling the concentration accordingly.

Figure 6.4.1 shows the change in the UV absorbance spectrum for 5 mM gly-gly. After 90 hours of irradiation, the absorption at 193 nm fell by a factor of about 15. Figure 6.4.1 also shows the UV-vis spectrum of a 10 mM aqueous solution of glycine to compare the irradiated spectrum with the “ideal” spectrum which would result if all peptide bonds were broken, forming two glycines for

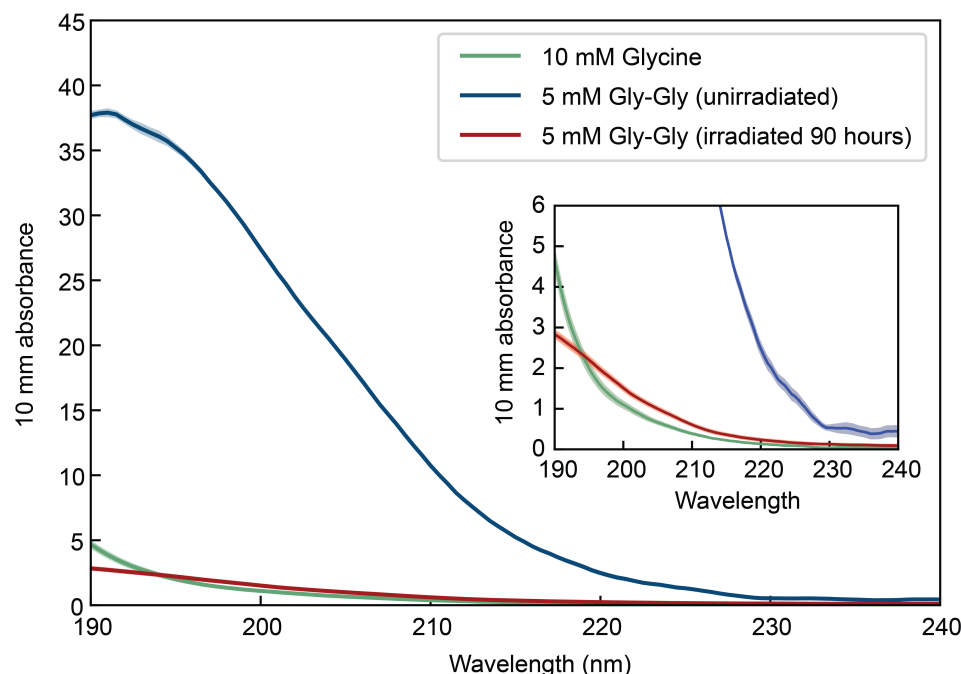


Figure 6.4.1: **Broad spectrum irradiation of gly-gly.** UV absorption spectrum of 5 mmol L⁻¹ aqueous gly-gly before and after 90 hours of irradiation with a broad spectrum light source. The measured spectrum of unirradiated 10 mmol L⁻¹ glycine is also shown for comparison. The shaded areas represent the standard deviation of repeated measurements. The inset shows the same plot with a smaller y-range, providing a more detailed view of the irradiated gly-gly and unirradiated glycine spectra.

every gly-gly, with no other light-induced chemical changes. The glycine spectrum is very similar to that of the irradiated gly-gly, suggesting that the majority of peptide bonds holding together the dipeptides were broken. This result is interesting in and of itself, as it demonstrates that the peptide bond is indeed fragmented following irradiation by a broad spectrum UV light source. However, one cannot make any conclusions about which wavelength is primarily responsible for the bond scission from this experiment alone, due to the broad spectrum emission of the light source.

6.5 Photolysis of the peptide bond using 193 nm light

We now turn our attention to monochromatic UV irradiation, beginning with 193 nm light. Light with 193 nm wavelength is near the absorption peak of the peptide bond and is commonly used in UVPD, so this wavelength is a good candidate for peptide photolysis. We measured the photolysis of gly-gly under low intensity, 193 nm laser irradiation. Additionally, the quantum yield of scission of gly-gly at 193 nm has been previously reported by Nikogosyan *et al* (albeit measured by a somewhat different technique), providing a point of comparison for our results [166].

Figure 6.5.1a shows our setup for irradiating and monitoring the scission of peptide bonds by 193 nm laser light *in situ*. A Ti:Sapphire laser (Chameleon Ultra II, Coherent) generated tunable IR light in 140 fs pulses at an 80 MHz repetition rate with a total output power of around 3.5 W. The IR beam is directed through a Faraday isolator to prevent back reflections and then into a harmonics module (APE Harmonixx FHG 3+1) which generates the fourth harmonic of the fundamental frequency. We tuned the IR laser to 772 nm so that the harmonics module would output a 193 nm beam. The power is significantly reduced with each harmonic generated, resulting in a typical output power of around 0.5 mW at 193 nm. The UV beam is directed into an acrylic enclosure that is continuously purged with N₂ to remove oxygen, which absorbs strongly at 193 nm. The beam passes through an aperture and is then directed towards a beam splitter using two UV mirrors. The splitter directs about 20% of the beam towards a reference power meter to track the laser power, which may drift over the duration of an experiment. The remainder of the beam passes through a 1 cm path length quartz cuvette (Spectrocell R-3010-S) which contains the sample solution. The sample is continuously stirred with a magnetic stir bar which is kept near the edge of the cuvette to avoid interfering with the beam. A second power meter measures the transmission of 193 nm light through the sample.

Figure 2.5.3 shows our setup for irradiating and monitoring the scission of peptide bonds by 193 nm laser light *in situ*. 193 nm laser light irradiated an aqueous solutions of 100 $\mu\text{mol L}^{-1}$ gly-gly and an aqueous solution of 200 $\mu\text{mol L}^{-1}$ glycine, both at pH 6.5, at an average power of 370 μW for one hour each. The transmission was measured continuously *in situ*. Every 10 minutes, the top of the purged enclosure was briefly opened, and a small aliquot of the solution was sampled from the cuvette using a micropipette to measure the full UV-vis in a separate spectrophotometer (Nanodrop ONE). Fig. 6.5.1b shows the evolution of the UV absorption spectrum of 100 μM gly-gly (pH 6.5) over one hour of irradiation with 193 nm pulsed laser light. There is an approximately 30% decrease in absorption at 193 nm Fig. 6.5.1c plots the measured absorbance at 193 nm from both the *in situ* transmission measurement and the UV-vis spectra as a function of irradiation time. The plot additionally shows the absorbance at 193 nm for a 200 $\mu\text{mol L}^{-1}$ solution of glycine measured by the same methods. Both the *in situ* transmission and offline UV-vis methods yield similar results, and agree that the absorption of gly-gly at 193 nm falls by about 30 % over 1 h of 193 nm irradiation while the glycine sample's absorbance remains unchanged.

We irradiated an aqueous solutions of 100 μM gly-gly and 200 μM glycine, both at pH 6.5, with 193 nm laser light at an average power of 370 μW for one hour. The transmission was measured continuously and a full UV-vis spectrum of the sample was recorded every 10 minutes in a separate spectrophotometer (Nanodrop ONE). Figure 6.5.1b shows the evolution of the UV absorption

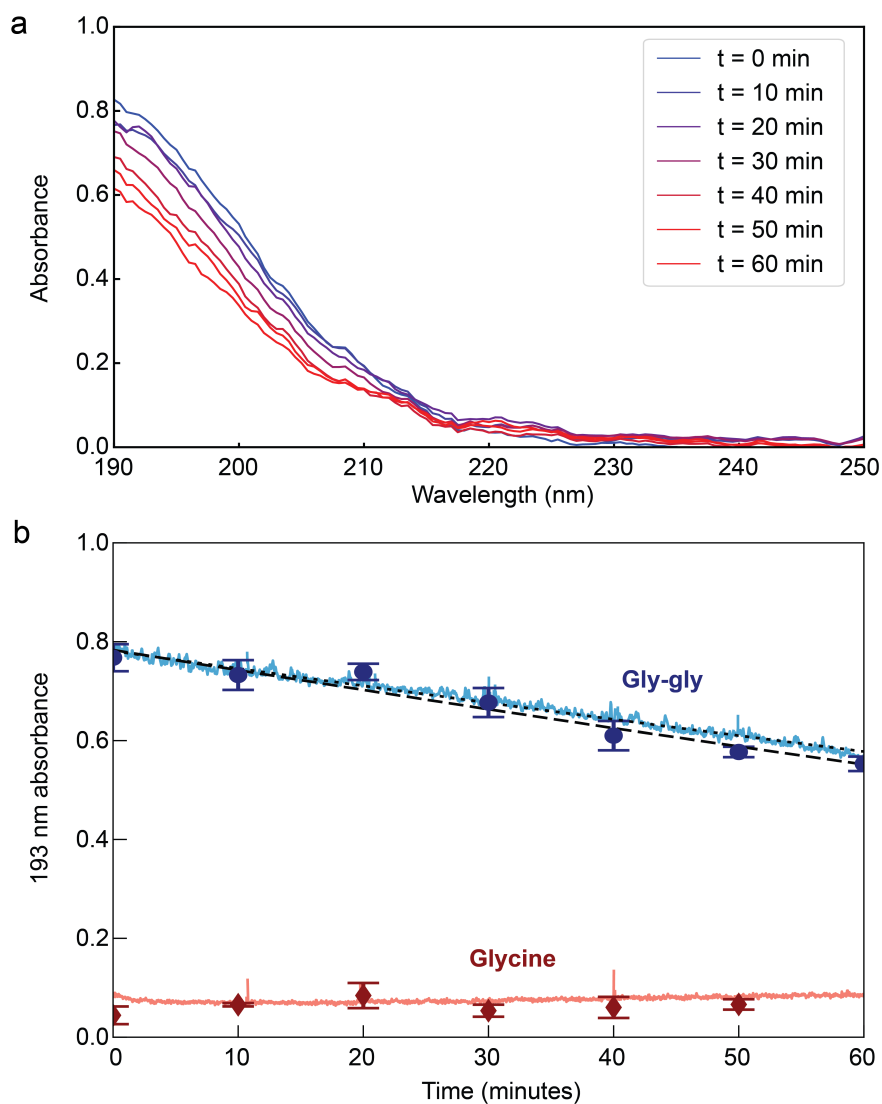


Figure 6.5.1: **193 nm irradiation results.** **1** UV-vis spectra of 200 μM gly-gly solutions at pH 6.5 collected at 10 minute intervals during irradiation with 193 nm pulsed laser light. **b** Absorbance of gly-gly (blue) and glycine (red) at 193 nm measured both *in situ* during irradiation (lighter solid lines), and from the UV-vis spectrum (circles). The quantum yield of scission is determined from the fits to both gly-gly measurements (black dotted line for *in situ*, black dashed line for UV-vis).

spectrum of 100 μM gly-gly (pH 6.5) over one hour of irradiation with 193 nm pulsed laser light. There is an approximately 30% decrease in absorption at 193 nm Figure 6.5.1c plots the measured absorbance at 193 nm from both the *in situ* transmission measurement and the UV-vis spectra as a function of irradiation time. The plot additionally shows the absorbance at 193 nm for a 200 μM solution of glycine measured by the same methods. Both the *in situ* transmission and offline UV-vis methods yield similar results, and agree that the absorption of gly-gly at 193 nm falls by about 30% over one hour of 193 nm irradiation while the glycine sample's absorbance remains unchanged.

We define the quantum yield of peptide bond scission, Φ_{sc} , as the ratio of the number of peptide bonds broken to the number of UV photons absorbed. In practice, we find Φ_{sc} by fitting Eq. (6.2.5) to the gly-gly absorbance data shown in Fig. 6.5.1c with both Φ_{sc} and the initial gly-gly concentration, c_0 , as fitting parameters. The fitting function requires the molar absorption coefficients of gly-gly and glycine at 193 nm as inputs, the values given in Table 6.1 were used. The *in situ* measurement gives $\Phi_{\text{sc}} = 1.51 \pm 0.23$ % and $c_0 = 108 \pm 13$ μM and the UV-vis measurement gives $\Phi_{\text{sc}} = 1.71 \pm 0.23$ % and $c_0 = 109 \pm 13$ μM . The two independent measurements agree within the experimental uncertainty, giving a mean value of 1.61 % with a combined uncertainty of 0.16 %. This value for the quantum yield of scission is lower than that reported by Nikogosyan *et al* by about 38 % [166]. In that work, the quantum yield of scission was determined by measuring absorption at 210 nm at only two time-points, before and after irradiation. In contrast, we determined the quantum yield of peptide bond scission by two independent measurements with multiple intermediate time points of the absorption at 193 nm, a far more sensitive wavelength for measuring the presence of peptide bonds.

6.6 Photolysis of the peptide bond using 222 nm light

We next investigated the photolysis of gly-gly following 222 nm irradiation. A 222 nm photon carries less energy than a 193 nm photon (5.6 eV compared to 6.4 eV), so it may be expected to have a lower quantum yield of bond scission as the reduced excess energy may be more easily dissipated by the molecule and surrounding solvent. At the same time, the lower energy of 222 nm photons may result in more specific peptide bond cleavage rather than inducing other chemical changes. It is additionally important to investigate the effect of 222 nm irradiation on peptides as this wavelength has found widespread use in disinfection lamps following the COVID19 pandemic.

Figure 2.5.1 shows a schematic of the experiment in which we irradiated samples with 222 nm light. A 222 nm KrCl excimer lamp irradiated 3 mL of aqueous 1 mM gly-gly (pH 6.5) at an irradiance of about 3.5 mW cm⁻² while periodically withdrawing and freezing 50 μL samples for later UV-vis spectroscopy. Fig. 6.6.1b shows the evolution of the UV absorption spectrum of the gly-gly solution over 63 hours of irradiation. The spectra show a monotonic decrease in absorption between 190 nm to 220 nm upon irradiation, similar to the changes seen upon irradiation with 193 nm light. It is interesting to note that at the irradiation wavelength of 222 nm, the absorption has a more complex behavior, as shown in Fig. 6.6.1c. The non-monotonicity may be due to other chemical changes besides peptide bond scission. 222 nm absorbance is therefore a poor choice for *monitoring* peptide fragmentation. Because we measured the full UV-vis spectrum at multiple times

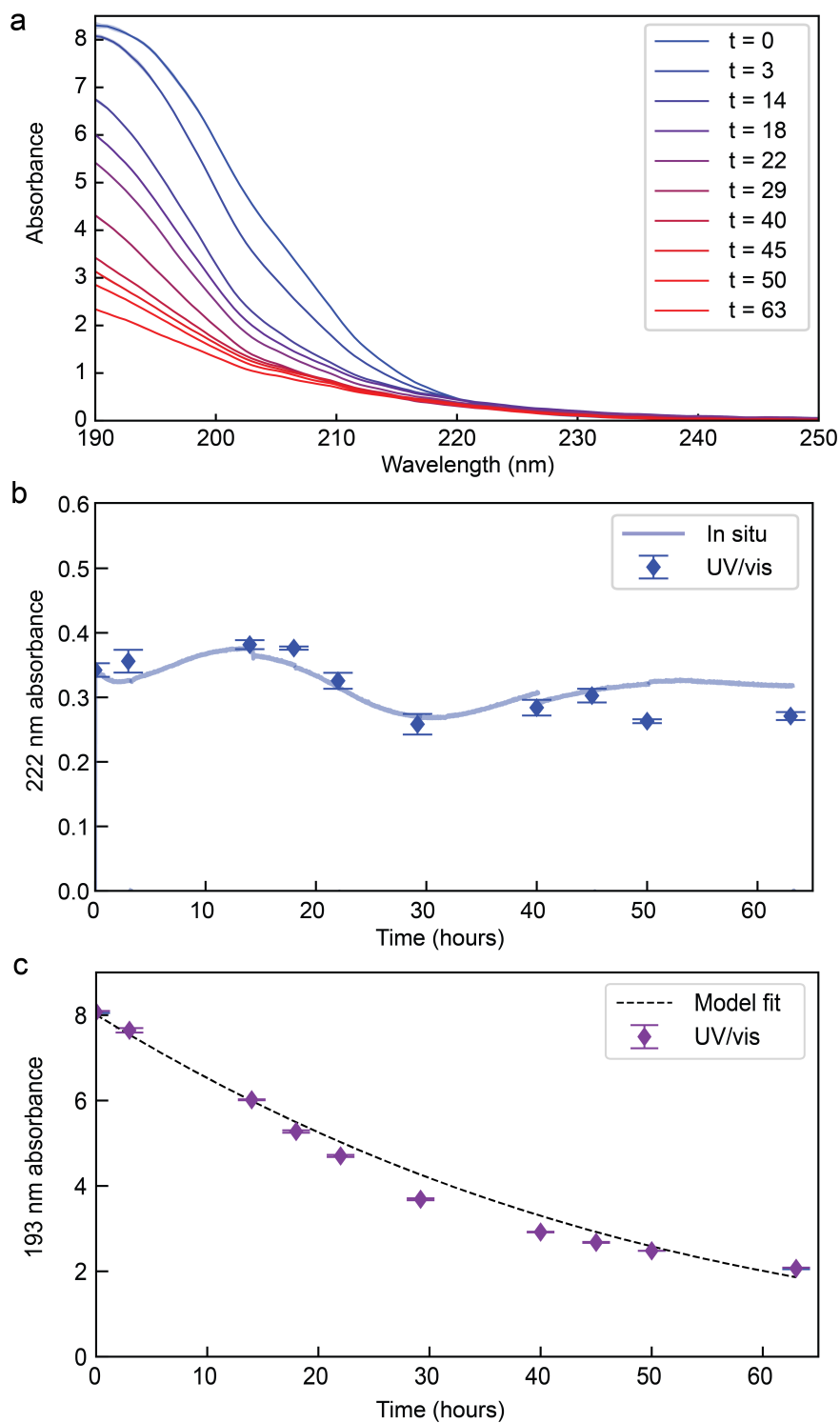


Figure 6.6.1: **222 nm irradiation results.** **a** UV spectra of 1 mM gly-gly at pH 6.5 collected following various durations (in hours) of irradiation with 222 nm light. **b** Absorbance of gly-gly at 222 nm from both the *in situ* and UV/vis measurements. **c** Absorbance of gly-gly at 193 nm from the UV/vis measurements and the model fit used to find the quantum yield.

during irradiation, we can extract the absorbance at 193 nm vs time (like in Fig. 6.5.1c) despite this not being the irradiation wavelength. Fig. 6.6.1d plots the measured absorbance at 193 nm from the UV-vis spectra as a function of time. These measured absorbance values are fit to Eq. (6.2.5) with the initial gly-gly concentration, c_0 , and the quantum yield of peptide bond scission, Φ_{sc} , as free parameters. In the case of 222 nm, the fitting function requires the measured molar absorption coefficients of gly-gly and glycine at *both* 193 nm and 222 nm; we use the values given in Table 6.1. The fit yields $\Phi_{sc} = 0.18 \% \pm 0.02 \%$ at 222 nm, approximately 1 order of magnitude smaller than the quantum yield found for 193 nm above. Despite the low quantum yield of scission, these results still demonstrate unambiguously that peptide bonds can be broken in solution by 222 nm light.

6.7 Implications for single molecule peptide sequencing

Knowledge of the quantum yield of peptide bond scission allows us to estimate the experimental requirements to efficiently fragment peptides for single molecule analysis. Combining the absorption and scission processes yields the average photodissociation rate for a particular species, $J\sigma_i\Phi_i$, where J is the photon flux ($\text{m}^{-2}\text{s}^{-1}$) and σ is the absorption cross section (m^2). The cumulative probability that a particular peptide bond has been broken following t seconds of irradiation is then given by

$$P(t) = 1 - e^{-J\sigma\Phi t}. \quad (6.7.1)$$

Fig. 6.7.1 shows a plot of $P(t)$ for four different incident power densities at 193 nm (Fig. 6.7.1a) and 222 nm (Fig. 6.7.1b). The plots were generated using the following values: $\Phi_{193} = 1.6 \%$, $\sigma_{193} = 2.75 \times 10^{-17} \text{ cm}^2$, $\Phi_{222} = 0.18 \%$, and $\sigma_{222} = 1.01 \times 10^{-18} \text{ cm}^2$. 193 nm light can break peptide bonds more than two orders of magnitude faster than 222 nm light at the same incident power density. This is due to its 25-fold higher absorption coefficient and 10-fold higher quantum yield of peptide bond scission. Commercially available 193 nm ArF excimer lasers which are widely used in UVPD experiments can reach time-averaged power densities on the order of 10^5 W m^{-2} - 10^6 W m^{-2} [314, 315, 316], so efficient cleavage of peptides within the ion source should be achievable on a timescale of 10s of milliseconds. 193 nm is a better choice of wavelength if high throughput is desired, as it breaks peptide bonds with markedly higher efficiency than 222 nm. Additionally, 222 nm light sources tend to have lower power densities [317, 318].

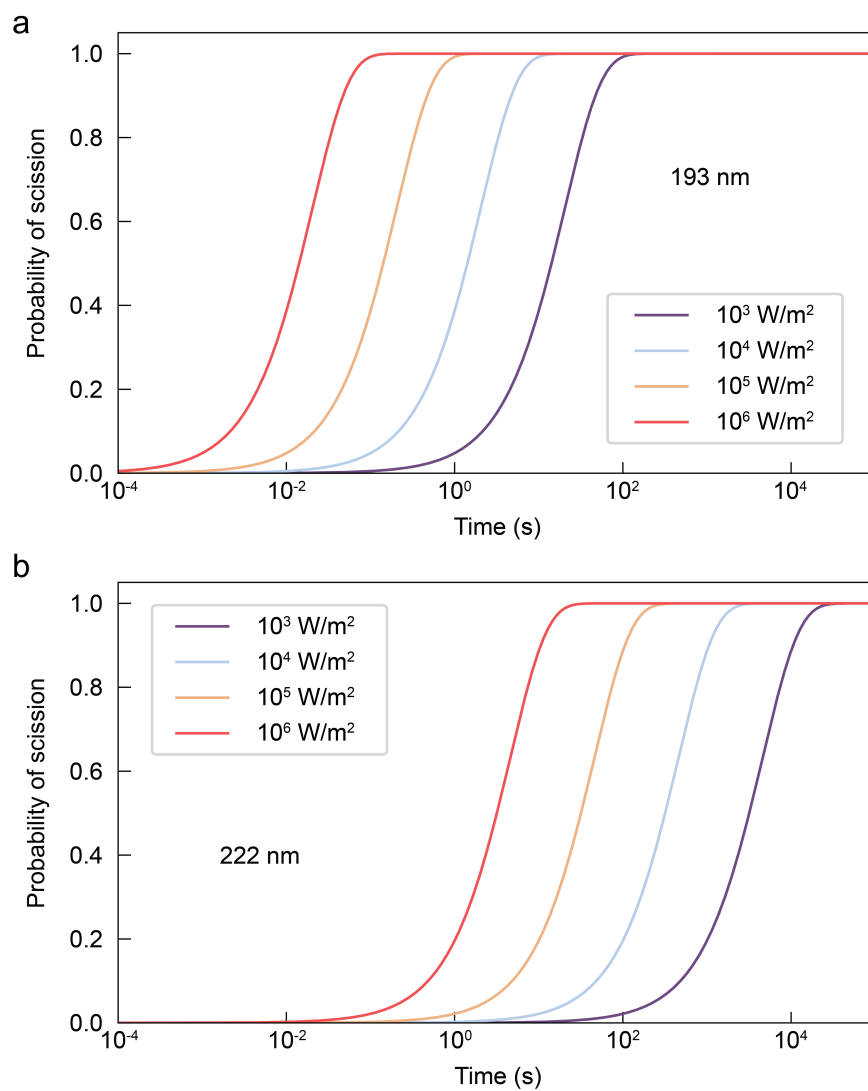


Figure 6.7.1: **Power requirements for peptide bond scission.** Plot of the probability of peptide bond scission as a function of time for different irradiances at 193 nm (a) and 222 nm (b).

6.7.1 Broader implications

The susceptibility of the peptide bond to UV photolysis has broader implications for fields such as origins-of-life and UV disinfection. For the first life to develop, the organic molecules involved in prebiotic chemistry had to persist long enough to interact and form more complex structures. UV photolysis of the peptide bond limits the expected lifetime of small peptides under early Earth conditions. Models of the solar photon flux incident at Earth’s surface 3.9 billion years ago indicate that approximately 10^{13} photons per second would strike each cm^2 of the Earth’s surface in the wavelength range of 200 nm - 230 nm [319]. Lower wavelengths would have been effectively screened by atmospheric H_2O and CO_2 . Prebiotic freshwater bodies were largely transparent to UV with wavelength greater than 200 nm [320]. Using Eq. 6.7.1 and an estimated quantum yield of 0.1 % - 1 % for this wavelength range, we estimate that the lifetime of a peptide bond on the surface of the early Earth is on the order of 10^6 s, or several days. This is far faster than the uncatalyzed hydrolysis of peptides, which occurs on a timescale of hundreds of years [321]. The UV-induced degradation of peptides appears to be underappreciated in the origin-of-life field, as it goes unmentioned in major reviews of prebiotic peptide chemistry [322, 323].

Additionally, the application of 222 nm UV light in disinfection processes should carefully consider the potential for protein damage. Studies of the germicidal effect of 222 nm irradiation typically focus on damage to nucleic acids [311, 324], and proteins are typically thought to provide protection by absorbing the light before it reaches the DNA in the nucleus [309, 310, 325, 324]. Damage to those same proteins is typically not considered in great detail, and may warrant further consideration in future studies of the germicidal properties and human safety profile of 222 nm light.

6.8 Conclusions

This study has demonstrated the feasibility of using ultraviolet (UV) light at 193 nm and 222 nm to induce peptide bond scission in aqueous solutions. Through a combination of in situ transmission measurements and offline UV-vis spectroscopy, we have shown that gly-gly can be fragmented by UV light, with single photon quantum yields of approximately 1.6 % at 193 nm and 0.18 % at 222 nm. These findings support the feasibility of our single-molecule protein sequencing approach. Additionally, power considerations favor the use of 193 nm over 222 nm to ensure rapid efficient bond cleavage. Future work that combines UV photolysis with mass spectrometry could shed additional light on the specific photo-chemical pathways involved and the products generated. Ensuring that

amino acid side chains remain largely intact while peptide bonds are cleaved will be essential for integrating UV photolysis into a single molecule peptide analysis MS workflow.

Conclusions and Outlook

"Hofstadter's Law: It always takes longer than you expect, even when you take into account Hofstadter's Law."

– Douglas Hofstadter

7.1 Summary

This thesis set out to address fundamental limitations in electrospray ionization for the purpose of improving the sensitivity of MS-based proteomics. Based on the established physics underlying ESI, we proposed the nanopore ion source as the best way to overcome these limitations. We also laid out a vision for single molecule protein sequencing by nanopore mass spectrometry, which requires a method to fragment peptides within a nanopore ion source prior to emission.

We developed and characterized the nanopore ion source, which features a quartz emitter with tip radius < 100 nm operating under high vacuum. Using quadrupole mass spectrometry, magnetic sector mass spectrometry, and sensitive current measurements, we demonstrated that the nanopore ion source can emit ions from aqueous solution directly into high vacuum. In particular, we demonstrated direct emission of amino acid and small peptide ions with and without post-translational modifications, precisely the kinds of ions that are central to MS-based proteomics. These ions are transmitted into the vacuum with near unity efficiency, approximately two orders of magnitude higher than conventional nanoESI. Multiple lines of evidence lead us to conclude that the nanopore ion source can operate in the pure ion emission regime, in which no charged droplets are emitted and all of the emission current leaves the electrified meniscus in the form of evaporating ions. We also

developed theoretical models to describe the basic thermal and electrical properties of the nanopore ion source. The models capture several critical features of the ion source’s behavior, most notably that water can remain in a liquid state at the nanoscale emitter tip despite being exposed to high vacuum, and that the emission current increases exponentially with voltage rather than having a sudden onset like conventional ESI sources. Interestingly, our IV model predicts that for nanoscale emitters, significant ion currents can be generated at voltages well below those required for Taylor cone formation. Finally, we presented measurements of the absorbance and quantum yield of peptide bond scission for the model dipeptide gly-gly in aqueous solution at 193 nm and 222 nm, providing critical information for the implementation of our envisioned single molecule protein sequencing approach.

7.2 Prospects for mass spectrometry

The ability to emit a stable current of ions directly from an electrified water/vacuum interface has not, to our knowledge, been previously demonstrated. This type of ion emission has been previously observed only in liquid metals, ionic liquids, and high concentration electrolyte solutions in low-volatility solvents like glycerol and formamide, all of which can have high conductivity and can form a stable interface with vacuum without freezing [230, 114, 155, 154, 151]. Ion sources which can generate purely ionic emission in vacuum have found widespread use in focused ion beam and space propulsion applications, but so far have seen limited use in mass spectrometry. Past attempts at achieving direct ion emission from aqueous solutions have suffered from issues related to freezing and electrical arcing [249]. The nanopore ion source is able to avoid all of these issues thanks to its size. The sharpness of the emitter tip enhances the local electric field, enabling ion evaporation at modest voltages, preventing electrical arcing, and the small area of the water/vacuum interface prevents evaporative cooling from leading to freezing. This central insight, that nanoscale emitters enable direct ion transfer from volatile solutions into vacuum, opens up new opportunities in mass spectrometry.

The nanopore ion source addresses the central limitation of ESI, its poor ion transmission efficiency. In ESI, a background gas is required to provide the thermal energy to desolvate droplets and liberate ions. This background gas is the source of ESI’s significant sample loss, as ions must enter the MS through a long and narrow capillary which reduces the gas load in the mass spectrometer [326]. The majority of ions are then lost to collisions with the walls of this transfer capillary. When ions are instead emitted directly into high vacuum, in which the mean free path of a small molecular

ion is on the order of 100 m or higher, there is nothing to obstruct the ions' path to the mass analyzer and detector. This makes achieving near unity ion transmission efficiency very simple, since the hard part is getting the ions into the vacuum in the first place. Direct ionization in vacuum also has the added benefit of drastically reducing the gas load and simplifying the mass spectrometer's pumping requirements.

The high ion transmission efficiency of the nanopore ion source holds particular promise for the burgeoning field of single cell proteomics. Current single-cell proteomics techniques are limited by their ability to detect low-abundance proteins, which are often key regulators of cellular function [327]. The nanopore ion source's near-unity transmission efficiency removes a major bottleneck for sample loss in the MS-based proteomics workflow, which could improve the detection limits for these low-abundance proteins by as much as two orders of magnitude. Along with advances in sample prep and MS measurement techniques that minimize sample loss [328, 51], the nanopore ion source may form part of an eventual workflow that is capable of truly comprehensive quantitation of the cellular proteome.

7.3 Prospects for single molecule protein sequencing

The nanopore ion source solves one of the primary technical challenges faced by our envisioned single molecule protein sequencing approach. Recall that we proposed sequencing individual proteins or peptides by first denaturing (unfolding) them within a nanopore ion source, breaking the peptide bonds between each amino acid, and then delivering the constituent amino acids directly into a mass spectrometer for identification (Fig. 1.1.4). The ability to deliver amino acid ions directly into a mass spectrometer with high efficiency and in a manner that preserves their order is foundational to the feasibility of this approach. Previous work from our group has demonstrated that electric field gradients within the ion source can prevent amino acid ions from diffusing passed one another if they are broken apart close enough to the meniscus [237]. In Ch. 3, we showed that the probability of an ion experiencing a collision following emission is on the order of 2%. Taken together, this implies that it should be possible to reconstruct the amino acid sequence from the time of arrival of each ion at the detector.

The next major technical challenge is breaking the peptide bonds between each amino acid within the ion source just prior to emission. Photolysis seems to us to be the most straightforward fragmentation strategy for this purpose. UV light with wavelength around 200 nm in particular appears ideally suited, as it can penetrate the water and quartz that comprise the emitter with

minimal absorption, it is strongly absorbed by the amide groups of the peptide backbone, and a single photon carries enough energy to break peptide bonds. A small difference in wavelength can have a very large effect on photochemical processes [329], so with limited and conflicting literature, we sought to determine the optimal wavelength for UV photolysis of peptides. We found that the quantum yield of scission for the peptide bond in gly-gly is approximately 1.6% at 193 nm and 0.18% at 222 nm. This order of magnitude difference in quantum yield, in combination with the dramatic difference in absorption cross section and wider availability of high intensity light sources, makes 193 nm a far more appealing choice of wavelength. While wavelengths below 193 nm have found use in UVPD-MS [43], strong absorption by water and quartz make these wavelengths unsuitable for in-source fragmentation.

7.4 Other applications

Beyond proteomics, the nanopore ion source may be impactful in a number of applications that have yet to be explored. In the field of ion propulsion for small spacecraft, for example, the nanopore ion source could enable the use of water as a propellant, presenting an abundant and more easily stored alternative to rare noble gases and ionic liquids [151, 239]. Nanofabrication and thin film deposition are widespread throughout semiconductor manufacturing; these applications might benefit from the ability to emit controlled ion beams for deposition and surface modification with a wide range of potential molecular species. Atom probe tomography (APT) is an analytical technique that provides three-dimensional compositional mapping at near-atomic resolution by ionizing surface atoms and projecting them onto a detector [240]. The nanopore ion source may be useful to the APT field by offering a stable liquid/vacuum interface, extending the technique to study aqueous solutions and dynamic processes within liquids. As explained in Ch. 5, the nanopore ion source acts as an ideal ion exchange membrane, only allowing the emission of ions of a particular polarity. This could open up new avenues of research in fuel cells and other energy conversion technologies, in which ion exchange membranes are essential for ion transport but often face limitations in efficiency and selectivity [330]. This is not an exhaustive list and is limited primarily by the breadth of the author’s knowledge and imagination, but it illustrates the enormous potential of the nanopore ion source in opening new avenues of research in diverse fields.

7.5 Limitations and suggestions for future work

As with any new technology, the first iteration of the nanopore ion source has a number of apparent limitations that remain to be fully explored and properly addressed. Currently, the main bottleneck when working with nanopore ion sources is reproducibility. In our hands, approximately 40% of the nanopore ion source emitters we test are able to generate stable ion emission from aqueous solutions in vacuum. That number jumps to $> 90\%$ when working with formamide solutions. We believe the variation in performance is largely due to differences in wetting at the emitter tip, as discussed in Ch. 5. Both under- and over-wetting of the tip will tend to increase the area of the meniscus, which will increase the likelihood of both droplet emission and freezing (more so in water than formamide). Approaches to increase the hydrophobicity of the emitter's exterior surface while keeping the interior surface hydrophilic should be explored. This may be achieved through a two-step process: first, plasma treating the entire emitter to make both surfaces hydrophilic, then immediately silanizing the exterior surface while maintaining high pressure inside the emitter to prevent silane vapor from reaching the interior surface.

The question about the nanopore ion source that I've been asked by MS practitioners more than any other is "how easily does it clog?". The answer is "not as easily as you might expect." We have been able to operate a single nanopore ion source for hundreds of hours in total without clogging while using 100 mM aqueous amino acid solutions. However, we have not yet tested the ion source with complex mixtures or solutions containing whole proteins which are likely to be much stickier and prone to clogging. While some ion sources do abruptly stop emitting, it is difficult to determine the cause due to their size and the minuscule flows involved. We reason that the nanopore ion source is less susceptible to clogging than would be expected from naively extrapolating the behavior of ESI sources since there is very little bulk fluid flow in the first place. Ions reach the meniscus by diffusion and electromigration more so than advection. Further research is required to understand the extent of clogging within nanopore ion sources, especially as it pertains to the complex mixtures of peptides and proteins used routinely in MS-based proteomics workflows.

Future work should also investigate the lower limits of solute concentration and conductivity at which stable ion emission from the nanopore ion source can be sustained. We have worked mostly with high concentration solutions (1 mM - 1 M) as they have tended more reliably generate ion emission and quickly interpretable spectra. Additionally, all available evidence suggests that higher conductivities are desirable for purely ionic emission [114, 148]. Even in the case that there is some minimum ion concentration or conductivity necessary to maintain emission, this does not

necessarily imply that analytes below this concentration limit would not be detectable. Any species with charge of the correct polarity should be ionizable and measurable by MS as long as the *total* ion concentration is above that minimum concentration. While salt is typically avoided in ESI due to the formation of salt adducts which complicate mass spectra [331], this does not seem to be an issue in the nanopore ion source. Therefore, one can imagine measuring an analyte ion at very low concentrations by doping the solution with some salt to maintain the minimum ion concentration or conductivity necessary for sustained emission; while most of the emitted ions will be salts, the analyte of interest should still be expected to make an appearance.

Those familiar with ESI will have likely noted that the currents generated by the nanopore ion source tend to be significantly lower than those of conventional ESI or nanoESI sources. It appears that the nanopore ion source operates most stably when the emission current is kept as low as possible, typically < 50 pA when working with aqueous amino acid or peptide solutions, or about 2-3 orders of magnitude lower than typical nanoESI emission currents [118]. These low currents suggest that, when integrated into an MS-based proteomics workflow, the nanopore ion source may result in lower throughput. The increased ion transmission efficiency could make up for the lower emission current in some cases, but it is worth considering additional ways of improving throughput. Parallelization of many nanopore emitters is one potential approach that may increase throughput without sacrificing sensitivity. Future work in this direction may find inspiration in the micro-fabricated arrays of emitters used in ionic liquid thrusters [332].

To accelerate the adoption of the nanopore ion source by the broader proteomics community, a crucial next step is integration with an upstream separation technique such as liquid chromatography or capillary electrophoresis (CE) [333]. These separation methods are essential in MS-based proteomics, because samples typically contain many thousands of different proteins with wide-ranging abundances. In the absence of separation, the resulting mass spectra would be overwhelmingly complex, with overlapping signals making it difficult to identify and quantify individual species. One possibility may be an integrated microfluidic device that features on-chip separation [334] as well as a nanopore emitter. Another intriguing (though admittedly speculative) possibility is that the nanopore ion source itself might offer an inherent separation capability. In capillary electrophoresis, ions are separated based on their electrophoretic mobility as they migrate through a narrow capillary under an applied electric field. The separation is significantly influenced by the charge on the capillary walls, which generates electroosmotic flow that carries analytes through the capillary [335]. The narrow tip of the ion source, which is made of the same silica material as the capillaries used in CE, could potentially generate a similar separation process.

Significant theoretical work remains to fully describe the behavior of the nanopore ion source. While the theoretical models described herein capture some of the important features of the ion source’s thermal and electrical properties, they are based on simplifying assumptions that limit their explanatory power. Future modeling efforts should focus on capturing the full complexity of ion transport within the emitter, likely requiring the use of the Poisson-Nernst-Planck equations to accurately describe the coupled electrokinetic and diffusive processes [336]. Comprehensive modelling of ion transport in similar conical nanopore geometries has been reported previously [337, 338], but the selective ion exchange that occurs at the meniscus of our ion emitters requires the use of unique boundary conditions. This approach would enable a more comprehensive understanding of phenomena such as concentration polarization, which may play a crucial role in determining the stability of ion emission. Furthermore, developing a model that accurately describes the shape and evolution of the liquid meniscus, similar to those developed by Higuera and Lozano’s group, could provide insights into the transition between purely ionic emission and mixed ion/droplet regimes. These more advanced models would deepen our understanding of the fundamental physics governing nanopore ion sources and also guide the optimization of their design and operating parameters for applications in mass spectrometry and beyond.

Finally, investigations should begin on the UV photolysis of peptides confined within the nanopore ion source to bridge the gap between UV photolysis studies in bulk solution and the application to single molecule peptide sequencing. A key challenge will be ensuring the delivery of UV light to the tip of the emitter inside the vacuum of a mass spectrometer. This will likely rely heavily on methods previously developed to couple UV lasers to MS instrumentation for UVPD [43]. Careful optimization of wavelength, pulse duration, and power will likely be necessary to maximize peptide bond cleavage while minimizing damage to amino acid side chains, preserving the sequence information. Another challenge will be determining the optimal rates of three distinct processes that must all work in concert: the translocation of peptides through the emitter, the photolysis of peptide bonds near the emitter tip, and the emission of ions from the tip. Ultimately, these efforts may culminate in a proof-of-principle demonstration of sequencing short peptides using this integrated nanopore ion source and UV photolysis based approach, paving the way for true single-molecule protein sequencing by mass spectrometry.

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