Soft Landing Mass-Selected Ions for Single Molecule Imaging

Xiaocui Wu, Dhaneesh Kumar, Jimin Ham, Shengpeng Huang, Kelvin Anggara

Soft Landing Mass-Selected Ions for Single Molecule Imaging

Xiaocui Wu, Dhaneesh Kumar, Jimin Ham, Shengpeng Huang, Kelvin Anggara

Table of Contents

Preface

Dear Reader,

As authors, we want you to learn something from this primer.

We thus owe it to you to be clear from the start on what this primer is about and how it can help you.

This primer focuses on bridging two seemingly distant technologies – mass spectrometry and single molecule imaging – with a technique called “soft landing.” This combination offers unique solutions and opportunities that could not be realized by either of them alone: it allows molecules you have worked so hard to prepare to be directly imaged one-molecule-at-a-time.

You may have heard of mass spectrometry as a technique that identifies molecules by measuring their molecular mass. You also may have heard of single molecule imaging techniques, such as scanning probe microscopy and transmission electron microscopy, which are two direct imaging techniques today capable of imaging, with atomic resolution, the structures of individual molecules adsorbed on surfaces in vacuum.

Now you will learn how to combine mass spectrometry and single molecule imaging into a single workflow.

“But, why?” you ask. Because, by doing so, we can now transfer molecules initially in solutions onto surfaces in vacuum so that they can be imaged one-at-a-time. Direct imaging of single molecules allows you to determine the diverse structures and interactions of complex molecules at the ultimate single molecule level. This is a feat that is beyond the reach of today’s analytical techniques – which analyze millions of molecules at a time (also known as ensemble averaging) and hence obscure the rich chemical information at the individual level.

To accomplish this, we discuss three key steps in this workflow: how to bring complex molecules from solutions into the gas phase as ions using electrospray ionization (Chapter 2), how to select them in the gas phase based on their mass-to-charge ratios (Chapter 3), and how to land them intact on surfaces for their direct single molecule imaging (Chapter 4). We write the primer to emphasize the main ideas and the mental models we use to understand, refine, and troubleshoot these three key steps.

Our hope is that this primer bridges the first time learners and the survey literature in the field, e.g., textbooks and reviews, which we provide as a reading list at the end of every chapter. Focusing on clarity over completeness, we write the primer not as an in-depth review of the field, but as a “travel guide” for prospective tourists to the field. For those familiar with mass spectrometry, you will learn a way to soft land and direct image the molecular ions you are studying in the gas phase. For those familiar with microscopy on surfaces, you will learn a way to prepare contaminant-free samples of complex molecules isolated from solution phase. For

newcomers to both fields, you will learn an analytical tool that allows molecules in solutions to be isolated in gas phase, landed onto surfaces in vacuum, and imaged one-at-a-time for their single molecule structural analysis.

This primer focuses on single molecule analysis of soft landed molecules isolated from solutions by electrospray ionization. We note however that this is not the sole application of soft landing technique, which by itself finds wide applications beyond those covered in this primer, such as surface deposition of metal or metal oxide cluster ions on self-assembled monolayers or electrode surfaces for thin film material fabrication. We further note that ions can be generated by techniques, alternative to electrospray ionization, such as laser ablation, matrix desorption, magnetron sputtering, etc.

We will be thrilled to hear your feedback, be that clarification on specific points or even your ideas on how to make things better. We ask that you to not hesitate to reach out to one of us.

To close, in the Biographical Memoirs for John B. Fenn describing his work on electrospray ionization of macromolecules to which he was awarded the 2002 Nobel Prize in Chemistry, Charles E. Kolb wrote:

Like Disney's Dumbo did before.
Elephant ions learned to fly.

It is time they learn how to land.

Best wishes,

The Authors

Author biographies

Xiaocui Wu is a postdoctoral researcher in the Nanoscale Science Department at the Max Planck Institute for Solid State Research. She is currently working on single molecule imaging of polysaccharides soft landed on metal surfaces by electrospray ion beam deposition. She received her B.Sc. and M.Sc. degrees in nuclear engineering from Sun Yat-sen University, China, and Ph.D. in physical chemistry from Université Paris Sciences et Lettres, France, in 2020. She continued her research as postdoctoral fellow at Technische Universität Ilmenau in Germany and then at University of Warwick as Marie Curie Cofund fellow in the United Kingdom before joining Max Planck Institute for Solid State Research.

Dhaneesh Kumar is a postdoctoral researcher in the Nanoscale Science Department at the Max Planck Institute for Solid State Research. He is currently working on single molecule imaging and spectroscopy, via scanning tunneling microscopy, of biomolecules soft landed on metal surfaces by means of electrospray ion beam deposition. He received his B.Sc. degree in Physics and Mathematics from the University of British Columbia, Vancouver, Canada. He obtained his Ph.D

in Physics from Monash University, Australia in 2021 where he focused on studying the structural and electronic properties of low-dimensional organic nanostructures on surfaces using scanning tunneling microscopy before joining Max Planck Institute for Solid State Research.

Jimin Ham is a postdoctoral researcher in the Nanoscale Science Department at the Max Planck Institute for Solid State Research. She is interested in imaging and material synthesis at the nanoscale. She is currently studying structures of single protonated molecules deposited by electrospray ion beam deposition with scanning tunneling microscopy, as well as modeling dynamics of two-dimensional materials followed by electron microscopy. She received B.Sc. and Ph.D. degrees in mechanical engineering from Hanyang University ERICA Campus, South Korea, where she studied epitaxial growth of inorganic crystals on two-dimensional materials by electron microscopy.

Shengpeng Huang is a Ph.D. student in the Nanoscale Science Department at the Max Planck Institute for Solid State Research, currently working on single molecule analysis of RNA structures. By combining electrospray ion beam deposition and scanning tunneling microscopy, he deposited mass-selected RNA molecular ions on surfaces and imaged the RNA molecules one-at-a-time to reveal their individual structures. He received his M.Sc. in Physics from the University of Lund, Sweden, in 2023, where he studied structures and electronic properties of bismuth islands grown on semiconductor surfaces using scanning tunneling microscopy and X-ray photoelectron spectroscopy.

Kelvin Anggara is a group leader in the Nanoscale Science Department at the Max Planck Institute for Solid State Research, working in the physical analytical chemistry of single molecules on surfaces. He received his Ph.D. in physical chemistry from the University of Toronto, Canada, in 2018, and he did his postdoctoral stint as the Alexander von Humboldt fellow at the Max Planck Institute for Solid State Research up until 2021. His research has been recognized by Karl Freudenberg Prize, European Research Council Starting Grant, Human Frontier Science Program Research Grant, among others.

Dedications and acknowledgements

We acknowledge support from ERC Project GlycoX (101075996) and Human Frontier Research Program (RGEC31/2023), and we thank ACS and the ACS In Focus team, Tricia Louvar and Sara Tenny, for this opportunity and for their support throughout the project. We acknowledge proofreading support on our manuscript from Cynthia Khor and Lili Zhang.

Chapter 1. What is electrospray ion beam deposition?

1.1. Introduction

"Break things and fix them" is one of the tried and tested ways to learn how things work.

For example, to learn how computers work, you could pick up a screwdriver, take apart your computer into its tiny components, and put them all back in a weekend.

But, have you wondered how you would go about learning how small systems — say, a cell — work? Trillions of molecules, a few nanometers (10^{-9} meter) in size, from proteins to carbohydrates, all *do something* to ensure the cell works as it should (Figure 1.1). Hidden within these molecules are the answers to many important questions: How do our immune systems work? Why and how cells turn cancerous? How do diseases emerge? Why do we age? The list goes on.

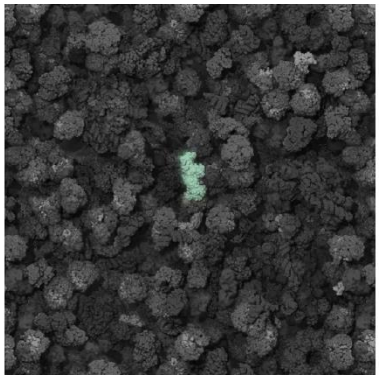
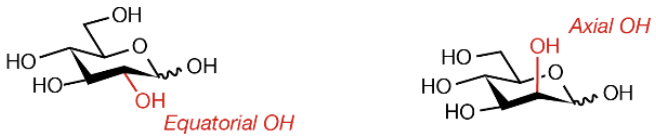


Figure 1.1 | A needle in a haystack. Molecular crowding makes it difficult to understand how complex systems work. The figure is a cartoon illustration of a single protein merely a few nanometers (10^{-9} m) in size (highlighted in green) among many other biomolecules in a cell. Credit: Reproduced from McGuffee SR, Elcock AH (2010) Diffusion, Crowding & Protein Stability in a Dynamic Molecular Model of the Bacterial

Cells are, of course, only one out of many complex systems that demand our thorough understanding. Today, complex systems, ranging from sustainable materials that power our civilization to catalysts that ensure our long-term food security, are all vital subjects in need of investigation.

Clearly, one way to understand how a complex system works is to break it down to its individual constituents, identify them, and understand how they work with one another to accomplish a task. This means that first we need to *identify every single molecule* that makes up a complex system – but how can we do this?

To answer this question, let us consider how these molecules differ. First, each of these molecules can have a different molecular formula (i.e., the number of every type of atom in a molecule), which gives each of them a specific molecular mass. For example, a cytochrome C protein ($C_{558}H_{878}O_{155}N_{148}FeS_4$) has a mass of 12323.37 Da, while a glucose molecule ($C_6H_{12}O_6$) has a mass of 180.06 Da. Second, while some molecules could have the same mass, they differ by how each atom connects to one another. For example, glucose and mannose sugars ($C_6H_{12}O_6$) both weigh 180.06 Da, with the only difference being that glucose has an equatorial OH while mannose has an axial OH (Figure 1.2).



Glucose
180.06 Da

Mannose
180.06 Da

Figure 1.2 | A molecule is a network of atoms. Molecules with the same mass can be differentiated by how their constituent atoms connect to one another.

This brings us to two new problems: First, how can we separate molecules by their masses? Second, how can we determine the way atoms connect to one another in a molecule?

On the first problem, let us recall that the mass of an object dictates the magnitude of its acceleration when a force is applied to it. Imagine this: what happens if you kick an empty bottle versus the same bottle filled with water? Clearly, the empty bottle travels further than the filled bottle, because the lighter, empty bottle experiences a greater acceleration than the heavier, filled bottle. Hence, it follows that by applying an equal force (be that mechanical, electrical, magnetic, etc.) to a mixture of molecules, we could expect that molecules with different masses to move with different acceleration that subsequently separates them in space. However, to accomplish this, mass separation of molecules must be performed in a clean vacuum environment to eliminate friction effects caused by atmospheric gas molecules colliding with molecules we are interested in. To meet this requirement, a method to deliver molecules from solution phase into vacuum is thereby needed.

On the second problem, one simple solution toward observing the connections between atoms is to see individual chemical bonds directly in a single molecule – a stunning feat that has so far been accomplished only by scanning probe microscopy (SPM) (Figure 1.3). To attain such a

resolution, molecules need to sit on an atomically clean and flat surface at temperatures below 10 K (-263 °C); the latter is essential to remove any thermal motion of the molecule and to give maximum stability to the microscope. The need for such a low temperature requires SPM imaging to be conducted in an ultrahigh vacuum (UHV, ~10^{-10} mbar) environment. This prerequisite thereby calls for a method to transfer the intact molecules we are interested in from ambient atmosphere onto an atomically clean surface in an UHV environment.

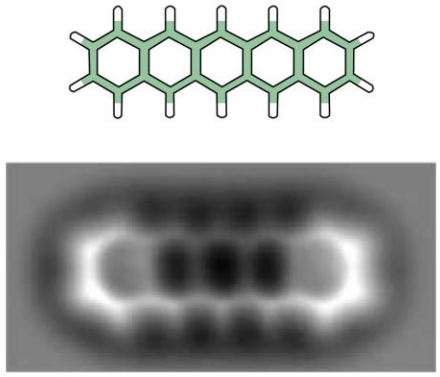


Figure 1.3 | Atomically resolved imaging of a single molecule. (Top) Model of pentacene molecular structure. (Bottom) Direct observation of chemical bonds in a single pentacene molecule was accomplished by scanning probe microscopy (SPM) at low temperatures (~4 K). Credit: From Leo Gross *et al.* The Chemical Structure of a Molecule Resolved by Atomic Force Microscopy. *Science* **325**,1110-1114(2009). DOI:10.1126/science.1176210 Reprinted with permission from AAAS.

Here we showcase a technique that meets the aforementioned demands, termed electrospray ion-beam deposition (ESIBD) (Figure 1.4). This technique enables single molecular species to be isolated from a complex system for their structural studies on surfaces by microscopy techniques. It accomplishes three important tasks:

(i) **Electrospray ionization:** delivery of molecules from solution to gas phase as ions;

(ii) **Mass selection:** separation and selection of molecular ions by their mass; and

(iii) **Deposition:** soft landing of the mass-selected ions on surface for direct imaging.

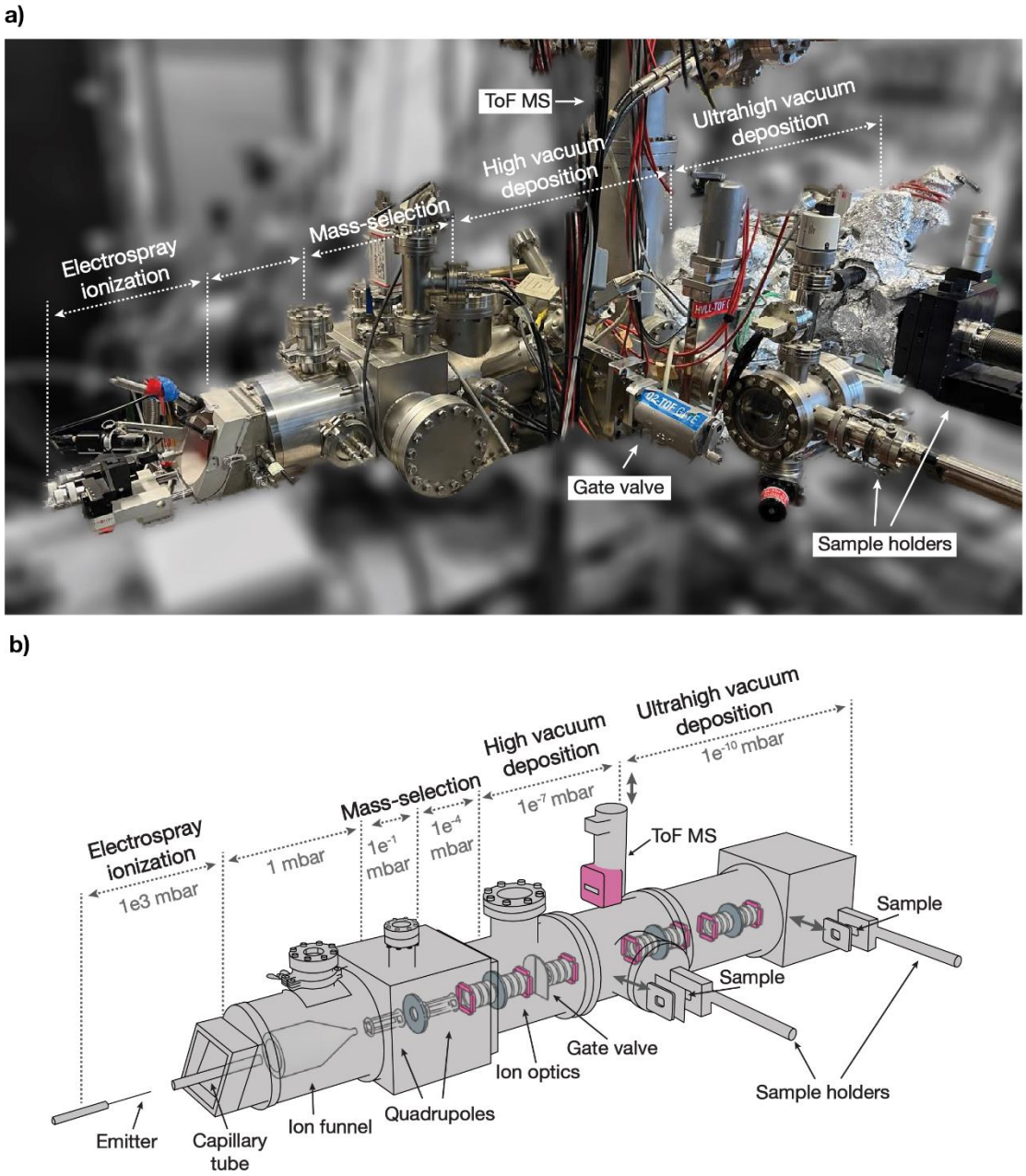


Figure 1.4 | Bringing molecules into vacuum. (Top) Experimental apparatus and (Bottom) schematic of the electrospray ion beam deposition (ESIBD) technique highlighting the three aspects of the technique: electrospray ionization, mass selection, and deposition. The deposition can be done at different pressures (e.g., high vacuum and ultrahigh vacuum).

Sidebar text box: See the equipment in an actual lab! Click this link to view a video showing the instrument set-up for electrospray ion beam deposition in a lab at the Max Planck Institute. https://www.youtube.com/watch?v=w-JBnzW6bdQ

To show how the ESIBD technique contributes to existing fields of science, we highlight two important applications of ESIBD (out of many in the literature): first, in enabling complex molecules from cells to be observed and analyzed at the single molecule level, and second, in enabling self-assembled structures from complex building blocks to be observed on surface. The two applications discussed here demonstrate ESIBD as a valuable complementary addition to present analytical workflows to determine the structure and interactions of complex molecules at the ultimate single molecule level.

1.2. ESIBD enables single molecule analysis of biomolecules

The marriage of ESIBD to SPM allows any molecule that can be electrosprayed to be imaged and structurally analyzed at the single molecule level on surface.

This approach is attractive for many complex molecules that remain intractable by today's analytical technology, such as the dense sugar layer that covers cells (e.g., lipopolysaccharides in bacteria, proteoglycans in human cells), the diverse RNAs that regulate the functions and dysfunctions of our cells, the polymers central to our efforts to build a healthier and more sustainable society (e.g., chitopolymers in biotechnology, marine polysaccharides in environmental technology, and conjugated polymers in sustainable electronics and photovoltaics technology), and many more.

Here, we will focus on one key application of ESIBD+SPM technique in analyzing biomolecules decorated with glycans (also known as carbohydrates, oligosaccharides, or sugars) at the single molecule level.

At this point, you may wonder: why should we care about glycans at all?

Glycans are one of the most understudied biomolecules, and yet the most ubiquitous ones in all living systems. All cells without exception are covered with a thick layer of glycan molecules on their surfaces that function like an ID card; they identify the type (e.g., human or bacterial) as well as the state of the cells (e.g., healthy or cancerous). The latter is particularly valuable as it presents an opportunity to detect cancerous cells simply by detecting specific glycans on cell surfaces, potentially enabling early cancer detection. Such early detections are highly desired as they increase the survival rates of cancer patients (late detection of cancer is associated with fatalities).

As an example, mucins are a family of bottlebrush-like molecules found on cell surfaces, each consisting of a protein chain decorated with glycans (Figure 1.5). These mucin molecules appear differently in healthy cells than in cancerous cells. In healthy cells, mucins are densely decorated with long glycans, while in cancerous cells, mucins are sparsely decorated with short glycans. In every mucin molecule, the position and structure of every glycan along the protein chain is thought to form a “barcode” that stores information that is so far readable only to biological systems – but not us. Hidden within these barcodes are the answers to how biological systems recognize each other, how our immunity works, how we achieve early cancer detection, and many more.

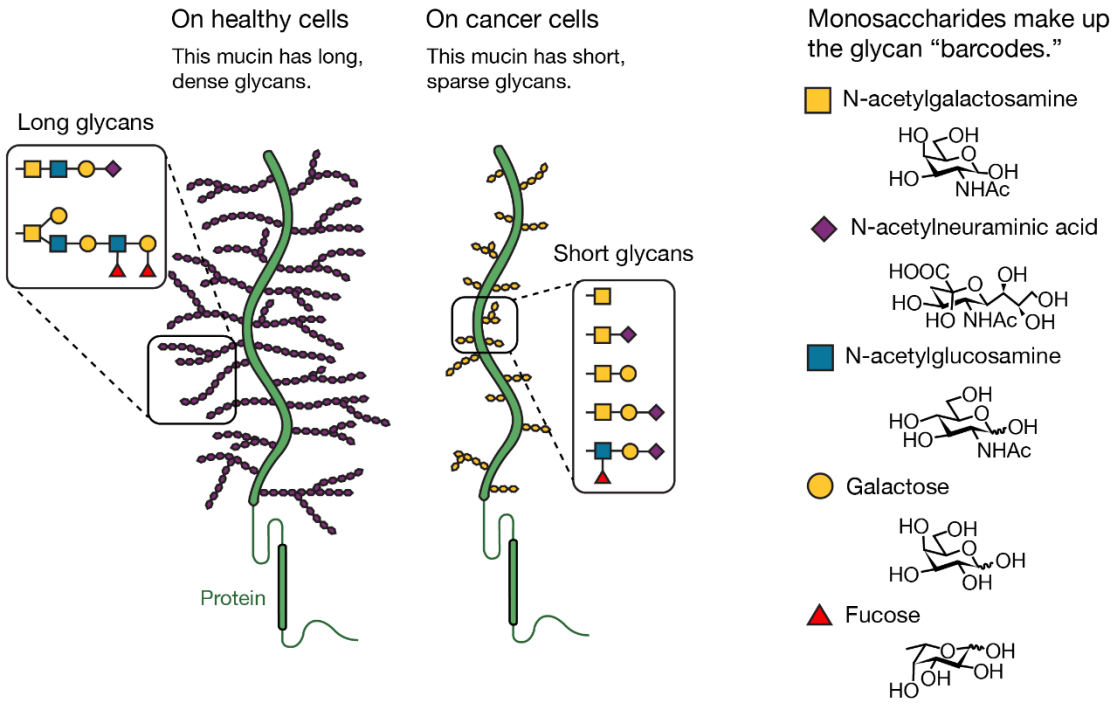


Figure 1.5 | Mucin molecules in healthy and cancerous cells. Mucins consist of a protein chain decorated by glycans, which are long and dense in healthy cells but short and sparse in cancerous cells. Examples of glycans present in mucins are given in the inset.

Now, given the immense value of mucins to our understanding of biology and to our therapeutics development, the question is: what stops us from reading the barcode in every mucin present in a cell?

The answer is technology. The technology available to us today to determine molecular structures (e.g., mass spectrometry or cryo-electron microscopy) relies heavily on averaging structural information obtained from millions of molecules (also known as ensemble averaging). This approach is highly incompatible for mucins, given that a single cell possesses a large variety of mucin molecules whose individual barcodes vastly differ from one another. As a result, ensemble-

averaged analysis of mucins returns an averaged barcode of mucins, obscuring the true composition of mucin barcodes in a single cell.

This is where ESIBD enters the fray.

The ESIBD technique allows mucin molecules to be deposited on a surface as isolated single molecules so that each mucin molecule could be directly imaged by SPM to reveal every glycan present along the protein chain. Thus, the mucin barcode is unveiled one molecule at a time (Figure 1.6).

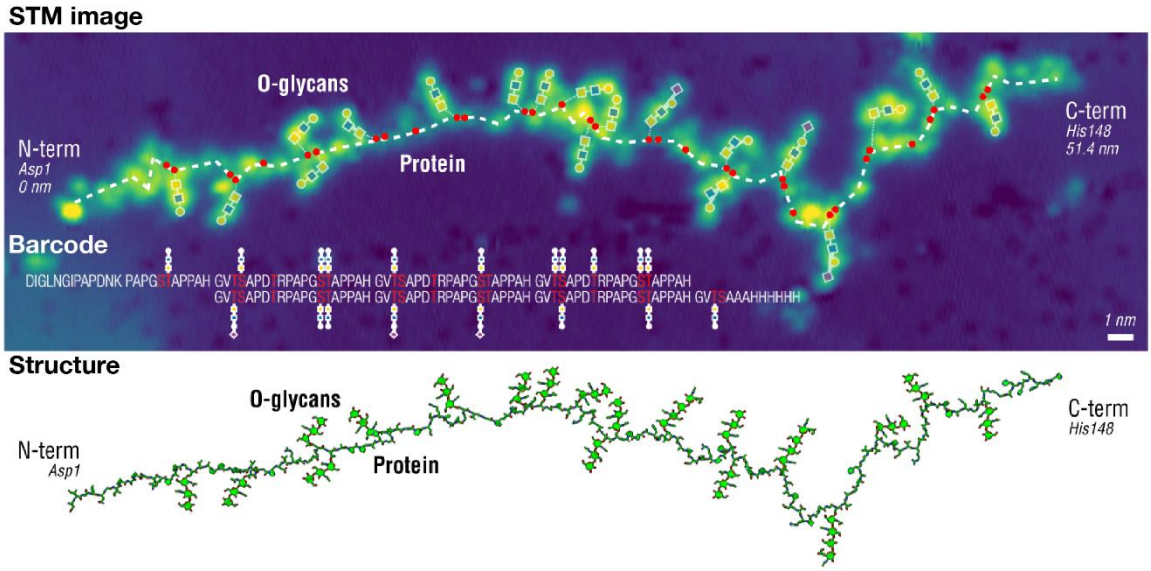


Figure 1.6 | Reading mucin barcode one molecule at a time. Direct imaging of a mucin reveals the location and structure of every glycan (marked by blue squares, yellow squares and yellow circles for N-acetyl glucosamine, N-acetyl galactosamine, and galactose, respectively) attached to the protein chain (marked by white dotted lines). The resulting mucin barcode is given as an inset to the STM image. Credit: From Kelvin Anggara *et al.*, Direct observation of glycans bonded to proteins and lipids at the single-molecule level. *Science* **382**,219-223(2023). DOI:10.1126/science.adh3856. Reprinted with permission from AAAS.

Figure 1.6 shows this very direct imaging of a single mucin molecule. This direct imaging reveals the protein chain of mucin (dashed line in Figure 1.6) and the *locations* where every glycan connects to the protein chain (marked by red circles in Figure 1.6). Not only that, but direct imaging also reveals the *structure* of every glycan attached to the protein chain, whether they are trisaccharides or tetrasaccharides. Both information—the location and structure of the glycans—unveils the barcode of the mucin (inset in Figure 1.6). By further imaging multiple mucin molecules on surface, a collection of mucin barcodes emerges, revealing the diversity of unique mucin barcodes as well as their relative composition – all at the ultimate single molecule level.

The success of the ESIBD+SPM technique in accessing individual mucin barcodes unlocks new opportunities to study the true diversity of mucin barcodes in healthy and cancerous cells. Such studies are anticipated to reveal the mucin barcodes that appear most often in healthy cells, but not in cancerous cells, and vice versa, thus defining the most sensible mucin barcode targets for pharmaceutical developments and therapeutic interventions.

Sidebar text box: Enhancing SPM imaging for biomolecular analysis. While SPM imaging triumphs in accessing structural information at the single molecule level that is so far inaccessible to present analytical techniques (e.g., mass spectrometry, ion mobility spectrometry, cryo-IR gas phase spectroscopy), SPM imaging still has to improve before it could become a practical analytical tool to analyze glycan-containing biomolecules (or any biomolecules in general). For instance, SPM sensitivity should be increased (e.g., using functionalized tip or using bias spectroscopy) to allow glycan isomers to be distinguished down to the atomic level, or SPM imaging and analysis should be automated as much as possible to improve the data throughput of the method.

1.3. ESIBD enables preparation of self-assembled nanostructures on surface

Beyond single molecule analysis, the ESIBD technique enables preparation of nanostructures on surfaces. This is accomplished by depositing the mass-selected building blocks at a surface for them to self-assemble into a functional material, allowing one to study how the building blocks interact with one another and what the properties of the resulting material are at the atomic scale.

The ESIBD technique is attractive in its potential to access new materials on surface via a vast choice of building blocks. The diverse electrospray-generated building blocks at our disposal and the freedom to specify the sequence in which we use them allow us to embark upon a journey to build new and specific materials with tailored properties.

This section summarizes one key achievement of the ESIBD technique in preparing two dimensional (2D) materials using mass-selected ions.

But why? Why do we need to care about 2D materials? What is so remarkable about them?

New materials enable new wonders. New materials enable better lives. Before concrete, towering skyscrapers were a mere dream. Before semiconductors, many electronic marvels that make our lives more comfortable and connected (think the internet!) would not exist. New materials have the power to revolutionize many aspects of our daily lives, shaping a better future for us.

Recent years have seen surging interests into a class of materials called 2D materials. Simply put, they are layered materials that are only few atoms thick, such as graphene – a one-atom-thick layer of carbon atoms arranged in a honeycomb pattern – or molybdenum disulfide (MoS_2) – a three-atom-thick layer of sulfur and molybdenum atoms arranged in a triangular pattern.

What makes 2D materials so attractive? One answer is that they exhibit rich quantum properties that are ripe for exploration and exploitation. These rich quantum properties result from electrons in 2D materials being confined to a remarkably small thickness (i.e., few atoms thick), causing the electrons to behave very differently when compared to electrons in bulk (3D) materials. One consequence of this confinement is that electron energies now depend on the shape and dimensions of the materials. This unlocks vast opportunities to engineer material properties by simply changing their geometries. One historic example is the emergence of superconductivity (i.e., the ability to conduct electricity without energy loss) when two layers of graphene are brought together at a specific “magic” twist angle of 1.1 degree. Until today, no superconductivity has been observed from a single layer of graphene or bulk carbon-based materials.

Despite the exciting discoveries and technologies that are emerging from the field of 2D materials, their studies are inherently limited by the choice of 2D materials accessible by today’s preparation methods, particularly in bottom-up preparation (where materials are built from their smaller building blocks). Building blocks used to prepare 2D materials at a surface are often limited to atoms or simple molecules that could withstand high temperatures, as they need to be thermally evaporated onto the surface. As an example, organic molecules, such as sugars, could no longer be used as building blocks as they would caramelize and burn when heated too strongly. The restrictions in the choice of building blocks ultimately limits the versatility of 2D materials that could be prepared on surface, thus creating barriers in exploiting the full potential of 2D materials in addressing our societal challenges.

This is where ESIBD enters the picture.

The ESIBD technique expands the variety of building blocks that can be used to prepare materials on surfaces to include thermally labile building blocks, such as inorganic clusters, organic

molecules, or even metal-organic frameworks. The ESIBD offers two advantages when compared to material preparation based on thermal evaporation: (i) the electrospray ionization offers a gentle transfer of complex, non-volatile molecules intact from solution into gas phase; and (ii) the mass selection offers precise selection of building blocks deposited on surface. Both benefits translate to a greater control of surface chemistry and thus unlocks access to a vast range of 2D materials on surface.

In the following example, the ESIBD technique allows the use of MoS clusters to prepare MoS_2 nanoribbons, which are inaccessible by preparation techniques based on thermal evaporation. These nanoribbons were visualized at the atomic level by scanning transmission electron microscopy (STEM) to be few micrometers in length and few nanometers in width (Figure 1.7).

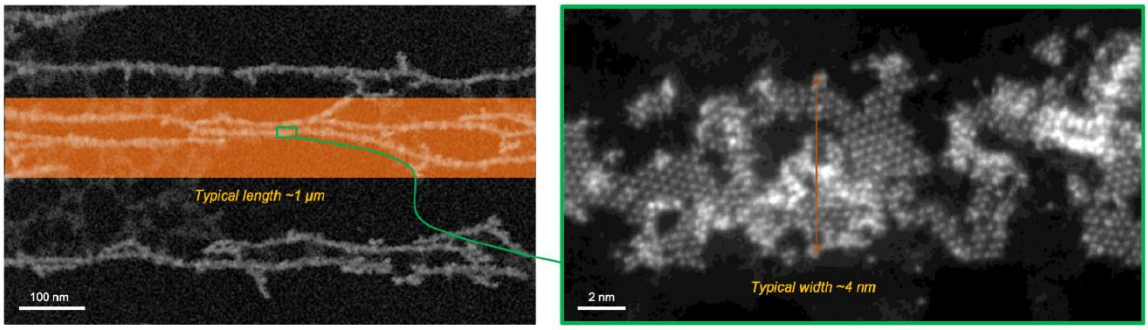


Figure 1.7 | Nanoribbons of molybdenum disulfides prepared by the ESIBD technique. MoS_2 nanoribbons (white strips) on graphene prepared by the ESIBD technique are visualized at the atomic scale using scanning transmission electron microscopy (STEM). Credit: Reproduced from X. Zhang, V. Srot, X. Wu, K. Kern, P. A. van Aken, K. Anggara, Controlled Formation of Nanoribbons and Their Heterostructures via Assembly of Mass-Selected Inorganic Ions. *Adv. Mater.* 2024, 36, 2310817. https://doi.org/10.1002/adma.202310817. CC BY 4.0 https://creativecommons.org/licenses/by/4.0/

The power of ESIBD technique is further demonstrated by the precise selection of building blocks in fine tuning the final nanostructures on surface. In this example, MoS_2 nanoribbons obtained by depositing small MoS cluster ($HMo_4S_{13}^{1-}$) were observed to be few nanometers thinner than that obtained by depositing large MoS cluster ($HMo_6S_{19}^{1-}$) (Figure 1.8). This observation showcases the advantage of precisely selecting and depositing building blocks on surface in finely tuning the kinetics and thermodynamics of the surface chemistry that ultimately yields the final nanostructures.

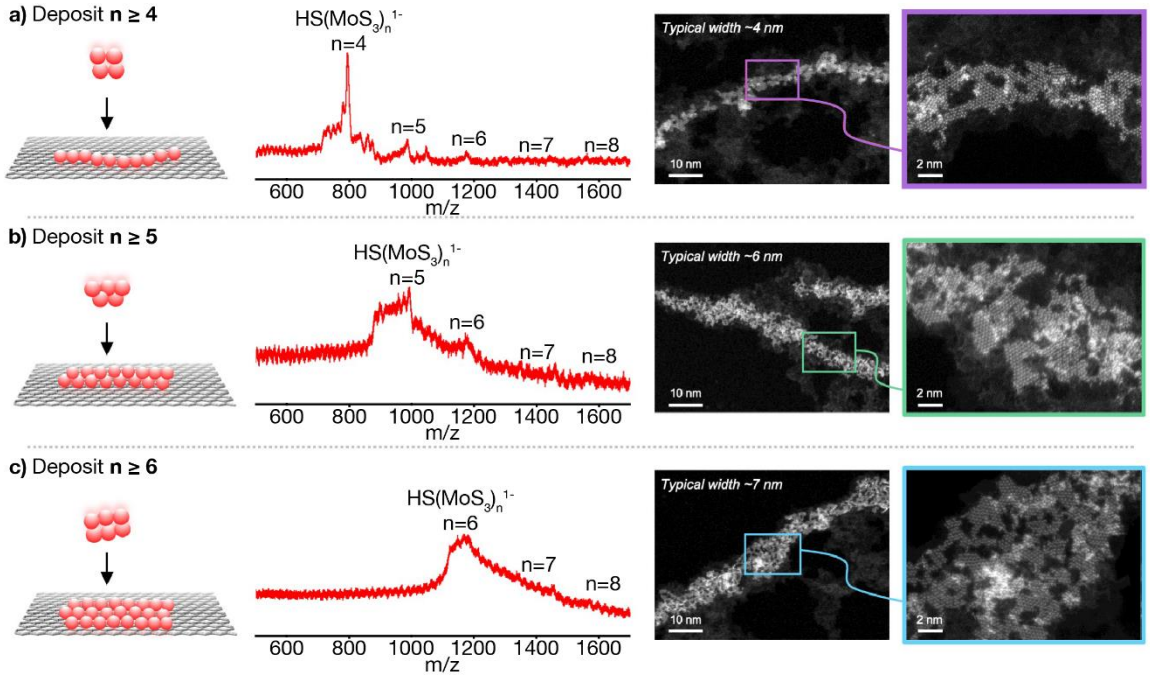


Figure 1.8 | Final nanostructures depend on deposited building blocks. Varying the deposited $HS(MoS_3)_n^{1-}$ ions: (a) for n ≥ 4, (b) for n ≥ 5, and (c) for n ≥ 6, results in an increase in the average width of the nanoribbons from 4.7 to 7.3 nm, respectively. Credit: Reproduced from X. Zhang, V. Srot, X. Wu, K. Kern, P. A. van Aken, K. Anggara, Controlled Formation of Nanoribbons and Their Heterostructures via Assembly of Mass-Selected Inorganic Ions. *Adv. Mater.* 2024, 36, 2310817. https://doi.org/10.1002/adma.202310817. CC BY 4.0 https://creativecommons.org/licenses/by/4.0/

In the future, due to the diverse building blocks accessible through electrospray ionization starting from biomolecules to inorganic clusters, the ESIBD technique is expected to be instrumental in accessing biomaterials as well as metal-organic framework materials with the goal of advancing our understanding in fields as diverse as photovoltaics, electronics, catalysis, and many more.

1.4 [INSIDER – how did you get started in the field?]

1.5. ESIBD roadmap

The two cases discussed highlights the diverse role of ESIBD as a preparative technology, first, in preparing microscopy samples and, second, in preparing complex materials on surfaces. While these achievements signal a promising start, more research efforts are needed to explore the full exciting potential of such preparative technology. One could thus speculate the directions upon which ESIBD could be improved.

1.5.1. Ion transmission at atmosphere-vacuum interface

Finding strategies to harvest as many ions as possible at the atmosphere-vacuum interface (especially in the region between 1000 to 1 mbar) remains a barrier toward the full potential of ESIBD technique. This is due to the fact that the motion of ions in this pressure regime is significantly affected by the gas flow dynamics (e.g., laminar vs turbulent flow and shockwaves), and is thus no longer predictable by simply calculating the forces exerted on the ions by electric fields from electrodes (Note: in pressures below 10^{-4} mbar, the motion of ions are governed by electric fields which simplify the design process of the ion steering and focusing elements). The difficulty in predicting the ion motions often leads to challenges in designing the best atmosphere-vacuum interface. Today, the best solution to this problem is the use of ion funnels which allows

ions to be transmitted into vacuum with an ion flow as high as few nA (nanoamperes). However, more focused efforts are required to push this transmission limit of the ESIBD technology to include molecules that are not known to ionize well.

1.5.2. Selection of ion shape

Given that two molecular ions with the same mass-to-charge ratio could have a different molecular geometry (e.g., a protein that could have various folding states or a molecule that could have various relative orientation among their functional groups), a profitable direction to develop the ESIBD technique is to include a scheme to perform ion selection by its respective shape. The principles to develop this are readily available from the sister field of gas phase ion mobility spectrometry (IMS). In IMS, ions are separated by their shapes by letting the ion drift through a chamber filled with inert gases with a constant electric field. Molecular ions with larger cross section (e.g., a star-shaped molecule) tend to travel slower than molecular ions with smaller cross section (e.g., a stick-shaped molecule).

1.6. Next chapters

The subsequent **Chapters 2 to 4** will further detail the ESIBD technique as follows:

(i) Chapter 2 – Electrospray ionization. Molecules are brought from solutions into vacuum as molecular ions by using a technique termed “electrospray ionization.” Electrospray generates micrometer-sized (10^{-6} m) droplets of the solutions that contain the molecules we are interested in. These microdroplets are the source from which molecular ions are obtained.

(ii) Chapter 3 – Mass selection. The molecular ions are subsequently focused into a beam of ions. This ion beam is subsequently transmitted to a mass selector which serves to transmit ions

with specific mass-to-charge ratio (*m/z*), effectively removing any unwanted ions from the ion beam.

(iii) Chapter 4 – Ion soft landing on surfaces. The purified ion beam is aimed at a clean surface for their surface deposition. Here the kinetic energy of incident ions is typically reduced to achieve intact deposition, that is, soft landing, of the molecules on a surface for their single molecule microscopy studies.

1.7. Read these next

1. Effects of crowded molecular environment in living systems: Diffusion, Crowding & Protein Stability in a Dynamic Molecular Model of the Bacterial Cytoplasm. PLOS Computational Biology 6, e1000694 (2010).

2. How to use SPM to visualize single chemical bonds in molecules: The Chemical Structure of a Molecule Resolved by Atomic Force Microscopy. Science 325, 1110 (2009).

3. An example of how ESIBD enables analysis of single biomolecules: Direct observation of glycans bonded to proteins and lipids at the single-molecule level. Science 382, 219 (2023).

4. An example of how ESIBD enables preparation of inorganic 2D materials: Controlled Formation of Nanoribbons and Their Heterostructures via Assembly of Mass-Selected Inorganic Ions. Advanced Materials 36, 2310817 (2024).

5. Detailed reviews describing the ion soft landing phenomena and instrumentation: (a) Collisions of ions with surfaces at chemically relevant energies: Instrumentation and phenomena. Reviews of Scientific Instruments 72, 3149 (2001). **(b)** Mass Spectrometry as a Preparative Tool for the Surface Science of Large Molecules 9, 473 (2016).

6. Soft landing applications in synthesis of multilayer materials: (a) From Isolated Ions to Multilayer Functional Materials Using Ion Soft Landing. Angew. Chem. Int. Ed. 57, 16270 (2018).

(b) Chemical Synthesis with Gaseous Molecular Ions: Harvesting $[B_{12}Br_{11}N_2]^-$ from a Mass Spectrometer. 62, e202308600 (2023).

Chapter 2. The electrospray ionization

2.1. Introduction: Giving wings to molecular elephants

Sidebar text box: Ionization strategies. Electrospray ionization (ESI) is one of the typical means to generate ions in gas phase. Other than ESI, typical ion sources include electron impact (EI) ionization, where electrons are collided at ~70 eV with molecules (M) to form positive molecular ions (M+); or matrix assisted laser desorption ionization (MALDI), where lasers are used to ablate and desorb molecules embedded in a matrix (usually aromatic acids) to give molecular ions at low charge states. We refer the reader to literature for an exhaustive survey of ionization methods (Finkelstein, J. Development of ionization methods. *Nat Methods* **12** (Suppl 1), 6–7 (2015). https://doi.org/10.1038/nmeth.3538; Hansell, C. Enter the matrix. *Nat Methods* **12** (Suppl 1), 17 (2015). https://doi.org/10.1038/nmeth.3527; Dellisanti, C. Electrospray makes molecular elephants fly. *Nat Methods* **12** (Suppl 1), 15 (2015). https://doi.org/10.1038/nmeth.3525)

The ESIBD technique uses electrospray ionization (ESI) to reliably transfer macromolecules from solution phase to gas phase. This chapter aims to present a mental model of the ESI process as a starting point to help you see: (i) which factors are critical to ensure successful delivery of molecules from solutions into the gas phase and (ii) what strategies are available at your disposal.

You may wonder at this point: Why electrospray? What is it? How does it work?

Before that, let us challenge you. Imagine some giant molecules (say, proteins) floating inside a big pool of water. We want to pull them out of the water – so that we can study them. How would you go about doing this?

Well, one way is to **disperse the solution into tiny droplets.** By doing so, we significantly reduce the number of solvent molecules (here, water) for every protein molecule. The smaller we can

make these droplets, the less effort it will take to further remove these solvent molecules from the droplets so that we end up with the solvent-free protein molecules we want.

This brings us to another problem: How can we make the tiniest droplet possible?

Think of a garden hose. You will notice that water exits the hose at a much higher speed if we squeeze the opening of the hose. By squeezing it even further, the water can shoot out so quickly that it breaks into droplets. The underlying physics is based on the continuity equation in fluid dynamics, which states that for an incompressible liquid (such as water) the flow rate (volume per time) entering the system (Q_{in}) must equal to the flow rate exiting it (Q_{out}).

$$Q_{\text{in}} = Q_{\text{out}} \qquad \text{(Eq. 2.1)}$$

Where the flow rate is defined as the flow speed (s) multiplied by the flow cross section area (A). Modifying the exit flow rate (Q_{out}) with this expression gives us:

$$\mathrm{Q_{in}} = (A_{\text{out}}) \cdot (s_{\text{out}}) \qquad \text{(Eq. 2.2)}$$

Accounting for the garden hose phenomena, this equation shows that, as we leave the tap open the same way (constant Q_{in}), reduction in the hose opening ($A_{\text{out}} \downarrow$) causes water to exit the hose at higher speed ($s_{\text{out}} \uparrow$).

One interesting consequence of fluid stream moving at high speed is that they break apart into smaller droplets, as the kinetic energy of the fluid is able to overcome the cohesive force that holds the liquid together. Hence, it follows that we need to dramatically reduce the opening diameter of the nozzle down to molecular sizes. How can we do this within practical limits?

One idea is to use electric fields to shape the liquid itself into a nozzle-like cone shape.

This cone shape shrinks the diameter of the liquid stream down to hundreds of nanometers, creating droplets whose sizes approach the sizes of macromolecules. This technique is called **electrospray ionization (ESI)**, and it remains to be one of the most reliable methods to transfer molecules from solutions into gas phase.

ESI offers two important advantages. First, many molecules have been demonstrated to be amenable to ESI as long as they could exist as ions. As a result, ESI has become one of the cornerstones of mass spectrometry, showing successful delivery of biological macromolecules (e.g., protein complexes) up to intact viruses and microorganisms into the gas phase (Figure 2.1). Second, ESI requires very little sample quantity, as low as 0.1 nanomoles (10^{-10} moles), making it a highly valuable method to analyze samples available in trace quantity, such as biological samples.

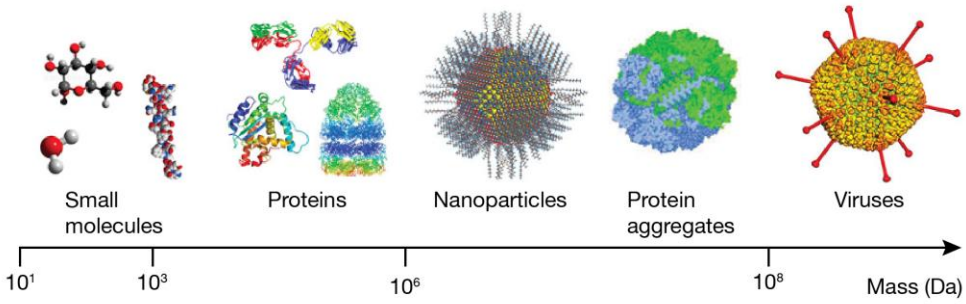


Figure 2.1 | Analytes amenable to ESI: from small molecules to big viruses. Examples of analytes accessible by ESI, ranging from small molecules (a few Daltons) to viruses (a few Megadaltons). Credit: Reproduced from Arnaud, C.H; Charge Detection Mass Spectrometry Measures molecular Structures Too Big For Regular Mass Spec; C&EN. **2019**, 97 (36). Copyright 2019 American Chemical Society.

In the following sections, a model of the ESI process is presented to lay out all factors critical to ensure successful delivery of molecules from solutions into gas phase. This includes discussion

614 on (i) how molecular ions emerge from the ESI-generated droplets, (ii) the choice of emitter
615 parameters, and (iii) sample solution parameters.
616

617 ## 2.2. How it works: From liquid jets to single ions

618

619 The ESI process transfers molecules from sample solutions into the gas phase as molecular ions.
620 The process begins with the application of high voltages (above 0.5 kV) to a capillary filled with a
621 sample solution, known as an “emitter.” The applied voltage shapes the liquid at the end of the
622 emitter into a cone-like structure (known as Taylor cone) that eventually breaks apart at its apex
623 into sub-micrometer-sized charged droplets (Figure 2.2). These droplets continue to shrink and
624 disintegrate until single molecular ions are formed (Figure 2.3).
625

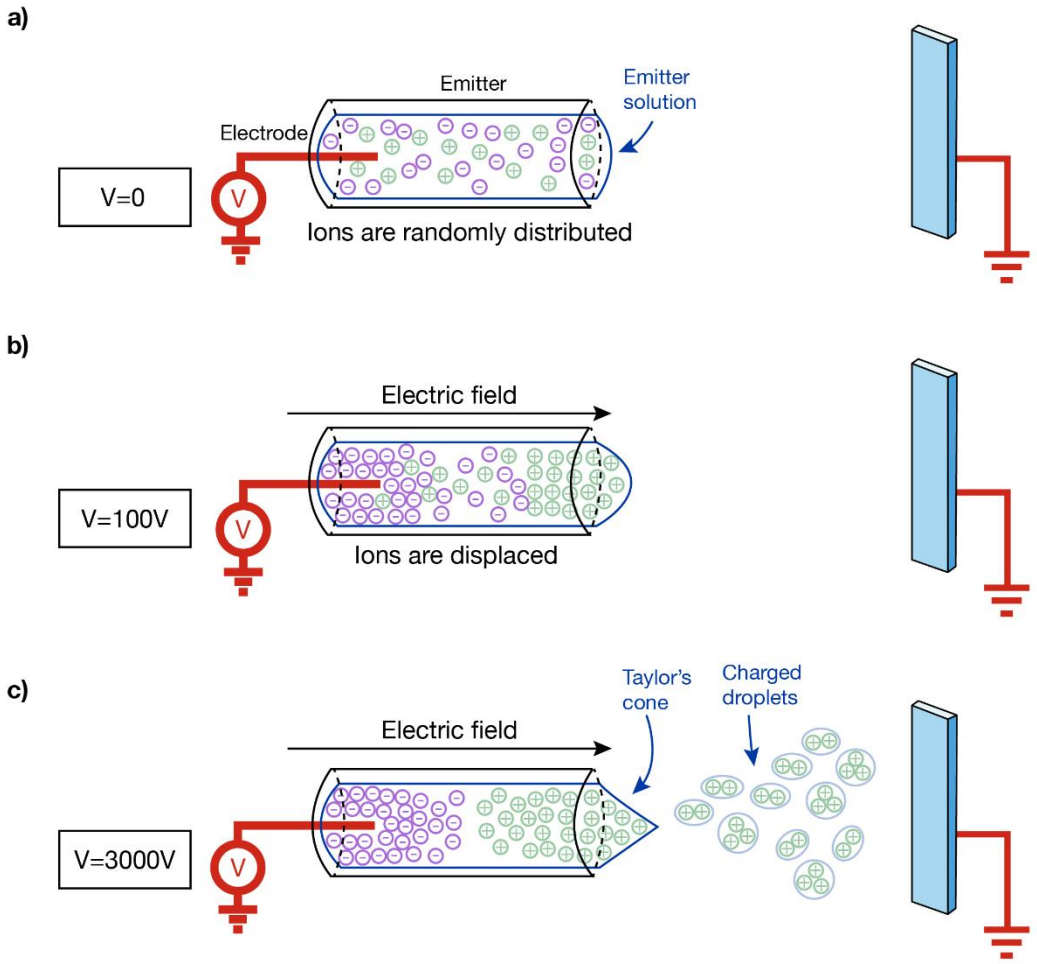


Figure 2.2 | Sending molecules from solutions into gas phase. ESI generates a stream of microdroplets from which molecular ions emerge. The example is shown here for ESI in positive mode, generating positively charged droplets which subsequently evolve into positively charged molecular ions. Negatively charged ions are generated when ESI is operated in negative mode.

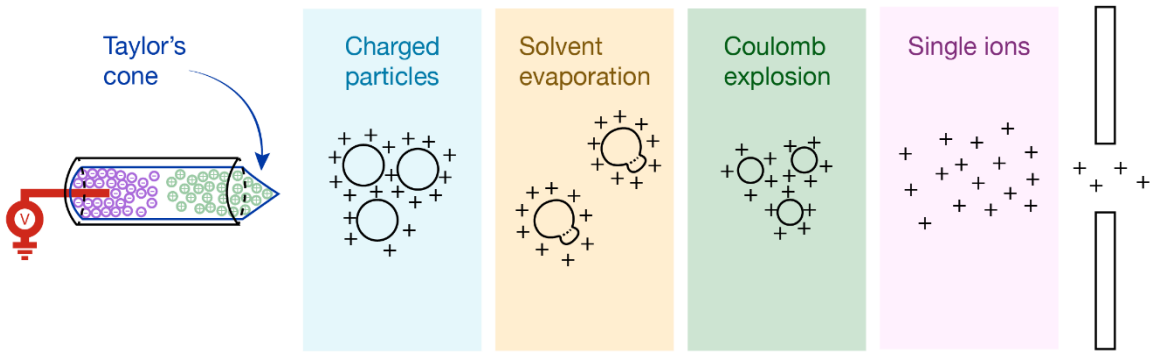


Figure 2.3 | Ion formation mechanism in electrospray ionization. The electrospray ionization process develops from microdroplet formation to molecular ions in gas phase. Here we only show an example of positive ions, but negative ions can also be generated depending on the polarity of the high voltage applied to the emitter.

Now let us take a closer look into every step of the ESI process.

First, let us see how the Taylor cone is formed. By applying a voltage through an electrode to the sample solution, say, a positive voltage, negative ions in the solution are brought closer to the electrode while positive ions in the solution are repelled as far as possible from the electrode toward the emitter tip (Figure 2.2). As the applied voltage is increased, more positive ions would accumulate at the tip, and eventually above a certain voltage threshold, a stable Taylor cone is formed, generating from its cone apex, a stream of droplets containing positive ions. But wait, with the positive ions leaving the liquid, does this mean the solution becomes more and more negative in charge? No. The liquid stays charge neutral due to the redox reactions occurring at the electrode-liquid interface, either by producing positive ions (e.g., H_3O^+) to replenish the positive ions that left the liquid or by turning the negative ions into neutral molecules, depending on the solution composition. Note that while the mechanism here is described for ESI in the positive mode, the same mechanism applies to the ESI process in negative mode (where the applied voltage is negative), which generates droplets containing negative ions.

Now, let us follow the fate of the ions in the droplets after they have left the emitter (Figure 2.3). In ESIBD, these droplets are delivered into vacuum via a heated tube. By heat convection, this heated tube helps to evaporate the remaining solvent in these droplets. As more and more of the solvent leaves the droplet, the droplet shrinks. The reduced droplet size brings the ions closer to one another, causing the repulsive forces between ions to skyrocket. Eventually, below a critical size, the repulsive forces between the ions are enough to overcome the surface tension of the droplet, causing the droplet to disintegrate into smaller offspring droplets in a process called "Coulomb fission." The same evaporation-fission cycle continues for the offspring droplets.

Unfortunately, the mechanism of how molecular ions emerge in the final step of the ESI process remains obscure due to the absence of direct experimental observation. Despite that, two models have emerged from computational studies of the ESI process. The ion evaporation model (IEM) proposes that ions are directly expelled from the shrinking droplets due to the increased repulsion between ions, while the charge residue model (CRM) proposes that repeated evaporation-fission cycles occur until one single molecular ion is left in every droplet, which further evaporates to yield single molecular ions. A special intermediate case called chain ejection model (CEM) has also been proposed to explain the ion formation mechanism of highly charged polymer ions. Our understanding of the ESI process would benefit from more experiments investigating the circumstances of the IEM, CEM, and CRM mechanisms.

Another mystery that calls for direct experimental observation is how Coulomb fission events change the composition of the ions in the droplets, and how this composition differs from the ion composition in the sample solution. As the droplets shrink to volumes that approach molecular sizes, molecules in these droplets may behave differently from how they would behave in bulk solutions. As a result, chemical equilibrium in such confined systems may differ radically from

chemical equilibrium in bulk solutions, leading to interesting phenomena that have only been very recently reported in literature such as acceleration of reaction rates in microdroplets.

2.3. ESI in practice

The performance of ESI in delivering molecules from solutions into gas phase could be measured by comparing how many ions arrive at a detector in vacuum (measured as ion current) to the consumption rate of the sample solution. This so-called ESI efficiency is an important figure of merit that assesses how well the ESI parameters work in delivering the molecules we are interested in from solutions into gas phase.

For example, if we are interested in measuring the ESI efficiency of a sample solution containing 10^{-5} M of a dye molecule, first we need to convert the observed ion current from the intact molecule (e.g., few picoamperes, 10^{-12} A) into the number of molecules arriving per unit time on the detector. Assuming that an intact dye carries a charge of +2, the charge carried by a single dye ion is thereby 3.2×10^{-19} C (= 2 × elementary charge), which gives us an arrival rate of around 3 million dye ions per second on our detector (obtained from dividing 10^{-12} A by 3.2×10^{-19} C). This arrival rate could now be compared to the consumption rate of sample solution (e.g., few nanoliters per minute). Given the concentration of 10^{-5} mol of dye molecules per liter, the total number of molecules consumed by the ESI process is thereby 1.6×10^{-16} mol per second (obtained from 10^{-5} M multiplied by 10^{-9} L/minute) – or around 100 million of molecules per second (where 1 mol = 6.022×10^{23} molecules). Comparing the ion current (3 million molecules per second) against the sample solution consumption rate (100 million molecules per second) brings us to 3% ESI efficiency.

The ESI efficiency depends on many factors, such as the emitter, the flow rate, the structure of analytes, the solvent, and many more. As these factors may lead to dramatic changes in efficiency, this section aims to provide practical guidance to navigate the art of finding the best parameters for a given analyte.

2.3.1. The emitter

First, let us discuss critical parameters in the ESI emitter, such as the material, the opening diameter, and the flow rate, as well as how these affect the ESI efficiency (Figure 2.4).

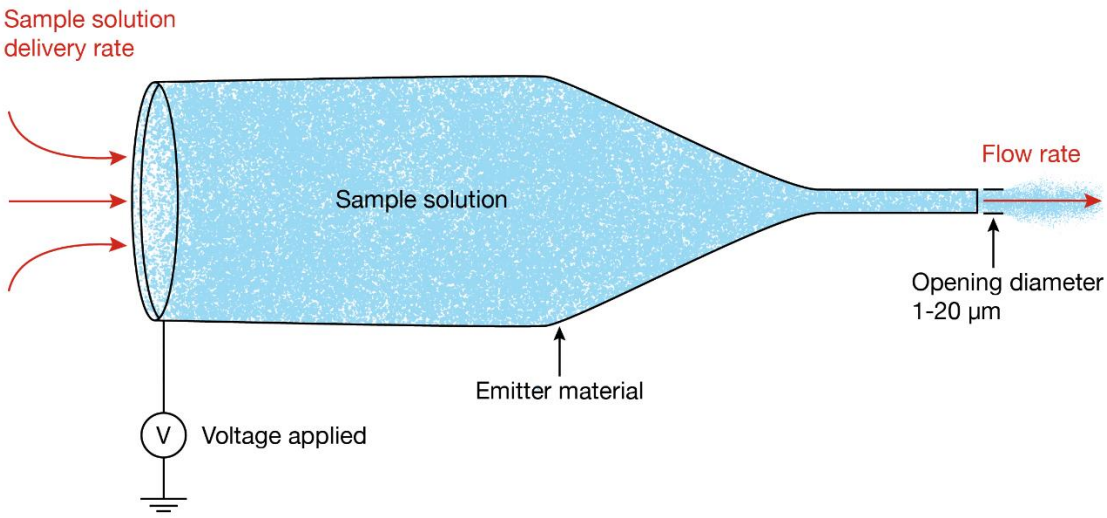


Figure 2.4 | Important parameters in an ESI emitter. ESI efficiency depends on the emitter material, the opening diameter, and the flow rate.

An ESI emitter can be made of many different materials, such as metal, glass, plastic, or even paper! Metallic emitters offer long lifetime and reusability; but they may be challenging to fabricate, especially in achieving small opening diameter (around 20 µm or smaller), and hence they are more often used for ESI at high flow rates (around few µL per minute). Nonmetallic emitters are

more fragile, but are low cost and easy to fabricate, and thus are more often used for ESI at low flow rates (around few nL per minute) which demands a very small opening diameter (around 1-2 µm or smaller). To deliver high voltages to nonmetallic emitters, we can either (i) put an electrochemically inert Pt wire in contact with the solution inside the emitter, or (ii) coat the emitter with conductive metal (e.g., Pt).

Why should we care about flow rate? The flow rate is critical in ESI as it dictates the size of droplets that come off the Taylor cone. Smaller droplets are thought to undergo less Coulomb fission cycles, causing a higher proportion of molecules from the sample solution to end up as molecular ions. Simply put, low flow rate gives smaller droplets – and smaller droplets give higher ESI efficiency.

Here are two common ways to get low flow rates: by lowering the solution delivery rate to the emitter; and by reducing the opening diameter of the emitter. First, in the case of *online* ESI where sample solution is constantly fed to the emitter by external forces, such as a syringe pump or a back-pressure, a low flow rate is obtained by lowering the pump speed of the syringe pump or lowering the gas pressure that pushes the sample solution toward the emitter tip. In the case of *offline* ESI where sample solutions are drawn from the emitter tip solely due to the electric field, the flow rate is already at the minimum. A further strategy to reduce the flow rate is to reduce the emitter opening diameter to few µm or lower. This is known to reduce the flow rates to as low as few nanoliters per minute.

While all presented strategies are known to increase ESI efficiency, care must be taken in practice. For example, a smaller opening diameter of an emitter makes it more prone to clogging, thus making long unsupervised ESI sessions unfeasible. Also, lowering the sample solution delivery rate may decrease the total ion current observed as the ESI efficiency may have reached

100% and the ion current is now bottlenecked by the amount of molecular analyte introduced to the Taylor cone.

However, one important benefit in reducing the opening diameter of an emitter is the reduction of threshold voltage needed to form a stable Taylor cone. An emitter with a few μm opening needs 0.5 to 1 kV to obtain a stable Taylor cone, while an emitter with a few tens of μm opening needs 3 to 4 kV. Low threshold voltage is highly beneficial to reactive and fragile molecules in sample solutions as it reduces the electrochemical contamination of the sample solution as well as the fragmentation of molecules in the sample solution, thereby increasing the amount of analytes delivered into the gas phase as ions.

2.3.2. The sample solution

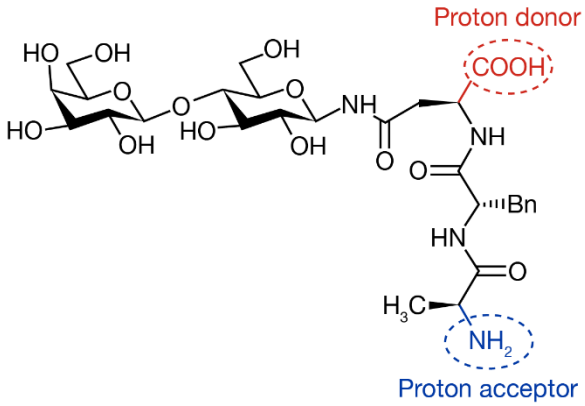
Apart from the emitter parameters, we can also play around with the sample solution parameters. Here we present strategies on how to optimize them.

One way to approach this problem is to first look at the chemical structure of molecule we would like to bring to the gas phase – and ask ourselves: “What ion species do I want to observe in the gas phase?”

Before we answer this, it is useful to know two classes of ions accessible by ESI: bare ions and adduct ions. For a given target atom/molecule (M), bare ions refer to M-ions without any additional partner; while adduct ions refer to M-ions decorated with additional partner atoms or molecules. Common bare ions are single ions (e.g., Rb^{+1}, Cs^{+1}, I^{-1}, Br^{-1}, etc.) and deprotonated molecules (e.g., $[M-H]^{-1}$, $[M-2H]^{-2}$, etc. – these refer to singly and doubly deprotonated M molecule with -1

and -2 charge, respectively), whereas common adduct ions are protonated ions (e.g., $[M+H]^{+1}$, $[M+2H]^{+2}$, etc. – these refer to singly and doubly protonated ions, respectively), ions decorated with bare ions (e.g., $[M+Na]^{+1}$, $[M+2Na]^{+2}$, $[M+Cl]^{-1}$, $[M+F]^{-1}$ etc. – these refer to M molecule decorated with 1 Na-ion, 2 Na-ions, 1 Cl-ion, and 1 F-ion, respectively), as well as hydrated ions (e.g., $[M+H_2O]^{+1}$, $[M+2H_2O]^{+1}$, etc.).

Given the types of ions commonly accessible by ESI, we now need to examine what ions are attainable from a given molecular structure. As an example, let us consider a glycopeptide molecule (Figure 2.5). By inspecting the structure, we see that the molecule has a carboxylic acid (RCOOH) group that could be deprotonated to give a $[M-H]^{-1}$ ion as well as an amine (RNH_2) group that could be protonated to give a $[M+H]^{+1}$ ion. Could we also expect to see a $[M-H]^{-2}$ ion as a result of deprotonation on both the RCOOH group (into a carboxylate, $RCOO^{-1}$) and ROH group (into an alkoxide, RO^{-1})? Not likely. The alcohol group (ROH) is a much weaker proton donor than the carboxylic acid group (RCOOH), making the population of such doubly deprotonated ions in solutions to be extremely small. As another example, let us consider a protein, which may have many amine and carboxylic acid groups in a single molecule, say 10 COOHs and 10 NH_2 groups. The structure thereby implies that all NH_2 groups could be protonated to give a $[M+10H]^{+10}$ ion or all COOH could be deprotonated to give a $[M-10H]^{-10}$, though ions with incomplete protonation or deprotonation could also be expected, such as $[M-2H]^{-2}$ that comes from the protein having 2 COOH groups deprotonated.



794

795 **Figure 2.5 | Planning the target ESI ion: an example glycopeptide molecule.** Ions of organic molecules
796 obtainable from ESI are commonly dictated by the number of proton acceptors and proton donors in the
797 molecule. Common proton donors are carboxylic acid groups (RCOOH), sulfate groups ($ROSO_3H$), and
798 phosphates ($ROPO_3H$); while common proton acceptors are lone pair–containing groups, such as amines
799 (RNH_2) and alcohols (ROH).

800

801 With all these choices, how would you decide which ions you should choose for ESIBD
802 deposition? While the answer ultimately depends on the goal of your deposition experiment, we
803 provide some important considerations based on ion composition and ion charge state. First, let
804 us compare bare ions to adduct ions. Bare ions are preferred in analytical experiment (esp. when
805 depositing unknown molecules) to remove as many contaminants as possible from the deposited
806 molecules and allow the clearest interpretation of the deposited molecules from the single
807 molecule imaging. On the other hand, adduct ions offer interesting possibilities to examine at the
808 single molecule level the effect of adduct formation on the host molecule (e.g., the effect of
809 hydration or sodium attachment on a molecule).

810

811 Next, let us consider the ion charge states (Figure 2.6). Ions with high charge states (e.g., protein
812 at +10 charge state) are structurally unfolded as the charges repel each other to maximize the
813 distance between them, whereas ions with low charge states (e.g., protein at +1 charge state) are

structurally folded, potentially retaining more structural similarity to their native structures in solutions. In the context of SPM imaging studies, unfolded molecules on a surface are suitable for sequence (primary structure) determination studies, whereas folded molecules on a surface are suitable for 3D-structure (secondary structure) determination studies.

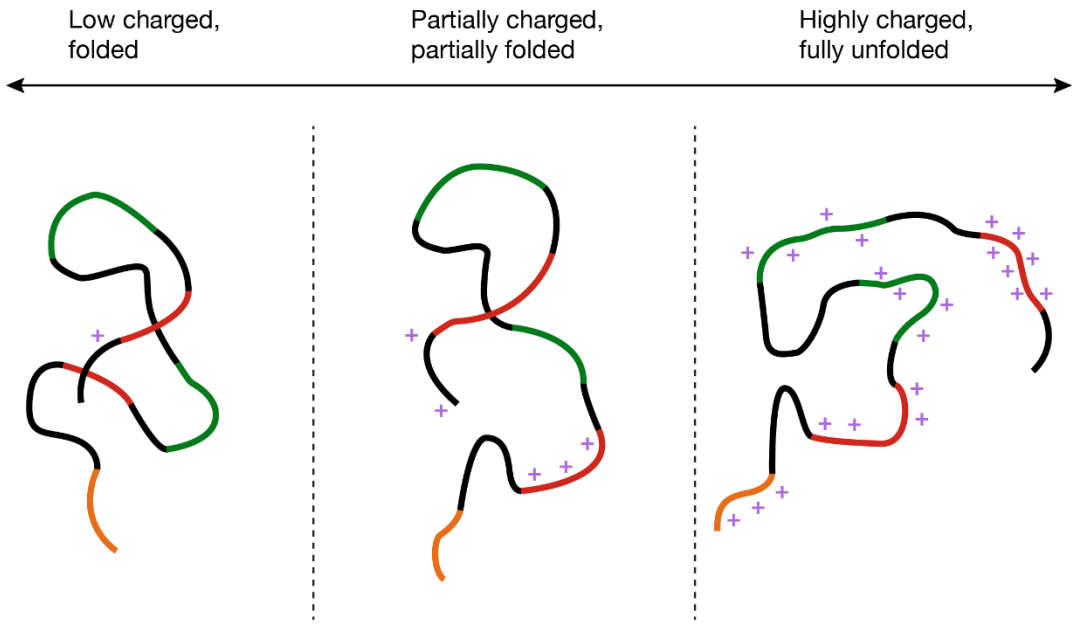


Figure 2.6 | Unfolding polymer in gas phase by charge repulsion. Molecules with low charge states give folded structures, while molecules with high charge states give unfolded structures.

Once we define the target ions, we ought to provide the best conditions to increase their population in sample solutions. Three common strategies are at our disposal: pH modification, additives, and solvent. First, volatile acids or bases are often added to the sample solution to shift the equilibrium in the solution toward the ions we desire. Common acids used to promote protonated ions are formic acid (HCOOH), acetic acid (CH_3COOH), as well as trifluoroacetic acid (CF_3COOH), while popular bases to promote deprotonated ions include ammonia (NH_3). Volatile buffers are also commonly used to maintain a stable pH of the solution such that the native

structures of the molecules are preserved when isolated in the gas phase. Popular buffers include ammonium acetate (NH_4CH_3COO) and ammonium bicarbonate (NH_4HCO_3). Next, additives are often added to sample solutions to promote formation of adduct ions, such as adding sodium salts (e.g., sodium formate, HCOONa) to promote formation of sodiated ions. Finally, solvent is a critical parameter in sample solution. Less polar solvent with low surface tension promotes formation of Taylor cone, generates smaller droplets, increasing the overall ESI efficiency. However, more polar solvents improve stability of ions in the solution (recall that salt is soluble in water, a polar solvent – but not in oil, a non-polar solvent, because the ions from salt are not well stabilized by non-polar oil molecules). The delicate balance between these two factors leads to an empirical list of common solvents used in ESI, such as 1:1 water:ethanol mix, methanol (CH_3OH), or acetonitrile (CH_3CN). For hydrophobic analytes, organic solvents such as toluene ($C_6H_5CH_3$) have seen success especially when mixed with methanol or acetonitrile. For biological molecules, ammonium acetate buffer solution is often used to retain the folded structure of the biomolecules from solution into gas phase.

2.4. Read these next

1. Computational studies of ESI mechanism: Unraveling the Mechanism of Electrospray Ionization. Anal. Chem. 85, 2 (2013).

2. Overview of chemical phenomena in microdroplets. Reaction Acceleration in Electrospray Droplets: Size, Distance, and Surfactant Effects. J. Am. Soc. Mass Spectrom. 30, 2022 (2019).

3. Use of electrospray to study labile species in solution: Evaluation of Electrospray Mass Spectrometry as a Technique for Quantitative Analysis of Kinetically Labile Solution Species. Anal. Chem. 71, 3785 (1999).

Chapter 3. The mass selection in vacuum

3.1. Introduction: Designing a filter for ions

From the previous chapter, we can see how we can send molecules from the solution into the gas phase. Generally, however, the molecular ion beam that is generated from the electrospray ionization process comprises a multitude of different molecular ionic species. To ultimately enable single molecular imaging, we will require a method to select from this ion beam a single molecular species of interest while simultaneously rejecting everything else. Typically, since we know the masses of the molecules that we are interested in, we can filter the ions, keeping only those ions that have the right masses. That is, **we want to mass select our molecules of interest.**

How do we go about filtering through all the molecules?

Well, since the molecules in our ion beam are charged, we can make use of a combination of electromagnetic fields to do this filtering. This is because charged particles (either atoms or molecules) respond in a particular way when exposed to these fields. As we will see later in this chapter, these charged particles respond to these fields in a way that depends on their mass and charge, or more specifically, their **mass-to-charge ratio** (m/z; m = mass number, z = charge number). The point is that with careful manipulation of these external fields, we can filter out, based on their m/z values, unwanted molecules while keeping those that we care about in our ion beam.

Sometimes, however, either by design or by chance, the molecules that we are interested in can exist in our ion beam in different charged states. They could have a charge of +1, +2, +3, and so

on in the same ion beam. Despite having the same mass (since it is the same molecule) they will have different *m*/*z* values, from their different charge states (same *m* different *z*). Thus, we can also potentially choose the charge state of the mass-selected molecule of interest.

In the end**,** mass-selection means that we can have an ion beam that is **free of contaminants** and that is **composed of a specific molecular species** that is **in a specific charged state** (Figure 3.1).

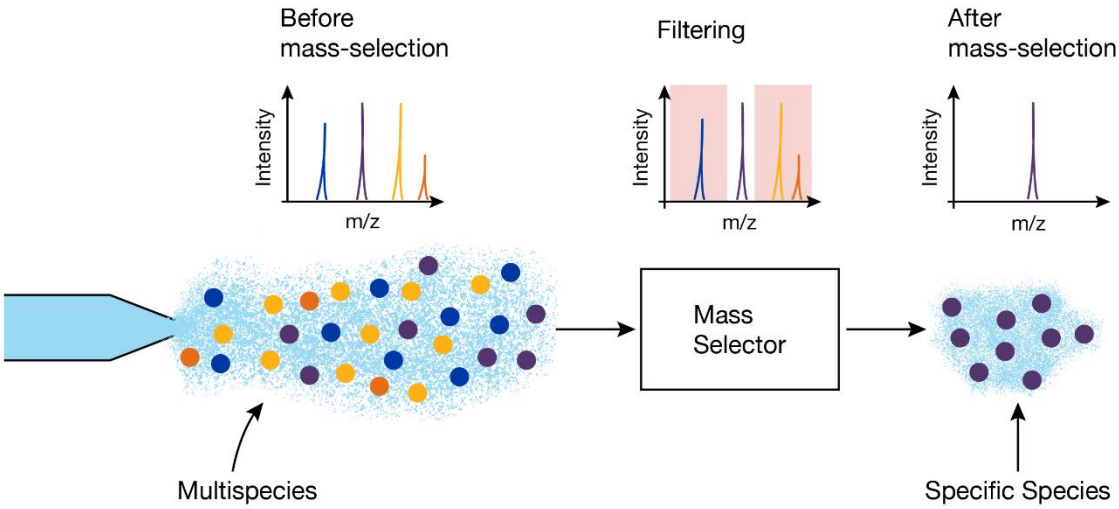


Figure 3.1 | Schematic of the mass-selection process. The ion beam, generated from the electrospray ionization process, may contain contaminants, multimolecular species, and/or molecules in multiple charged states (e.g., +1, +2, +3, -1, etc.). Passing the ion beam through a mass-selector allows for the purification of the ion beam by filtering out unwanted components, leaving us with an ion beam with a specific molecule in a specific charged state.

To ensure reliability and robustness, even for weak ion beams, the mass selection is generally performed under vacuum conditions (with pressures << 1 mbar). This ensures that ion trajectories are determined and controlled solely by external electric and magnetic fields, which is critical for precise mass-to-charge ratio selection. The ability to guide the ion beam into a mass-selector is

also crucial as otherwise nothing comes out from the mass selection process as nothing goes in to begin with. Both aspects, establishing vacuum conditions and guiding ion beams, are discussed in this chapter before finally discussing *m*/*z* mass-selection.

3.2. Vacuum basics

From the kinetic theory of gases, one can calculate the average distance that a molecule can travel before colliding with other molecules. This average distance between successive collisions, also known as the mean free path, necessarily depends on the pressure, which in turn depends on the number of molecules in a given volume. In ambient pressure, the mean free path of molecules is on the order of nanometers (see Table 3.1) whereas in pressures of ~10^{-7} mbar, the mean free path can reach kilometers. Under ultrahigh vacuum (pressures of ~10^{-10}), this mean free path can reach hundreds of kilometers. What this means is that under ambient pressures, the trajectory of one molecule is almost impossible to predict as it can undergo many collision events in a short distance and time period. Under high to ultrahigh vacuum conditions, molecules travel much farther without undergoing collisions, that is, their trajectories can remain unchanged for much longer (except for when they hit the walls of the chamber they are confined to! See Figure 3.2).

Pressure (mbar)	Mean free path
~1000 (ambient pressure)	~60 nm
~100 (low vacuum)	~10 μm
~ 10^{-2} (medium vacuum)	~10 mm
~ 10^{-7} (high vacuum)	~1 km
~ 10^{-10} (ultrahigh vacuum)	~10^{3} km

Table 3.1 | Typical mean free path of particles in differing pressures at room temperature.

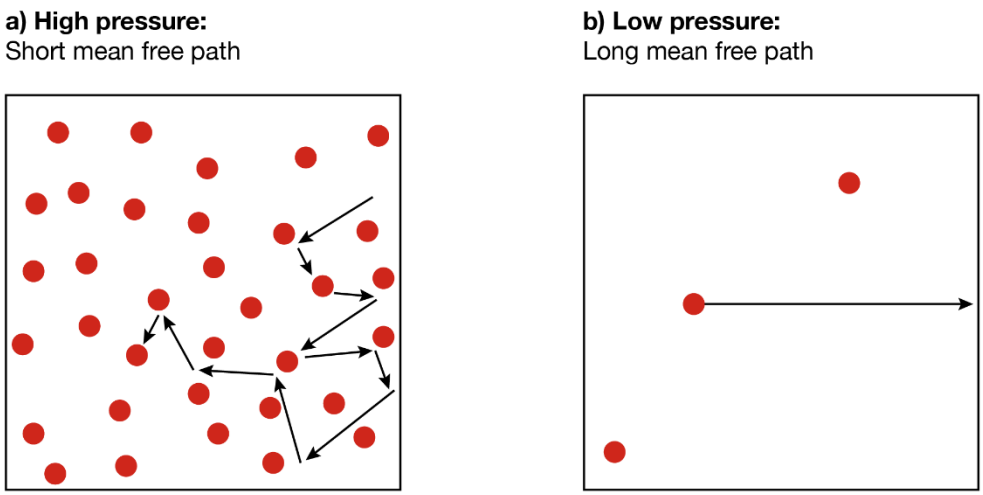


Figure 3.2 | Mean free paths of particles in differing pressures. Particles in high pressure regimes undergo frequent collisions with each other and therefore acquire a short mean free path. Whereas particles in a low-pressure regime rarely undergo collisions and acquire a long mean free path.

In essence, under high and ultrahigh vacuum conditions, one can treat particles as isolated objects free from influence from other particles. This has several advantages: (1) the analysis of the trajectories of charged particles in vacuum due to external electric and/or magnetic fields is made easier as collisions between particles (which affect the trajectories of the particles) can be reasonably ignored; (2) higher ion transmissions (e.g., through required apertures) can be achieved due to the severe reduction in random particle collisions that would alter ion trajectories; (3) unwanted chemical reactions between particles (due to collisions) can be controlled and severely mitigated; and (4) the breakdown voltage between two conductive electrodes in vacuum can be made very high (see Paschen's law).

Sidebar text box: What is Paschen's Law? Paschen's Law describes the relationship between the breakdown voltage required to initiate electrical discharge (such as a spark) between two electrodes. The law shows that for a given gas and electrode material, the breakdown voltage depends on the gas pressure and the separation between the electrodes. The breakdown voltage increases as the pressure decreases

well below ambient pressures. At lower pressures, the gas becomes less conductive because there are fewer gas molecules to ionize to cause an electrical discharge between electrodes. However, discharge can still occur even at lower pressures if the gap between the electrodes is sufficiently small.

3.2.1. Establishing vacuum

Creating a vacuum essentially means removing gas molecules from an enclosed environment to reduce its pressure well below atmospheric pressure. In connection to high-vacuum or ultrahigh vacuum single molecule imaging methods, stainless steel chambers (with polished clean interiors so that little foreign contaminants can desorbed from surfaces) are employed as a vessel from which air will be evacuated. Various kinds of vacuum pumps can then be connected to these chambers to pump out internal gas and achieve the desired pressures. These vacuum pumps can be broadly categorized into: (1) positive displacement pumps; (2) momentum transfer pumps; and (3) entrapment pumps.

Positive displacement pumps work by mechanically trapping and expelling gas molecules to reduce the pressure of the chamber. These kinds of pumps include rotary vane pumps, scroll pumps and diaphragm/membrane pumps. These pumps are commonly used to achieve low to medium vacuum (1 mbar to ~10^{-3} mbar) within a chamber. Scroll and diaphragm pumps are also often used as a backing pump (see below).

Momentum transfer pumps rely on high-speed motion to direct gas molecules out of the chamber. A popular example includes turbomolecular pumps which are extremely effective for achieving high to ultrahigh vacuum pressures (10^{-3} mbar to 10^{-10} mbar). These pumps however require backing pumps to ensure that forevacuum level, that is the pressure level at the exhaust of the

turbomolecular pump, remains low (1 mbar to ~10^{-3} mbar) to effectively maintain the high to ultrahigh vacuum conditions in the chamber.

Entrapment pumps are pumps that capture gaseous molecules by condensing or chemically binding them. Such pumps include cryopumps which can achieve high to ultrahigh vacuum by trapping gas on cold surfaces as well as getter pumps which uses reactive materials to chemically absorb gas.

While the kinds of pumps are an important factor to consider in establishing vacuum, other factors to note are the pumping speed of the pump, e.g., turbomolecular pumps can come in small or large sizes with small turbomolecular pumps expelling air at a lower rate than larger turbomolecular pumps which can expel air at a higher rate. The size of the chamber that needs to be pumped is also an important consideration. Smaller chambers can achieve a lower vacuum level compared to a larger chamber, assuming both are pumped with an identical pump. Chamber leaks can also limit the ultimate vacuum level that can be achieved. It is important to ensure that the chamber is vacuum sealed correctly, i.e., no leaks are present. However, there are cases where intentional leaks are introduced (see next section) which therefore must be considered when establishing the appropriate vacuum level.

3.2.2. Differential pumping

The electrospray ionization process which transfers molecules in solution to gas phase happens typically in ambient conditions. On the other hand, mass selection, surface deposition, and single molecule imaging (e.g., with scanning tunneling microscopy or transmission electron microscopy) are performed in a chamber under vacuum. We therefore need a way to bridge these two extreme

pressure regimes without severely compromising the vacuum integrity of the chamber. We achieve this by means of differential pumping which is a technique to maintain different pressure levels in adjacent sections of a vacuum chamber system.

This approach involves dividing the vacuum system into separate regions with vacuum-tight partitions, each evacuated by its own dedicated pump (Figure 3.3). Narrow apertures or small orifices connect these regions (these act as intentional leaks between the regions), restricting the flow of gas between them. The reduction in gas flow between the regions ensures that each region can maintain its designated pressure level. This way, we can design a set-up such that one end of the vacuum chamber system is opened to ambient air (where we can introduce the gas phase ions from electrospray ionization process) and the other end of the vacuum chamber is held at ultrahigh vacuum for depositing these molecules on a surface, for example.

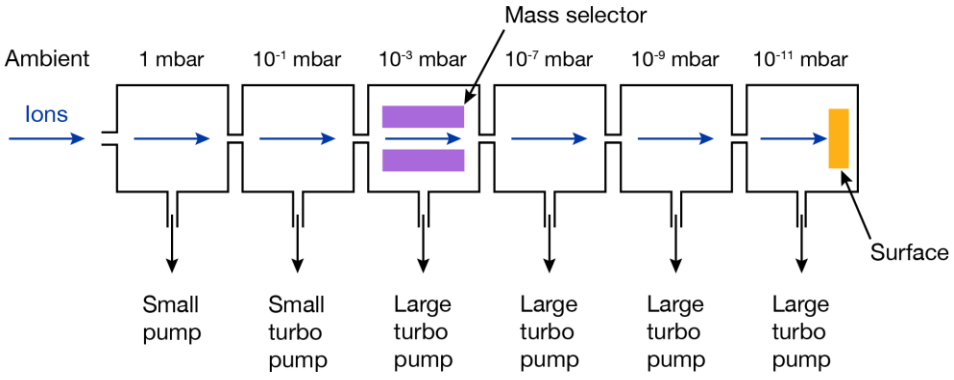


Figure 3.3 | Differentially pumped vacuum system. This schematic shows six vacuum chambers that are each pumped by a dedicated vacuum pump and are connected to each other via a small orifice. The pressure levels in the vacuum chambers decrease from the leftmost chamber to the rightmost chamber. The leftmost chamber has an opening to ambient air. This enables the introduction of ions from the electrospray ionization process directly into vacuum from low to ultrahigh vacuum conditions.

This differentially pumped chamber set-up maintains the vacuum integrity of each region while allowing for the feasibility of having an opening to ambient conditions as large as a few millimeters. This is not without its challenges as properly setting up a differentially pumped system requires precise engineering of the apertures between regions and pump placements to achieve the desired pressure gradient between the different regions. This also means increased system complexity and cost due to the need for multiple pumps. Despite these challenges, differential pumping is an indispensable tool enabling complex systems to operate across vastly different pressure ranges.

3.3. Ion manipulation in vacuum

For the purposes of mass-selection and surface deposition, it is essential that we can manipulate the trajectories of ions in vacuum. Proper trajectory control ensures that ions are guided efficiently through and between vacuum chambers, focusing them into narrow beams or directing them toward specific targets (e.g., a mass-selector or a surface). This helps to minimize any ion losses in the whole electrospray ionization beam deposition process. Additionally, trajectory manipulation compensates for initial ion dispersion, misalignment, or environmental disturbances, enabling accurate analysis or experimentation.

3.3.1. Kinetic energies of ions

The use of any electromagnetic fields to manipulate the trajectory of an ion invariably depends on how fast these ions are moving in vacuum, more specifically, it depends on their kinetic energies. When ions transit from a low-vacuum chamber (~10^{-1} mbar) into a high-vacuum (~10^{-3} mbar) chamber, the kinetic energy of these ions is largely determined by and somewhat proportional to

the voltage (electric field) applied at the aperture between the chambers. In the low-vacuum chamber the mean free path of the ions, on the order of hundreds of micrometers, is significantly shorter than the dimensions of the chamber, which means ions can still undergo many collisions with residual gas molecules. The effect of these collisions results in the loss of the ions' kinetic energy until the ions acquire an average kinetic energy proportional to the temperature of the residual gas (as a mental model, note that it is difficult for a person to run through a crowded hallway and in fact, the person's speed is somewhat limited to the average speed of the crowd in that hallway).

Now, a weak DC electric field applied across the low-vacuum chamber to high-vacuum chamber interface helps ensure that the ions maintain enough momentum to transition effectively from the low-vacuum to the high-vacuum region. As ions enter the high-vacuum region, the mean free path of the ions increases dramatically, allowing the ions to travel unimpededly. Once in high-vacuum, the ions retain the kinetic energy imparted by that weak electric field as collisions with gas molecules in the high-vacuum regions are negligible.

3.3.2. Electrostatic lenses (Einzel lenses)

Einzel lenses are essential tools in ion optics for focusing ions without significantly altering their kinetic energy. They use electrostatic fields created by three cylindrical electrodes arranged coaxially along the ion's trajectory. The central electrode is usually held at a different electric potential than the two outer electrodes, allowing the lens to manipulate the trajectories of ions passing through them (Figure 3.4).

As an example, for a positively charged ion approaching the lens, the middle electrode is held at a higher electric potential compared to the two outer electrodes. As this positive ion enters the lens and exits the first electrode, it decelerates axially due to the increase in the electric potential (from the middle electrode) and is pushed outward radially. Upon entering the second electrode, the ion continues to decelerate axially (but at a slower rate) and is pulled radially inward. As the ion exits the second electrode, it starts to accelerate axially while still being pulled inward radially. As the ion enters the third electrode, its acceleration slows, and upon leaving the lens, the ion leaves with the same kinetic energy as it had before. As a result, an ion beam, depending on the applied potentials, can be focused into a tighter beam upon leaving the lenses. The point of focus of the ion beam (i.e., the focal point) can be adjusted by adjusting these potentials. Since the ion beam necessarily diverges beyond this point, successive Einzel lenses can be used to ensure that the ion beam is refocused.

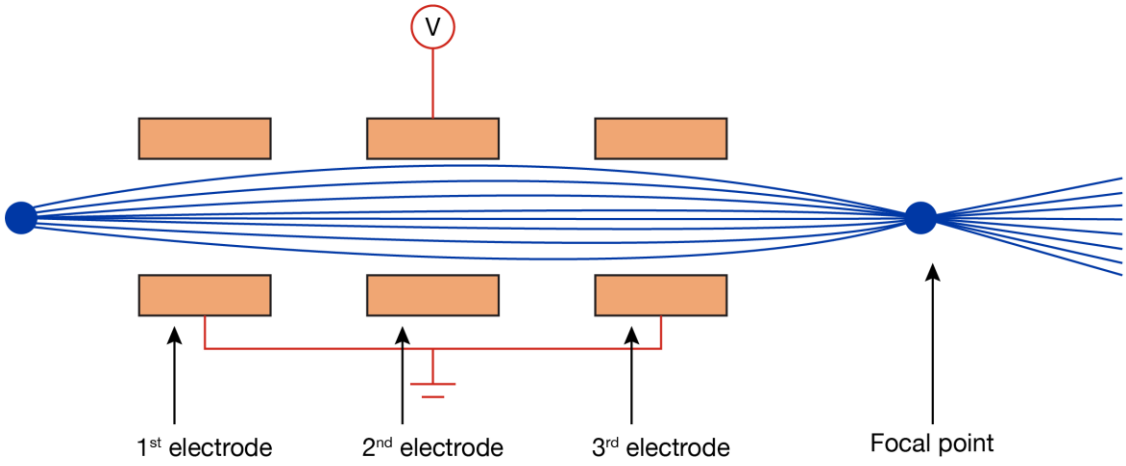


Figure 3.4 | Focusing ion beams with Einzel lenses. The schematic shows a cross section of a three-electrode Einzel lens set-up. The outer two electrodes are held at the same potential, in this case they are grounded while the middle (second) electrode is held at a different potential. For a properly applied potential to the middle electrode, an ion beam entering the lenses can be made to focus to a focal point upon exiting the lenses.

While these lenses do not alter the kinetic energy of the ions passing through, the focusing effect of these lenses depends on the kinetic energy of these ions. Typically, ions with higher kinetic energy travel faster through the lenses and are less affected by the focusing field than those ions with a lower kinetic energy. For ions with higher kinetic energy, often higher potentials are needed to achieve the same degree of focusing. Tuning these potentials is therefore important to achieve the desired results. In fact, applying incorrect potentials can lead to ion beam divergence instead of convergence upon the ion beam leaving the lenses. In some scenarios, it is even possible to completely prevent ions from traversing the lenses resulting in a termination of the ion beam at the lenses itself.

If properly tuned, a series of these lenses placed along the intended ion beam path will ensure accurate guiding of the ion beam to its target and minimal loss in ion transmission throughout the vacuum chamber.

3.3.3. Electrostatic steering plates

Steering plates are pairs of parallel, flat electrodes used to deflect and align ion trajectories by creating uniform transverse electric fields relative to the ion beam path (Figure 3.5). Simply, two plate are place on opposite sides of an ion beam. By applying a potential difference between the plates, a transverse electric field is generated which exerts a force on the ions (perpendicular to their motion), deflecting them toward the desired direction. The degree of deflection depends on the ion’s charge, mass, velocity as well as the electric field strength. Having two additional plates in along the other axis provides for fine adjustments of the ion beam trajectory in the up-down or left-right axes. These steering plates can be used to correct for misalignments or to direct ions into the narrow apertures connecting multi-chamber vacuum systems.

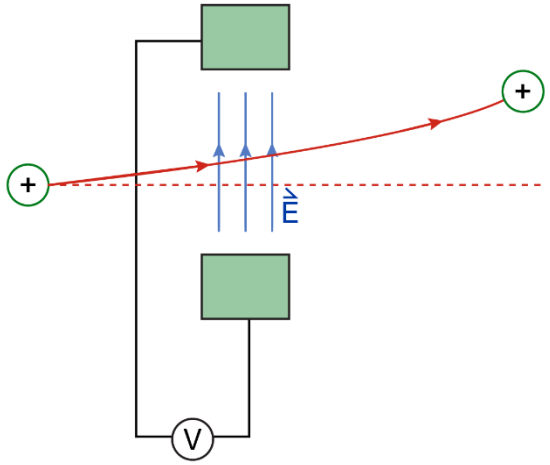


Figure 3.5 | Ion deflection with steering plates. The schematic shows a sideview of how a positively charged ion's trajectory can be deflected upwards (relative to unperturbed trajectory; red dashed line) by applying an electric field between the two electrodes (green).

3.4. Quadrupole mass selection

While there are different ways to perform mass selection—such as with a double focusing mass selector—a popular method for mass selection, especially in the context of soft landing, involves the use of a quadrupole mass selector. Its relatively low cost, straightforward design and ease of use make the quadrupole mass selector often a preferred choice in many circumstances. To this end, we focus on their principles of operation in this section. We note that the results of the mass selection process are often then checked with mass analyzers such as time-of-flight mass spectrometers, orbitraps, or Fourier transform ion cyclotrons, to name a few. The operating principles of these mass analyzers are not covered here but can be reviewed in the further readings section at the end of this chapter.

3.4.1. *m*/*z* mass selection: how does it work?

In general, mass selection based on the mass-to-charge ratio, *m*/z, of molecules depends on the combination of two physical laws. The first is Newton's Second Law (Eq. 3.1):

$$\mathbf{F} = m\mathbf{a} \qquad \text{(Eq. 3.1)}$$

$\mathbf{F}$ is force that an object of mass (m) requires to move it with some acceleration ($\mathbf{a}$). The second law is the Lorentz Force Law (Eq. 3.2):

$$\mathbf{F} = z(\mathbf{E} + \mathbf{v} \times \mathbf{B}) \qquad \text{(Eq. 3.2)}$$

This law says that a particle with some charge (z) when exposed to some electric field ($\mathbf{E}$) and some magnetic field ($\mathbf{B}$) experiences a force ($\mathbf{F}$), on it that: (1) is proportional to the electric field ($\mathbf{E}$); and (2) is proportional to the cross product of the particle's velocity ($\mathbf{v}$), with the applied magnetic field ($\mathbf{B}$).

If we now substitute Eq. 3.1 into Eq. 3.2, and for simplicity we set $\mathbf{B} = 0$, we get:

$$\mathbf{a} = \frac{\mathbf{E}}{m/z} \qquad \text{(Eq. 3.3)}$$

That is, if we know at some point in time that a particle with mass, m, charge, z, is travelling with velocity, $\mathbf{v}$, and it is exposed to some electric fields ($\mathbf{E}$), then we can use Eq. 3.3 to figure out the particle's trajectory for future times. The heart of mass selection comes from the fact that the trajectory of the particle depends, with all else being equal, on its mass-to-charge ratio (m/z) as seen in the denominator on the right-hand side of Eq. 3.3. If we are deliberate and creative with our choices for $\mathbf{E}$, we can get only molecules with the desired m/z to move through a defined path while forbidding other molecules with different m/z moving along that same path. Simply put, this is all the physics we need to build an ion filter with electric fields. We say creative, as we are not restricted to using just *static* or constant fields, as Eq. 3.3 is also valid for time-dependent and space-varying fields $\mathbf{E}(\mathbf{r}, t)$**,** which can give rise to non-trivial particle trajectories.

3.4.2. Quadrupole arrangement and principles

A quadrupole mass selector typically refers to an arrangement of four collinear cylindrical metal rods (Figure 3.6). Each opposing pair of rods are connected electrically such that voltages can be established between each pair of opposing rods. We apply some constant voltage, U, in addition to a time-dependent voltage, $V\cos(\omega t)$ (ω is the frequency of the time-dependent voltage), to the rods in this arrangement. These voltages create an electric field in the space between the rods which can be approximated by the following equation:

$$\mathbf{E} = \frac{U + V\cos(\omega t)}{r_0^2}(-x\hat{\mathbf{x}} + y\hat{\mathbf{y}}) \qquad \text{(Eq. 3.4)}$$

where r_0 is the (shortest) distance from the center axis of the quadrupole to the surface of the rods. Likewise, x and y are the distances from and perpendicular to this center axis, respectively. Note that the electric field along the center axis is zero. That is, there is only a non-zero electric field in the xy plane.

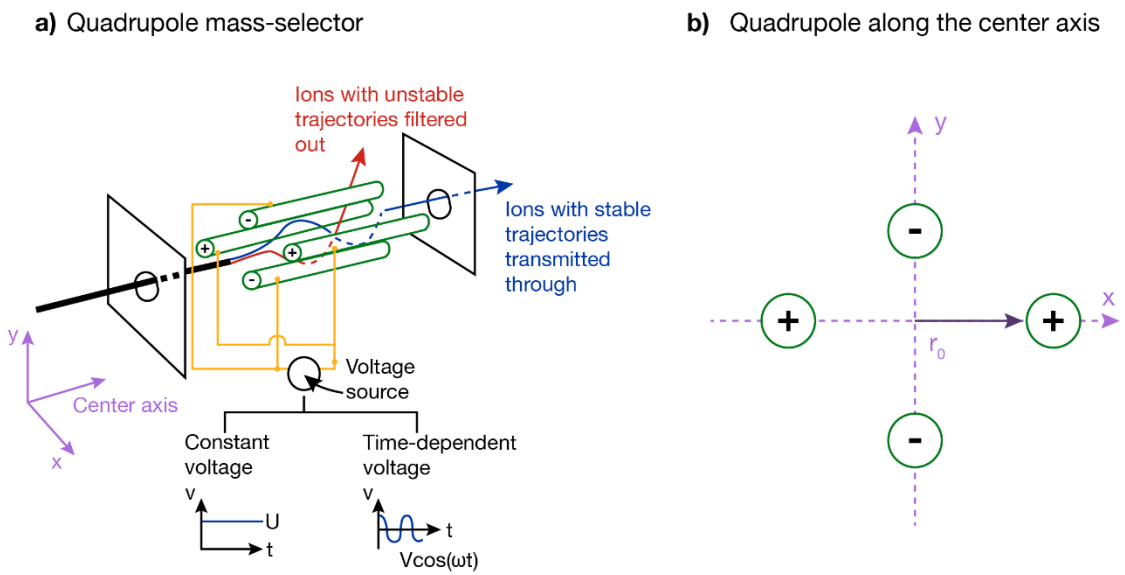


Figure 3.6 | Schematic diagram of a quadrupole mass-selector. (Left) This instrument consists of four long cylindrical rods arranged parallel to one another with each two opposing rods in electrical contact with one another. Depending on the constant and time-dependent voltages applied between the rods, particles can have either a stable trajectory through the quadrupole or an unstable trajectory and are filtered out. (Right) A view of the quadrupole along the center axis.

Substituting Eq. 3.4 into Eq. 3.3 will lead to differential equations known as Mathieu's differential equation:

$$\frac{\mathrm{d}^2u}{\mathrm{d}\xi^2} + [a_u + 2q_u\cos(2\xi)]u = 0 \qquad \text{(Eq. 3.5)}$$

Here, we have rewritten our original equation with $\xi = t/2$, $a_u = (4zU/\omega^2 r_0^2 m)$, $q_u = (2zV/\omega^2 r_0^2 m)$, and where u can represent either x or y.

What does solving this equation give us?

Well, if we set $u = x$, the solution to this equation allows us to determine the values of $a_{u=x}$ and $q_{u=x}$ where a particle has a stable motion along the x-axis. In this context, a stable motion just means that the particle motion along the x-axis remains within the quadrupoles. But, for a particle to be able to exit the quadrupole on the other side, we need it to have stable motion along both the x-axis and the y-axis. So, we need to solve Eq. 3.5 for $u = y$ as well, giving us the values of $a_{u=y}$ and $q_{u=y}$ where a particle has a stable motion along the y-axis. Plotting the region of values for a and q for which the particle has a stable motion along the x-axis or the y-axis results in a plot like that shown in Figure 3.7 (left). Where the regions overlap gives the values for a and q for which the particle has a stable motion along *both* the x-axis *and* the y-axis (regions I, II, III and IV in Figure 3.7). Outside these regions, the particle will end up leaving the quadrupole partway and hit some wall somewhere (Figure 3.6). Now, if we know these values for a's and q's (such that the particle has a stable motion) we can calculate what voltages, U and V, we need to apply to the quadrupoles for a desired molecule with specific m/z value to go through our quadrupole (we use $a = (4zU/\omega^2 r_0^2 m)$, $q = (2zV/\omega^2 r_0^2 m)$).

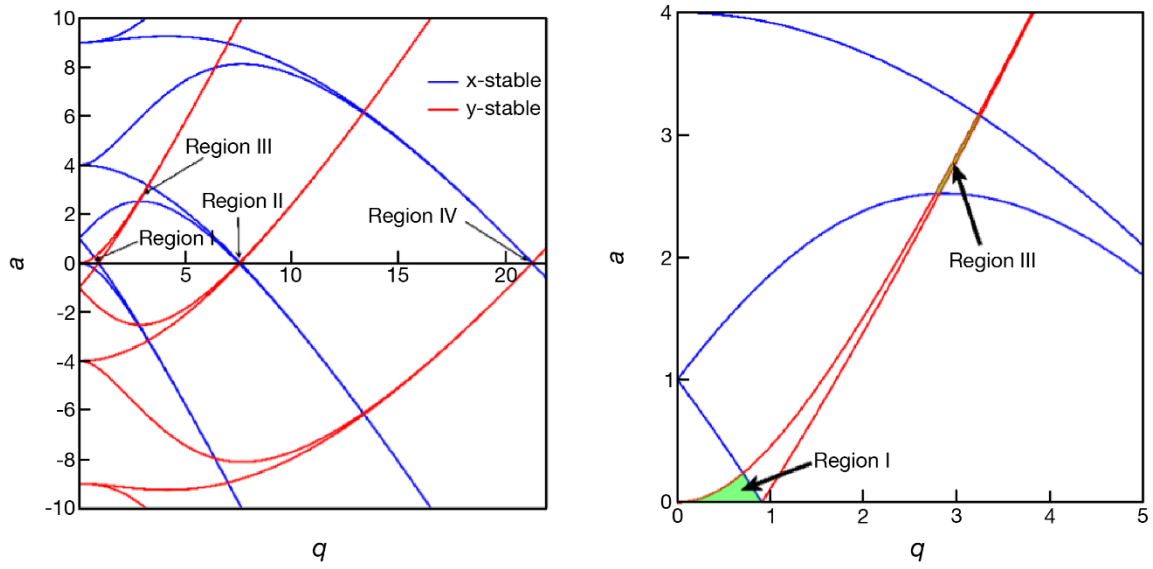


Figure 3.7 | $a - q$ stability diagram. The plot shows the values of a and q for which the trajectory of an ion through a quadrupole is stable along the x-axis (x stable) and the y-axis (y stable). The regions where the x-stable and y-stable plots overlap give the stability regions where the ion trajectory is stable along both the x-axis and the y-axis simultaneously. These overlap regions, regions I, II, III, and IV, refer to the first, second, third, and fourth stability regions, respectively. Right: zoomed-in stability diagram near the origin.

3.4.3. First stability region

In practice, we often only focus on the first stability region (region I in Figure 3.7). Since both a and q are proportional to U and V, respectively, large voltages will need to be applied to the quadrupoles to operate within the other stability regions (regions II, III or IV). Of course, this depends on the frequency, ω, of the applied voltage. Typically, frequencies of 0.5 – 2 MHz are used. In this case, it is difficult to maintain large voltages (e.g., ~1kV) at such high frequencies due to hardware electronic limitations. Furthermore, large voltages should be avoided to avoid any electrical arcs forming between the quadrupoles in vacuum (Paschen's law).

Keeping to the first stability region, we can control the range of m/z's to let through the quadrupole. We do this by controlling the ratio of the voltages, U/V, that we apply to the quadrupoles. The U/V ratio defines a line that cuts through the first stability region (Figure 3.8), where the intersection points between the line and the region of stability defines the lowest and highest m/z the ions can have to be transmitted through the quadrupole.

A small U/V ratio means that we have a line with a less steep slope cutting the region of stability (Figure 3.8, blue line), since $a/q = 2(U/V)$. The intersection of the blue line with the region of stability gives the large range of m/z to be transmitted through. If we now set U and V such that U/V is large, the line cutting the region of stability has a steeper slope (Figure 3.8, green line). Here, the green line cuts a smaller portion of the region and therefore allows only molecules with

a small range of m/z through the quadrupole. By making the ratio U/V higher, we can narrow the window of m/z that molecules are allowed to have if they want to make it to the other side of the quadrupole. However, setting U/V too high effectively forbids any molecule from exiting the quadrupole (Figure 3.8, red line).

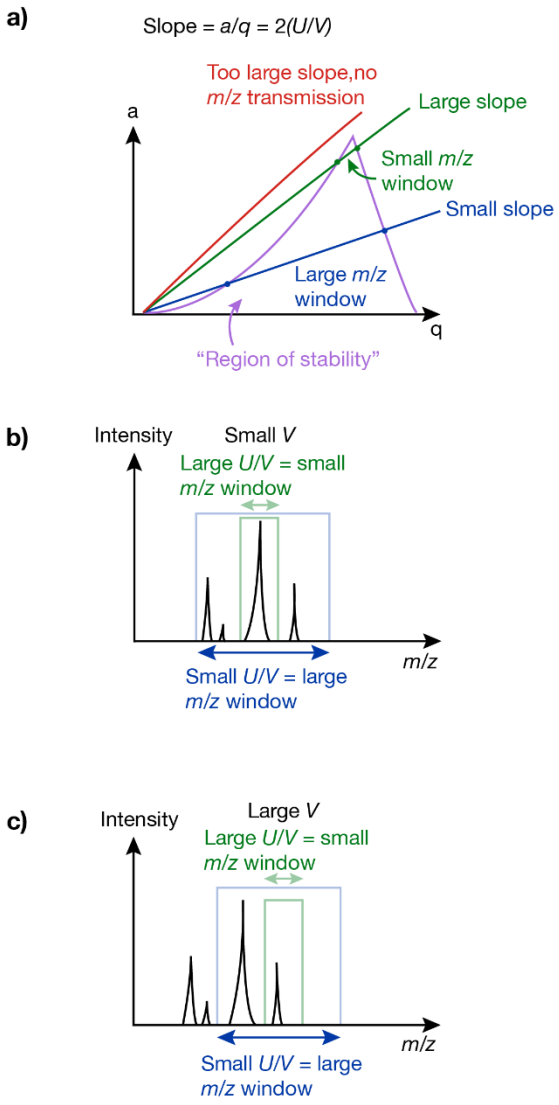


Figure 3.8 | Parameters that allow and prohibit ions to pass through a quadrupole. (a) By adjusting the ratio of applied voltages to the quadrupoles, $\mathrm{U/V}$, we can control the range of $\mathrm{m/z}$ values for which molecules can travel through the mass-selector. A higher $\mathrm{U/V}$ means a very narrow $\mathrm{m/z}$ window. While the

ratio $\mathrm{U/V}$ changes the width of the $\mathrm{m/z}$ window, the value of the time-dependent voltage, V (keeping $\mathrm{U/V}$), shifts the window to (b) lower $\mathrm{m/z}$ for smaller V and to (c) higher $\mathrm{m/z}$ for larger V.

Experimentally, we set a high U/V ratio such that the width of the m/z window that is allowed through is small (it can be as small as ± 1 atomic mass unit per charge) and then we adjust V (while keeping U/V constant) until our window is centered on the desired m/z that we want to transmit through the quadrupole. A lower V means a smaller m/z while a higher V means a larger m/z (Figure 3.8; bottom). The maximum applied V therefore defines the maximum m/z that we can measure for a fixed frequency, ω.

3.4.4. Trade-offs

In practice, the straightforward design and easy maintenance of the quadrupole device make it often the go-to when it comes to mass-selection. The small form factor (sizes typically on the order of centimeters) makes it convenient to use in a complex vacuum system. Though, some consideration should be made with regards to how compact the quadrupole should be. A small r_0 will not allow sufficient space for ions to separate in space. Furthermore, the theoretical treatment of the quadrupole implicitly assumes that the quadrupoles are infinite in length where the charged particles are exposed to an infinite number of cycles of a time-varying voltage, $V\cos(\omega t)$. In reality, the finite length of the quadrupole can have a real impact on the resolution of the mass-selection: longer quadrupoles will improve the resolution of mass-selection, however, simultaneously, decrease the transmission of molecules through the quadrupole.

Further deviations from theory include the fact that the mass windows do not have a sharp m/z cutoff but rather a sloped cut-off. This means that a high-resolution operating mode of the quadrupole can inevitably lead to a decrease in ion transmission through the quadrupole and

therefore lead to low ion current at deposition stage and hence prohibitively long deposition time. Beyond the design of the quadrupoles, the operation and m/z window of the quadrupoles relies not just on the values of the voltages applied but how stably these voltages can be maintained. Thus, while the design of the quadrupole is simple, the electronics required to maintain a highly stable voltage signal to the rods can become a limiting factor. The more stable the signal, the more expensive the electronic hardware required.

3.5 Read these next

1. Detailed discussion on vacuum physics from theory to practice: Modern Vacuum Physics. CRC Press (2004).

2. A textbook including principles and operations of mass analyzers: Mass spectrometry: a textbook. Springer Science & Business Media (2006).

3. Detailed discussion into quadrupole-based mass separation: Computer Simulation of the Mass Filter for a Finite Length Quadrupole. Commun. Mass Spectrom. 11, 184 (1997).

Chapter 4. The soft landing

4.1. Introduction: Parachuting ions onto surfaces

Alright, now that we have sent our molecules flying and have mass selected them, it is time to land them on surfaces! In other words, we need to catch a flying object on a surface.

How would you do this? How would you catch a fast flying object?

Well, clearly, we first need to know how *fast* we are talking about. Typical ions in an ESIBD instrument fly with a ~50 eV of kinetic energy.

This is *fast*.

For a 100-Da molecule (e.g., a small organic molecule), this is 9800 *meters per second*. For a 10-kDa molecule (e.g., a small protein), this is 980 *meters per second*. As a reference, an airplane that brings you around the world flies at a mere 230 *meters per second*. Ions are thereby flying at incredible speeds in an ESIBD instrument, and we need to find ways to bring them onto a surface – stationary and intact.

Now, as we want the ion to be *stationary* on surface at the end of the day, this means that we need to reduce the speed of the ions flying at these incredible speeds to zero. While this can certainly be achieved by simply putting a surface in the flight path of the ions, this will end up badly for the ions, as they will crash on the surface and break apart into fragments. Think about this: imagine what happens when your phone falls from the third-floor balcony onto the ground?

Well, you will witness the breakup of your phone into pieces – and worry if it can be turned on again after you reassemble them.

So, how can we bring the ions onto a surface without destroying them?

Well, we need a strategy to lower the speed of incoming ions before they come in contact with the surface. By doing so, we will land them intact on surface – we will **soft land** them on surface.

The ESIBD technique relies on soft landing to deposit molecular ions intact on surface to ensure that the species adsorbed on surface is identical to the species mass-selected in the gas phase. However, soft landing is merely one regime out of the vast possibilities offered by the ESIBD technique as it allows ions to be deposited on surface with energies ranging from near zero to few hundred eVs (depending on the design of the deposition stage).

Hence, in the following sections, we present a model of ion deposition to flesh out all important parameters in bringing molecules from gas phase onto surfaces. This includes how to change the kinetic energies of ions approaching the surface, how to understand molecular excitations caused by molecules colliding with a surface, and how to decide what kinetic energies and surface conditions to use to achieve specific outcomes.

4.2. How it works: Setting the touchdown speed and direction

First, let us look at what is involved in a molecule-surface collision. When two objects collide, their collision outcome is dictated by the collision energy, collision angle, and impact parameter. The collision energy tells you how much kinetic energy is involved in the collision, the collision angle

tells you the direction by which these two objects approach one another, and finally the impact parameter tells you the distance by how much the two objects miss one another.

Now, in the context of molecule-surface collision, the surface is considered to be at rest while the molecule approaches the surface from the gas phase. Hence, the collision energy refers to the kinetic energy of the molecule approaching the surface; the collision angle refers to the direction of how the molecule approaches the surface; and the impact parameter refers to the contact site of the molecule on surface, whether it lands directly above a surface atom or on the space between two surface atoms, and so on.

Today, collision energy and angle are two parameters we can control reliably in ESIBD experiments, while no strategy exists to control impact parameter of molecule-surface collisions. We thereby describe how to control the collision energy and angle in ion deposition experiments below.

4.2.1. Collision energy

Collision energy in a molecule-surface collision refers to the kinetic energy of the molecule approaching the surface i.e., the *translational* kinetic energy of the molecule. But, why should we care about it? Taking real world examples, you can already imagine what happens if you accidentally drop your phone on the floor from your sofa versus from your third-floor balcony. From the sofa, nothing happens. But from the balcony, your phone shatters into pieces. Control of collision energy is hence key to control the collision outcome.

The collision energy is most commonly varied in ion-surface collision by using electric field to accelerate or slow down the incident molecular ion.

Consider an example, if we want to slow down a positive ion with 50 eV kinetic energy, we should have the ion travel from a point with 0 V potential (point A) to another point with higher potential (say, +40 V – call it point B). In this case, the ion will decelerate due to the electric field caused by the 0 to +40 V potential difference (Figure 4.1). In effect, this converts the ion's kinetic energy (KE) into the electrostatic potential energy (PE) of the ion as much as:

$$\Delta PE = z\left(V_{final} - V_{initial}\right) \quad \text{(Eq. 4.1)}$$

Arranging this equation to yield final ion kinetic energy gives the following expression:

$$KE_{final} = KE_{initial} - z\left(V_{final} - V_{initial}\right) \quad \text{(Eq. 4.2)}$$

Hence, for a positive ion with z = +1e, going from point A (V_{initial} = 0 V) to B (V_{final} = +40 V) converts 40 eV of the ion kinetic energy to its electrostatic potential energy, leaving the ion at point B still moving forward with 10 eV of kinetic energy (Figure 4.1, green ion). For an ion with z = -1e, the opposite happens! The ion will be accelerated instead, such that at point B, the ion moves forward with 90 eV of kinetic energy (Figure 4.1, purple ion). Altogether these examples show how ion kinetic energy can be lowered or increased simply by changing the electric potential surrounding the ion. Details on how to implement this in an experiment are outlined in section 4.4.

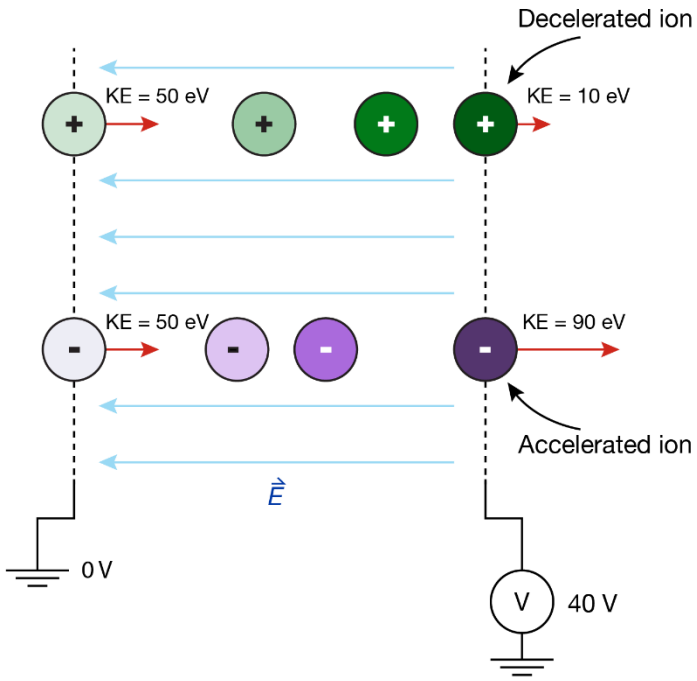


Figure 4.1 | Acceleration and deceleration of ions by electric field. Positive ions are decelerated when brought to a region with a higher electric potential, while negative ions are accelerated when brought to a region with a higher electric potential.

4.2.2. Collision angle

Collision angle in a molecule-surface collision can be defined by the approach angle of the ion toward the surface relative to a reference, such as the surface plane. Now, again, why do we need to care about collision angle? Going back to real world examples, do you recall what happens when we throw a stone to a pond at a near-parallel angle versus at a perpendicular angle? At a near-parallel angle, the stone will skip across the water, while at a perpendicular angle, the stone will sink immediately. This case demonstrates the importance of controlling collision angle to influence the collision outcome.

The simplest way to vary collision angle is by rotating the surface so that the ion beam impinges the surface at a desired angle.

4.3. What happens to ions during and after landing?

Now, let us consider what molecular motions and surface motions (i.e., dynamics) are initiated when a molecule collides with a surface.

At the fundamental level, collision is an event that distributes energy between collision partners. Hence in a molecule-surface collision, initial translational energy of a molecule approaching a surface is distributed into the kinetic energies of diverse molecular and surface dynamics. But, what dynamics are exactly excited from this?

Before we jump in, let us clarify what “molecular dynamics” and “surface dynamics” mean. Here, molecular dynamics refers to any internal motion in molecules, such as molecular rotation, bond vibration, bond bending, bond twisting, bond breaking, and so on. Surface dynamics refers to internal motion on surfaces, such as rippling (think of water ripples formed when a stone hits a pool of water), shearing, disintegration, and so on. Which molecular and surface dynamics are excited by a collision ultimately depends on the initial parameters of the molecule-surface collision, that is, collision energy, angle, impact parameters, and of course the identity of the incoming molecule. It is these collision-induced dynamics that dictate the outcome of any molecule-surface collision.

Now let us follow the journey of a molecular ion from gas phase onto a surface.

The first event that happens even before the molecule touches the surface is electron based. As a molecular ion approaches a surface within distances that allow electron tunneling (~1 nm or lower), a molecular ion may end up adsorbed on the surface as a charged molecule – or as a neutral molecule. Electron transfer can happen from the surface to the incoming molecular ion or vice versa depending on how the work function of the surface compares to the energy level of molecular orbitals. Here, work function refers to the energy required to remove an electron from a surface.

If the lowest unoccupied molecular orbitals (LUMOs) of the incoming ion are lower in energy than the occupied states of the surface, then one or more electrons could be transferred from the surface to the incoming ion, effectively increasing the number of electrons in the ion. Alternatively, if the highest occupied molecular orbitals (HOMOs) of the incoming ion are higher in energy than the unoccupied states of the surface, then one or more electrons could be transferred from the incoming ion to the surface, effectively decreasing the number of electrons in the ion. When both conditions are not satisfied, the ion retains the number of electrons it has in the gas phase when it adsorbs on the surface. We understand that through these molecule-to-surface or surface-to-molecule electron transfers, a molecular ion from the gas phase may end up as a neutral molecule on a surface.

Next, let us look at the ion dynamics as it touches the surface.

The dynamics could be largely categorized into impulsive and thermal dynamics. Impulsive dynamics (also known as non-equilibrium dynamics) refers to molecular motions where energy is largely present in a few specific types of molecular motions, while thermal dynamics (also known as equilibrium dynamics) is understood as motions where energy is distributed equally among all possible molecular motions. Following the first contact between an incoming molecule and a

surface, the first few picosecond (10^{-12} s) of the dynamics is governed by impulsive dynamics, which eventually weakens and are replaced by thermal dynamics within a few nanoseconds (10^{-9} s). But, what exactly are these impulsive dynamics and thermal dynamics in the context of molecules and surfaces?

From the molecular viewpoint, the main impulsive dynamics are compression and shearing, whose proportions depend on the collision angle. When molecules collide perpendicular to the surface, they experience compressive forces. Alternatively, when molecules collide near parallel to the surface, they experience shear forces. Such molecular compression and shearing may break covalent bonds or non-covalent interactions (e.g., hydrogen bonds) to give mechanically induced chemical reactions or conformation changes, depending on the extents of compression and shearing. These extents depend on the energy of the molecule-surface collision. On top of inducing compression and shearing, higher collision energies could cause molecules to bounce back to the gas phase, while lower collision energies could help molecules to adsorb on surfaces. The exact values of these energies strongly depend on the surface reactivity, as we will detail in section 4.4.

Following the impulsive dynamics operative within first few picoseconds, thermal dynamics begin to dominate. Energy associated with molecular compression and shearing begins to spread to other motions, where, within few nanoseconds, this energy spreads almost uniformly among every atom in the system (molecule and surface). Following the complete redistribution of collision energy, the adsorbed molecules are fully governed by their dynamics on surface such as their diffusion on surface, their rotation on surface, and so on, detailed in section 4.4.

From the surface viewpoint, the collision creates pressure waves that travel away from the collision site, along the surface plane and also into the bulk of the surface. These waves are

identical to ripples formed in a pool of water when you throw a stone into it. As collision energy increases, these shockwaves increase in amplitude, which at some point, is strong enough to break bonds between the surface atoms. Notably the extent of energy that can be adsorbed by the surface depends on the surface rigidity, detailed in section 4.4. As an analogy, a bouncy ball responds very differently when dropped on a pool of sand or on a ceramic floor.

4.4. Soft landing in practice

Now that we have a model of molecule-surface collision in our mind, it is time to dive into real practice. At this point, we should have a mass-selected ion beam aimed toward a surface. Three decisions need to be made before starting a deposition experiment: (i) the landing energy of ions, (ii) the deposition time, and (iii) the surface choice.

4.4.1. The landing energy

Setting the landing energy, that is the collision energy of the ions onto surface, involves two steps: (i) sweep the surface bias to measure the kinetic energy of ions, and (ii) set the appropriate surface bias so that the ions land on the surface with the desired energy. Step (i) is accomplished by measuring the ion current arriving from the ion beam aimed perpendicularly toward a surface while changing the voltages applied to that surface (called retarding voltages). By increasing the retarding voltages, we expect the ion current reaching the surface to decrease to zero at some large retarding voltage as now all ions are completely repelled by the surface (Figure 4.2a). This measurement of the ion current as a function of retarding voltage gives a sigmoidal curve (Figure 4.2b). The first derivative of this curve yields the energy distribution of the ions in the ion beam (Figure 4.2b), allowing us to determine the most probable kinetic energy of the ions as well as its

spread. Next, on step (ii), the desired landing energy is set by applying an appropriate voltage to the target surface. For example, if we have an $[M+2H]^{+2}$ ion flying at 100 eV kinetic energy and we want to land this ion with 1 eV landing energy at the surface, then we need to set +49.5 V on the surface, following equation 4.2.

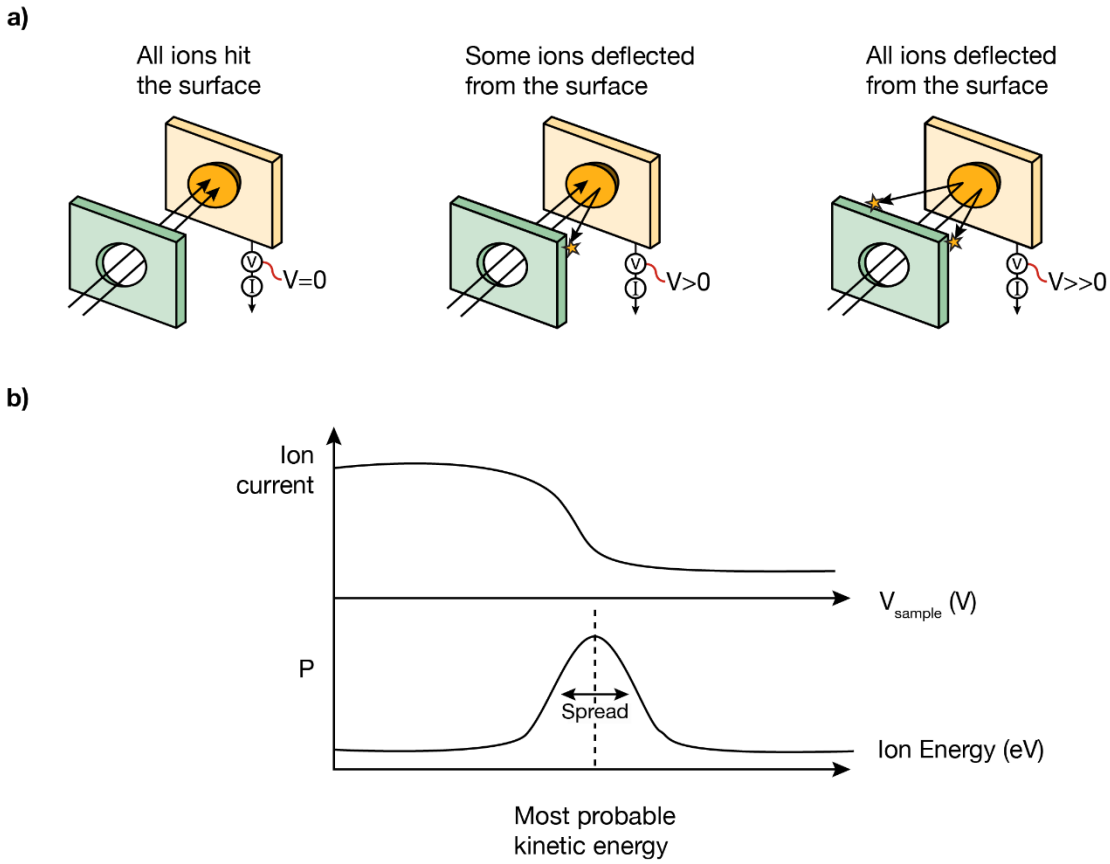


Figure 4.2 | Instrument setup for ion deposition. Panel (a) shows the effect of increasing retarding voltage on the ion motion (here assumed to be positive ions). Panel (b) shows a typical sigmoidal curve of current vs retarding voltage obtained from an energy measurement, and the first derivative of this sigmoidal curve which yields the distribution of ion kinetic energy per ion charge.

The landing energy is critical to the outcome of the molecule-surface collision event (Figure 4.3). However, the choice of collision energy should consider the size of the molecular ion that you

want to deposit. Given that kinetic energy is proportional to the molecular mass and, by that extension, to the number of atoms, the choice of kinetic energy should be normalized to either the molecular mass or the number of atoms. So far, for molecular ions landing on metal surfaces, landing energies of ~4 meV/atom (or ~300 m/s) allow gas phase molecules to preserve their structures upon landing on surface, ~40 meV/atom (or ~980 m/s) could cause molecules to change their conformation upon molecular landing, and ~80 meV/atom (or ~1500 m/s) are sufficient to induce chemical reaction upon molecular landing.

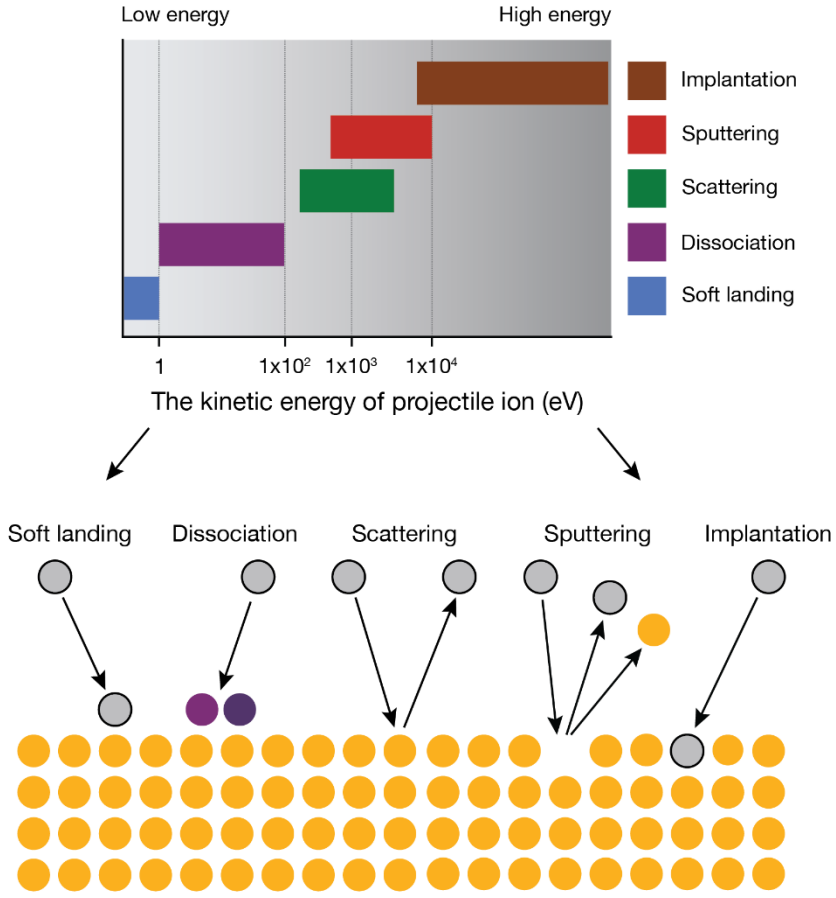


Figure 4.3 | Ion-surface collision effects. The different ion-surface collision outcomes are illustrated according to the level of kinetic energy. Credit: Adapted with permission of Annual Reviews, from Jacobs, D.C.; Reactive Collisions of Hyperthermal Energy Molecular Ions with Solid Surfaces. *Annual Review of*

Physical Chemistry. **2002,** *53,* 379-407. DOI: 10.1146/annurev.physchem.53.100301.131622; permission conveyed through Copyright Clearence Center.

4.4.2. The deposition time

Once we know the landing energy, it is time to decide how much ions you want on a surface. Do you want to have a sub-monolayer, monolayer, or even multilayer coverage? These considerations will give you a target coverage denoted by the number of molecules per unit area, which can be used to calculate how much total ions you should deposit on surface.

Let us consider an example. Suppose that you want to have a sub-monolayer coverage of $[M+H]^{+1}$ molecules of around 1 molecule per 100 x 100 nm^2. For a circular surface with a radius of 0.2 mm, this corresponds to a total of 1.2 x 10^{11} molecules on the surface. With an ion current of 20 pA arriving at the surface, this corresponds to a deposition time of 17 minutes (0.28 hour).

4.4.3. The surface choice

Finally, the choice of the surface comes into play. Four parameters are worth considering here: (i) surface conductivity; (ii) surface thickness, (iii) surface reactivity, and (iv) surface temperature.

First, the surface conductivity must be taken into account as we are landing ions on surfaces. Insulating surfaces may lead to charging issues. As more ions arrive on the surface, the electric potential starts to build up to the point that it may be enough to repel the incoming ions, preventing these additional ions from landing on the surface. Hence, the use of conductive surfaces (e.g., metal surfaces) is important to ensure deposition of significant ions on the surface as well as to allow measurement of ion current from ions that arrive on the surface.

Second, surface thickness is critical to ensure compatibility with the goal of the deposition experiment. If you want to image the deposited molecules with SPM, *thick* metal surfaces are often used in UHV-based SPM while graphite (i.e., HOPG – highly ordered pyrolytic graphite) surfaces are common for ambient SPM. If you want to image the deposited molecules with electron microscopy, *thin* film substrates are used such as freestanding single layer graphene or amorphous carbon.

Sidebar text box: Scanning probe microscopy (SPM) versus electron microscopy (EM). SPM and EM have similarities and differences in their sample requirements due to their unique imaging mechanisms. In both SPM and EM, the sample should be made as rigid as possible to prevent any mechanical vibration that create artifacts in the imaging process. An SPM image is formed by moving a tip over a surface at a very small (<1 nanometer) tip-surface distance to measure a signal (e.g., electric current, force between tip and surface, etc.) at every X and Y coordinates on the surface. In the case of EM imaging, an image is formed by scattering a beam of electrons aimed toward a sample. Any vibration from loose parts of the sample (e.g., a loose screw on sample plate or a loosely held crystal on a sample plate) would create artifacts in both SPM and EM images. In SPM, vibrations would change the tip position and the tip-sample distance, while in EM, vibrations would change the area where the electron beam hits the sample. On top of these, common EM imaging mode (i.e., image generated by electrons that penetrate through a sample) requires the sample to be as thin as possible (ideally below 50 nanometers, and no more than 100 nanometers). For more details, we refer the reader to the literature (Bian, K., Gerber, C., Heinrich, A.J. *et al.* Scanning probe microscopy. *Nat Rev Methods Primers* **1**, 36 (2021). https://doi.org/10.1038/s43586-021-00033-2; Ayache, J., Beaunier, L., Boumendil, J. et al. *Sample Preparation Handbook for Transmission Electron Microscopy.* Springer, New York, NY (2010). https://doi.org/10.1007/978-1-4419-5975-1).

The thickness of the surface also determines its mechanical rigidity, influencing the outcome of the deposition. To imagine this, think of what happens when you land on a trampoline (thin, flexible surface) versus when you land on a ceramic floor (thick, rigid surface). For example, gas phase structures of proteins are preserved by landing them on flexible, single atom thick graphene; while some structural changes occur when folded proteins are landed on rigid crystal surfaces of metals

or insulators. Notably, hard surfaces could also be coated with soft matter (such as polymers or self-assembled monolayers) to cushion molecules landing on these surfaces.

Second, surface reactivity is important in dictating what happens to the deposited molecules after they arrive on the surface. Molecules adsorbed on a surface could undergo non-reactive events, such as diffusion or conformation change, as well as reactive events, such as dissociation or reaction with other adsorbed molecules to give new molecular species on surface (Figure 4.4). Consider highly reactive silicon surfaces, e.g., Si(100) or Si(111), against inert metal surfaces, e.g., Au(111). In contrast to Au surfaces, silicon surfaces are extremely reactive due to presence of dangling bonds, causing the surface to form covalent bond with any atoms or molecules adsorbed on it, effectively anchoring them on the surface. As a *heuristic*, here we sort common surfaces in surface science experiments from the most reactive to the least reactive: Si, Ir, Pd, Cu, Ag, Au, NaCl, graphene, graphite. For noble metal surfaces, (110) facet tends to be the most reactive while (111) facet tends to be the least reactive, with (100) facet lies somewhere in between.

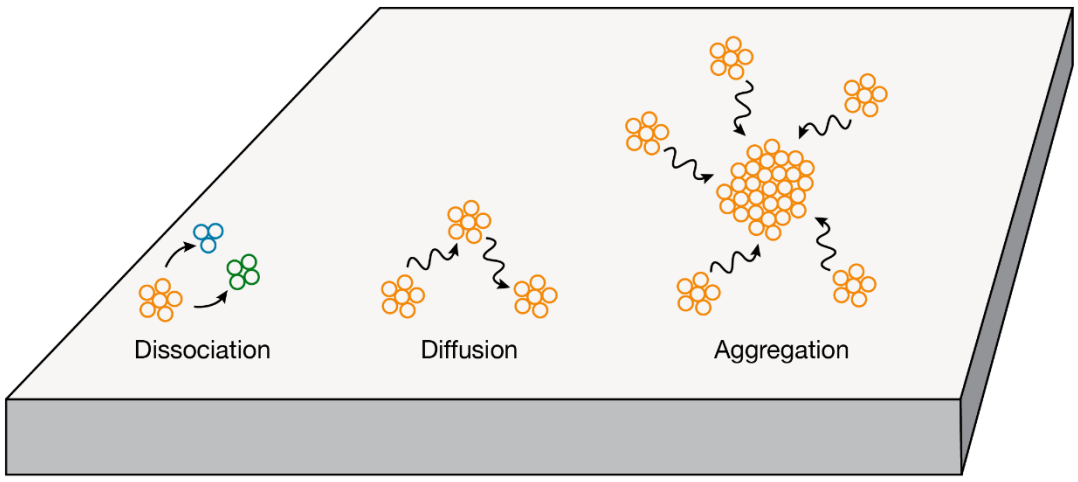


Figure 4.4 | What do molecules do on surfaces? Molecules on a surface could diffuse or aggregate into molecular islands. These adsorbed molecules could also dissociate or react with other molecules on the surface to give new molecular species.

The choice of surface ultimately depends on the goal of the experiment. For single molecule analysis by SPM, an ideal surface should be sufficiently reactive to limit molecular mobility on surface by increasing its diffusion barrier (otherwise, molecules will diffuse and form 2D molecular islands on surface), but also sufficiently inert so that the deposited molecules do not chemically react further into something else. Our experience finds that Cu(100), Cu(111), and Ag(110) hits the balance required for such experiments. For material synthesis, an ideal surface should be inert to encourage high mobility of deposited molecules so that they form large islands of assembled molecules. Common surfaces used here are inert surfaces, such as graphite, graphene, Au(111) surfaces, or surfaces decorated with self-assembled monolayers.

Finally, surface temperature is an important parameter that dictates whether adsorbed molecules have sufficient energy to overcome the barrier for various molecular processes on surface (Figure 4.4). For single molecule analysis, deposition of ions on cold surfaces is often desired to deactivate any molecular diffusion on surface. Given that activation barriers for molecular diffusion are often smaller than that for chemical reactions, deposition on cold surfaces also ensures that molecules do not further react chemically after being adsorbed on the surface, thus ensuring that the identity of molecules on surface remains the same as that in the gas phase. For material synthesis on surfaces, elevated temperature may be desired to encourage molecules to diffuse and react with one another on surfaces, and also to give enough energy for molecules on surface to arrive at their most thermodynamically stable states.

Overall, the combination of surface thickness, surface reactivity, and surface temperature provide a wealth of parameters to achieve your experiment goals.

4.5 [Insert Insider: How do you use soft landing of mass-selected ions in your lab?]

4.6. The future of the field

This primer has described the role of ESIBD in empowering the field of single molecule microscopy and surface science. The soft landing technology has also found remarkable success in advancing material sciences (see the Insider Q&A video in section 4.5). Also, very recently, ESIBD has found success in interfacing with the field of cryo-electron microscopy, allowing the three-dimensional gas phase structures of proteins to be imaged in real space. We anticipate that ESIBD could find ripe areas of exploration when coupled with recent ion manipulation techniques in tandem mass spectrometry.

Building on top of section 1.4, we hereby speculate several additional directions where explorations remain scarce. (i) Soft landing on insulators. This could be realized by performing the ion landing in non-vacuum environment (e.g., in 1 mbar of Ar), allowing ions on surface to be "solvated" with noble gases to form a "Coulomb crystal" like assembly on surfaces – or alternatively, concurrent deposition of positive and negative ions could be envisioned. (ii) Optical control of ion state prior to soft landing. Quantum state of molecular ions could be manipulated via laser pulses prior to their soft landing, thereby attaining quantum state-dependent soft landing. (iii) Soft landing on liquids. This could lead to nanostructures at gas-liquid interfaces, which could allow unique properties of gas-liquid interface to be realized (e.g., nanostructures formed by soft landed ions at the gas-liquid interface could be used to control evaporation rates of different dissolved gases in liquids). These speculative directions paint an exciting future that awaits the soft landing technology.

4.7 [Insert INSIDER - What is the future of this field?]

4.8. Read these next

1. Detailed discussion on ion-surface collision dynamics: (a) Reactive collisions of hyperthermal energy molecular ions with solid surfaces. Annu. Rev. Phys. Chem. 53, 379 (2002). **(b)** Fast Molecular Compression by a Hyperthermal Collision Gives Bond-Selective Mechanochemistry. Phys. Rev. Lett. 126, 056001 (2021). **(c)** Exploring the Molecular Conformation Space by Soft Molecule–Surface Collision. J. Am. Chem. Soc. 142, 21420 (2020). **(d)** Landing Proteins on Graphene Trampoline Preserves Their Gas-Phase Folding on the Surface. ACS Cent. Sci. 9, 151 (2023).

2. Fundamentals of surface chemistry: Introduction to Surface Chemistry and Catalysis. ISBN: 978-0-470-50823-7 (2010).

3. Other areas of ion soft landing: Soft-Landing of Peptides onto Self-Assembled Monolayer Surfaces. J. Phys. Chem. A. 4, 1678–1687 (2006).

4. Proof of principle of ESIBD+cyroEM: Cryo-EM of soft-landed β-galactosidase: Gas-phase and native structures are remarkably similar. Sci. Adv. 10, eadl4628 (2024).

5. Ion manipulation techniques in gas phase: The Omnitrap Platform: A Versatile Segmented Linear Ion Trap for Multidimensional Multiple-Stage Tandem Mass Spectrometry. J. Am. Soc. Mass Spectrom. 33, 1990 (2022).