

SINGLE MOLECULE SCIENCE

**Physical Principles
and Models**

Dmitrii E. Makarov



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Preface

This book is on chemical physics and chemical kinetics as viewed through the prism of single-molecule measurements. Traditional chemical kinetics describes how (large) amounts of chemicals evolve in time. A single-molecule measurement zooms in on the elementary processes that cause such time evolution. This book provides an introduction to the mathematical tools and physical theories needed in order to understand, explain, and model single-molecule observations. This book will not teach you how to actually perform single molecule measurements (although the physical principles behind some of the more popular single-molecule techniques are discussed here), but a vast body of already existing literature is concerned with the experimental protocols.

This text does not assume any background beyond undergraduate chemistry, physics, and calculus. Every effort was made to keep the presentation self-contained and to derive or justify every result starting from a limited set of fundamentals (such as several simple models of molecular dynamics and the laws of probability), although the mathematical rigor of some of the proofs may not be up to a purist's standards. The essential concepts used throughout the book (such as the Boltzmann distribution or the rules for working with probabilities) are explained in the two Appendices. Tedious derivations, topics requiring more advanced math, as well as the discussion of issues that are tangential to the main subject are relegated to the sections designated as "Further Discussion." Reading the Further Discussion material is not necessary in order to follow the main body of the book except that some of the mathematical formulas would have to be taken for granted.

The selection of the topics is somewhat geared toward the single-molecule methods used by biophysicists. Within such a narrower scope, the coverage of the relevant models and theoretical ideas is extensive. But given that single-molecule techniques are now widely used across many fields of science, overall cohesion took priority over the book's breadth. For example, I had to (reluctantly) leave out any discussion of single-molecule electronics as a very different set of theoretical ideas and methods would have to be introduced to describe it. Furthermore, of a variety of alternative theoretical approaches to single-molecule phenomena, the discussion is limited to the ones that I felt provided the most intuitive and the least mathematically demanding picture. As a result, some of the topics belonging to the standard repertoire of a theoretical chemical physicist (such as the Fokker-Planck equation) were not included in the book. Finally, some topics were left for the reader to explore as the Exercises randomly dispersed throughout the text.

I am fortunate to have many colleagues and collaborators who have shared their insights with me. Jin Wang was the first person to introduce me to the then emerging field of single molecules about two decades ago and Maria Topaler was my first collaborator on this topic. As a member of Horia Metiu's research group I was lucky to work on a range of problems, from single-photon statistics to kinetic models of protein folding and unfolding, which shaped up my current understanding of those

phenomena and got me interested in the field of biophysics. Kevin Plaxco's work on protein folding was another major influence which convinced me to focus on biophysics problems (and to subsequently collaborate with Kevin on a variety of topics).

Benjamin Schuler, besides being a wonderful collaborator, took a particular interest in this book project and provided detailed comments and invaluable advice on almost every chapter. My thinking on many of the topics described in this book was further influenced by many discussions with colleagues, particularly with Ioan Andricioaei, Stanislav Avdoshenko, Paul Barbara, Christopher Bielawski, Johnathan Brantley, Frank Brown, Alan Campion, Srabanti Chaudhury, Ryan Cheng, Yuri Dahnovsky, Atanu Das, Giovanni Dietler, Olga Dudko, William Eaton, Ron Elber, Kilho Eom, E.L. Florin, Irina Gopich, Alexander Grosberg, Helen Hansma, Paul Hansma, Alexander Hawk, Graeme Henkelman, Wilson Ho, Lei Huang, Gerhard Hummer, Tamiki Kamatsuzaki, Serdal Kirmizialtin, Anatoly Kolomeisky, Sai Konda, Christy Landes, Ronald Levy, Hongbin Li, Pai-Chi Li, Andreas Matouschek, Liviu Movileanu, Mauro Mugnai, Abraham Nitzan, Henri Orland, Garegin Papoian, Baron Peters, Steve Presse, Gregory Rodin, Peter Rossky, Michael Rubinstein, Omar Saleh, Reza Soheilifard, Andrea Soranno, John Stanton, John Straub, Attila Szabo, Devarajan Thirumalai, Brian Todd, David vanden Bout, Eric vanden Eijnden, Arthur Voter, Zhisong Wang, X. Sunney Xie, Haw Yang, and Giovanni Zocchi. Of course, I am solely responsible for any misinformation that may result from reading in this book.

1 A Brief History of Thought and Real Single-Molecule Experiments

No one has ever seen or handled a single molecule. Molecular science, therefore, is one of those branches of study which deal with things invisible and imperceptible by our senses, and which cannot be subjected to direct experiment.

James Clerk Maxwell, *Molecules*

At the time of writing, the existence of molecules has been viewed as proven for about a century. Although various philosophers had argued that matter must consist of small, indivisible particles for millennia, early scientific evidence for molecules came around the early 19th century after chemists noticed that, in order to form chemical compounds, their components or “elements” have to be mixed in certain simple proportions. For example, carbon was known to form two different oxides. To make the first one out of one gram of carbon, it would have to be combined with 1.333 grams of oxygen. In contrast, the second oxide would require 2.666 grams of oxygen. John Dalton explained such observations by positing that chemical compounds consist of molecules, which, in turn, are formed from atoms. The molecule of the first oxide (CO, in modern notation) consists of one carbon atom and one oxygen atom, while the second one (CO₂) has one carbon and two oxygen atoms, which immediately explains why it requires twice as much oxygen. Furthermore, one readily concludes that the ratio of the mass of the oxygen and the carbon atom must be equal to $1.333 \approx 4/3$. By analyzing such proportions in various chemical compounds, an internally consistent list of *relative* atomic masses was established. For example, if the hydrogen mass is taken as the atomic mass unit, then the masses of carbon, nitrogen, and oxygen are, respectively, equal to 12, 14, and 16. Unfortunately, the *absolute* mass of an atom cannot be determined in this way. Further refined by his followers, notably by Amedeo Avogadro who proposed the existence of diatomic molecules such as O₂ or H₂, Dalton’s ideas became widely accepted as the 19th century progressed. Despite overwhelming indirect evidence for Dalton’s atomic theory, however, the lack of any direct observations of atoms and molecules led many prominent scientists to view molecules as convenient mathematical devices rather than real physical entities.

Remarkably, estimates of physical properties of molecules such as their mass, speed, or size had been deduced from experimental observations long before molecules could be observed. Those estimates were based on the bold proposition that macroscopic properties of gases originate from random motion of their constituent molecules. First put forth around 1738 by Daniel Bernoulli and currently known as “kinetic theory of gases,” this theory, in particular, explains the pressure that a gas exerts on the walls of its container as a result of incessant bombardment by the gas molecules. Let u_x be the component of the molecule’s velocity measured, at the instant just before the

molecule strikes the container wall, along the axis x that is perpendicular to the wall. Assuming a perfectly elastic collision, u_x changes its sign once the molecule bounces off the wall. As a result, a momentum $mu_x - (-mu_x) = 2mu_x$, where m is the molecule mass, is transferred to the wall. According to Newton's second law, the total force exerted by the gas on the wall equals the momentum transferred to the wall by all the molecules per unit time. The molecules that strike the wall during a short time interval Δt are those that happened to be close enough to reach the wall. Specifically, they must be within the distance $u_x \Delta t$ from the wall. The number of such molecules is $\rho A u_x \Delta t / 2$, where ρ is the number of molecules per unit volume, A is the area of the wall, and the factor $1/2$ accounts for the fact that half of the molecules contained within the volume $A u_x \Delta t$ are actually moving away from the wall and should not be counted. The number of molecules that hit the wall, per unit time, is thus $\rho A u_x / 2$, resulting in a force that is normal to the wall and equal to $2mu_x \rho A u_x / 2 = m \rho A u_x^2$. Since the velocities of different molecules are different, this result has to be averaged over all the molecules. Using angular brackets to denote an average quantity and recognizing that the gas pressure P is the force per unit area, we find

$$P = \rho m \langle u_x^2 \rangle = \frac{1}{3} \rho m \langle u^2 \rangle.$$

Here u denotes the total velocity of a molecule and the following obvious identity is used,

$$\langle u^2 \rangle = \langle u_x^2 + u_y^2 + u_z^2 \rangle = 3 \langle u_x^2 \rangle,$$

with u_y and u_z being the components of the velocity along two axes orthogonal to x . Finally, notice that

$$\rho_m = \rho m$$

is the mass density (i.e., the mass per unit volume) of the gas. Therefore, all one needs to know in order to estimate the root mean square velocity of a molecule is the gas pressure and its mass density:

$$\langle u^2 \rangle^{1/2} = \sqrt{3P/\rho_m}.$$

The result depends on the gas in question and, typically, ranges from hundreds to thousands meters per second. To a physicist of the 19th century, it must have been shocking to conclude that his skin is bombarded by little projectiles each moving as fast as a bullet from a gun.

But what perhaps was even more surprising was that those projectiles did not travel very far on the average: collisions with other molecules cause them to frequently change their course, with the resulting net motion being very slow. In a lecture reported in a 1873 issue of *Nature* [1], James Clerk Maxwell opened a bottle of ammonia. If each ammonia molecule traveled along a straight line, it would have reached the walls of the lecture hall in a fraction of a second. Yet, as a result of collisions and the ensuing erratic motion, it took an appreciable time before the audience could smell the chemical. A more quantitative version of Maxwell's lecture demonstration enabled another 19th century physicist, Johann Josef Loschmidt, to estimate the size of a molecule, from which the number ρ of molecules per unit volume (often referred to as the Loschmidt number) and, consequently, the molecular mass could further

be deduced. Loschmidt's experiments with gas diffusion allowed him to estimate the mean free path, i.e., the average distance λ a molecule travels before colliding with another molecule. Without going into details of the actual measurements, let us show how λ can, in principle, be estimated from Maxwell's demonstration.¹ The trajectory of a selected ammonia molecule consists of connected straight-line segments, each having a random direction. Although all the segments do not have to be of the same length, an order-of-magnitude estimate of the mean segment length λ can be obtained if we pretend that all of them have a length equal exactly to λ . Mathematical properties of such trajectories, also referred to as random walks, are discussed in Appendix A. Using the results from the appendix, the mean square distance traveled by the ammonia molecule away from the bottle is given by

$$\langle r^2 \rangle = n\lambda^2,$$

where n is the number of steps in the walk (i.e., the number of segments), which can be estimated as the length of the trajectory of a molecule traveling during a time t with a typical velocity u (which we have already estimated) divided by the length of each straight segment:

$$n \approx ut/\lambda.$$

Thus we have

$$\langle r^2 \rangle \approx \lambda ut.$$

If r is taken to be the length of the hall where Maxwell lectured, then

$$t = \frac{r^2}{\lambda u}$$

can be taken as a crude estimate of the time it takes the ammonia smell to spread uniformly over the room. Of course, some of the molecules may travel the distance r and reach the listeners' noses sooner than the mean time t predicts so a more careful analysis would require consideration of the probability distribution of r derived in Appendix A. Given our unrealistic assumption of perfectly still air, however, such more refined estimates will not be pursued here. The mean free path can now be estimated, once the size of the lecture hall r and the smell spreading time t are measured, as

$$\lambda \approx \frac{r^2}{ut}.$$

It is clear that the mean free path must be related to the molecular size d for, if the molecules were infinitely small, they would never collide with one another. This relationship can be crudely estimated if we think of all molecules as hard spheres of diameter d . Imagine tracking the path of a selected molecule. During some time interval t , it will collide with every molecule whose center came within the distance d from the molecule's path. Since the length of the path is $l = ut$ then the number of the molecules that act as obstacles is simply $\pi d^2 l \rho$, where, again, ρ is the number

¹ In practice, our estimate would not be very realistic as it requires the air in the lecture hall to be perfectly still.

of molecules per unit volume of the gas. The average distance traveled between two collisions is, therefore,

$$\lambda = \frac{l}{\pi d^2 l \rho} = \frac{1}{\pi d^2 \rho}. \quad (1.1)$$

If one of the two quantities, ρ or d , is known, then the other one can be calculated from Eq. 1.1. However the experimental information considered so far does not appear to allow independent estimation of either of the two. Loschmidt realized that yet enough relationship between d and ρ can be established by comparing the volume occupied by a gas, V_g , and the volume of the same material in the liquid form, V_l . Suppose the material contains N molecules. Then, by definition, we have

$$\rho = \frac{N}{V_g}. \quad (1.2)$$

On the other hand, Loschmidt reasoned, the typical distance between the molecules of a liquid must be comparable to the molecular size d . Indeed, close proximity of molecules is supported by the fact that liquids are nearly incompressible. The volume of the liquid then can be estimated as

$$V_l \approx c N d^3. \quad (1.3)$$

Even if we believe that molecules are truly spheres, the exact numerical proportionality coefficient c is not easy to calculate unless the molecules are packed in an orderly fashion (which we know is not the case for a liquid). For an order-of-magnitude estimate, this numerical factor will simply be omitted. For consistency, we will also drop π from Eq. 1.1. Taking the ratio of Eqs.1.2 and Eqs.1.3, we now arrive at the sought after independent relationship between ρ and d :

$$\rho d^3 \approx \frac{V_l}{V_g}. \quad (1.4)$$

Rewriting Eq. 1.1 as $\lambda \approx \frac{d}{d^2 \rho}$ and using Eq. 1.4, we find

$$\lambda \approx d \frac{V_g}{V_l}.$$

This gives an estimate of the molecular size,

$$d \approx \lambda \frac{V_l}{V_g},$$

which, to within a numerical factor, agrees with that of Loschmidt. Specifically, using experimental data on gas diffusion, he estimated d to be in a nanometer range, in remarkably good agreement with our modern knowledge. The number of molecules per unit volume can now be estimated using Eq. 1.1, which gives:

$$\rho \approx \frac{1}{\lambda d^2} \approx \frac{1}{\lambda^3} \left(\frac{V_g}{V_l} \right)^2.$$

Maxwell's estimate for this number, which is now called the Loschmidt constant, is $1.9 \times 10^{25} \text{ m}^{-3}$ [1]. This estimate is remarkably close to the modern value of $\approx 2.69 \times 10^{25} \text{ m}^{-3}$ at 0°C and one atmosphere.

A different piece of evidence for molecules as building blocks of all materials came from the discovery of incessant random motion (Brownian motion) exhibited by small material particles suspended in water, which is usually attributed to the Scottish botanist Robert Brown. In 1905–1906, Albert Einstein and, independently, Marian Smoluchowski developed quantitative theories of Brownian motion based on the premise that it originates from the bombardment of the Brownian particles by the surrounding molecules of water. Some of the predictions of their theories will be described in Chapter 4 of this book. In particular, the Einstein-Smoluchowski equation, Eq. 4.17, relates the viscous drag experienced by a Brownian particle and its diffusion coefficient, which both can be measured experimentally. This equation contains the physical constant k_B that relates the energy of a molecule to temperature and is known as the Boltzmann constant.² As a result, the value of the Boltzmann constant k_B can be estimated. On the other hand, kinetic theory of gases predicts the Boltzmann constant to be related to the universal gas constant R , which is the experimentally measured proportionality constant in the ideal gas equation of state,

$$PV_m = RT,$$

where V_m is the volume occupied by one mole of an ideal gas at pressure P and temperature T . The relationship between the microscopic constant k_B and the macroscopic constant R is given by

$$k_B = R/N_a,$$

where N_a is the number of molecules in one mole, referred to as Avogadro's number. Once k_B is known, Avogadro's number can also be estimated.³ Finally, the mass of a molecule can now be estimated by dividing the mass of one mole by Avogadro's number. Experiments conducted by Jean Baptiste Perrin in 1908 confirmed the predictions of the theory of Brownian motion and yielded estimates of k_B , N_a and other microscopic properties. Despite the lack of direct observation of molecules, those experiments were generally viewed as the final proof that molecules are real.

The preceding discussion attempted to describe a century or so of efforts in gathering and interpreting circumstantial evidence for molecules and atoms. But when was a molecule first observed? In response to this question, my colleagues gave diverse answers. Should we count jewelers as single-molecule experimenters given that, technically speaking, a diamond crystal is a single molecule? Probably not. The detection of microscopic (particularly, radioactive) particles has been demonstrated as early as in the beginning of the 20th century, when Hans Geiger, Ernst Marsden, and Ernest Rutherford performed their famous experiments with alpha particles and when C.T.R.

²For example, the mean translational kinetic energy of a molecule at a temperature T is $3k_B T/2$. See Appendix B for further details.

³Note that Avogadro's number is related to the Loschmidt constant through $\rho = \frac{N_a P}{RT}$ and so Loschmidt's estimate should be regarded as the first estimate of N_a as well. Also note that Max Planck independently estimated the value of k_B from his theory of black body radiation, which was published in 1900 and which is regarded as the beginning of the quantum theory.

Wilson invented the cloud chamber. Notwithstanding those early advances, the birth of single-atom and single-molecule measurements is usually attributed to several developments that occurred in the 1970–1980s. In the field of electrophysiology, reversible jumps in the conductance of cell membranes were interpreted as arising from the conformational changes in single ion channels [4,5], i.e., pore-forming membrane proteins that can selectively control the passage of ions through cell membranes. In a distinctly unrelated field of quantum optics, Kimble et al. observed fluorescence photons emitted by individual atoms in an atomic beam [2,3]. Their experiment analyzed temporal correlations among photons emitted at different times and confirmed the “photon antibunching” effect predicted earlier by quantum theory: unlike the photons emitted by multi-atom sources, photons from a single atom tend to “repel” one another so that one photon is unlikely to be immediately followed by the next. The photon antibunching effect, which will be further discussed in Chapter 7 of this book, is now routinely used to verify whether observed light has a single-molecule origin.

The experiments of Kimble et al. employed an atomic beam but were performed under the conditions such that no more than a single atom was typically in the observation volume at any moment. Later studies showed that individual atomic ions could be confined within electromagnetic traps for a long time [6]. Those experiments employed radiation pressure from tunable lasers to slow down the motion of atoms thereby cooling them to extremely low temperatures at which they could not escape the applied electromagnetic field. In contrast to atoms, however, cooling molecules with lasers remained elusive because, in addition to their translational motion, molecules also undergo rotation. This problem was circumvented by a different approach, in which the molecules of interest were trapped within a solid [7,8]. Achieving a single-molecule level of resolution within this technique was facilitated by a phenomenon that is normally considered a nuisance: because of the interaction of the “guest” molecule with a “host” solid, each molecule sees a somewhat different local environment and, as a result, absorbs and emits light of somewhat different wavelength. This results in the so-called inhomogeneous broadening effect, where the observed spectral lines are much broader than those expected from an individual molecule. But what was considered to be a handicap in the bulk spectroscopy of solids turned out to be a blessing in disguise for the single-molecule spectroscopists: when the concentration of guest molecules in the solid was low enough the laser light wavelength could be tuned so as to selectively excite only one guest molecule within the observation volume while the surrounding guest molecules remained “dark.”

Other important developments that occurred in the 1980s included the invention of the scanning tunneling microscope (STM) and the atomic force microscope (AFM), which allowed imaging and manipulation of individual molecules at surfaces. While early single-molecule spectroscopy studies required low temperatures, this limitation was circumvented in the 1990s (see ref. [9] for a review), paving the way for single-molecule studies of biochemical processes and living systems. As a result, the single molecule field saw explosive development in the 1990s, when numerous new experimental methods were developed and used in chemistry, physics, molecular biology, and materials science. By now, single-molecule experimental techniques have evolved from being technological marvels to nearly routine tools, although many

exciting developments, particularly concerning the improvement in temporal and spatial resolution, are still underway.

Finally, it should be noted that the behavior of systems containing one or few molecules has also been the subject of considerable theoretical thought, which often preceded experimental observations. In the early 1960s, for example, Terrell L. Hill published a book [10] that anticipated the future need for extending thermodynamics—which is conventionally formulated for systems of infinite size—to microscopic objects. The advent of computer simulations in the second half of the 20th century has further led to the development of Molecular Dynamics and Monte Carlo methods, which track the fate of individual molecules on a computer. Although the original purpose of those studies was not necessarily to observe the behavior of individual molecules, virtual single-molecule computer experiments became a natural by-product. As the reader will see from the following chapters of this book, recent discoveries made through single-molecule experiments continue to go hand in hand with theoretical advances in molecular science.

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2 How the Properties of Individual Molecules Are Measured

... Why are atoms so small? Clearly, the question is an evasion. For it is not really aimed at the size of the atoms. It is concerned with the size of organisms, more particularly with the size of our own corporeal selves. Indeed, the atom is small, when referred to our civic unit of length, say the yard or the metre.

Erwin Schrödinger, *What is Life?*

2.1 TYPICAL SIZE OF A MOLECULE

The distance between the oxygen atoms in the O_2 molecule is about $R_{O-O} \approx 1.2 \times 10^{-10}$ meters. One Angström (denoted $1\text{\AA}$) is equal to 10^{-10} meters and one nanometer (1 nm) is 10^{-9} meters. The distance R_{O-O} is thus equal to $1.2\text{\AA} = 0.12\text{ nm}$. To appreciate how small this length is, imagine drawing this molecule on your notepad as two circles (representing the oxygen atoms) connected by a line. If the distance between the circles is, say, 10 centimeters, then it represents the oxygen molecule magnified by about billion (10^9) times. For comparison, the size of your drawing magnified by another billion times would be comparable to the distance from the Earth to the Moon.

Some molecules are considerably larger than the simple diatomics such as O_2 . The DNA that encodes the human genome, for example, is a molecular chain that, if fully stretched, would have a length of a few centimeters. Many polyatomic molecules fall somewhere in between these two extremes. In particular, many molecules of life have linear dimensions of a few nanometers. We will consider this to be the “typical” molecular size.

The tremendous disparity between microscopic, molecular length scales and the typical dimensions of the macroscopic devices we use to make measurements presents a tremendous hurdle to single-molecule observation. Interestingly, human sensory systems can, in certain cases, successfully overcome this hurdle and provide us with an interface to the microscopic world: For example, our eye is capable of detecting individual photons!¹

¹ Our brain, however, is designed to ignore single photons and sets the detection threshold to several photons

2.2 OPTICAL DETECTION OF AN INDIVIDUAL MOLECULE

Our eye analyzes the wavelength and the intensity distribution of the light emitted, reflected, or scattered by an object thereby providing us information about its shape and color. Typically, the light we see originates from an astronomic number of molecules. Shapes and colors of small objects that cannot be resolved with the naked eye are conveniently examined with a magnifying glass or a microscope. Could we then simply use a very powerful microscope to look at the structure of a single molecule?

Unfortunately, the wave nature of light imposes a fundamental limit on the resolution of an optical microscope. Specifically, light diffraction causes any small object observed through a microscope to appear blurred. Consequently, spatial details of an object can be discerned only if the length scale of such details is greater than, roughly, the wavelength λ of light used to observe the object. The wavelength of visible light is in the range 400–700 nm, orders of magnitude greater than a typical molecular size. Therefore, visible light scattered or emitted by a molecule carries little information about its geometry or structure. Nevertheless, this light can be used to detect *the presence* of a molecule in a spot whose size is comparable to λ .² This approach could be promising if we could come up with a sample containing 1 molecule per λ^3 , or, equivalently,

$$\frac{1}{\lambda^3} \approx \frac{1}{(500\text{nm})^3} \approx 10^{19} \frac{\text{molecules}}{\text{m}^3}.$$

For comparison, air contains approximately 2.5×10^{25} molecules per cubic meter while liquids or solids typically contain roughly 10^{28} molecules per cubic meter. It then appears that observing a single molecule in a condensed phase is impossible, as many other molecules are bound to fall within the same observation spot and interfere with the image.

Fortunately, there is a solution to this problem. Molecules can either absorb or emit light. When light is absorbed, the energy of a photon, $h\nu$, is utilized to drive the molecule from a low energy state E_1 to a high energy state E_2 ,

$$E_2 = E_1 + h\nu, \quad (2.1)$$

where $\nu = c/\lambda$ is the frequency of light, c is the speed of light, and the proportionality coefficient between the photon energy and its frequency, $h \approx 6.626 \times 10^{-34} \text{ J} \times \text{s}$, is known as Planck's constant. Likewise, a transition from a higher energy level E_2 to a lower level E_1 may be accomplished by photon emission. The wavelength of the emitted or absorbed light, therefore, depends on the energy levels $E_1, E_2, \dots$ of the molecule which, in turn, depend on its chemical identity. Different molecules have different light absorption/emission wavelengths. Textile industry routinely takes advantage of this fact: By incorporating various “dye” molecules within, e.g., cotton fibers, colorful t-shirts can be readily produced. Likewise, chemists take advantage of different “colors” of the molecules to distinguish among them. The scientific term

² Moreover, the location of the molecule within such a spot can be determined with an accuracy that is better than λ using the so-called superresolution methods. The idea behind superresolution is that a diffraction-blurred image of a molecule is nonuniform and, moreover, is centered around the molecule's true location

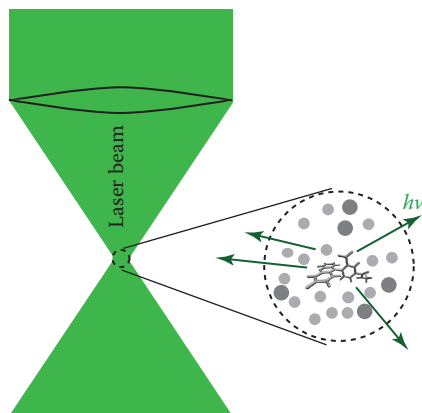


FIGURE 2.1 Single-molecule fluorescence spectroscopy experiment: A laser beam is focused in a tight spot, typically of about a micrometer in size. The wavelength of light is chosen so as to excite the fluorescent molecule of interest and the photons emitted by this molecule are detected with a single-photon detector (not shown). The concentration of the fluorescent molecules is so low that, typically, no more than one such molecule is found within the observation spot. This molecule may be surrounded by many other chemical species but those do not fluoresce (in the wavelength range probed) and so they do not impede the observation of the fluorescent molecules under study.

for this method is *spectroscopy*. A typical single-molecule spectroscopy setup is illustrated in Figure 2.1 and involves a laser beam focused into a small spot. The minimum possible size of the spot is, again, determined by light diffraction and, crudely, is comparable with the wavelength λ . Thus we estimate the volume of the spot to be

$$V \approx \lambda^3 \approx (0.1 - 1) \times 10^{-18} \text{m}^3 = (0.1 - 1) \text{ femtoliters.}$$

The wavelength of laser light is chosen so as to drive certain kind of molecules (that we want to study) to a higher energy state. As a result, those molecules can re-emit light through the process called fluorescence. By properly tuning the wavelength of light, the molecules of interest can be driven to emit light while the surrounding molecules remain dark because they have no energy levels satisfying Eq. 2.1. To observe the fluorescence light emitted by a single molecule, all we have to do now is to ensure that the concentration of the fluorescent molecules is low enough that no more than one of them is located within the detection volume V . This can be achieved, e.g., by preparing a very dilute solution of the fluorescent molecules, which contains fewer than $1/V \approx 10^{18} - 10^{19}$ molecules per cubic meter. Chemists prefer to measure concentrations of molecules in moles per liter and use the capital letter “M” to indicate these units. One mole per liter corresponds to Avogadro’s number of molecules contained in one liter of solution,

$$1\text{M} \approx 6.02 \times 10^{23} / 10^{-3} \text{m}^{-3} = 6.02 \times 10^{26} \text{m}^{-3}.$$

In terms of these chemical units, the fluorescent molecules must have a concentration of less than 1–10 nM. It should be emphasized that the total number of molecules in