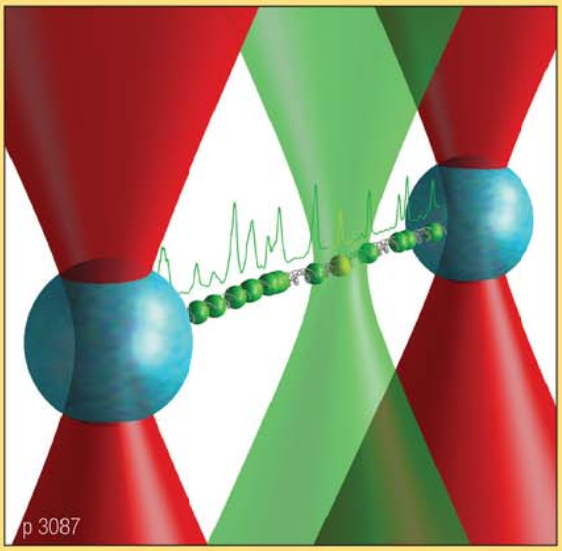
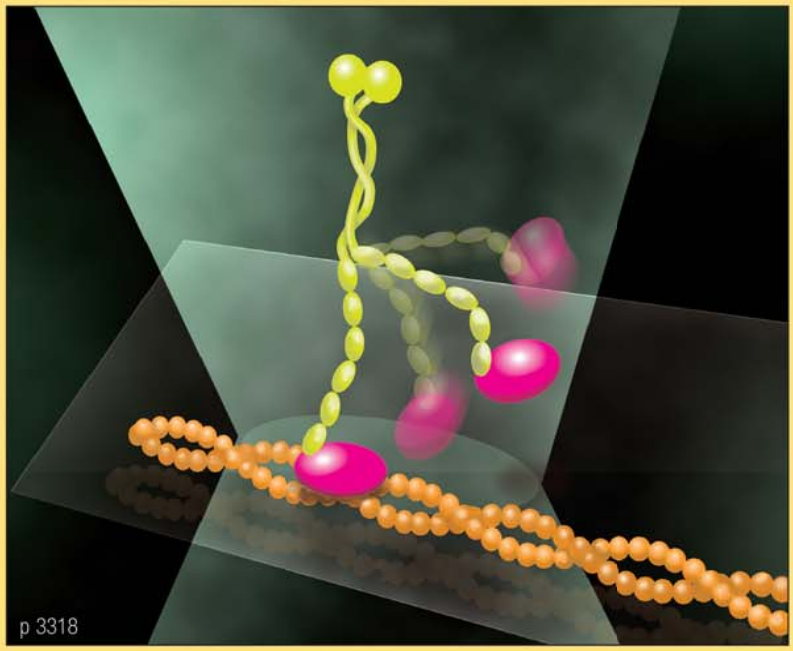
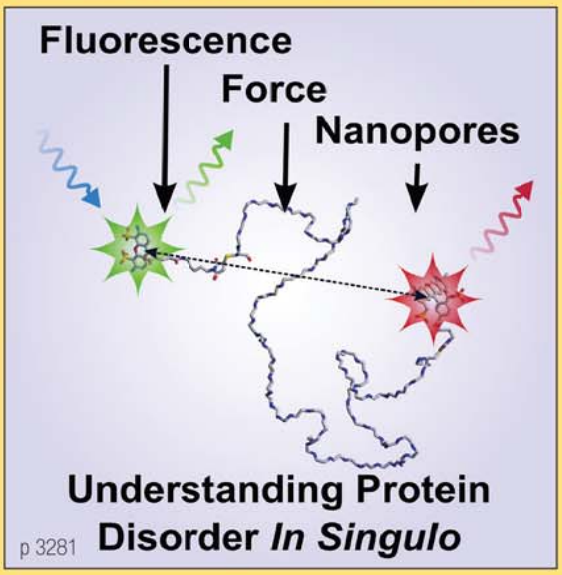
CHEMICAL REVIEWS

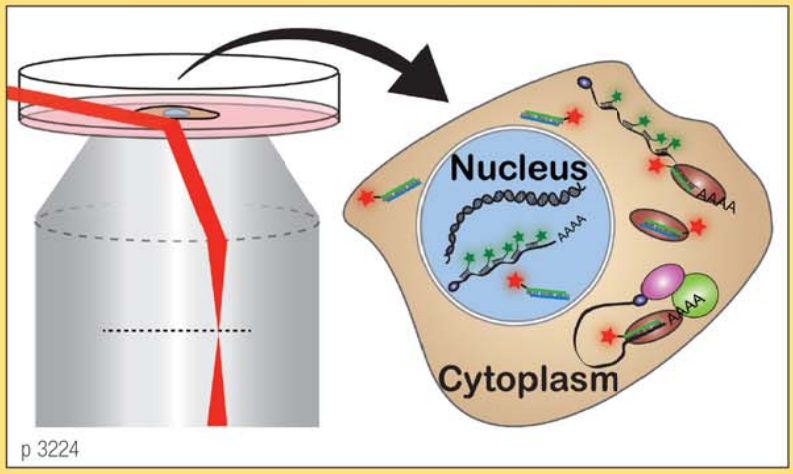
MARCH 26, 2014 VOLUME 114 NUMBER 6 pubs.acs.org/CR

SINGLE MOLECULE IMAGING AND MECHANICS: SEEING AND TOUCHING MOLECULES ONE AT A TIME









ACS Publications
MOST TRUSTED. MOST CITED. MOST READ.
www.acs.org

CHEMICAL REVIEWS

Editorial

pubs.acs.org/CR

Introduction to Single Molecule Imaging and Mechanics: Seeing and Touching Molecules One at a Time

All things keep on in everlasting motion
Out of the infinite come the particles
Speeding above, below, in endless dance
— Titus Lucretius Carus (De Rerum Natura)

One of the first concepts of molecules dates back to the pre-Socratic Greek philosopher Empedocles, who intuited around 450 B.C. that the four "elements"—fire, earth, air, and water—experience "forces" of attraction ("love") and repulsion ("strive") that allow them to mix and separate in ways that induced the origin and development of life.[1] These ideas were refined by Democritus, who conceived the existence of atoms or indivisible particles and whose ideas were preserved for posterity by the Latin poet Lucretius.[2] This knowledge was lost with the end of the Roman Empire, and reemerged only after almost two millennia in the 1661 treatise "The Sceptical Chymist", wherein Robert Boyle hypothesized that matter is composed of clusters of "corpuscles" capable of arranging themselves into groups. By the 1770s, the theory of molecules as particles endowed with hooks and barbs holding them together was generally referred to as "Cartesian Chemistry" in honor of Descartes' work on the subject.[3] It would take another 200 years to develop sophisticated microscopes that could visualize single molecules based on either their optical absorption[4] or fluorescence,[5] and mechanically manipulate and detect them through the use of magnetic tweezers,[6] optical tweezers,[7] and atomic force microscopes.[8]

In the years since the publication of these pioneering studies, the initial skepticism about the power of single molecule methods has been replaced with their enthusiastic adoption by a growing number of scientists. This special issue of *Chemical Reviews* has assembled some of the leading experts in the field with the goal of having them present the current state-of-the-art of these single molecule methods and their applications. As will become apparent throughout these contributions, the ability to interrogate individual molecules and follow their molecular trajectories provides direct access to the detailed molecular mechanisms underlying biomolecular processes.[9,10] In particular, single molecule studies naturally avoid the ensemble or population average that characterizes bulk methods, and can provide direct access not only to the mean values but also the higher moments of the kinetic coefficients that characterize a dynamic process. Furthermore, single molecule methods can give access to nonuniform kinetic behavior as well as transient and rare or unlikely molecular states that are all but impossible to study in bulk. Similarly, complex spatiotemporal distributions and properties of ensembles of single molecules in an individual cell or specimen can be revealed. Constant refinement of the microscopy tools available often has led and will continue to lead to the rapid emergence of entirely new areas of inquiry. Accomplishments have come in rapid succession over the past two decades, and the future of single molecule approaches appears assured as they are applied to samples of increasing complexity, including whole organisms.

In this thematic issue, the assembled 11 reviews represent a snapshot of many of the techniques and insights emerging from the field. Naturally, the selection of articles is limited by our own biases and the availability of authors. While we focus on current applications in the molecular biosciences, single molecule methods can equally be applied to scientific problems in, for example, materials science. It is therefore in a spirit of humble awe of what is already possible and what may still become feasible in years to come across all fields of science that we offer this inherently limited snapshot in hopes that it may help inspire new applications, technical advances, and recruitment of the brightest young minds.

First, **Greene** and colleagues remind us that DNA often plays an active, dynamic role in directing the biological functions of DNA/protein complexes. After establishing a conceptual framework for the interactions between DNA and its effector proteins, the authors review how their fluorescence microscopy based single molecule DNA curtains have opened a window onto biologically important aspects of this framework.

Next, **Wuite** and colleagues introduce us to optical tweezers as a complementary tool to probe DNA/protein interactions. They highlight the plethora of assays that have been developed to perform single molecule force measurements that quantify the mechanical properties of DNA. Upon binding protein partners, these forces are changed in a way that often provides unique mechanistic insight into the mechanics and energetics of DNA/protein complexes. On occasion, optical tweezers observations are combined with fluorescence microscopy to more completely monitor force-induced changes. This set of tools has led to insights across a whole variety of biological processes involving the replication, maintenance, and expression of genetic information.

Ando and colleagues then introduce us to recent advances in atomic force microscopy (AFM) that have allowed them to directly image any biopolymer at near video rates without the need for attaching fluorophore labels or handles for tweezing. High-speed AFM relies on a tiny cantilever tip bouncing over an atomically flat surface that is coated with the single molecules of interest, typically bound to a scaffold or membrane. The range of dynamic samples visualized so far spans from motor proteins like myosin walking on actin and membrane-bound energy-conversion enzymes such as F_1-ATPase and bacteriorhodopsin to self-aggregating proteins and cellulose-digesting enzymes, and even includes live cells. In an outlook, the authors share their perspective of how this nascent imaging tool may further evolve.

Direct observation of the dynamic spatiotemporal distributions of proteins in the cell is the topic of the review by **Lippincott-Schwartz** and colleagues. These and other authors in the field have developed a set of superresolution techniques

Special Issue: 2014 Single Molecule Mechanisms and Imaging

Published: March 26, 2014

dx.doi.org/10.1021/cr500059w | *Chem. Rev.* 2014, 114, 3069−3071

that break the classical diffraction limit of optical microscopy by utilizing the property of single molecule fluorescence emitters to generate a diffraction-limited point-spread function (PSF) when imaged with a conventional microscope. Through fitting each PSF observed in an image with a mathematical function that determines its center position at typically 10- to 20-fold higher resolution than the classical limit (as determined instead by the width of the PSF), a superresolved image is reconstructed using successive images in techniques memorably named PALM (photoactivated localization microscopy) and STORM (stochastic optical reconstruction microscopy). In this manner, both PALM and STORM separate in time what cannot otherwise be separated in space. In combination with ever more potent fluorophores that can be turned on and off so that only a sparse subset is visible in each image to avoid overlap, PALM and STORM have led to the discovery of unknown nanoscale cellular structures and dynamic processes.

Transitioning into applications of single molecule tools, **Bustamante** and colleagues describe how a process as complex as transcription—the copying of DNA into RNA—is being studied at the single molecule level. These authors show how all three phases of both bacterial and eukaryotic transcription—initiation, elongation, and termination—are being mechanistically dissected in unprecedented detail through the use of fluorescence microscopes, magnetic tweezers, and optical tweezers. Important concepts are beginning to emerge that are painting an ever sharper picture of the process of gene expression and its regulation.

RNAs as the products of transcription, in particular nonprotein coding RNAs (ncRNAs), have risen to prominence, particularly since the human genome project was completed around the turn of the millennium. The discovery that almost none (only ∼1.2%) of our genome codes for proteins, but instead for ncRNAs, has led to a frantic rush to decipher ncRNA functions and harness them for rational gene expression regulation. **Walter** and colleagues exhaustively illuminate the rapidly advancing *in vivo* studies of ncRNA mechanisms and functions using single molecule fluorescence microscopy as a relatively noninvasive tool sensitive to cellular spatiotemporal distributions of these important molecular species.

Kaiser and Tinoco then take the next step in gene expression and illustrate how the translation of mRNAs can be monitored by force, typically using optical tweezers. The ribosome is a molecular motor that often has to forcefully unfold secondary structure in its mRNA substrate. Thus, optical tweezers turn out to be particularly suitable for probing the molecular mechanisms underlying the mechanochemical transduction by ribosomes. The authors show that despite force measurements intrinsically operating under nonequilibrium conditions, equilibrium parameters such as thermodynamics and zero-force kinetics can also be derived.

Once proteins are translated by the ribosome, they may either fold or remain in a (partly) disordered state. **Brucale, Schuler, and Samori** review how intrinsically disordered proteins (IDPs) are increasingly studied by single molecule tools, including fluorescence microscopy, AFM, optical tweezers, and nanopores, where the translocation of a single protein molecule blocks an ion current that can be sensitively measured. Taken together, these complementary approaches have started to reveal important features of the structural and dynamic behavior of IDPs that often can be translated into mechanistic terms using computational methods.

Next, **Yanagida** and colleagues provide a comprehensive overview of single molecule studies of motor proteins, such as myosin, kinesin, and dynein that have uncovered how such nanoscale motors can transport cargo directionally within the noisy environment of the cell. It turns out that these proteins convert chemical energy into directed motion by harnessing and biasing the intrinsic thermal motions that dominate the cell. Elegant optical tweezers, scanning probe, and single molecule fluorescence microscopy experiments, sometimes in combination, have revealed critical parameters such as motor step size, forces generated, and—through them—mechanistic details of transport processes that can, for example, explain muscle action.

Blehm and Selvin then detail how multiple kinesin and dynein molecules can indirectly interact through a tug-of-war over a common cargo that is transported bidirectionally on an actin track. This interaction can be studied with single molecule tools from the "bottom up", by building complexes from components *in vitro*, or from the "top down", by direct localization in the cell. Both measurements and theoretical considerations have begun to yield evidence for local regulation of directionality through tug-of-war among the molecular species and global regulation through the availability and functional modification of the respective motor proteins.

Its intrinsic dynamics are critical to the function of any biopolymer. In the final review, **Brooks** and colleagues show how *in silico* single molecule modeling tools such as normal-mode analysis (NMA) and enhanced-sampling molecular dynamics (MD) simulation can reveal the large-scale motions of dynamic protein machines and resolve structural rearrangements observed in another single molecule imaging technique, cryo-electron microscopy.

We wish to thank all authors for their commitment to their contributions, which has made it possible for this issue to come together. Writing a review that is both comprehensive and comprehensible is no easy task, and some authors may have been surprised to experience how that task grew larger—not smaller—as progress was made (we certainly did!). We also need to give a big thank you to Associate Editor Rob Kuchta for inviting us to guest edit this issue and to Saundra Richter for helping us push it over the finish line. Scientific advances are always built on previous knowledge that must not be lost, and we hope that this collection of reviews becomes a stepping-stone for the next advances in the field.

Nils G. Walter*

Department of Chemistry, University of Michigan, Ann Arbor

Carlos Bustamante*

Departments of Chemistry, Physics and Molecule & Cell Biology, University of California, Berkeley

AUTHOR INFORMATION

Corresponding Authors

*E-mail: nwalter@umich.edu.
*E-mail: carlosb@berkeley.edu

Notes

Views expressed in this editorial are those of the authors and not necessarily the views of the ACS.

Biographies

Nils G. Walter was born in 1966 in Frankfurt am Main, Germany. He received his "Vordiplom" (B.S.) and "Diploma" (Masters) from the Technical University of Darmstadt after performing research with Hans-Günther Gassen on the physiochemical characterization of a protein dehyrogenase enzyme. He earned his Dr. rer. nat. while studying molecular *in vitro* evolution of DNA and RNA using fluorescence techniques with Nobel laureate Manfred Eigen at the Max-Planck-Institute for Biophysical Chemistry, Göttingen. For his postdoctoral studies, he turned to RNA enzymes under the guidance of John M. Burke at the University of Vermont in Burlington, Vermont. He is currently a Professor of Chemistry at the University of Michigan in Ann Arbor, Michigan. His research interests focus on noncoding RNA through the lens of single molecule techniques. He currently directs the unique Single Molecule Analysis in Real-Time (SMART) Center at Michigan.

Carlos J. Bustamante received his Ph.D. in Biophysics from his work with Prof. Ignacio Tinoco. After a short postdoctoral training at the Lawrence Berkeley National Laboratory, he started his own laboratory in 1982 at the University of New Mexico, where he became interested in the physical forces at work in biological processes. In 1991, he moved to University of Oregon, where he continued to develop methods for single molecule detection. He moved to University of California at Berkeley in 1998, where he is currently a Raymond and Beverly Sackler Chair of Biophysics. His laboratory has applied novel methods of single molecule detection, such as scanning force microscopy, and manipulation, such as optical and magnetic tweezers, to study the structure and function of nucleoprotein assemblies and to characterize the mechanochemical coupling of molecular motors. The biological processes of interest in his laboratory include DNA translocation, transcription, translation, protein folding, and protein degradation. Carlos Bustamante is a Howard Hughes Medical Institute Investigator.

REFERENCES

(1) Parry, R. In *The Stanford Encyclopedia of Philosophy*; Zalta, E. N., Ed.; Stanford University: Palo Alto, CA, 2012.
(2) Esolen, A. M. *Titus Lucretius Caro "On the Nature of Things: De rerum natura"*; The Johns Hopkins University Press: Baltimore, MA, 1995.
(3) Kim, G. *Affinity, That Elusive Dream: A Genealogy of the Chemical Revolution*; MIT Press: Cambridge, MA, 2003.
(4) Moerner, W. E.; Kador, L. *Phys. Rev. Lett.* **1989**, *62*, 2535.
(5) Orrit, M.; Bernard, J. *Phys. Rev. Lett.* **1990**, *65*, 2716.
(6) Smith, S. B.; Finzi, L.; Bustamante, C. *Science* **1992**, *258*, 1122.
(7) Moffitt, J. R.; Chemla, Y. R.; Smith, S. B.; Bustamante, C. *Annu. Rev. Biochem.* **2008**, *77*, 205.
(8) Bustamante, C.; Erie, D. A.; Keller, D. *Curr. Opin. Struct. Biol.* **1994**, *4*, 750.
(9) Bustamante, C. *Annu. Rev. Biochem.* **2008**, *77*, 45.
(10) Walter, N. G.; Huang, C. Y.; Manzo, A. J.; Sobhy, M. A. *Nat. Methods* **2008**, *5*, 475.