Under the Microscope: Single Molecule Symposium at the University of Michigan, 2006

ABSTRACT:
In recent years, a revolution has occurred in the basic sciences, which exploits novel single molecule detection and manipulation tools to track and analyze biopolymers in unprecedented detail. A recent Gordon Research Conference style meeting, hosted by the University of Michigan, highlighted current status and future perspectives of this rising field as researchers begin to integrate it with mainstream biology and nanotechnology. © 2006 Wiley Periodicals, Inc. Biopolymers 85:106–114, 2007

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Inspired by recent breathtaking advances in single molecule detection and analysis, the University of Michigan hosted a two-day symposium on May 18 and 19, 2006, under the rally cry: "At the Single Molecule Frontier: Integration in Biology and Nanotechnology". The focus of the meeting was to bring basic scientists, engineers, and clinical researchers from inside and outside the university together to discuss current status and future prospects of applying single molecule technologies to the most relevant current problems in biomedicine and nanotechnology. A total of 20 speakers, seven from leading institutions around the country and 13 from within the University of Michigan, gave a broad overview of the current status of the field. In a final panel discussion, the future of single molecule tools was debated. Here we review some of the highlights from the meeting.

Session 1—Single Molecule Biophysics

During recent years, much effort has been invested in developing a broad range of biophysical techniques to study the molecular properties of individual DNA and RNA molecules and an ever expanding array of structural proteins and enzymes.[1–19] Many of these techniques flourished with advances in fluorescent probes (fluorescent proteins, semiconductor nanocrystals, dendrimers), labeling techniques, and detection methods, and allowed fluorescent imaging and spectroscopic techniques to be applied to mainstream questions in modern biology. Fluorescence resonance energy transfer (FRET), for example, has recently played a key role in following directly the dynamic structural changes of biomolecules at the single molecule level, while total internal reflection fluorescence microscopy (TIRFM), with its low background signal, has facilitated imaging applications that made it possible to track individual fluorescently-labeled molecules or particles with nanometer precision. A second class of measurement techniques, including optical tweezers, atomic force microscopes, microneedles, and magnetic traps, is based on directly perturbing and/or characterizing the mechanical properties of individual molecules to analyze the molecular mechanisms they engage in. Truly ground-breaking and insightful discoveries using these single molecule approaches have rapidly expanded, in some cases even provided first insights, into the molecular mechanisms of molecules like molecular motors, DNA and RNA, DNA binding proteins, various catalytic enzymes, and structural proteins. The presentations of the first session of the symposium, chaired by Edgar Meyhofer (University of Michigan, Mechanical Engineering and Biomedical Engineering), were founded in these biophysical techniques and approaches, but pushed forward with technical innovations, new assays, and exciting applications.

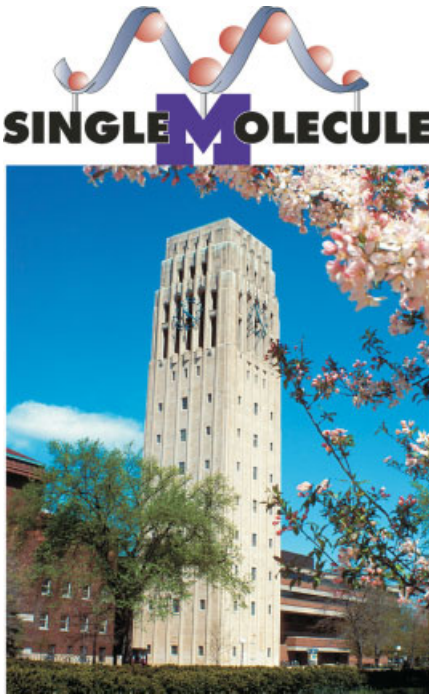

FIGURE: The Single Molecule Symposium hosted by the University of Michigan in the spring of 2006 was designed to bridge basic science, engineering, and medical disciplines and to entice researchers to more broadly integrate the power of single molecule tools into biology and nanotechnology.

Paul Barbara (University of Texas Austin, Chemistry and Biochemistry),

Director of the Center for Nano- and Molecular Science and Technology at UT Austin and newly elected National Academy member, spoke about his work on the kinetics of a nucleic acid binding protein, the nucleocapsid (NC) protein, that chaperones opening of DNA hairpins from the human immunodeficiency virus HIV-1.[20,21] The NC protein plays a central role in HIV-1 replication, as it stabilizes the enveloped virion, interacts with specific DNA and RNA hairpins to chaperone the formation of stable nucleic acid duplexes, and destabilizes the secondary structure of bound nucleic acids. Barbara and coworkers in Austin and at the University of Minnesota focus on the role of the NC protein in the conformational dynamics of transactivation response region (TAR) DNA, a hairpin structure that must anneal to its complementary RNA such that retrotranscription of the viral genome can proceed. TAR DNA was believed to exist in a closed (C-form) and a partially open (Y-form) conformation that interconvert with simple two-state kinetics on the time scale of a few milliseconds. Using single molecule FRET, Barbara and colleagues now report that the reversible transitions from the C- to the Y-form are dynamically complex with kinetics ranging from a few to longer than 250 ms, suggesting the existence of multiple structural and kinetic pathways.[21]

Jens-Christian Meiners (University of Michigan, Physics and Biophysics) reported on single molecule DNA studies that are aimed at advancing our understanding of the polymer physics of DNA and at revealing mechanical aspects of DNA that are important in its biological function.[22,23] A key emphasis of Meiners's presentation was the continued need to push the development of new and improved single molecule techniques and tools. A scanning-line optical trap, as developed in his lab, represents an intriguing example of such advances.[24] Because this particular laser trap does not exhibit the typical limitations and artifacts that are associated with active feedback force clamps, it is possible to readily exert constant forces, with high bandwidth over micrometer-long distances. Using this new technique, his group was able to extend studies of the relaxation of λ-DNA to the sub-millisecond time and femtonewton force domains, revealing new equilibrium and non-equilibrium dynamics that can be interpreted in the framework of polymer statistical mechanics. This work lays the foundation for understanding the dynamics of DNA-protein complexes in vivo.

Nils Walter (University of Michigan, Chemistry) reported on the single molecule properties of noncoding (nc)RNAs from pathogens.[25–27] These RNAs are of significant interest, because they frequently act as enzymes (ribozymes) and play important roles in the regulation of genetic information from the pathogen. Using the hairpin ribozyme, which plays a key role in the viroid infections of plants, as a model system, Walter and his coworkers analyzed dynamic properties via single molecule FRET spectroscopy and molecular dynamics (MD) simulations. Their principal findings are that the hairpin ribozyme exploits coupled hydrogen bonding networks in the catalytic core that allow structural communication between different ribozyme domains.[27] Interestingly, they also find stable heterogeneities in subpopulations of single hairpin molecules with distinct dynamics.[25] The populations are so stable that they can even be isolated by native gel electrophoresis. As heating and annealing eventually interconverts the isolated hairpin ribozyme subpopulations, Walter speculated that this surprising diversity must be related to distinct folds of the individual molecules represented by these subpopulations.

In the last presentation of the session, Duncan Steel (University of Michigan, Electrical Engineering and Computer Science and Physics and Biophysics) made a compelling case of why single molecule spectroscopic studies should be applied to the analysis of protein folding diseases. Well-known examples include amyloidogenic diseases like Parkinsons and Alzheimers, which are characterized by the aggregation of precursor proteins in effected cells that eventually lead to cell death. Key to understanding the molecular disease mechanism will likely be the oligomerization/aggregation process starting from only a few precursor molecules. Single molecule spectroscopic studies provide probably the only direct means to test this important hypothesis. Steel presented critical preliminary data that suggest that these challenging studies of tracking individual precursor molecules and small aggregates will be feasible. However, he also described significant technical challenges that relate to the photobleaching and fluorophore labeling as well as surface immobilization. Solving these problems will not only be central to this exciting new application of single molecule spectroscopy, but will also advance many other assays and broaden the applicability of single molecule measurements.

Session 2—Modeling of Single Molecule Behavior

It is well-recognized that the recent availability of data from single molecule experiments now opens new opportunities for molecular modelers in explaining and predicting experi-

mental observations. The talks in the second session, chaired by Noel Perkins (University of Michigan, Mechanical Engineering), were motivated by the opportunity to explore this close interplay between quantitative modeling and quantitative experimentation at the single molecule level.

The enormous ranges of length and time scales in single molecule science conspire to create formidable challenges to modelers. The challenge was reflected in the talks given in this session that embedded modeling methods that span multiple length/time scales. These include the atomistic-level descriptions championed by MD simulations, to continuum approximations for long biopolymers (notably DNA), to the models of statistical mechanics (e.g., bead- and worm-like chains). Clearly, high-resolution MD models are essential for developing a fundamental understanding of the short-length actions created by inter-atomic potentials. However, MD methods remain confined to short length (nm) and time (ps to ns) scales, whereas many biological processes occur on far greater length and time scales including, for example, the lipid–protein interactions, DNA packaging, and DNA looping systems described by the speakers in this session. Thus, lower resolution models arising from continuum and statistical mechanics provide valuable insights at these longer length/time scales that would be otherwise unreachable by MD methods alone. It is also likely that advances in modeling will be found at the intersections of these modeling techniques as the field of single molecule analysis further develops. The modeling session therefore could be understood as a snapshot of the current status of the field.

Rob Phillips (California Institute of Technology, Applied Physics and Mechanical Engineering) discussed the mechanics of DNA bending and its central role in the biological functions of the molecule in several organisms.[28,29] Employing continuum (rod) mechanics and statistical mechanics models of the duplex, Phillips explored model predictions and experimental data for DNA packaging and ejection in viral capsids[30–32] and DNA looping by regulatory proteins. For the latter system, the Lac repressor-DNA complex was proposed as the 'hydrogen atom of gene regulation' due to its role as a canonical gene-regulatory mechanism.

Ioan Andricioaei (University of Michigan, Chemistry and Bioinformatics) illustrated the use of atomic-resolution MD simulations[33,34] in studying the transition of the canonical B-form of DNA to the so-called P-form originally predicted by Linus Pauling. Experimentally and computationally, this unusual transition can be triggered under rather large distortion of the duplex by combined twist and extension. The B-to-P transition was successfully captured by the presented MD simulations on the length-scale of a dodecamer at 100–1000 pN forces. Subsequent thermodynamic averaging led to a reversible pathway in the free energy landscape that correctly mapped onto the phase diagram predicted from single molecule experiments.

Ronald Larson (University of Michigan, Chemical Engineering) considered both MD and course-grain models of short peptides interacting with lipid mono- and bi-layers.[35–37] The dynamics of peptide-layer insertion were simulated and revealed the strong influence of rapidly changing hydrogen-bonding interactions on peptide position and orientation in the lipid bilayers. Insertion was accelerated upon the addition of an applied electrostatic potential. Special attention was drawn to the possible 'hydrophobic mismatch' between the peptide and the lipid bilayer,[37] which was accommodated by peptide tilting, bilayer bending, and the 'snorkeling' of the positively charged lysine side chains among the negatively charged lipid headgroups.

Session 3—From Single Viruses to Molecular Motors in Cells

Despite impressive achievements in the compilation of molecular inventories and the identification of essential molecular interactions, we still lack a fundamental understanding of what it means to be alive. How does the aggregate behavior of molecules translate into the properties of the living cell? Many cell biologists strive to identify emergent properties of complex living systems and to analyze their fundamental ingredients. They are confident that the laws of physics will explain the beautiful complexities of cells, but they do not presume to be ready to describe those complexities from first principals. Rather, by studying molecules in the context of the living cell they identify the most important effects that describe molecular behaviors. This requires analyses of the stochastic behavior of molecules far from thermodynamic equilibrium, either in vitro with purified molecules, in permeabilized models of cells, or inside intact living cells. Several presentations and a great amount of discussion throughout the symposium centered on the possibilities for analyzing single molecules in the context of the living cell. The third session, chaired by Joel Swanson (University of Michigan, Microbiology and Immunology), began to show how answers of classic cell biology questions are emerging from the analysis of single molecule behavior.

Xiaowei Zhuang (Harvard University, Chemistry and Chemical Biology and Physics) described microscopic studies of influenza virus dynamics inside infected cells.[38–41] Such experiments do not per se require the ability to detect or resolve single fluorescent molecules in cells. Instead, high resolution fluorescence microscopy was used to compare the localization of virus particles, labeled with multiple fluorophores, with that of fluorescent protein chimeras that are involved in viral entry or that mark various membranous compartments. Quantitative analyses allowed dissection of the mechanism of clathrin-based endocytosis of viruses, as well as the identification of the endocytic compartments from which viruses escape.[41] The Zhuang lab has also developed novel optical switches, combinations of fluorophores that can be reversibly driven into long-lived dark triplet states.[42] These should facilitate imaging and analysis of single molecules inside living cells.

Edgar Meyhofer (University of Michigan, Mechanical Engineering and Biomedical Engineering) described work aimed to explain the behavior of the mechanochemical motor protein kinesin-1, as it interacts with microtubules, in vitro and in vivo.[43–45] A variety of technical approaches, including single molecule laser trapping, total internal reflection fluorescence (TIRF) microscopy, and nanofabrication of analytical chambers, were employed to analyze the dynamics of single kinesin-1 molecules. Quantitative models of kinesin-1 were tested in living cells by expressing and visualizing kinesin-1 chimeras containing multiple tandem Venus (fluorescent protein) molecules. Together, the studies laid out a feasible framework for analyzing the diverse behaviors of selected single molecules in the context of the living cell.

Kristin Verhey (University of Michigan, Cell and Developmental Biology) presented a different approach to the analysis of kinesin-1 function in cells,[46,47] one that complemented the approaches of the Meyhofer group. Molecular and cell biological studies identified cargo proteins that associate with kinesin-1 in a regulated manner inside cells, as well as posttranslational modifications of tubulin that bias kinesin-1 activities to selected microtubule tracks in single cells. Collaborative studies between the Verhey and Meyhofer groups analyzed fluorescent kinesin-1 molecules by TIRF microscopy, revealing the existence of preferred linear tracks, presumably alongside microtubules, inside cells.

The collaborative studies of the Verhey and Meyhofer groups provided a successful model for how complex behaviors of organelles in living cells can be analyzed productively by a cross-disciplinary approach combining thorough quantitative analysis of single molecule behavior in vitro with state-of-the-art molecular biology and fluorescence microscopy in vivo.

Session 4—Single Molecule Bionanotechnology

It is widely recognized that there is a considerable "Nano-Gap" in biology and medicine: Biochemistry provides a wealth of insight into interactions between small molecules up to the size of proteins, and cellular biology sheds light on the function of entire living cells. But a substantial gap remains in our understanding of the function of large sub-cellular structures. The gap in medicine is even larger: there are hardly any therapeutic measures on the scale between drugs and surgery.

The reason for this gap is twofold: the inaccessibility of sub-cellular structures to experimental interrogation in vivo, and the complexity of models, as the tight coupling to the molecular and cellular scale requires sophisticated multiscale modeling. Nanotechnology can help bridge this gap by engineering structures that can interact with biological entities on the nanoscale to interrogate, manipulate, or simulate these biological systems. The fourth session, chaired by Jens-Christian Meiners (University of Michigan, Biophysics and Physics), gave examples of what "bionanotechnology" tries to accomplish.

Harold Craighead (Cornell University, Applied and Engineering Physics), the Co-Director of the Nanobiotechnology Center at Cornell University, presented his approach to using "Nanostructures for Single-Molecule Detection and Analysis."[48–50] He reported the use of nanofabricated metallic structures as small apertures for near-field optical fluorescence excitation to study biomolecular interactions in extremely small reaction volumes that are of the order of 10^{-20} l. He also reported the use of microfabricated flow channels for single-molecule studies of DNA, and pointed to the importance of entropic forces for the conformation of these biopolymers.[49] These experiments may not only be of fundamental interest for understanding biological systems and interactions on the nanoscale, but could also lead to new diagnostic techniques, such as single-molecule DNA sequencing.

In the second talk of the session, Alan Hunt (University of Michigan, Biomedical Engineering) presented his work on "Microtubule Dynamics on the Nanoscale: Stability persists without a GTP-cap."[51,52] A study of the dynamics of polymerization and depolymerization of microtubules that uses microfabricated barrier structures[53] and optical tweezers to make measure-

ments with nanometer resolution indicates that the stability of the microtubule tip is mechanically determined. The microtubule tip does not require a GTP-tubulin cap, explaining the previously not-well-understood ability of the evolving tip to confer intrinsic variability to microtubule growth rate and thus plasticity to cellular morphology and motility.

In the last talk of the session, the director of the Michigan Nanotechnology Institute for Medicine and Biology, James Baker Jr. (University of Michigan, Biologic Nanotechnology), presented work on the use of PMAM dendrimers as a platform for single-molecule experiments.[54–57] These dendrimers are highly branched synthetic macromolecules that can be synthesized in a well-controlled fashion with a wide variety of attached targeting, therapeutic, and imaging groups, making them promising candidates for the nanotherapeutics of the future.

Session 5—Single Polymers In Vivo And In Vitro

Session 5, chaired by Roger Sunahara (University of Michigan, Pharmacology), included a diverse array of speakers spanning the fabrication of polymeric nanotubes and thin fibers to the utilization of nanostructures for micromanipulation. The session epitomized the state of the art where nanopolymers and nanotechnology are being utilized to advance our understanding of the bases of disease. The span of polymers discussed in this session ranged from pure carbon to deoxyribonucleic acid to actin and microtubules.

Richard Superfine (University of North Carolina-Chapel Hill, Physics and Astronomy), the Director of the Center for Computer Integrated Systems for Microscopy and Manipulation at UNC, provided an exciting seminar that interfaced nanotechnology, including single molecule force spectroscopy,[58] with the etiology of lung disease. As a member of the Virtual Lung Project at UNC, Superfine uses biophysical approaches to track the movement of lung cilia. His team uses single molecule tracking via bead technology and nanorods to follow the force generated by beating cilia. They employ magnetic bead rheology to investigate the viscoelastic properties of mucous, the transport of which is accomplished by the whip-like activity of cilia. The ultimate goal is to elucidate the consequences of inadequate ciliary activity in lung disease.

Jason Khan (University of Maryland College Park, Chemistry and Biochemistry) utilizes both simulations and biophysical approaches to predict and determine DNA conformations during Lac repressor binding.[59,60] Khan studied one of nature's most interesting polymer structures: DNA. He and his collaborators utilize single molecule FRET to determine spatial relationships between the Lac repressor and the ends of Cy3 and Cy5 double-labeled DNA fragments. He has also taken advantage of the relative ease of generating polymers of varying length (varying nucleotide basepairs). Taken together, these data suggest that the DNA is arranged in a closed-form loop conformation.[61] The work of Kahn, together with that of Rob Phillips in Session 2 on the Lac repressor-DNA complex, lays the foundation to study more complex, higher-order eukaryotic promoters.

Jerome Lynch (University of Michigan, Civil and Environmental Engineering) specializes in the fabrication of nano-and micro-scale polymers designed primarily to function as strain sensors.[61] It is anticipated that the development and refinement of the process technology will contribute toward more environmentally stable carbon-nanotube thin films for structural monitoring applications. The efforts of Lynch and his research team may soon culminate in the application of such nanostructures to biology.

Session 6—The New Deal; Live Cells and Membranes, Part 1

In vitro single molecule studies are experiencing many challenges, yet through innovative experimental design to overcome these challenges, new knowledge about molecular behavior is rapidly emerging. The greatest challenge, however, is to take these techniques into the living cell. Here, there is the potential to observe reaction dynamics, molecular forces, active transport, gene expression, and other critical cellular functions at the single molecule level under "native" conditions, where the complexity is presumably much greater and richer, but the impact of the measurements is directly biologically relevant. Although there has been initial success, many of the challenges in this area still remain. For fluorescence, it will be essential to work on natural fluorophores (e.g., flavins) or to link bright fluorescent proteins (e.g., green fluorescent protein, GFP, and its variants) to specific proteins. Labeling with extrinsic probes, as is frequently done in vitro, is more challenging in vivo if one is to avoid disruption of cellular function. Many groups are moving in this direction, so the hope is that over the next few years, we will see major advances in this area. Session 6, chaired by Duncan Steel (University of Michigan, Electrical Engineering and Computer Science and Physics and Biophysics), gave a current snapshot of the state-of-the-art in this area.

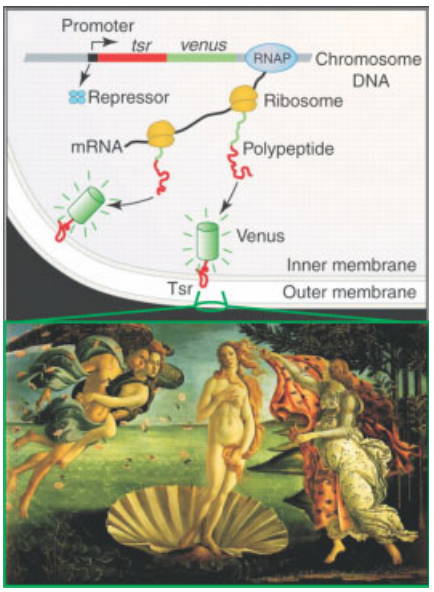

FIGURE 1 The Birth of Venus as a single protein molecule. Reproduced from Ref. 73 with permission from AAAS (www.sciencemag.org).

Sunney Xie (Harvard University, Chemistry and Chemical Biology) first spoke of techniques to follow enzyme kinetics without the limitations posed by the usual problems of chromophore bleaching. In particular, Xie's group was recently able to monitor thousands of enzymatic turnovers of individual β-galactosidase molecules by monitoring the release of fluorescent products.[62] By careful data analysis they detected catalytically distinct conformers of the enzyme that interconvert much slower than the turnover rate, which is likely a widespread biological phenomenon, as it is also observed for the hairpin ribozyme (see Session 1). Reassuringly, the Michaelis–Menten equation still holds even for such fluctuating single enzyme molecules. The Xie group also recently observed conformational changes of single T7 DNA polymerase molecules when elongating a DNA template, providing a direct rare view of the birth of a DNA molecule. Finally, Xie spoke of his new techniques to follow gene expression in the cell.[63,64] In one approach, the group expressed in *Escherichia coli* (under a repressed condition), a fast maturing fluorescent protein (Venus) fused to the membrane-targeting peptide Tsr so as to detect the membrane-localized Venus with single-molecule sensitivity. Each stochastically transcribed messenger RNA molecule was found to lead to the translation of a burst of only a few protein molecules, demonstrating the potential of single molecule imaging in live cells to witness the "Birth of Venus" as an example for the cellular expression of a low-copy number protein (as shown in Figure 1).[63]

Roger Sunahara (University of Michigan, Pharmacology) showcased a new technique to study membrane bound proteins that reconstitutes the protein in a native-like membrane disk. The approach entails a discoidal phospholipid bilayer that is stabilized at the edges by a protein shell of apo A-I[65]. Sunahara showed that a G-protein coupled receptor (GPCR) can be incorporated into the lipid bilayer, creating the morphology of a sushi roll, while maintaining the biological integrity of the GPCR. Such a nanoscale assembly lends itself to single molecule analyses of otherwise difficult to study cell membrane components.

Mark Banaszak Holl (University of Michigan, Chemistry) spoke of his work, in conjunction with the Michigan Nanotechnology Institute for Medicine and Biological Sciences, on the observation that polycationic dendrimer nanoparticles produce nanoscale holes in supported lipid bilayers and cell membranes.[66–68] Together with Brad Orr (University of Michigan, Physics), Banaszak Holl's group uses atomic force microscopy and patch clamp techniques to characterize these holes, which have severe implications for the applicability of dendrimers for biomedical, industrial, and consumer products, which is expected to greatly increase over the next 10 years.

Session 7—The New Deal; Live Cells and Membranes, Part 2 & Panel Discussion: Quo Vadis—The Future of Single Molecule Tools

Dr. Martin Philbert (University of Michigan, School of Public Health) presented his work in collaboration with Raoul Kopelman (University of Michigan, Chemistry) on intracellular optical nanosensors capable of detecting multiple ions, O_2, and NO.[69–72] These PEBBLEs (photonic explorers for bioanalysis with biologically localized local embedding) are nanometer-sized particles (20–100 nm) in which a variety of fluorescence-based sensor molecules are embedded.[72] Typically, both a reference dye and a sensor dye are included to permit ratiometric measurements. In some cases, a coating such as polyethylene glycol is used to improve biocompatibility. An advantage of the PEBBLE method over standard fluorescent dyes is the reduced adverse interaction of dye molecules with intracellular components. Examples of the use of PEBBLEs in studying neurotoxicity were presented, in which PEBBLEs were microinjected into neurons to detect alterations in ion concentrations, NO, and O_2 near mitochondria.

Following this last presentation, many speakers took part in a panel discussion on the future of single molecule studies. The participants were: Harold Craighead (Cornell), Jens-Christian Meiners (U. Michigan), Jason Kahn (U. Maryland), Martin Philbert (U. Michigan), Rob Phillips (Caltech), Richard

Superfine (UNC Chapel Hill), and Xiaowei Zhuang (Harvard).

A number of questions were posed to the panel and the following answers were given:

1. What technical or conceptual hurdles currently limit our ability to answer key questions using single molecule approaches? The need for super-bright, super-stable fluorophores—and preferably the ones that can be genetically encoded—is motivated by the necessity to reduce measurement noise. In addition, Richard Superfine raised the possibility of new approaches to reduce measurement noise beyond the capabilities of the averaging methods currently in use. It was also suggested that multifunctional probes could provide more than just position information. Panel members pointed out that, to advance, single molecule science must be accessible to newcomers. Such accessibility will be facilitated by ways to overcome the inevitable steep learning curve, perhaps by the creation of summer training courses. Other major limiting hurdles were identified as the limit in spatial resolution of current detection methodologies and the fact that some current techniques are not sufficiently 'noninvasive'.
2. What research areas/questions that should be amenable to single molecule analysis have not yet been addressed by single molecule methods? The panel felt that to better answer the outstanding, important, yet "messy" biological questions with the existing tools, it will be important to bring people with different expertise together; exciting biological questions will then automatically become the focus, rather than the single molecule tools themselves.
3. What is the current reach of the single molecule field? Is it largely a "boutique" field limited to a small number of academics or does it have wider practical implications as well? The panel discussed the potential for single molecule sequencing of DNA, as one of the most profound implications of single molecule technologies, with many applications including rapid personalized genome analyses. It was pointed out that there are several start-up companies aimed at addressing this problem.
4. What is its potential reach and what would be needed to get there? Currently, single molecule experiments are very slow and personnel intensive. It may be beneficial to develop automation of such studies for faster throughput in a national resource center. In parallel, improvements in data analysis would be needed to prevent a new bottleneck. In addition, education of students and postdoctoral fellows was pointed out to play a key role in expanding the reach of single molecule tools. Graduate training needs to be rethought to insure both deep training in a specialty and broad training in both the biological sciences and the technology/physics/engineering that have led to the development of single molecule approaches. It was generally felt that investments need to be made into centers of expertise to enable the broad integration of single molecule techniques into biology and nanotechnology.

In summary, the symposium showcased tremendous advances and the great potential of the single molecule field. It is clear that interactions between the basic physical sciences (physics and chemistry), engineering, and the biomedical sciences will be critical to the next stage of the field in moving beyond a technique-focused emphasis to one driven by key biological and nanotechnological problems. Establishing and fostering such connections will be important for institutions that want to nurture a focus in the exciting emergent area of single molecule studies.

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