Future of Biomedical Sciences: Single Molecule Microscopy

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ABSTRACT:

The behavior of single molecule defines whether a cell lives, dies, or responds to a specific drug treatment. Single molecule microscopies have begun to reveal the number, location, and functionalities of molecules outside and inside living cells. This issue of Biopolymers presents a first set of reviews that aim to highlight the accomplishments and future prospects of single molecule microscopies. © 2006 Wiley Periodicals, Inc. Biopolymers 85:103–105, 2007.

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The future of biomedical sciences in the 21st century will be intimately coupled to the further development and expanded use of single molecule microscopies. What justifies such a seemingly bold statement? This question is answered by another question: What is the ultimate goal of biomedical sciences? The answer here may be more obvious—For a complete understanding of the life, disease, and death of a cell (and thus the organism it represents or is part of) we ideally need to know three observables about every distinct molecule in the cell: (1) the number of its kind present; (2) the precise location of each member of the ensemble of identical molecules; (3) the functionality of each member of the ensemble. How can such a complete survey of the number, location, and functionality of all molecules in a cell be accomplished? Only by single molecule microscopy, since most molecules (at least all biopolymers) in a cell are present in very low copy numbers, in the range of 1–1000.[1] Why do techniques ultimately not suffice that amplify the signal by combining and averaging over multiple cells? It is now well understood that patients with formally the same disease and even individual cells of the same tissue show a distribution of behaviors (for example, at the onset of cancer lies typically a single or very few malignant cells) so that averaging does not tell the true story and is at risk of missing the important outliers.

A single molecule symposium at the University of Michigan this past spring 2006 entitled "At the Single Molecule Frontier: Integration in Biology and Nanotechnology",[2] as well as several coinciding meetings such as the very successful first Gordon Research Conference on "Single Molecule Approaches to Biology," and the NIGMS Conference on "Frontiers in Live Cell Imaging", heralded the breakout of single molecule microscopies from the confinement of just a few specialist laboratories onto the center stage of biomedical sciences and nanotechnology. The account of the Michigan meeting given in this issue[2] is intended to be representative of the accomplishments and future prospects of the young single molecule field. Among the accomplishments—possible only because single molecule techniques reveal observables that otherwise are lost in the ensemble average—are the discoveries of kinetic heterogeneity at the individual molecule level,[3–5] as well as of rare reaction intermediates,[6] and the application of force to single biopolymers to elucidate their mechanical and thermodynamic properties[7–9] or directly observe the mechanical action of motor proteins.[10,11] The future prospects for the application of single molecule microscopies seem boundless, but the most important impact in the biomedical sciences may be expected from

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their potential to provide complete surveys of the molecular composition, including three-dimensional spatial location and functional properties, of all molecules over time in a living cell. Many modern techniques in genomics,[12] transcriptomics,[13] proteomics,[14] and metabolomics/metabonomics[15] as well as high-throughput drug screening[16] already bring the same techniques to bear on single cell samples as are the basis for single molecule microscopies. Yet they typically do not yet reach single molecule detection sensitivity and may not work in living cells. To overcome these limitations it is critical that the envelop of single molecule microscopies be further pushed and active collaborations between basic scientists, engineers, and clinical researchers be forged. Only then can we harvest all necessary information and feed it into systems biology tools for a complete understanding of how cells live and die and how drugs may prevent the latter.

Biopolymers has rededicated itself to provide the vigorous forum that the changing needs of the Biochemical and Biophysical research communities demand and deserve.[17] Serving the community by launching a succession of reviews in the area of single molecule microscopies is a step in this direction. This issue carries the first five articles in a series that will continue over the coming years. Following the Central Dogma of Biology we begin with reviews on DNA that underscore how single molecule techniques help define mechanical, kinetic, and thermodynamic properties in the absence and presence of DNA binding proteins and drugs. The review by Garcia et al.[18] starts out with a description of the mechanical properties of DNA and shows how these properties play a critical role in many biological functions from viral and eukaryotic DNA packaging to regulation of gene expression. The review by Mannion and Craighead[19] further expands on the mechanical and structural properties of DNA and shows how these can be modulated and studied using fluorescent detection at sub-diffraction resolution by the application of nanofluidics. The review by van Oijen[20] reveals how the mechanical differences between single- and double-stranded DNA stretched in a microfluidic flow can be exploited to study the biological function of DNA binding nucleases and polymerases at the single molecule level. The review by McCauley and Williams[21] describes how optical tweezers can denature DNA in the absence and presence of protein and drug ligands, opening a direct observation window onto the thermodynamics of ligand binding. Finally, the review by McDowell et al.[22] gives a glimpse of the utility of computational tools to describe the dynamic properties of nucleic acids, in this case of molecular dynamics (MD) simulations of single RNA molecules, as an example for the powerful synergy between modern computational and experimental single molecule approaches.

The reviews in this first single molecule centered issue of *Biopolymers* present but a narrow look onto the full repertoire of capabilities of single molecule microscopies, and none of them focus on living cells. Single molecule experiments on living cells are only beginning to emerge,[23–25] while further technical hurdles such as the diffraction limit of optical microscopy are being removed,[26–28] so that the necessarily limited selection showcased here and in future issues is only a first step in highlighting the bright future for single molecule studies of biopolymers.

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