

Lester Wolfe Workshop in Laser Biomedicine

Bio-Optics of DNA: Shedding Light on Structure and Dynamics

Tuesday, November 14, 2006
4:00-6:00pm

Massachusetts Institute of Technology
Building E25 (Whitaker College), Room 111
45 Carleton St,
Cambridge, MA

Refreshments served at 3:30pm

The central importance of DNA to all of biology has led researchers to study its structure and function at the single molecule level. This symposium will cover some optical approaches for studying and manipulating DNA molecules, particularly concerning laser tweezers and fluorescence resonance energy transfer techniques.

Introductory remarks

Brian Seed, Harvard Medical School and Massachusetts General Hospital

Actions on a Single DNA: Real-Time Movies From *In Vitro* to *In Vivo*

Sunney Xie, Harvard University

Analyzing DNA Dynamics and DNA Protein Interactions Using Nanopores and Single Molecule Fluorescence

Amit Meller, Boston University

Watching Tweezed Hairpins with FRET

Matthew Lang, MIT

Sponsored by the GR Harrison Spectroscopy Laboratory, MIT; MGH Wellman Center for Photomedicine; Harvard-MIT Division of Health Sciences and Technology, and Center for Integration of Medicine and Innovative Technology (CIMIT)

Summary

Forum Title: Lester Wolfe Workshop in Laser Biomedicine Bio-Optics of DNA: Shedding Light on Structure and Dynamics

Brian Seed

Brian Seed opened the workshop by extolling the necessity of understanding DNA structure and dynamics.

Sunney Xie

Professor Sunney Xie presented his lab's studies on single molecule biology and the methods used to visualize biological processes one molecule at a time. The technique he described is called "single molecule time resolved spectroscopy." This method enables one to visualize individual molecules directly and analyze their behavior, both *in vitro* and *in vivo*. Professor Xie emphasized the notion that most biological processes in cells are stochastic. He used the central dogma in biology, in which DNA transfers information to RNA, which transfers that information in to the synthesis of proteins, as a heuristic to demonstrate the power of his method for gaining a richer understanding of basic biology. He began by describing the means to visualize the transfer and verify the mechanism by which a single RNA polymerase protein slides on a single DNA molecule. (Previous work by Ryter and Schultz in EMBO v.17, 7414 [1998] postulated this mechanism but could not directly demonstrate it using the crystallographic techniques described in their paper.) Professor Xie was able to demonstrate this behavior in an elegant series of experiments in which real-time production of an individual protein was visualized in an individual *E. coli* cell (Science [2006] 311:1600). Using as a model system the Lac repressor, which can maintain a low level of gene expression, and a fast-maturing yellow fluorescent protein whose transcription is controlled by the Lac repressor and can be detected as a single molecule, he was able to demonstrate the system's stochastic nature, both spatially and temporally. He found that the protein molecules are produced in bursts, with the number of molecules produced following a geometric distribution. A detailed mathematical analysis of the results demonstrated the stochastic nature of the system. For more information on this topic, visit Professor Xie's home page at <http://bernstein.harvard.edu/XieHome.html>

Amit Meller

Professor Meller discussed several ways to analyze the dynamics of DNA using single molecule detection. The application he described was DNA moving through a nanopore; his ultimate goal is to develop this methodology into a means for rapidly sequencing DNA one molecule at a time. At the beginning of this seminar, Dr. Meller stated that nanopores can be used to study the dynamics and biophysical characteristics of DNA in general; for example, he has demonstrated that one can determine the orientation of a ssDNA (5' to 3' or 3' to 5') molecule as it traverses a

molecular pore. He went on to describe how nanopores can be used for single molecule DNA sequencing, acknowledging the difficulty of discriminating each DNA base pair as it traverses a pore. Finally, he described new technology based on the possibility of decorating a short ssDNA fragment in such a way as to produce a longer, periodically structured DNA molecule, modifying the DNA sequence so that it could be read using his nanopore technology. The exact technology was not fully elaborated upon in this seminar. For further information, see Professor Meller's home page <http://www.bu.edu/dbin/bme/faculty/?prof=ameller> and the NIH CRISP data base for the abstract of Dr. Meller's newly funded NIH proposal on DNA sequencing http://crisp.cit.nih.gov/crisp/CRISP_LIB.getdoc?textkey=7193034&p_grant_num=1R01HG004128-01&p_query=&ticket=26508078&p_audit_session_id=189254294&p_keywords=

Mathew Lang

Professor Lang presented elegant experiments in which he simultaneously used optical trapping and single molecule fluorescence to characterize the molecular dynamics of biological molecules (reviewed in Lang et. al [2003] *Journal of Biology* 2(1):6). He used the term *mechanome* (akin to genome) to emphasize that there exists an untapped reservoir of information on the mechanisms and dynamics of biological molecular interactions; understanding these interactions is necessary for our complete understanding of the behavior of biological systems. Professor Lang described a method for measuring nanometer scale interaction, which he calls Interlaced Optical Force-Fluorescence Spectroscopy (Brau et. al [2006] *Biophysical Journal* 91:1069). In accordance with the theme of the workshop, he stressed the importance of single molecule studies as a way of making direct observations of biologically relevant processes; conventional measurement techniques lose important molecular detail in the averaging of many events. Applied to DNA dynamics, these new single molecule techniques have led to the discovery of new mechanistic pathways, conformations, and intermediate states. One formidable challenge in this field has been that of simultaneously measuring the fluorescence of a labeled DNA molecule and maintaining in an optical tweezer without photobleaching. Professor Lang's solution was to excite fluorescence and activate the optical tweezers in rapid alternation. The quick shifts would prevent the two processes from affecting each other. Using this method, he was able to mechanically unzip a 15 base pair DNA fragment that was fluorescently labeled, yielding information on the dynamics of the process a single molecule at a time. For more information on Professor Lang's research, visit his web page at: <http://web.mit.edu/~langlab/>