

## SPOTLIGHT



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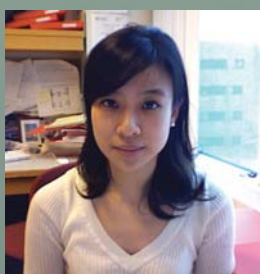
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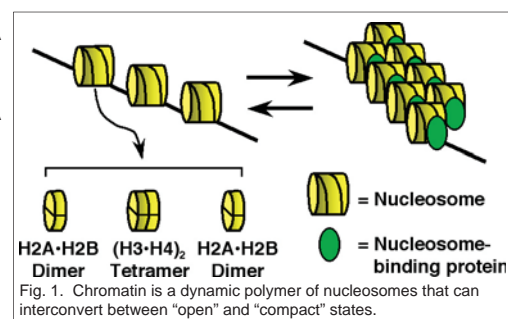


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## Chromatin Structure, Function, and Dynamics: From Mononucleosomes to Polytene Chromosomes

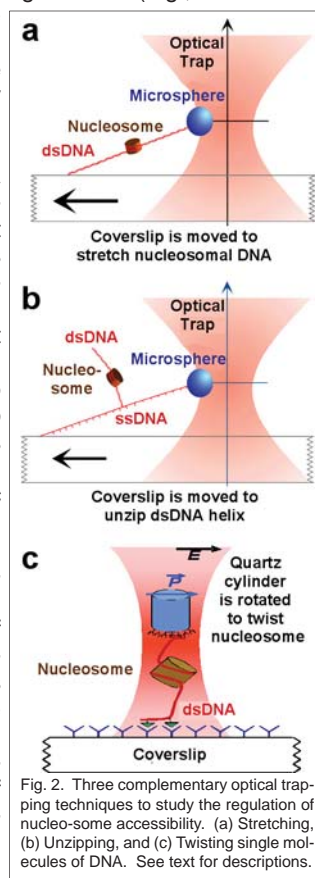
**Team:**  
**W. Lee Kraus** (Molecular Biology and Genetics, Cornell; AFM, nucleosome dynamics)  
**John T. Lis** (Molecular Biology and Genetics, Cornell; Multiphoton microscopy, in vivo imaging, transcription factor dynamics)  
**Michelle D. Wang** (Physics, Cornell; Optical trapping, single-molecule nucleosome dynamics)  
**Watt W. Webb** (Applied Engineering and Physics, Cornell; Multiphoton microscopy, in vivo imaging, transcription factor dynamics)

Chromatin is the physiological template for nuclear processes involving genomic DNA, including transcription, replication, and repair. The repeating unit of chromatin, the nucleosome, is a protein-DNA structure comprising two copies each of four core histones (H2A, H2B, H3, and H4) forming a histone octamer around which 1.7 turns of genomic DNA are wrapped (Fig. 1) [1]. The electrostatic attraction between the highly positively charged histone core and the highly negatively charged DNA ensures a stable, yet accessible, structure. Chromatin is a dynamic polymer whose biochemical and biophysical properties play important and specific roles in determining the structure and function of chromatin in vivo. The dynamic properties of chromatin include: (1) structural alterations within a nucleosome (e.g., removal of H2A/H2B dimers), (2) mobilization of nucleosomes along a length of DNA (e.g., translational repositioning), and (3) inter-nucleosome interactions leading to the compaction of nucleosomes into higher-order structures (e.g., chromatin condensation) [1]. These aspects of chromatin play an important role in the regulation of genomic processes, such as gene transcription by sequence-specific DNA binding factors.



The complexity, size, and dynamic nature of chromatin present a challenge for detailed structural studies, especially in ensemble-averaged molecular populations. Much of the previous information about chromatin structure has come from: (1) X-ray crystallographic studies of mononucleosomes, which provide a static structural view, (2) biophysical techniques typically run under extreme chemical or thermal conditions, which are far from the physiological ideal, and (3) indirect biochemical assays involving nuclease access to nucleosomal DNA. The studies of the Chromatin Structure, Function, and Dynamics group bring together three different approaches from nanobiotechnology to explore the structure and dynamics of chromatin, as well as factors that influence chromatin: (1) single molecule optical trapping (Wang lab), (2) atomic force microscopy (AFM) (Kraus lab), and (3) dynamic multiphoton microscopy (MPM) (Lis and Webb labs).

Optical traps are devices capable of manipulating and detecting sub-nanometer displacements for sub-micron dielectric particles, making them very useful for the manipulation and study of single molecules of DNA or protein attached to those dielectric particles [2]. Single molecule optical trapping techniques can be used to probe dynamic events directly and quantitatively under physiological conditions, without the averaging and smearing effects associated with measurements taken from populations of molecules. The Wang lab is using three complementary optical trapping techniques to probe the dynamic structure of nucleosomes, as well as the effects of the SWI/SNF chromatin remodeling complex on nucleosome positioning. These include stretching, unzipping, and twisting single molecules of DNA (Fig. 2) [3-6]. Twisting, a novel approach developed by the Wang lab, has recently been used to generate and measure both torque and force on an optically trapped particle [7, 8] (Fig. 2c).





# from the director

## Harold Craighead

The Nanobiotechnology Center continues to attract new faculty members. In the past twelve months fourteen faculty have joined the Center, with the majority coming from the Life Sciences, continuing the trend of recent years as the advances in Nanobiotechnology research are successfully addressing important biological, biomedical and biotechnological questions.

Many of these new members are actively collaborating in our research program. For 2008 we are directly supporting twenty nine research projects, six of these are new initiatives in areas such as: Recreating Three-Dimensional Cell Niches with Microfluidic Tumor Cells; Cantilever Array Sensors and 3-D Matrices for Modeling the Bone-Cartilage Interface.

We plan to continue to build on our successes in bringing together life scientists, physical scientists and engineers to advance biomedical science, improve imaging, diagnostics, therapeutics and drug discovery. Our researchers are committed to continuing these advances and to developing ever stronger partnerships with our colleagues at Weill Medical College.

Our Ninth Annual Nanobiotechnology Symposium will be held on Monday October 27th, 2008. This all day meeting showcases Nanobiotechnology research at Cornell and our partner institutions. It is an excellent opportunity to learn of recent advances from invited speakers, to discuss related topics in depth at our poster session and to explore opportunities for future collaborations. We invite you to attend, and hope to see you there. Further details will be available on our website soon.

## Research, continued from previous page ...

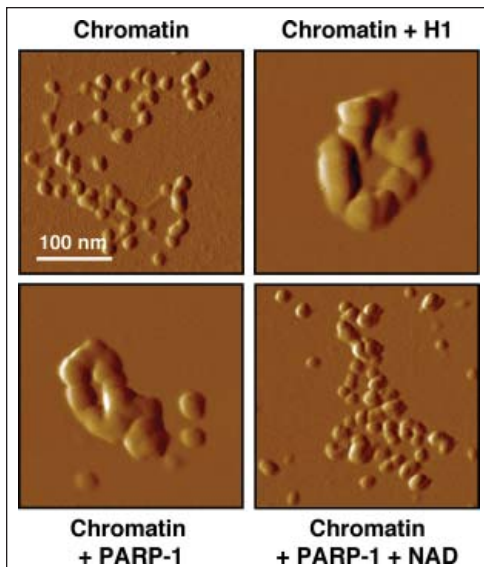


Fig. 3. PARP-1 and H1 promote the condensation of polynucleosomal arrays, as revealed by atomic force microscopy (AFM). Chromatin was assembled in vitro using an ~11 kb plasmid in the presence or absence of a saturating amount of PARP-1 ( $\pm$  NAD<sup>+</sup>) or H1. Scan probe oscillation amplitude images are shown. A length scale is shown. The images show (clockwise from top left) (1) free chromatin in an extended or "open" conformation, (2) chromatin bound by H1, which adopts a compacted or "closed" conformation, (3) chromatin bound by PARP-1, which also adopts a compacted or "closed" conformation, and (4) the release of PARP-1 from chromatin in the presence of NAD<sup>+</sup>, a metabolic signaling molecule.

Atomic force microscopy (AFM) is an imaging technique that provides three-dimensional views of biological molecules with nanometer resolution and can also be extended to the determination of the molecular identities of specific components within complexes or mixtures [9]. Furthermore, in many biological applications of AFM, the samples can be analyzed under physiological conditions (e.g., aqueous, buffered, unfixed). The Kraus lab is using AFM to analyze protein interactions with chromatin, specifically the compaction of polynucleosomal arrays by the nucleosome-binding proteins PARP-1 and H1 [10] (Fig. 3). These studies have been performed using the Picoplus AFM (Molecular Imaging) at Cornell University's AFM facility in Duffield Hall. In addition, the Kraus lab is planning to use advanced AFM approaches (e.g., immuno-AFM, simultaneous AFM and fluorescence imaging; [11]) to achieve simultaneous molecular recognition and dissociation force measurements.

Multiphoton microscopy (MPM) can provide real-time views of gene regulation in living tissue that were of unprecedented clarity and resolution [12]. The deep-penetrating and low background of MPM is ideal for examining an extraordinary nuclear structure provided by naturally amplified (500X) *Drosophila* polytene chromosomes (Fig. 4). The ability to visualize specific loci in giant polytene by MPM can be coupled with the introduction into the *Drosophila* germline of stably incorporated transgenes expressing specific protein factors that are tagged with various fluorescent, 'GFP', polypeptides. The GFP-tagged chromatin proteins can be observed by MPM at specific genes with high temporal and spatial resolution. The Webb and Lis laboratories are using MPM to investigate the dynamics of histone core and linker proteins at specific genes in vivo by combining GFP-

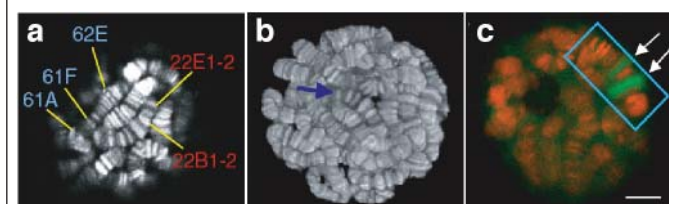


Fig. 4. Multiphoton imaging of *Drosophila* polytene nuclei in living cells. (a) One optical section of a polytene nucleus stained with Hoechst33342. Recognized bands on chromosome arm 2L (red) and 3L (blue) are noted. (b) 3D reconstructed polytene nucleus. Blue arrow indicates the ends of chromosomes. (c) The optical sections of polytene nuclei containing an EGFP-tagged subunit of RNA polymerase II (green) co-stained with the DNA stain Hoechst (red). Scale bars are 5  $\mu$ m.

tagging of these proteins and the optical sectioning power of multiphoton imaging in the polytene nuclei of *Drosophila* salivary gland cells. In addition, they are using MPM to study the dynamics of HSF, a sequence-specific DNA-binding factor, and RNA polymerase II at heat shock loci [12, 13]. Photobleaching recovery experiments in transcriptionally inactive regions have revealed that both the canonical and variant histone proteins are stably incorporated into chromatin, yet some histones are lost rapidly from specific genetic loci during transcription activation. In addition to the standard GFP tagged proteins, the Lis lab is developing the use of photo-activateable GFP (paGFP) and other photochromic proteins to determine the fate of histone proteins that package a gene that is inactive and which subsequently becomes transcriptionally activated. These will be useful for addressing the question of whether histones completely dissociate into the nucleoplasm or rather are preserved in some manner on the DNA template so they can be replaced when transcription ceases.

The integration of these three approaches allows us to examine chromatin and associated factors in an unprecedented way both under highly defined conditions with single molecules and in the complex native environment of living cells, all at nano-scale resolution.

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# Education at the Nanobiotechnology Center

## Celebrating Women in Science Dinner

December 7, 2007 was the first Celebrating Women in Science dinner. The middle school and high school girl's science clubs joined a number of women from Cornell, Lockheed Martin, Kodak, Xerox, and Welch Allyn. The professional women shared their stories about their own career and family goals; while the students had an opportunity to discover the diversity in science professionals and receive encouragement to look at science careers. All who participated reported that this evening was a positive experience. Many of the professional women stated that they had no idea such outreach activities were taking place and hoped to participate in additional activities.

The students finished the evening by asking for the scientist autographs in their guide books. It was a great event where being a scientist was genuinely celebrated.



Amanda Haas, Engineer at Lockheed Martin talking with students (12-7-07)

## NBTC, CCMR, and CNF join to offer Scanning Electron Microscope experience



Judy Cha showing the students how to focus the microscope and identify individual atoms.

With impressive facilities and active outreach programs, these three centers have come together to develop and offer a most interesting science activity. Student groups now have the ability to come learn about the science of the SEM as well as have the opportunity to prepare samples and run the instruments. The first testing of this field trip involved 12- 7th and 8th grade students from Groton Middle School but we hope to offer this as a Friday Evening or weekend field trip.

## Onondaga Nation School field trip to Strong Museum of Play

The education staff continues to provide science enrichment activities for the ONS through science clubs and field trips. This year's field trip took the 2nd – 8th graders to the Strong Museum in Rochester. The museum is currently hosting Grossology, an exhibit on how the body works. The students learned that each person produces one liter of snot each day and about the molecules that create foul smells.

The education staff had the privilege of meeting a new Cornell grad, the butterfly guy, at the museum. Tad Yankoski is a recent graduate of the Entomology department reported that his many outreach activities as a part of his undergraduate work made his current employment possible.



ONS Student learning about stomach contents

## Research, continued from previous page ...

The Chromatin Structure, Function, and Dynamics group in the news:

<http://www.news.cornell.edu/stories/Feb08/Parp1.bpf.html>

<http://www.news.cornell.edu/stories/Jan08/Webb.Lis.html>

<http://www.news.cornell.edu/stories/July07/helicases.kr.html>

<http://www.news.cornell.edu/stories/Sept06/webb.multiphoton.html>

[http://www.news.cornell.edu/Chronicle/05/2.10.05/cellular\\_enzyme.html](http://www.news.cornell.edu/Chronicle/05/2.10.05/cellular_enzyme.html)

- Jennifer Weil, NBTC Director of Education

# education

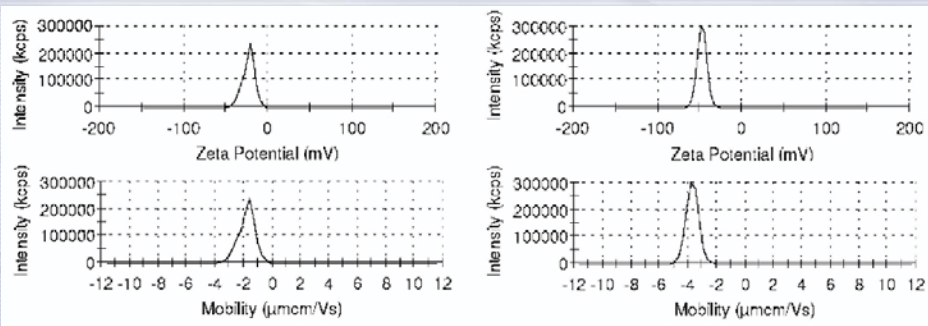
## Malvern Zetasizer Nano-ZS

[www.nbtc.cornell.edu/facilities.htm](http://www.nbtc.cornell.edu/facilities.htm)



The Zetasizer Nano-ZS instrument provides the ability to measure particle size, zeta potential and molecular weight in liquid using Dynamic Light Scattering. The Zetasizer allows these measurements to be taken much more rapidly than with traditional methods.

The instrument also enables determination of the protein melting point and the ability to perform trend measurements (size vs time, size vs. temp etc.)

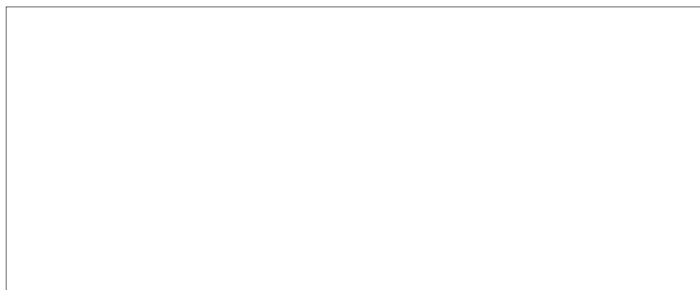


The NBTC Malvern Zetasizer enabled Ian Hosein of the Liddell group to examine changes in zeta potential that occurred by modifying ZnS photonic crystals with a polyelectrolyte coating. This coating technique led to stabilization of the nanospheres in aqueous solution. (Langmuir, 2007).

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