Dynamic DNA Packaging Across Kingdoms: Chromatin & Beyond

Asilomar Conference Grounds, Pacific Grove, CA

July 5 - 8, 2011

Study Book, Part 2 – Poster Abstracts

Wednesday, July 6  2:00 – 5:00 PM
Thursday, July 7  2:00 – 5:00 PM

Sponsored by the Biophysical Society,
With additional support from the Mechanobiology Institute at the National University of Singapore.
WEDNESDAY POSTER SESSION

2:00 PM – 5:00 PM, Room Heather

Posters for presentation on Wednesday, July 6, should be set up Tuesday, July 5, between 7:00 PM and 11:00 PM and removed by 11:00 PM on Wednesday, July 6.

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**THURSDAY POSTER SESSION**

2:00 PM – 5:00 PM, Room Heather

Posters for presentation on Thursday, July 7, should be set up Thursday, July 7, between 7:00 AM and 8:30 AM and removed by 11:00 pm on Thursday, July 7.

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The Effect of the Temperature and Capsid Structure of the DNA Packing and Ejection Process

Afaf Al Lawati, Issam Ali, Muataz Al Barwani.

Sultan Qaboos University, Muscat, Al Khodh, Oman.

We use coarse-grained molecular dynamics simulations to study the effect of temperature and viral capsid tail on the packing and ejection of viral genome into and out of spherical bacteriophage capsids. We find that temperature in our simulations does not have much effect. We also find that packing is more efficient with the presence of a tail. On the other hand, the tail makes the ejection slower.

We use molecular dynamics simulations in which the DNA/RNA are coarse-grained into 100 beads. The beads are joined by FENE springs and exclusion volume interactions are included via the Lennard-Jones potential. We add an extra “bending” interaction to model the semiflexibility of DNA. The capsid is modeled as a hard sphere. [1]. The chains are coupled to the fluid via stochastic rotation dynamics model [2], which divides the fluid into point particles that randomly collide and go off at random directions.

We study the effect of the following factors on the dynamics of viral genome packing/ejection:
1- Temperature
2- Tail of the phage

**There is not much effect of the temperature in the packing process although the experiments shows that lower temperatures possibly lead to opening – closing of the head-tail connector changes in the conformation of the tail, which should slow down packing appreciably. This is not modeled in our simulations.**

**It is easier to pack with the tail in both good solvent (neutral DNA) and in a solvent where DNA charge is important. This is because the opposing entropic force outside the capsid is diminished by the tail, whereas the ejection becomes slower with the tail.**

(2) A, Malevanets and R. Kapral (1999), JCP, vol 110, 8605
Electrostatic Effects of the Ion Atmosphere on Nucleosome Core Particle Attraction

Kurt Andresen¹, Chongli Yuan², Xiangyun Qiu³.

¹Gettysburg College, Gettysburg, PA, USA, ²Perdue University, West Lafayette, IN, USA, ³George Washington University, Washington, DC, USA.

Nucleosome-nucleosome attraction is known to be a vital aspect of chromatin compaction. The attraction is primarily electrostatic and is effected by the addition of divalent (or higher valence) ions. The specific mechanisms of this compaction, however, have yet to be determined. In particular, there has been recent debate about the role of the nucleosome tails in nucleosome-nucleosome attraction. Using buffer equilibrium atomic emission spectroscopy, we have determined the ion composition of the nucleosome in both wildtype and various tail-modified mutants under a variety of ionic conditions. These data should be vital in determining the role of the ions, tails, and, more generally, the electrostatics involved in nucleosome compaction.

DNA-histone Interaction as a Common Currency for Quantifying Eukaryotic Gene Regulatory Evolution

Gregory Babbitt.

Rochester Institute of Technology, Rochester, NY, USA.

A fundamental task remaining in molecular evolutionary biology is the development of effective methodology for detecting the action of natural selection on eukaryotic gene regulation. The lack of clear rules of functional organization, as well as the lack of any abstract representational "code" in regulatory DNA sequence, has made this a challenging task. Because regulatory DNA is functionally defined by DNA-protein interaction, often involving competition for access to DNA between various transcription factors and the histone core, future molecular evolutionary investigations of gene regulation might be better framed using a biophysical rather than bioinformatic approach. The determination and modeling of biophysical parameters defining DNA-histone interaction can provide a first step towards a common currency upon which the effect of selection on gene regulation can be measured.

Here we present a likelihood-based statistical approach for detecting natural selection acting upon the sequence-dependent biophysical modeling of nucleosome dynamics. We compare this method to two of our previously published methods for detecting natural selection acting on chromatin organization. We present strong evidence that natural selection acting to preserve nucleosome formation is driving a well-known bias on presumably neutral variation in yeast genomes. We also demonstrate relaxed selection against the accidental binding of transcription factors at sites that co-occur with functionally conserved chromatin contexts. We propose that sequence-based methods to detect the evolutionary signature of functional chromatin organization may prove invaluable for locating functional regulatory regions in large genomes with low gene density (i.e. human genome) as well as for investigating population genetic variation affecting age-associated human diseases involving gene regulatory dysfunction.

Recent Relevant Publication
**Architecture of the Chromatin Factor HMGN2-Nucleosome Complex as Revealed by Methyl-TROSY NMR**

Hidenori Kato¹, Hugo Van Ingen², Bing-Rui Zhou¹, Hanqiao Feng¹, Michael Bustin¹, Lewis E. Kay², Yawen Bai¹.

¹National Cancer Institute, Bethesda, MD, USA, ²The University of Toronto, Toronto, ON, Canada.

Packaging of the eukaryotic genome into chromatin is regulated by numerous chromatin factors and enzymes that bind to nucleosomes. High mobility group nucleosomal (HMGNs) proteins are abundant vertebrate proteins that decompact chromatin condensed by the linker histone H1, thereby enhancing transcription activity of the chromatin template. HMGNs bind specifically to nucleosomes independent of post-translational modifications of histone tails, using a conserved nucleosome-binding domain (NBD). The structural basis of this interaction is of particular interest, since structural information on interactions involving unmodified nucleosomes is still very limited. Here, we report the architecture of the HMGN2-nucleosome complex determined by a combination of methyl-transverse relaxation optimized nuclear magnetic resonance spectroscopy (methyl-TROSY)¹¹ and mutational analysis. In this complex, the HMGN2 NBD (residues 19-42) anchors to the nucleosome core with an extended conformation while the regions N- and C-terminal to the NBD are disordered. The NBD binds to both the histone core and nucleosomal DNA, predominantly through electrostatic interactions. The conserved positively charged C-terminal part of the NBD binds DNA near the point where it enters and exits the nucleosome. This places the C-terminal tail of HMGNs in a position so as to interfere with the binding of linker histone H1, facilitating chromatin decompaction. A conserved Arg/Ser-rich motif in the N-terminal region of the NBD interacts with the acidic patch in the H2A-H2B dimer, explaining how phosphorylation in this motif during mitosis leads to the dissociation of HMGNs from chromatin through electrostatic repulsion. In addition to providing insights into the mechanism of HMGN function, our methyl-TROSY approach is readily applicable to the study of other important nucleosome-binding interactions in chromatin.

**Kinetic Proofreading in Chromatin Remodeling**

Ralf Blossey.

CNRS, Villeneuve d'Ascq, Nord, France.

Chromatin remodeling, the ATP-consuming repositioning of nucleosomes, has recently been suggested to be another biological example in which kinetic proofreading scenarios can be present. After a general proofreading scenario which couples the recognition of histone modifications to chromatin remodeling was put forward (Blossey & Schiessel, 2008), a proofreading scenario for chromatin repositioning was suggested for the ISWI system, based on experimental data (Narlikar, 2010). In this presentation I explain the general kinetic proofreading scenario that couples histone modification recognition to remodeling, and as an example for transcription initiation illustrate its role in the activation of the INF-beta gene. I then discuss a proofreading model for the ISWI system which couples tail recognition and repositioning. Finally, I show how the kinetic proofreading scenario can explain recent in-vivo observations on the dynamics of chromatin remodeling in human cells (Ertel et al, 2010).
Expression Noise and Chromatin Dynamics

Hinrich Boeger, Changhui Mao, Christopher R. Brown, Elena Falkovkaia, Eva Robinson.

UCSC, Santa Cruz, CA, USA.

Extensive structural analysis of the chromatin transition at the inducible PHO5 promoter of yeast has led to the conclusion that remodeled promoter chromatin is generated by a random process of nucleosome removal and reformation. Since nucleosomes interfere with transcription factor binding at promoter elements, it must be assumed that the induced promoter randomly switches between transcriptionally active and inactive states, which is expected to have a significant effect on the noise of expression. Indeed, the functional relationship between the mean and the noise of expression (noise profile) bears the signature of this random switching. Since the magnitude of the expression noise depends on the kinetic parameter values of the expression process, noise measurements allow for making inferences of about the steady-state kinetics of chromatin remodeling in vivo. The transcriptional activator of PHO5 controls two functional transitions of the expression process, but both transitions precede the initiation of transcription. The activator thus regulates the level of PHO5 expression exclusively by controlling the transition probability from transcriptionally silent into active promoter states, but not the holding time of active states or the rate of transcription within such states.

Dynamics of Bacterial Chromosome

Krystyna Bromek1, Eileen Nugent1, Avelino Javer1, Bianca Sclavi2, Marco Cosentino Lagomarsino3.

1University of Cambridge, Cambridge, Cambs, United Kingdom, 2LBPA, ENS, Cachan, Paris, France, 3Genomic Physics Group, CNRS, Paris, France.

Bacterial chromosome was for a long time believed to be a simple polymer devoid of structure. In recent years it became obvious however that dependent on cell cycle, growth conditions or external stimuli the structure of the nucleoid, DNA with associated proteins, can dramatically change.

Here we investigate the changes of the organisation into macro- and supercoiled domains of E. Coli nucleoid dependant on the growth conditions. We utilise laser tweezers, bright field fast imaging as well as epifluorescence from GFP tagged chromosomal loci to map the dynamics of particular parts of the chromosome.
Chromatin Condensation Affects Nuclear Mechanics and Pluripotency of Embryonic Stem Cells

Kevin Chalut, Markus Hopfler, Jochen Guck.

University of Cambridge, Cambridge, CAMBS, United Kingdom.

We investigated how cell and chromatin mechanics reflect pluripotent stem cell (PSC) state, i.e. pluripotent or committed to differentiation. In mouse embryonic stem cells, we used a GFP reporter for the transcription factor Nanog, which is essential for pluripotency, along with well-defined culture conditions to control and monitor pluripotency. As culture conditions are changed, and Nanog expression changes, the PSCs exit a stable pluripotent state into an unstable pluripotent state from which they are free to differentiate. We examined the structure and mechanical properties of PSCs as a function of pluripotent state with several biophotonics techniques. These techniques include digital holographic microscopy for monitoring whole cell structural changes and confocal microscopy to assess chromatin structure and dynamics. We also used atomic force microscopy and optical stretching to establish the PSC mechanical phenotype. We found that, surprisingly, as the PSCs enter an unstable pluripotent state, the actin cytoskeleton matures yet the whole PSC becomes softer. We developed a technique, using the optical stretcher, to assess nuclear mechanics without removing nuclei from the cells. We showed that the reason for the increasing softness of PSCs in an unstable pluripotent state (despite the more well-developed actin cytoskeleton) is that nuclei become softer and this is reflected in the whole cell mechanics. We found that the reason nuclei become softer is due to a change in the condensation of chromatin within PSC nuclei. The level of chromatin condensation is correlated with pluripotent state and is reflected in nuclear mechanics and chromatin remodeling dynamics. I will discuss how these results help to better understand the role of chromatin structure and dynamics in pluripotency and differentiation, and also show how these results provide a toolbox for monitoring pluripotency with marker-free biophotonics.
The Biochemical, Biophysical and Structural Characterization of PARP-1 and Its Complexes with DNA Damage Models

Nicholas J. Clark¹, Wayne G. Lilyestrom¹, Michael A. Kramer¹, Susan Krueger², Karolin Luger³.

¹Colorado State University, Fort Collins, CO, USA, ²National Institute of Standards and Technology Center for Neutron Research, Gaithersburg, MD, USA, ³Howard Hughes Medical Institute, Fort Collins, CO, USA.

Poly(ADP-ribose)polymerase 1 (PARP-1) is a highly abundant, multi-domain protein that binds both DNA and chromatin, and ADP-ribosylates itself as well as histones and histone-modifying enzymes in response to DNA damage. It plays important roles in transcription regulation and DNA damage repair, and is a target for cancer therapies. While the structures of most of the individual domains of PARP-1 are known (e.g. the N-terminal zinc finger DNA binding domain, and the C-terminal catalytic domain), their arrangement in the context of a full length protein in absence and presence of DNA are unknown. In particular, it is unknown to what extent the different types of DNA damage stimulate the enzymatic activity of PARP-1, whether this correlates with binding affinity, and how DNA damage recognition at the N-terminal end is transmitted to the C-terminal catalytic domain.

We have used a combination of biochemical, thermodynamic and biophysical approaches to probe the stoichiometry and affinity for several DNA damage model substrates, and measured the resulting activation of enzymatic activity. Small Angle Neutron, X-Ray- and light scattering experiments were used in a hybrid approach with molecular modeling to reveal the first structures of the full-length PARP-1 protein and its complexes with DNA. Our data supports a model in which the interaction with damaged DNA conveys a conformational change that results in the activation of the enzymatic activity of PARP-1. Together, these studies will result in a comprehensive picture of how PARP-1 is activated by DNA damage.
Communication between sequentially distant sites along DNA is important in gene regulation and expression. These long-range interactions require deformations of the DNA, such as its tight wrapping around nucleosomes in chromatin. Indeed, the observed communication between transcription factors bound to widely spaced sites along nucleosome-decorated DNA is markedly greater than that along free DNA. In order to gain insight into how histone proteins and the constituent DNA contribute to these effects, we have developed a simple, structurally based model of chromatin and performed Monte Carlo simulations of nucleosome-decorated DNA chains. Our coarse-grained representation takes account of the local structure and deformability of histone-bound and free (linker) DNA at the base-pair level and the electrostatic interactions of representative DNA and amino acid atoms. We can then extract from our simulations the variation in global chromatin properties, such as the end-to-end distance and fiber radius, for DNA linker lengths at single base-pair resolution. Our model also makes it possible to simulate chromatin with or without nucleosome histone tails and hence to study their effects on the chromatin local structure and global properties. The simulated probabilities of long-range contacts mirror the enhancement of gene expression induced by the histone tails observed in recent experiments performed on model biochemical systems. Our structure-based model makes it possible to relate specific changes in the chromatin local structure to global properties of the fluctuating fibers. For example, we have found that long-distance communication depends not only on the flexibility of the fiber but also on the local arrangement of the nucleosomes. We also perform a detailed analysis of the inter-nucleosome interactions in order to unravel the details of distant communication on DNA as well as to develop a simpler coarse-grained model of chromatin applicable to the study of longer, biologically relevant fragments.
DNA Packing and Architecture of Mammalian Chromosomes


University of Texas, Retired, Houston, TX, USA.

Light microscopy, a Direct-View Stereo Transmission Electron Microscope developed by the author, and other techniques, were used to study whole (not sectioned) chromosomes, nuclei, sperm, and cells. A model of chromosome structure, presented in 1980, was later refined (Lange, Cole, & Ostashevsky, Advances in Radiation Biology, Vol. 17, pp 310-421, 1993). Since this Multi-Stranded Backbone model conflicted with views of single strand chromosomes and genomes, and includes structures not reported elsewhere, we waited for other imaging studies to confirm or contradict our work. Meanwhile, linear genome constructs faced problems matching overlaps of long base-pair repeat sequences. Since our model has many long repeats, and no contradicting images emerged, we present further refinements based on the following inputs: stretched mitotic chromatids display quasi-parallel multi-strands; 30-fold extensions show 16 nucleosomal strands; histone-depleted 100-fold extensions show 16 DNA strands. These extensions alternate with BACKBONE RIBBONS of 8 parallel “MINI-RIBBONS”. Each Mini-Ribbon binds TWO PARALLEL DNA STRANDS by continual bridging by 7-nm TOROID LINKERS. Backbone Ribbons attach to 176 nm long “Half-Ribs” made of eight 22 nm particles. In intact Chromatids sequential Half-Ribs associate side-by-side to form Primary Split-Ribs with attached Backbone Ribbons looping outward as Lateral Extensions either to the right or left side of the Backbone. Secondary Ribs stabilize outgoing and returning Lateral Extension strands. Termini of ½ μm long Lateral Extension Loops are pairing / association sites for many cell functions. Abbreviated Lateral Loops continue as 16 Nucleosomal Loops that supercoil in mitotic chromatids. Primary Ribs are spaced every 100 nm along Interphase Backbones with Inter-Rib Segments forming Replication Origins. Mitotic Backbones shorten 4-fold by zig-zag foldings stabilized by Condensation Protein overlays. The model provides rationales for structures and processes in mitosis, meiosis, cell cycle organization, embryogenesis, chromosome damage and repair, and genome evolution.
Overlapping but Distinct Nucleosome Positioning Signals in Human and Maize Genomic DNA


Florida State University, Tallahassee, FL, USA.

Human and maize are representatives from two different kingdoms whose genomes share similar size and complexity. Both genomes are efficiently and compactly organized into chromatin whose fundamental subunit is the nucleosome: ~165 base pairs of DNA organized around a histone octamer. We have used the recent completion of the maize genome sequence to experimentally measure nucleosome distribution at 400 transcription start sites in maize. We have used these experimentally determined nucleosome distributions to train a maize-based support vector machine to identify the nucleosome formation potential of any given sequence of DNA. In parallel, we have measured nucleosome positions at 425 human transcription start sites, and used these data to train a human-based support vector machine to identify the nucleosome formation potential of any given sequence of DNA. By comparing results from human- and maize-based models we are able to identify common and distinct primary sequence features driving nucleosome distribution in each of these organisms. The sequence features responsible for distinguishing the nucleosome forming from the nucleosome inhibitory sequences in the training set are primarily nucleosome forming for the human-trained model and nucleosome inhibitory for the maize-trained model. The models generally perform well in cross-kingdom comparisons, but differences highlight key evolutionary differences in chromatin regulatory strategies. These results suggest maize and humans share overlapping but distinct sequence features driving nucleosomal organization.

Maximum Entropy Approach to Genome Conformation Reconstruction from C-based Technologies

Philipp M. Diesinger¹, Miriam Fritsche², Dieter W. Heermann³, Mark Bathe¹.

¹MIT, Cambridge, MA, USA, ²Institute of Theoretical Physics, University of Heidelberg, Heidelberg, Germany.

3D genomic architecture is increasingly being revealed to play an important role in genetic regulation by mediating physical interactions between distant regulatory elements such as promoters and enhancers. C-based technologies provide information on genome conformation by measuring mean interaction frequencies between specific genomic sites in a population of cells. Inverting these data to compute genome conformation explicitly offers the opportunity to improve our understanding of gene regulation by genomic conformation. Toward this end, here we present a Monte Carlo-based approach to compute chromatin conformational ensembles consistent with interaction frequency matrices measured using C-based technologies. The procedure computes the unique, maximum entropy ensemble of conformations consistent with the data, thereby differing from minimization-based approaches that solve for a single, mean genome conformation from the population-averaged data. The resulting conformational ensemble is analyzed to identify local genomic structures, interactions frequencies between distant sites that are not interrogated experimentally, as well as correlations in pair-wise interactions mediated by fluctuations in genomic architecture.
Asymmetry in Chromatin Patterns in Eukaryotic Daughter Cells

Thomas Donndelinger.

St. Alphonsus Medical Center Nampa, Nampa, ID, USA.

With a reengineered tissue processing methodology, allowing for a more detailed histological result, we have sought to analyze the pattern of chromatin packaging phylogenetically and histologically at 1000-1600X magnification. The results indicate predominant binary features of chromatin in daughter cells. These differences were interrogated with antibodies against altered chromatin modifications. Anti-phosphorylated histone H1 antibodies would demonstrate a difference in chromosome sets from prophase through metaphase to telophase. Antibodies to histone H4 acetylated at K16 demonstrated a difference in the interphase chromatin. In interphase cells the nuclei differ in size with the slightly smaller daughter cell nuclei demonstrating more coarsely clumped chromatin on the nuclear membrane and inside the nucleus. The cytoplasm displayed a difference in the degree of granulation and of protein expression. These differences were examined in protozoans, diatoms, dictostylium, simple metazoans, insects, and animals. This process was observed never to be absent in malignancies with the more undifferentiated tumors displaying greater difference. Similar observations were made in malignant cell lines. With the advent of time-lapse fluorescence real time microscopy these differences are becoming apparent in cell cultures as pairing. Cell suspension can be separated into two populations in flow cytometry assuming the Gaussian distribution normally seen is a summation of two curves.

Our concepts on cell biology, cell division, and chromatin come from the 19th century’s understanding and tools. With a new approach to artifact free histology and a reference to ultra-structure gray scaled at 1000-1600X magnification, as is standard, suggests that these observations have brought the light and dark cells of ultra-structure to the light microscope. These fundamental features of cell biology suggest we reexamine our fundamental concepts of chromatin and cell operating systems. Could we be looking at a binary operating system in chromatin and cell biology?
**Board 15  15-Pos**

**Effect of DNA Damage and Histone H3 K14 Acetylation on Nucleosome Dynamics**

Mingrui Duan, Michael J. Smerdon.

Washington State University, Pullman, WA, USA.

In eukaryotic cells, DNA repair occurs on nucleosomes, which consist of histone octamers and wrapped DNA. The association of DNA with histone octamers makes it difficult for repair machinery to gain access to DNA lesions. Nucleosomes exist in a dynamic equilibrium in which the DNA unwraps and rewraps, especially near the ends of nucleosomes. This ‘template dissociation’ from the histone octamer exposes damaged DNA, which may facilitate recruitment of repair machinery. We have used FRET and restriction enzyme accessibility to study nucleosome dynamics following UV damage, and find that UV photoproducts in DNA enhance spontaneous unwrapping in a dose-dependent manner (1). In addition, it was reported that H3K9 and/or H3K14 are hyperacetylated at the MFA2 promoter in yeast cells after UV irradiation (2). To study the effect of these histone modifications on the spontaneous unwrapping of nucleosomes, we have generated homogenous histone H3 specifically acetylated at lysine 14 (H3K14ac). Our results indicate that H3K14ac does not change the dynamic unwrapping of nucleosome core DNA. Thus, H3K14ac may affect higher order chromatin structure and/or act as a signal to recruit repair factors following UV irradiation.


**Board 16  16-Pos**

**Examining the Structural Pertubations of DNA by H-NS**

Victoria Fairweather, Sylvie Rimsky.

CNRS, Cachan, France.

Bacterial DNA of Gram-negatives is a highly complex entity and is compacted into what is called the nucleoid. The environment, cell cycle and stresses evoke overall changes in the structure of the negatively supercoiled chromosome. Many auxiliary proteins, the nucleoid associated proteins, are necessary to modulate this complex system of which the H-NS protein is one of the major players and is involved in the organisation of bacterial chromatin. H-NS manages to act both as a structural protein of the bacterial genome, compacting nucleoid, as well as acting as a silencer of transcription.

We wish to elucidate the characteristics of H-NS - and H-NS-like proteins – when they act as structural components and when they are involved in transcriptional regulation. This is of importance as both H-NS and its paralogues are implicated in the virulence of E.coli strains. Employing UV-laser footprinting coupled with capillary electrophoresis we are able to examine the effects of H-NS on the DNA structure both in vivo and in vitro, thereby examining closely the effects of the protein on promoter regions and on stretches of the DNA where it acts more as a structural component of the bacterial chromosome.
Heterochromatin to Euchromatin Transition within Human Interphase T-Lymphocytes.

John Frenster.

Stanford University, Atherton, CA, USA.

Repressed heterochromatin 30 nm microfibrils are transformed into active euchromatin 10 nm microfibrils during culture with $^3$H-Phytohemagglutinin ($^3$H-PHA).

Methods:
Human volunteers donated peripheral blood samples for in vitro culture with $^3$H-PHA. Buffy coats of contributed samples were incubated for 0, 15 min. 1, 2, 4, 12, 24, 48, and 72 hours with $^3$H-PHA, and were then studied by electron microscopic radioautography to quantitate location, number, and sizes of $^3$H-PHA foci within incubated granulocytes, monocytes, and lymphocytes.

Results:
Incubated granulocytes quickly disintegrated and had disappeared by 1 hour of culture.

Incubated monocytes displayed delayed uptake of $^3$H-PHA within the cytoplasm at 1 hour, but no nuclear $^3$H-PHA even at 72 hours. Monocytes gradually transformed into mature macrophages at 72 hours.

Incubated lymphocytes displayed very early uptake of $^3$H-PHA into the heterochromatin portion of the cell nucleus as early as 15 min. of culture, with a gradual tunneling of condensed heterochromatin beginning at 24 hours, accompanied by RNA synthesis at 24 hours, DNA synthesis and disappearance of all heterochromatin at 48 hours, and enlarged cell and increased size of nucleolus. Cell mitosis and cell division began at 48 hours, with increased cytoplasmic membrane folding and activity.

Previous studies have reported a return to the prominent heterochromatin repressed state upon removal of PHA from the lymphocyte culture media.

Conclusions:
Condensed repressed heterochromatin undergoes a progressive transition to the extended active euchromatin state within the cell nucleus of T-lymphocytes during $^3$H-PHA stimulation.
Board 18     18-Pos

Micro-Dynamics of Chromosomal Loci

Avelino Javer Godinez1, Marco Grisi1, Eileen Nugent1, Krystyna Bromek1, Chiara Saggioro2, Bianca Sclavi2, Marco Cosentino Lagomarsino3, Pietro Cicuta1.

1Cambridge University, Cambridge, Cambridgeshire, United Kingdom, 2LBPA, ENS de Cachan, Paris, France, 3Genomic Physics Group FRE3214 CNRS, Paris, France.

Previously believed to behave as an amorphous polymer, the bacterial chromosome has recently been shown to have structure at various length scales. 4 different macrodomains have been identified in E.Coli, and it has been estimated that it possibly contains more than 400 independent supercoiled domains. These results have renewed interest in studies of bacterial chromosome organization and its effect on the physiological state of the bacterium. With tag proteins, specific DNA sequences can be pinpointed, allowing the study of the dynamics of particular chromosome regions in live cells in real time. This has revealed new details of chromosome segregation, and has lead to the hypothesis of physical models of chromosome structure (2010 publications by Wiggins et al., Theriot et al.). In this work we analyze 15 different bacterial strains (the Boccard collection), where in each strain a particular chromosome position is labeled. The loci are continuously tracked by fluorescence imaging. The mean loci displacement with time is well described by a power law, and we discuss the motility and the exponent for each individual strain and under different growth conditions.

Board 19     19-Pos

DNA Looping in Topologically Constrained Domains


University of Texas at Dallas, Richardson, TX, USA.

Many aspects of DNA recombination, replication, and gene expression involve binding of specific proteins to multiple sites along the same DNA molecule. These interactions lead to the formation of a topologically closed DNA loop between protein-recognition sites, whose energetics depend on the structure and the flexibility of the intervening DNA, the degree of supercoiling, and the binding of accessory proteins such as HU and Fis in bacterial systems or histones and HMG proteins in the case of eukaryotic cells. The biophysical techniques that are available for characterizing in-vitro or in-vivo DNA looping in topologically closed DNA molecules are limited at present. Using a novel technique for incorporating multiple fluorophore probes in covalently closed DNA molecules, we investigate the helical-phase dependence of lac-repressor-mediated looping by FRET. Our results suggest that details of DNA tertiary structure influence equilibria between different conformations of the lac-repressor tetramer.
Chromosome Structure in Mammalian Mitotic Prophase - An Intermediate Stage with Intermingled Sisters and a Single Peripheral Core

Zhangyi Liang¹, Denise Zickler², Nancy Kleckner¹.

¹Harvard University, Department of Molecular & Cellular Biology, Cambridge, MA, USA, ²Université Paris-sud, CNRS, Laboratoire de Génétique, Orsay, France.

The structure of prophase chromosomes was examined in mammalian cells, both human and muntjac and compared with structures of later-stage chromosomes. Chromatin of sister chromatids was differentially labeled by BrdU. Chromosome structure was analyzed by silver staining and immunostaining for TopoII, cohesin and condensin(s). At early and mid prophase, sister chromatids exhibit intermingled chromatin and comprise a single long, thin morphological unit. At mid-prophase a single axial structure appears along chromosomes. In late prophase, double-core and single-core components coexist, with concomitant widening and progressive individualization of sister chromatid chromatin in double-core regions, even along the same single chromosome, supporting that sister chromatid separation is a continuous process. At this stage, single-core segments often exhibit a folded structure similar to that seen along each individual chromatid at prometaphase and metaphase. At both early- and mid-prophase, single core segments, whether straight or folded, are peripheral to the chromatin halo, not centrally located within the chromatin. Topoisomerase II inhibitor arrests sister-chromatid separation at the mid-prophase state at both the chromatin and core levels, precluding development of the folded pattern along single chromosome cores; thus, chromosomes exhibit only straight, single axes located peripherally to the chromatin. We propose that the folded single axis seen in normal, untreated mid-prophase chromosomes is an intermediate state between intermingled sister chromatids and separated ones. We further propose that in single-core regions, straight and folded, sister chromatid chromatin is organized into cooriented parallel linear loop arrays. This interpretation is supported by strong similarities with meiotic prophase chromosomes, which are known to exhibit such organization. How such a structure might develop and how it might transit to that seen at prometaphase/metaphase will be discussed.
**Board 21  21-Pos**

*Anaerobiosis-induced Nucleoid Associated Protein Dan Polymerizes Along dsDNA to Form Rigid Helical-like Co-filament that Is Able to Concatemerize*

Ci Ji Lim\(^4,2,3\), Sin Yi Lee\(^1\), Akira Ishihama\(^3\), Jie Yan\(^1,2,3\).

\(^1\)National University of Singapore, Department of Physics, Singapore, \(^2\)Mechanobiology Institute, Singapore, \(^3\)Center for Bioimaging Sciences, Singapore, \(^4\)NUS Graduate School for Integrative Sciences and Engineering, Singapore, \(^5\)Hosei University, Department of Frontier Bioscience, Tokyo, Koganei, Japan.

Dan is a newly identified nucleoid associated protein during E.coli anaerobiosis state. It is a positive regulator of an operon involved in expression of enzymes used in anaerobic metabolism pathway. Using single-molecule imaging and force manipulation techniques, we show that Dan is able to polymerize along dsDNA to form a rigid helical-like protein scaffold, causing DNA stiffening. We also show that the resulting Dan-DNA co-filament is able to cause DNA condensation, while intrinsically stiffens the DNA via the rigid protein scaffold. This reveals the mechanistic actions of Dan on DNA, indicating its ability in promoting genomic DNA packaging in E.coli, while forming rigid protein islands for gene regulatory functions. H-NS, a gene silencer, presumably forms a rigid protein scaffold along DNA to block gene access. Dan being able to coat DNA, is however a positive gene regulator. This suggests that Dan performs its gene regulatory function possibly by being a gene silencer “silencer”.

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**Board 1  22-Pos**

*Single Molecule Twist Measurements on Chromatin During Nucleosome Eviction*

Nicholas Luzzietti, Stefan Hahnewald, Ralf Seidel.

TU Dresden, Dresden, Sachsen, Germany.

Single-molecule force spectroscopy experiments are a helpful tool to study the mechanics of single nucleosomes. Typically discreet 25 nm-long abrupt DNA length changes occurring at around 20 pN of force are reported and explained as nucleosome eviction events. However, these steps represent only the release of approximately the last turn of DNA wrapped around the protein octamer. Unwrapping states that precede the eviction and that determine the state of the fully folded nucleosome in absence of tension are rarely resolved. To monitor full DNA unwrapping from nucleosomes in a more controlled manner we couple force spectroscopy experiments with direct twist measurements. We use reconstituted recombinant chromatin fibres, on long arrays of the Widom601 sequence (48 repeats) and magnetic tweezers to unzip the nucleosomes from the fibres. In addition we track the rotations of the magnetic bead that is used to exert the forces. This allows us to directly measure the release of twist during nucleosome eviction and to better assign the different unwrapping states. We compare the results obtained for canonical nucleosomes with nucleosomes containing H2AvD (H2AZ orthologue in D. melanogaster).
**Board 2  23-Pos**

**Human Rif1 Regulates DNA Replication and Transcription**

Satoshi Yamazaki, Hisao Masai.

Tokyo Metropolitan Institute of Medical Science, Tokyo, Japan.

DNA replication is spatially and temporally regulated during cell cycle. Many chromatin regulators are involved in this regulation. Rif1, Rap1 interacting protein 1, was originally identified in budding yeast as a regulator of telomere maintenance, and is widely conserved during evolution. Its human homologue may have little role in normal telomere maintenance but has been implicated in intra-S-phase checkpoint induced by double-strand breaks or replication stress. Rif1 also has been shown to be required for maintaining pluripotency of mouse ES cells and embryonic development. We have identified Rif1 as a bypass mutation of hsk1Δ (homologue of Cdc7 kinase) cells in fission yeast and have shown that replication initiation is deregulated on a genome-wide basis in rif1Δ cells. To gain further insight into the mechanisms of how human Rif1 may function in regulating DNA replication and transcription, we examined the effect of Rif1 depletion in human cancer and normal cells.

Rif1 exclusively localizes in nuclei and tightly binds to chromatin during interphase. Depletion of Rif1 induces global change of transcription pattern as well as spatial pattern of DNA replication. Thus, Rif1 may be an important chromatin factor that exerts global control over DNA replication and transcription. We would like to present our latest data on the functions of Rif1 and discuss how it might affect both transcription and replication.

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**Board 3  24-Pos**

**Fractal Globule and Other Polymer Models of Chromatin Packing in Eukaryotes**

Leonid Mirny.

MIT, Cambridge, MA, USA.

We study how a DNA molecule of about 2 meters long is folded inside a cell nucleus of about 5 microns in diameter. Packing and processing of DNA by the cells pose several intriguing problems for polymer physics. Recently developed experimental methods of chromatin conformational capture provided a comprehensive view at DNA organization inside the cell. Analysis of these data demonstrated that statistical characteristics of this organization are not consistent with well-known polymer states such a random coil or an equilibrium globule, but are consistent with the “fractal globule” state first proposed by Grosberg et al in 1988. The fractal globule is a dense, self-similar, and unknotted conformation of a polymer. Using theory and simulations, we study physical properties of the fractal globule, demonstrating that it constitutes a non-equilibrium state, which can serve as an attractive model for DNA architecture inside a cell. Comparison of available data on DNA organization in human, yeast and bacterial cells suggest that the same physical principles lead to formation of different DNA architectures in these organisms.
The Stability of the Nucleosome Is Controlled by the Charge State of Its Globular Core: a Comprehensive Study

Alexey V. Onufriev¹, Andrew Fenley².

¹Virginia Tech, Blacksburg, VA, USA, ²UCSD, San Diego, CA, USA.

DNA wrapping/unwrapping transition in the nucleosome is studied theoretically, based on the available atomic structure of the complex. It is shown that while the nucleosome itself is muchmore stable than a typical protein, (predicted stability is 34 kcal/mol), small changes in the charge of the globular histone core can completely destabilize or significantly loosen the core-DNA association. In contrast, charge alterations in the tail regions have virtually no effect on the nucleosome stability. Thus, it is suggested that alteration of the charge state of the globular core can be utilized as a mechanism to control the nucleosome stability. All possible single site acetylations and phosphorylations in the globular core are studied, and their effect on the nucleosome stability is quantified. Spatial regions where charge-altering PTMs will have the most effect on the core-DNA association strength are identified. Correlations with in-vivo studies are discussed.

Nanoscale Disorder in Nuclear Architecture at Different Cell Phase Stages as Measured by Partial Wave Spectroscopy

German Picasso¹, Yolanda Stypula¹, Jun Soo Kim², Vadim Backman¹, Igal Szleifer¹.

¹Northwestern University, Evanston, IL, USA, ²Ewha Womans University, Seul, South Korea.

In recent years it has become clear that the nano-architecture of the cell nucleus plays a crucial role in many aspects of genome maintenance, such as DNA repair, gene silencing and expression, as well as in diseases such as cancer. Our group has developed a new technique that can probe the spatial organization of nuclear components at the nanometer scale. This technique called Partial Wave Spectroscopy (PWS) uses light scattering to measure the disorder strength (Ld) of one-dimensional channels across the cell. This quantity is directly proportional to refractive index variations which is a linear function of the local density of intracellular solids (proteins, lipids, DNA, RNA, etc). Thus the spectrum of a 1D scattering signal contains information about spatial variations of density at length scales that are well below the wavelength.

In this study we have used PWS to probe the architecture of the nucleus of colon cancer cells as they go through the cell cycle. This methodology allowed us to obtain maps of chromatin condensation for cells of different levels of cancer aggressiveness, as well as at different stages in the cell cycle. This in turn permitted a direct comparison of a statistical parameter of nuclear architecture (Ld) between the interphase nucleus and the mitotic nucleus. In order to identify the structural entities contributing to changes in disorder strength in the nucleus we developed a theoretical model using computer simulations of chromatin condensation. From this work we concluded that increased values of chromatin compaction will correspond to increased readings of Ld.
Probing DNA Structure and Energetics in Vitro and in Vivo with X-Ray Scattering

Xiangyun Qiu.

George Washington University, Washington, DC, USA.

We have been using x-ray scattering, a well-established (i.e., ancient) technique, to investigate the structure and energetics of DNA/RNA assemblies under wide-ranging conditions. Despite tremendous advances, challenges remain as to unify the contentious theories and uncover the true physics. More critically, transferring the quantitative knowledge in vitro to biological milieu has been limited and far from satisfactory. Our current research focuses on bridging two of the many existing gaps between in vitro and in vivo. First, the behavior of nucleic acids in vivo is regulated by various proteins (e.g., packaging, bending, and twisting), which hardly resembles the in vitro reconstitution with simple cations. Second, the sequences (genetics) and modifications (epigenetics) of nucleic acids are of utmost importance in biology, whereas biophysical interrogations usually treat DNA/RNA as a charged uniform cylinder.

In accordance, our first aim is to quantitatively characterize nucleic acids systems that closely mimic biology, from simple ions to complex proteins, from DNA in a test tube to tightly packaged DNA in viruses and cells under stress, and to the organization of chromosomes. Our second aim is to quantify the effect of the sequence and epigenetic modification of nucleic acids on the strength and specificity of their interactions. Some progress has been made thus far. We have first measured the DNA pressure in dsDNA bacteriophage lambda, probed the energetics of isolated nucleoids, quantified the structure and dynamics of nucleosome particles, and discovered the anomalous condensation behavior of repeat sequences forming triple-strand DNA.

Understanding Sequence Dependent Effects upon the Mechanics of DNA

Krishnan Raghunathan¹,², Joshua Milstein¹, Jens-Christian Meiners¹,².

¹Department of Physics, University of Michigan, Ann Arbor, MI, USA, ²LSA-Biophysics, University of Michigan, Ann Arbor, MI, USA.

The sequence composition of DNA, despite being of vital importance in understanding its mechanical properties is often neglected in understanding its elastic properties. To study the effect of sequence on the bendability of DNA, we have designed sequences with varying elasticity but similar curvature. By stretching short sequences of DNA with an axial constant force optical tweezer, we found the persistence length to be dependent on the relative AT to GC content. The persistence length varied almost two fold between sequences containing 67% and 40% AT. Thus, bending a GC rich sequence of DNA is energetically more expensive compared to an AT rich sequence. Furthermore, we carried out Tethered Particle Motion (TPM) experiments to understand the effect of sequence dependent elasticity on the kinetics of protein-mediated DNA loop formation. However, from our prior experiments, we know that DNA loop formation is sensitive to even femtonewton forces and hence, it becomes imperative to follow DNA dynamics in a cellular environment. To this end, we have demonstrated a novel single molecule technique to follow the dynamics of DNA inside Dictyostelium discoideum. This technique can be extended to follow the dynamics of DNA looping inside a cell.
Diffusion-driven Contacts in Model Interphase Chromosomes: A Computer Simulation Study.

Angelo Rosa¹, Everaers Ralf².

¹SISSA, Trieste, Friuli-Venezia Giulia, Italy, ²Ecole Normale Superieure of Lyon, Lyon, France.

At early interphase and soon after nucleus formation, eukaryotic chromosomes are observed swelling from the rod-like, compact shape typical of mitosis [Alberts et al, Molecular Biology of the Cell], and rapidly reorganizing in discrete nuclear regions called ‘chromosome territories’ which play a crucial role in gene expression and gene regulation [Cremer & Cremer, Nature Rev Genet (2001)]. Unfortunately, because of the complexity of the nuclear environment a complete understanding of chromatin organization at finer time- and length-scales is still missing. A promising tool, direct detection of chromatin-chromatin interactions by Chromosome Conformation Capture (3C) [Lieberman-Aiden et al, Science (2009)] is marking a revolution in our understanding of chromatin organization. Nevertheless, this technique do not provide any information on the undergoing fiber dynamics. A complementary approach to ‘wet-lab’ experiments, computer (in-silico) polymer models are able to overcome the experimental limitations mentioned above. Recently, we have shown that the observed chromosome structure and dynamics are surprisingly well described by a generic polymer model of decondensing chromosomes [Rosa & Everaers, PLoS Comput Biol (2008); Rosa et al, Biophys J (2010)]. In particular, we concluded that the bulk of (long) chromosomes remains ‘trapped’ in a meta-stable, quasi-equilibrium state characteristic of unconcatenated ring polymers in dense solutions which can be described as a ‘fractal’ globule [Grosberg et al, Europhys Lett (1993); Lieberman-Aiden et al, ibid].

Here, we apply this framework to investigate the spatial and temporal behavior of diffusion-driven contacts between distant chromatin sites in model interphase chromosomes. In particular, we present novel results concerning quantitative predictions for times of first-contact between chromatin sites, which should be amenable to experimental validation.
Physics of the Force-induced DNA Melting: Peeling vs. Inside Melting

Ioulia Rouzina¹, Micah J. McCauley²*, Mark C. Williams².

¹University of Minnesota, Minneapolis, MN, USA, ²Northeastern University, Department of Physics, Boston, MA, USA.

It has been known for 15 years that application of a stretching force to the opposite ends of torsionally unconstrained double stranded (ds) DNA leads to its abrupt 1.7-fold elongation at the force Fo~65 pN. Two alternative explanations for this force-induced overstretching transition in B DNA include force-induced melting (FIM), in which its strand separate in a manner analogous to thermal DNA melting, and a transition from B-form dsDNA to another longer double-stranded form, denoted S–DNA. Recently several experimental studies have been reported in support of each of these two models. It has become clear that the observed DNA overstretching transition indeed occurs in two very different modes. One mode involves strong hysteresis of the DNA stretching and release, indicating that the two DNA single strands (ssDNA) re-anneal slowly, and suggesting that the transition involves peeling of the two strands. The rapid kinetics of the second mode of DNA overstretching was attributed to a B- to S-DNA transition. Here we consider a model in which the second mode of overstretching represents internal dsDNA FIM. We discuss how the switch between the peeling and internal FIM modes is affected by solution ionic strength, temperature, DNA sequence and the DNA pulling rate. We apply this model to explain the currently available experimental data. In addition we present new data on the pulling rate dependence of the DNA overstretching transition at different solution ionic strengths. We show that this dependence provides additional support for the co-existence of the two types of the DNA FIM.

The Effects of Histone Mutations and Modifications on the Thermodynamics of Chromatin Remodeling


Yale University, New Haven, CT, USA.

In every eukaryotic cell, DNA must be packaged into chromatin so that it physically fits within the nucleus. However, DNA must also be accessible during transcription, recombination, replication and repair. To that end, chromatin accessibility is highly regulated through the use of post-translational modifications. Although there have been several studies of nucleosome unwinding under force, the free energy of this process has not been measured, and no studies have quantified the effects of mutations and modifications of the core histones. We will present the results of single-molecule optical force microscopy measurements, using homogenous arrays of nucleosomes. We have developed methods that will allow us to determine the free energy associated with nucleosome unwinding with the goal of quantifying the effects of mutations and modifications on the thermodynamics chromatin assembly and disassembly.
Although static structures are known for many biomolecules, the functions of biomolecules are governed in space and time ultimately by their dynamic character. Using a confocal fluorescence microscope the newly developed multi-parameter fluorescence detection (MFD) enables us to simultaneously collect all fluorescence information such as intensity, lifetime, anisotropy in several spectral ranges) from picoseconds to seconds and to circumvent the classical pitfalls of the FRET method in ensemble measurements. In addition, we developed single-molecule (sm) super-resolution (SR)-FRET as a quantitative procedure for the proper consideration of the position distribution of the dye and of linker and a very high accuracy of FRET based structure determination. To achieve super-resolution (RMSD < 0.2 nm) in FRET experiments with dyes flexibly linked to DNA, it is indispensible considering all possible dye positions to achieve correct positioning in our newly developed rigorous FRET position system (FPS). We applied FPS to mononucleosomes, DNA and RNA. These novel FRET-based detection and analysis methodologies allowed us to resolve several structural subpopulations with Ångström resolution und derived FRET based structural models for several species from the same measurement.

**Trapping DNA-Protein Binding Reactions with Neutral Osmolytes for the Analysis by Gel Mobility Shift and Self-Cleavage Assays**

Nina Y. Sidorova, Shakir R. Muradymov, Stephen Hung, Donald C. Rau.

NIH, Bethesda, MD, USA.

The electrophoretic mobility shift assay (EMSA) is a popular tool in molecular biology for measuring DNA-protein interactions. EMSA is sensitive and provides flexibility both in sample volume and sample concentration. The main disadvantage is that EMSA is a separation technique, and the process of physically separating DNA-protein complex from free species can perturb the equilibrium. We take advantage of our observation that neutral osmolytes can strongly slow down the rate of DNA-protein complex dissociation to develop a method that uses osmotic stress to ‘trap’ mixtures of DNA-protein complexes. Adding high concentrations of neutral osmolytes blocks dissociation of already formed complexes while an excess of the specific sequence oligonucleotide competitor prevents the association of free protein with target DNA effectively preventing further reaction and enabling analysis of the products.

Many DNA-protein complexes are not stable enough and dissociate while in the gel matrix giving smeared bands that are difficult to quantitate. We demonstrate that the addition of the osmolyte triethylene glycol to polyacrylamide gels dramatically stabilizes labile restriction endonuclease EcoRI complexes with nonspecific DNA enabling quantitation of binding. The technique can be readily used for any RNA and DNA-protein complex as well as for multimeric transcriptional complexes provided these complexes are sensitive to osmotic stress.

We then apply the osmotic stress approach to develop a solution self-cleavage assay that uses the nuclease activity of restriction endonucleases to measure their specific binding to DNA. We demonstrate that at sufficiently high concentrations of neutral osmolytes the cleavage reaction can be triggered at only those DNA fragments with initially bound enzyme. We compare here the self-cleavage and gel mobility shift assays applied to the DNA binding equilibrium and kinetics of EcoRI, EcoRV, and BamHI restriction endonucleases.
Theoretical Modeling of Protein Accessibility to the Chromatin Fiber

Elena Koslover, Mario Díaz de la Rosa, Peter Mulligan, Andrew Spakowitz.

Stanford University, Stanford, CA, USA.

Gene expression is orchestrated by a host of regulatory proteins that coordinate the transcription of DNA to RNA. Regulatory proteins function by locating specific sequences of DNA and binding to these sequences to form the transcription initiation complex. The eukaryotic genome is tightly packaged into a dense chromatin fiber. This packaged structure acts both to store the massive genome and to facilitate the accessibility of the genome to regulatory proteins. The interplay between the packaging proteins and the regulatory proteins is critical in normal cellular function and plays a pivotal role in a number of human diseases. We present a theoretical study on the dynamic accessibility of the chromatin fiber, providing an overview of several approaches to this multi-faceted problem. Our modeling efforts address both the structural properties of packaged DNA and the dynamic processes involved in target-site localization of regulatory proteins. Modeling of the 30-nm fiber reveals the impact of local nucleosome configurational properties on the fiber geometry, and we predict the mechanical properties of the assemblies and the resulting dynamic accessibility. We also discuss the role of linker histones and variant histones in the fiber assembly. Using our 30-nm fiber models, we address large scale condensation by epigenetic factors and discuss the role of histone methylation in the determination of heterochromatin and euchromatin states. Upon establishing our chromatin model, we study the dynamic processes involved in the target-site search of regulatory proteins within the complex chromatin structure. This effort combines our structural models with our protein-transport models in order to provide a novel perspective on regulatory-protein function.
Both prokaryotes and eukaryotes store their genomic DNA in an environment, which balances the physical properties of double-stranded DNA with the mechanical effects of DNA-protein interactions. We have been utilizing a combination of techniques such as chromatin reconstitution (Nucleic Acid Res., 35: 2787-2799, 2007) and direct visualization of the nucleoid/chromatin fibers by various advanced atomic force microscopy (AFM) including fast-scanning AFM and recognition imaging to obtain a visual glimpse of dynamic aspects of these fibers (Ultramicroscopy, 110: 682-688, 2010). The origin of genome packing remains a mystery; it seems to go back to the very beginning of life itself. Since then, the principle of higher-order genome construction and architecture has been maintained and shared among three domains of living things. The major differences reside in the structural protein components of the architecture. Bacteria and Eukarya utilize HU and histones as the most fundamental structural proteins, respectively, forming the nucleoid in bacterial cell and the nucleosomes in the nucleus. On the other hand Archaea employ either HU or histones depending on their phylogenetic origins. Nevertheless, AFM revealed that the hierarchies of nucleoid/chromatin fibers look extremely similar among different species in different domains. Our recent structural investigations on both bacterial and eukaryotic genome foldings have revealed a step-wise folding of the genome DNA; from 10 nm to 30 nm fibers, and to 80 nm and further condensed beaded structures, depending upon the growth conditions and differential contribution of nascent single-stranded RNA. In some archaea (e.g., Thermococcus kodakarensis), besides histones, TK0471/TrmBL2 is an abundant chromosomal protein. Micrococcal nuclease-digested T. kodakarensis chromosome can be separated into chromosome fragments, a beads-on-a-string and a thick fibrous structure, enriched with histone and TK0471/TrmBL2, respectively; i.e., the archaea chromosome is organized into heterogeneous structures.
Single-Molecule Measurements of NAP-assisted Nucleosome Assembly on DNA

R. Vlijm¹, J. S. J. Smitshuijzen¹, A. Lusser², C. Dekker¹.

¹Kavli Institute of Nanoscience, Dept. of Bionanoscience, Delft University of Technology, Delft, Netherlands,
²Division of Molecular Biology, Innsbruck Medical University, Innsbruck, Austria.

Bulk studies have identified many proteins involved in the assembly and (re)positioning of nucleosomes, and point to the significance of the order in which the histones bind to DNA and its effect on supercoil density. The dynamics of protein-assisted nucleosome formation are however not well understood.

We study NAP (nucleosome assembly protein) assisted nucleosome formation at the single-molecule level using magnetic tweezers. This method allows the application of a well-defined stretching force and supercoiling density on a single DNA molecule, and to study in real time the change in linking number, stiffness and length of the DNA during nucleosome formation.

We observe a decrease in end-to-end length when NAP and core histones are added to the dsDNA. We confirm the formation of complete nucleosomes by measuring the change in linking number of DNA, which does not occur for non-specifically bound histones. By rotating the magnets, the supercoils formed upon nucleosome assembly are removed and the number of completely assembled nucleosomes can be counted.

We thus have established a single-molecule assay to study nucleosome assembly in real time and determine the number of formed nucleosomes.
Computer Simulation of Chromatin: Modeling the Influence of Global and Local Modifications

Gero Wedemann¹, René Stehr¹, Robert Schöpflin¹, Oliver Müller¹, Nick Kepper², Ramona Ettig², Karsten Rippe².

¹Univ. Appl. Sc. Stralsund, Stralsund, MV, Germany, ²Deutsches Krebsforschungszentrum, Heidelberg, Germany.

The three-dimensional structure of chromatin is a key factor for controlling the DNA accessibility for protein factors, DNA replication and repair. We utilized computer simulations in order to investigate the data of different experiments. The used coarse-grained model incorporates the elastic fiber properties of the DNA as well as a detailed description of the electrostatic and internucleosomal interactions. Ensembles in thermal equilibrium were generated using Metropolis-Monte Carlo-protocols. The simulations were capable to explain different experimental data of reconstituted polynucleosomes and chromatin from chicken erythrocytes quantitatively. Our results indicate that crossed-linker chromatin fibers with more than three nucleosome stacks were energetically unfavorable and unstable due to high electrostatic repulsion of linker DNA. Higher mass densities were achieved with interdigitated fiber conformations. A two-start helix fiber conformation was identified for chromatin without linker histone and low mass density with features that correspond to the tetranucleosome crystal structure. We examined the influence of different modifications on the three-dimensional structure of chromatin. Notably, the fiber structure is controlled by the strength of internucleosomal interactions, the nucleosomal repeat length and the local geometry of DNA entry and exit at the nucleosomes in very different ways. A systematic investigation revealed that these factors control the shape of the fiber as well as the interactions of distant parts of the fiber. Secondly, we investigated the effect of local modifications such as the displacement of single nucleosomes to model the effects of nucleosome-repositioning by remodeling complexes. Dependent on the size of the displacement the geometry of the fiber changed significantly. This serves as a tentative explanation for the effects of different remodeling complexes on processes such as DNA transcription.
Using Near UV Spectroscopy of Fluorescent Base Analogues to Study the Nucleic Acid Framework of ‘Macromolecular Machines’ of Gene Expression

Julia Widom¹, Kausiki Datta¹, Davis Jose¹, Wonbae Lee¹, Neil P. Johnson², Peter H. Von Hippel¹, Andrew H. Marcus¹.

¹University of Oregon Department of Chemistry and Institute of Molecular Biology, Eugene, OR, USA, ²Institut de Pharmacologie et de Biologie Structurale, Toulouse, France.

Specific local conformational changes in the DNA framework of functioning ‘macromolecular machines’ of gene expression are critical elements in many biological processes, such as chromatin remodeling and DNA replication, recombination and repair. Spectroscopy provides many ways to probe these conformational changes. The site-specific labeling of DNA with fluorescent base analogues, such as 6-methyl isoxanthopterin (6-MI, which replaces guanine) or 2-aminopurine (2-AP, which replaces adenine), allows the labeled positions to be probed selectively at wavelengths above 300 nm, where the other protein and nucleic acid components of these macromolecular machines are transparent. Base analogue dimers, in which two adjacent canonical bases are replaced by analogues, have proven to be especially sensitive structural probes. Spectroscopic techniques such as fluorescence and circular dichroism have been applied to these dimer probes and have yielded a wealth of information about DNA conformations and conformational changes in transcription and replication complexes. Nonlinear spectroscopic techniques such as 2-dimensional fluorescence spectroscopy (2D FS) have the potential to provide more quantitative structural information about these complexes, and can be extended to provide kinetic information as well. We present examples of the use of base analogue dimers to obtain structural information about the mechanisms of action of protein-DNA complexes. In addition, we present linear dichroism measurements on the highly fluorescent 6-MI probe that we are using to characterize its electronic transitions. We have made and will describe 2D FS measurements on dimers of zinc tetraphenylporphyrin in phospholipid membranes that demonstrate the structure-determination methods that we are presently gearing up to apply to functional protein-DNA complexes in solution.
DNA Stiffening and Bridging by Pseudomonas Aeruginosa Nucleoid-associated Protein MvaT and MvaU

Ricksen S. Winardhi¹⁴, Wen B. Fu², Yanan Li², Sandra Castang³, Simon L. Dove³, Jie Yan⁴⁵.

¹NUS Graduate School for Integrative Sciences and Engineering, National University of Singapore, Singapore, ²Department of Physics, National University of Singapore, Singapore, ³Division of Infectious Diseases, Children’s Hospital, Harvard Medical School, Boston, MA, USA, ⁴Mechanobiology Institute, National University of Singapore, Singapore, ⁵Centre for Bioimaging Sciences, National University of Singapore, Singapore.

MvaT and MvaU are nucleoid-associated proteins found in Pseudomonas aeruginosa, which have been identified as H-NS related proteins. Previous study using AFM imaging has shown that similar to H-NS, MvaT is able to bridge the DNA. Here we use transverse magnetic tweezers and AFM imaging to study the protein-DNA interactions. The results reveal that DNA stiffening occurs in complexes formed by both MvaT and MvaU, which coexist with DNA bridging. The importance of stiffening is highlighted by the absence of stiffening in MvaT mutants, which lack their functionality in vivo. AFM imaging revealed bridged filaments formed by MvaT and MvaU. The filaments formed by MvaT at 1 monomer to 1 bp ratio show corrugated structure with a periodicity of ~36 nm, which is probably due to bridging prior to DNA twisting. More proteins will further compact this structure into globular aggregates. Our results unravel the full binding mechanism of MvaT and MvaU to DNA and we show that they are different compared to H-NS, at least from their mechanical behavior.
Studies on Ionic Atmosphere and Its Dependence on Internal Pressure of DNA Array Using All-Atom Molecular Dynamics Simulations

Jejoong Yoo, Aleksei Aksimentiev.

University of Illinois at Urbana-Champaign, Urbana, IL, USA.

Protein-free DNA packaging has been the process of outstanding interest for biological and physical scientists as this phenomenon involves processes of fundamental scientific interests. From the biological point of view, the process is intriguing because viral DNA is tightly packed inside capsid simply by pressure. Apart from the biological context, DNA strands can also be packaged by osmotic pressure and/or condensing agents. Due to the relative simplicity, these protein-free systems served as a model for investigating the physical nature of the DNA-DNA interaction and its biological implication. By using experimental methods such as osmotic pressure and cryo-electron microscopy, how DNA strands are arranged and how the internal pressure depends on various conditions are fairly well understood. However, our understanding is limited because it is not currently possible to measure the DNA and ion distributions in detail inside the DNA array at physiologically relevant conditions. To elucidate what explicitly occurs inside the DNA array, we carry out all-atom molecular dynamics simulations of a 64 parallel DNA array confined in a harmonic well, which mimic the confinement by the viral capsid or osmotic pressure. The forces of the harmonic apply only to DNA to mimic DNA arrays, in which water and small solutes move freely. These simulations characterize the DNA and ion packing in atomistic detail, and semi-quantitatively reproduce experimental internal pressure as a function of inter-DNA distance and/or ionic conditions. Strikingly, our simulations indicate that DNA and salt ions are less homogeneously distributed than the experimental measurements, and we discuss how we can reconcile our prediction with the experimental data in order to enhance our understanding of the DNA-DNA interactions.

The Effects of Histone Tails on the Conformational Flexibility of a Nucleosome

Chongli Yuan, Ian T. Smith, Isabel Jimenez-Useche.

Purdue University, West Lafayette, IN, USA.

Post-translational modifications on the tails of histone proteins are known to play significant roles in regulating gene expression. In addition to various enzymatic reactions mediated by those tail modifications, histone tails can directly modulate chromatin structure and limits the accessibility of functionally important genes. This study utilizes a time-dependent fluorescence lifetime approach to reveal the effects of histone tails on the conformation and the dynamics of a nucleosome. Tails of H2A and H3 proteins are shown to contribute to the compactness of a nucleosome and to exhibit distinctive dynamic behaviors under different solution conditions. These findings can help to elucidate the roles of histone tails on the physical interactions that sustain a chromosome assembly and shed light on the functional roles of individual histone tails.
The RSC Chromatin Remodeling ATPase Translocates DNA with High Force and Small Step Size

George Sirinakis¹, Cedric R. Clapier², Ying Gao¹, Ramya Viswanathan², Bradley R. Cairns², Yongli Zhang¹.

¹Yale University, New Haven, CT, USA, ²University of Utah School of Medicine and HHMI, Salt Lake City, UT, USA.

ATP-dependent chromatin remodeling complexes use the energy of ATP hydrolysis to reposition and reconfigure nucleosomes. Despite their diverse functions, all remodelers share highly conserved ATPase domains, many shown to translocate DNA. Understanding remodeling requires biophysical knowledge of the DNA translocation process: how the ATPase moves DNA and generates force, and how translocation and force generation are coupled on nucleosomes. Here we characterize the real-time activity of a minimal RSC translocase ‘motor’ on bare DNA, using high-resolution optical tweezers and a ‘tethered’ translocase system. We observe on dsDNA a processivity of ~35 bp, a speed of ~25 bp/sec, and a step size of 2.0 (± 0.4, s.e.m.) bp. Surprisingly, the motor is capable of moving against high force, up to 30 pN, making it one of the most force-resistant motors known. We also provide evidence for DNA ‘buckling’ at initiation. These observations reveal the ATPase as a powerful DNA translocating motor capable of disrupting DNA-histone interactions by mechanical force.
Exploring the Effect of Sequence on Nucleosome Affinity via Mesoscale Simulation

Gordon S. Freeman¹, Vanessa Ortiz², Juan J. De Pablo¹.

¹University of Wisconsin-Madison, Madison, WI, USA, ²Columbia University, New York, NY, USA.

In this work we present a coarse-grain model for a nucleosome containing 147 DNA basepairs wrapped around the histone core. DNA sequences are described by a coarse-grain model in which each basepair is represented by three interaction sites (the “Three Sites per Nucleotide” coarse-grain model for DNA). We examine an artificial sequence designed to promote nucleosome affinity, a native nucleosomal sequence (PDB: 1kx5), and a nucleosome-free region known to have low nucleosome affinity. The histone complex is represented by a charged surface that reproduces the topology and local electrostatic environment of the histone core. Advanced sampling techniques are employed to determine the free energy of the complexes.

Using this model we explore the effect of DNA sequence motifs on the local flexibility of DNA in the nucleosome complex. In particular, we note differences in local bending and hybridization between sequence motifs known to promote high affinity for nucleosome formation versus a nucleosome-free sequence containing poly-A motifs. We also use this coarse-grain construct to identify sequence motifs that result in a preferred rotational orientation of DNA with respect to the histone complex. Consistent with previous experimental findings, we identify a sequence-specific rotational preference that is thought to play a key role in determining nucleosome positions in vivo.

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³Freeman et al., *Journal of Chemical Physics*, submitted.