412-Pos Board B167
Recovering Chromatin Conformations from Contact Probabilities
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The packaging of chromatin within the nucleus of eukaryotic cells is achieved through several levels of spatial organization. The lowest levels give rise to nucleosomes and the 30-nm chromatin fiber while the higher levels involve folding of the chromatin fiber into chromosomes. These higher levels, often referred to as the higher order organization of chromatin, are still poorly understood but are actively being investigated through a new class of experiments known as chromatin conformation capture (3C), and its high-throughput derivative called Hi-C. These experiments detect contacts between different genomic loci, yielding contact probabilities (CPs) that may be used to elucidate the higher order organization of chromatin. Here we present a computational method for recovering chromatin conformation ensembles from reference CPs (Meluzzi D and Arya G. Nucleic Acids Research. 2013 41:63). The conformations are generated by simulating a bead-chain polymer model that represents the 30-nm chromatin fiber. Selected parameters of this polymer model are optimized iteratively until the CPs estimated from the conformation ensembles match the reference CPs. To minimize the size of ensembles required to reliably compute the CPs, we have developed a method that estimates CPs by fitting the extended generalized lambda distribution to simulated inter-bead distances. We show that our overall approach enables the recovery of conformation ensembles for genomic lengths on the order of 1 Mbp and that these ensembles can be used to investigate the shape and spatial properties of biologically relevant chromatin domains.

413-Pos Board B168
Nucleic Acid Superstructures: Assembly Stories
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We will tell three stories, namely about chromosome folding, RNA auto-assembly and nanoparticles aggregation, to illustrate how physics can inspire biology as biology can inspire physics.
DNA molecules that compose the genome of any living organisms are condensed into supercoiled nucleoproteic filaments that compose nucleoid structures floating in the cytoplasm of prokaryotes and archea or discrete chromosome territories distributed in the nuclei of eukaryotes. In the first story, we will discuss some issues related to this compaction process, including the recent characterization of a DNA-condensing role for Hfq (mostly known as a prokaryotic RNA chaperone), the topological challenged faced by eukaryotic chromatin during transcription and the potential fractal organization of chromosome territories.
RNA is a molecule that serves both as a catalyst (like proteins) and as information storage (like DNA). In this second story, we will show recent evidence that small RNAs could auto-assemble in prokaryotic cells, and discuss the potential roles of such a phenomenon.
When mixed together, nanoparticles made of nanoceria coated with short polyacrylic acid moieties and cationic-neutral block copolymers form clusters through an electrostatic complexation process. In this third story, we will show how chromatin assembly protocols inspired us to propose a new pathway based on controlled desalting kinetics that enabled to control the size of the clusters from 100 nm to over 1 μm.
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414-Pos Board B169
Chromatin as a Dynamic Platform for Protein-Protein Interactions
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Post-translational modifications (PTMs) of histone proteins play a crucial role in regulating chromatin function. Effector proteins interact with histone PTMs through specific interaction domains (reader domains)[1]. Thereby, effectors are dynamically recruited to their target chromatin regions from a soluble pool[2]. It is currently not well understood how low-affinity interactions between histone PTMs and reader domains (with dissociation constants in the range of 5-100 micromolar) can result in rapid and specific effector recruitment. A possible model involves kinetic capture of effectors by a high local concentration of histone PTMs. Fast rebinding kinetics increase the effector residence time and result in local effector accumulation.
To shed light on these recruitment processes, we are investigating the molecular mechanism of dynamic protein localization through histone PTMs. Therefore, we are developing an in vitro system which allows us to measure the complex binding kinetics of chromatin factors to chromatin fibers carrying defined combinations of histone marks. Employing chemical protein semi-synthesis[3] and DNA engineering we construct specifically modified chromatin fibers of a distinct architecture. A total internal reflection fluorescence microscopy approach then allows us to determine effector dwell times dependent on the chromatin modification status, chromatin conformation and solvent conditions.
Quantitative analysis of dynamic chromatin processes in vitro is required to gain a better understanding of these processes in the complex environment of a nucleus. We thus expect that our analyses will significantly advance our knowledge of key aspects of the molecular mechanisms at work in histone PTM mediated effector recruitment in transcription, repression and DNA repair.
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415-Pos Board B170
Transposition of Native Chromatin for Fast and Sensitive Mulitmodal Analysis of Chromatin Architecture
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Eukaryotic genomes are hierarchically packaged into chromatin, and the nature of this packaging plays a central role in gene regulation. Major insights into the nucleoprotein structure of chromatin have come from high-throughput, genome-wide methods for separately assaying the chromatin accessibility ("open chromatin"), nucleosome positioning, and transcription factor (TF) occupancy. However, published protocols for these existing methods require millions of cells as starting material, complex and time-consuming sample preparations, and cannot simultaneously probe the interplay of nucleosome positioning, chromatin accessibility, and TF binding. These limitations 1) average over and ''drown out'' heterogeneity in cellular populations, 2) often require cells to be grown ex vivo to obtain sufficient biomaterials, modulating the epigenetic state in unknown ways, and 3) often prevent application of these assays to well-defined clinical samples, precluding generation of ''personal epigenomes'' in diagnostic timescales. Here we describe an Assay for Transposase Accessible Chromatin using sequencing (ATAC-seq) based on direct in vitro transposition of sequencing adapters into native chromatin - as a rapid and sensitive method for integrative epigenomic analysis. ATAC-seq captures open chromatin sites using a simple 2-step protocol from 500 to 50,000 cells, and reveals the interplay between genomic locations of open chromatin, DNA binding proteins, individual nucleosomes, and higher-order compaction at regulatory regions. Using this method, we discover classes of DNA binding factor that strictly avoid, can tolerate, or tend to overlap with nucleosomes. The method enabled serial daily epigenomes of resting human T cells to be observed using standard blood draws. We show that ATAC-seq is compatible with FACS, enabling studies on carefully sorted and rare subpopulations from primary tissues. The combination of speed, simplicity, and low input requirements of ATAC-seq will enable new gene regulatory insights into biology and medicine.

416-Pos Board B171
Long Distance Chromatin Interactions
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The dynamics of chromatin folding has an important role in regulating gene expression in higher eukaryotes. Detailed studies of several model loci, such as beta-globin, have shown that the formation of loops mediated by the interaction between specific regulatory elements that are located hundreds of kilobases away is crucial in gene control. This observation gives rise to an important question: how can a set of widely spaced elements communicate in order to regulate a specific target gene.
Recently a computational model has been proposed by Mukhopadhyay et al to explain the formation of long-range chromatin loops. This model is based on attractive nucleosome-nucleosome interaction, allowing the monomers of a chromatin polymer to stick to each other temporarily when in the vicinity of each other. This model shows the enhanced long range contact behavior