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Bivalent Recognition of a Single Nucleosome by the Tandem PHD Fingers of CHD4 is Required for CHD4-Mediated Repression

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CHD4 is a catalytic subunit of the NuRD (nucleosome remodeling and deacetylase) complex essential in transcriptional regulation, chromatin assembly and DNA damage repair. CHD4 contains tandem plant homeodomain (PHD) fingers connected by a short linker, the biological function of which remains unclear. Here we explore the combinatorial action of the CHD4 PHD1/2 fingers and detail the molecular basis of their association with chromatin. We found that the inter-domain organization of PHD1/2 leads to specific targeting of a single nucleosome in a multivalent manner, concomitantly engaging both of the histone H3 tails. This robust synergistic interaction displaces HP1γ from pericentric sites, inducing changes in chromatin structure and dispersion of the heterochromatic marks H3K9me3 and H4K20me3. We demonstrate that recognition of the histone H3 tails by the PHD fingers is required for repressive activity of the CHD4/NuRD complex. Together, our data elucidate the molecular mechanism of multivalent association of the PHD fingers with chromatin and reveal their critical role in the regulation of CHD4 functions.

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Improved Single-Molecule Imaging Based on Photon Counting with an EMCCD Camera

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New breakthroughs in digital imaging have historically opened opportunities to scientists in bio-medical research. One important landmark of the last decade has been the development of the electron-multiplying charge coupled device (EMCCD), an imaging detector that uses an avalanche phenomenon to amplify the weak photo-electron signals. EMCCDs are well suited for ultralow light applications in that a single photo-electron can be amplified sufficiently to allow the possibility of photon counting. However, as with all electronic detectors, the noise increases greatly with an increase in gain, diminishing the overall sensitivity and dynamic range and thus compromising images.
Recently, a new camera has been developed by Nüvü Cameras that significantly reduces the noise generated during the read-out process (up to 10 times) and allows more efficient photon counting. This major reduction of the noise threshold represents an opportunity for various low-light imaging applications including single-molecule studies.
We present a demonstration of its applicability in a total internal reflection fluorescence microscopy (TIRFM) assay to visualize single biomolecules. In molecular biology, it is well established that most proteins exert their biological roles in complex with other proteins. Thus, detecting individual components and the structure of these complexes is essential to understand the mechanism of biological events. To this aim, we employ TIRFM imaged in photon counting mode for visualization of single centromeric nucleosomes, containing a histone variant known as CENP-A. We show that this technique is well suited to TIRFM and that it is capable of detecting single fluorescently labelled CENP-A in the nucleosome with an enhanced signal to noise ratio. The desired and improved properties of the assay such as high sensitivity and photon counting shall pave the way for comprehensive studies of single molecules in this and other biological contexts.

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Chromatin Structure and Dynamics: Histone Variants and Remodeling Complexes

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The organization of DNA into nucleosome can be seen as a barrier for the transcription factors binding to their target DNA sequences and interferes with several basic cellular processes. ATP-remodeling machines and the incorporation of histone variants into chromatin are used by the cell to overcome the nucleosomal barrier and modulate DNA accessibility by the control of nucleosome dynamics. In this work, we use a single molecule technique (Atomic Force Microscopy, AFM) to visualize isolated mono- and oligo-nucleosomes and quantify their structure and dynamics at thermodynamical equilibrium and out of equilibrium.
We have studied the impact of H2A.Bbd histone variant incorporation at the mono-nucleosome and oligo-nucleosome level, and we have shown that this variant modifies both the structure and the dynamics of the nucleosome altering the ability to form a higher structure organization of the chromatin. Using a polymer physics model, we demonstrated that the behavior of variant chromatin can be quantitatively explained by the mono-nucleosome flexibility [1].
We are also interested in the mechanism of nucleosome remodeling by SWI/SNF and RSC on mono- and di-nucleosomes. Focusing on the di-nucleosomes, AFM imaging showed only a limited set of distinct configurational states for the remodeling products. No stepwise or preferred directionality of the nucleosome motion was observed [2]. Analysis of the corresponding reaction pathways allows deciphering the mechanistic features of RSC-induced nucleosome relocation. The final outcome of RSC remodeling of oligosomal templates is the packing of the nucleosomes at the edge of the template, providing large stretches of nucleosome depleted DNA.
[1] F. Montel, H. Ménoni, M. Castelnovo, J. Bednar, S. Dimitrov, D. Angelov & C. Faivre-Moskalenko; Biophys. J. 97, 544-553 (2009).
[2] F. Montel, M. Castelnovo, H. Menoni, D. Angelov, S. Dimitrov & C. Faivre-Moskalenko; Nucleic Acids Res. 39, 2571-9 (2011).

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Nucleosome Dynamics Studied by Single Pair FRET and Computer Simulations

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DNA in nucleosomes is sterically occluded and nucleosomes must open to allow full DNA access. We studied this process by single pair FRET (spFRET) and coarse-grained molecular dynamics. spFRET shows evidence for a new structural intermediate preceding histone dissociation, in which the (H3-H4)2 tetramer/(H2A-H2B) dimer interface is split open. This is followed by H2A-H2B dimer release from the DNA and, lastly, (H3-H4)2 tetramer removal. This open intermediate state could be demonstrated in Xenopus and yeast nucleosomes; histone variants such as H2A.Z may change the mechanism of opening. We estimate that the open state is populated at 0.2 - 3 % under physiological conditions, and could have significant in vivo implications for factor-mediated histone exchange and for DNA accessibility. DNA dynamics on the histone core are studied by a new coarse-grained model, replacing amino acids and DNA bases by single beads interacting through empirical potentials calibrated from all-atom MD. These simulations show a strong influence of the presence of the histone tails on DNA unwrapping, and an open state that can be stabilized by the displacement of the H3 tail into the gap between the DNA and the histone core.

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Effects of CpG Methylation on the Structure and Assembly of a Nucleosome

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We studied the mechanism of nucleosome assembly with a histone chaperon Nap1 and the effects of CpG methylation on the structure and the assembly of nucleosomes with fluorescence techniques. Based on single molecule and ensemble fluorescence measurements, we found that CpG methylation induces overwrapping of nucleosomal DNA accompanied by a topology change. Real-time monitoring of nucleosome assembly with the Selex 601 sequence and Nap1 revealed that nucleosome assembly takes place largely in four steps. Reduced kinetic rates for DNA wrapping during the assembly upon CpG methylation strongly suggest that DNA becomes rigid with methylated CpG dinucleotides. Free energy profiles of the nucleosome assembly process were also constructed based on the results. According to the profiles, all the intermediate states are kinetically stabilized but thermodynamically destabilized upon CpG methylation. The decreased kinetic rates upon CpG methylation may be due to the elevated transition state energy caused by the restricted conformational space of the rigidified DNA in the transition states. Based on these results, we propose a hypothesis where rigidified DNA upon CpG methylation inhibits nucleosome disassembly by destabilizing the transition states.