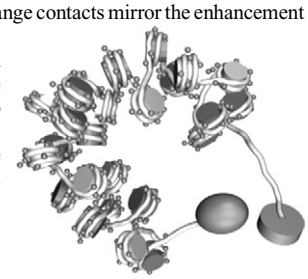
evolutionary conservation. Thus, H3 is fundamentally different in comparison to many proteins studied to date, including highly conserved proteins like actin, tubulin and histone H4. The discovery of the uniqueness in conservation of H3 goes against the conventional view that buried H3 residues are highly conserved just to maintain the stability of the nucleosome. This discovery thus points to either new additional functions of histone H3 that have not been uncovered or a unique conservation mechanism that goes beyond survival pressure.

351-Pos Board B151
The Impact of Nuclear Architecture on EGFP Diffusion Revealed by Pair Correlation Analysis
Elizabeth Hinde, Francesco Cardarelli, Michelle A. Digman, Enrico Gratton.
The diffusion of molecules within the nucleus is obstructed by the steric constraints imposed by the nuclear environment. The extent to which nuclear architecture directs the diffusive route taken by these molecules is of significant interest. No methods proposed thus far have the capability to measure overall molecular flow in the nucleus of living cells. Here we apply the pair correlation function analysis (pCF) to measure molecular anisotropic diffusion in the interphase and mitotic nucleus of live cells. In the pCF method we cross correlate fluctuations at several distances and locations within the nucleus, enabling us to define migration paths and barriers to diffusion. We use monomeric EGFP as a prototypical inert molecule and measure its flow in and between the different nuclear environments.
For the interphase nucleus we observe two disconnect molecular flows associated with high and low DNA density, which cause the chromatin to behave as a channeled network. Upon more detailed analysis in time, rare bursts of EGFP molecules are detected crossing the channel barriers. The intermittent nature of this transit suggests an intrinsic localized change in chromatin structure which periodically turns on and off. For the mitotic nucleus we observe the chromosomes to impart a markedly different mechanism toward regulation of the equivalent transit. That is, we found EGFP flow between the different nuclear environments of a mitotic nucleus to be continuous and delayed. The continuity of obstruction in time suggests the chromosomes to regulate diffusion by acting as a physical barrier. These two distinct diffusive routes were concomitantly observed in the *C. elegans* germ line. This is the first *in vivo* demonstration of cell cycle dependent diffusion of an inert molecule as regulated by nuclear architecture with high spatial and temporal resolution.

352-Pos Board B152
Computational Study of Nucleosome Positioning and Stability
Rajib Mukherjee, Thomas C. Bishop.
Nucleosomes are a highly conserved molecular mechanism for packing genetic material into the cell nucleus. Biologic functionality requires that the histone core be able to fold virtually any sequence of DNA into a nucleosome. However all nucleosomes are not created equal. *In vitro* histones occupy preferred locations on lengths of DNA greater than 147bp (i.e. positioning) and exhibit preferential binding in mixtures containing different 147bp long oligomers (i.e. affinity). *In vivo* nucleosome positioning is also observed. But the physical basis for stability and relationships between positioning and affinity remain unclear. For this purpose we have simulated over 300 nucleosomes using all atom molecular dynamics (MD) to investigate nucleosome energetics, structure and dynamics as a function of DNA sequence.
The 336 nuclesomes modeled represent 16 segments of DNA, one from each chromosome of *Saccharomyces Cerevisiae*. Each segment contains the highest occupied and least variable nucleosome positioning sequence for the parent chromosome. Each segment is 167bp long and includes: the observed 147bp positioning sequence and 10bp on each side. Every 147bp subsequence of each segment is threaded onto the octamer core as observed in xray structure 1kx5 and solvated, yielding 21 mononucleosomes for each of the 16 segments. Each mononucleosome is subjected to MD simulation. Here we report how the simulations are accomplished and focus attention on the segments for which all 21 mononucleosomes have been simulated for at least 20ns. Helical parameter analysis is used to investigate variations in structure and dynamics of nucleosomal DNA as a function of sequence. Results are compared to values obtained from a systematic MD study of nearest-neighbor effects on base pair step conformations and fluctuations of free DNA. The goal is to quantify variations in structure, dynamics and energetics of nucleosomal DNA as a function of sequence.

353-Pos Board B153
Long-Range Interactions in Chromatin
Nicolas Clauvelin, Wilma K. Olson, Vasily Studitsky.
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 nucleosomes and the constituent DNA and histone proteins 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 and the electrostatic interactions of representative DNA and amino-acid atoms. The simulated probabilities of long-range contacts mirror the enhancement of gene expression detected in model biochemical systems. The structure-based model (see figure) makes it possible to relate specific changes in nucleosome structure to global properties of the fluctuating chromatin chains. Analysis of the inter-nucleosome interactions is helping to unravel the details of distant communication on DNA as well as develop a simpler coarse-grained model of chromatin applicable to the study of longer, biologically relevant fragments.



354-Pos Board B154
Modeling the Effects of Structure and Epigenetic Modifications in Heterochromatin Condensation
Peter J. Mulligan, Elena F. Koslover, Andrew J. Spakowitz.
Proper gene silencing requires physically restricting access to those genes, such as in condensed nuclear regions called heterochromatin. Cells epigenetically inherit which genomic regions are heterochromatin at least in part through methylation of histone 3 at lysine-9 (H3K9). We have developed a thermodynamic model of heterochromatin condensation via bridging interactions of heterochromatin protein 1 (HP1) bound to nearby nucleosomes. HP1 binds at H3K9, with enhanced binding when the site is methylated. After taking low energy chromatin fiber structures from a model based on the energetics of the wrapped DNA, we find the distances between HP1 binding sites on multiple nearby fibers. Dimerization and interaction of HP1 is known to occur in vivo, so nearby bound HP1 have a favorable coupling energy which enhances the interaction between these chromatin fibers. We have fine tuned our model to experimental measurements of HP1 binding to offer predictions about the effects of fiber structure and lysine-9 methylation on fiber condensation. After comparing a range of low energy fiber structures, we find that similarly compact chromatin structures prefer to associate together. In addition, we show that the differing amount of methylation in chromatin can enhance local concentrations of HP1 similar to in vivo measurements. These results demonstrate how local chromatin structure and epigenetic modifications can impact large-scale chromatin organization in the nucleus.

355-Pos Board B155
The Tandem PHD Fingers of Chd4 Bind Synergistically to Histone H3
Catherine A. Musselman, Ann Kwan, Robyn Mansfield, Joel Mackay, Tatiana G. Kutateladze.
CHD4 is an ATPase dependent chromatin remodeler and a major subunit of the NuRD (nucleosome remodeling and deacetylase) complex, which is involved in transcriptional regulation and development. CHD4 contains a tandem of plant homeodomain (PHD) fingers, two chromodomains and an ATPase module. The PHD construct is found in many chromatin remodeling complexes and transcription factors, and is often found to be important for the targeting of these complexes to chromatin. Here we determine the specificity of the individual PHD domains of CHD4 for the unmodified tail of histone H3. We use NMR, mutational analysis and modeling to characterize the molecular basis of the interactions, revealing that both domains have an almost identical mode of interaction with the H3 tail. Analysis of the tandem PHD construct reveals that, when linked, each domain retains a distinct binding mode, however we find a synergistic effect on the binding affinities. Together our data reveal a unique mechanism of cooperative binding by multiple effector modules and suggest a multivalent targeting of CHD4 to chromatin that could manifest as either intra- or inter- nucleosomal.

356-Pos Board B156
3D Microscopy of Chromatin Movements in Living Yeasts Reveals the Physical Parameters That Govern its Dynamics
Houssam Hajjoul, Mathon Julien, Bancaud Aurélien, Goiffon Isabelle, Bystricky Kerstin.
Understanding the details of how chromatin folds and the physical parameters governing its behavior are among the most intriguing challenges in modern cell biology. Recent insights on chromosome conformation in yeast nuclei were gained owing to high-throughput molecular biology techniques [1], but the physical parameters governing chromosomes dynamics remain unelucidated.
We have recently developed an original fast 3D fluorescence microscopy technique for studying chromatin in living yeast [2], which allows sampling its dynamics with temporal resolutions of 15 ms. We reasoned that this