

specific binding of the PIC components to nucleosomes. We also show that nonconsensus binding has genome-wide influence on transcriptional frequency.

#### 2519-Pos Board B211

##### Specific DNA Sequence Search Dynamics of p53 and Effect of Acetylation of its C-Terminal Domain: Multi-Scale Simulation Study

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A transcription factor p53 is composed of ordered Core and Tetramerization domain and of intrinsically disordered N-terminal domain, C-terminal domain and the linker region, which prevent to elucidate the functional mechanism based on static structural analyses. In order to model the linker region supposed to play an important role, first, we performed all atom (AA) multi-canonical MD simulation of this 40 amino acid residues long region. Second, we decided the parameters of CG model so that the contact map of the CG structural ensemble was marginally same as that from the AA simulation. The SAXS profile obtained from the CG simulation using these parameters reproduces the previous experiment. Finally, we conducted the CG simulation of the system containing a full-length p53 quaternary structure and a 100 bp DNA including a p53 binding site. In the production simulation, p53 spontaneously binds to its binding site and forms a complex structure that is consistent with the electron microscopic model obtained previously. The result also revealed that p53 slides along nonspecific dsDNA with its CTD before it binds to REs, whereas, upon acetylation of the CTD, it repeats dissociation from and association to dsDNA, suggesting that previously suggested inhibitory effect of the CTD on binding of p53 to its binding site and the nullification of the inhibitory effect upon acetylation are largely attributed to the change of main search mechanism from sliding to 3D diffusion. Taken together, the result of this multi-scale simulation shed light on the problem that is difficult for conventional experimental technique to address due to the flexibility of this protein.

#### 2520-Pos Board B212

##### A Gripping New Mechanism of Drug Resistance in HIV-1 Reverse Transcriptase

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HIV-1 Reverse Transcriptase (RT) converts viral RNA into dsDNA that is integrated into the human genome. Nucleoside RT inhibitors (NRTIs) are highly effective in the treatment and prevention of HIV. NNRTI resistance mutations arise from therapy, yet the mechanism(s) of how these mutations inhibit polymerization by RT is unclear. We examine the role of NNRTI on the dynamics between RT and its Template/Primer (T/P) substrate using a combination of molecular modeling, single-molecule and bulk fluorescence techniques and provide an unprecedented glimpse into the dynamics of RT-T/P interaction as well as the intramolecular conformation of RT itself while bound to its substrate. The data suggests a unique mechanism of resistance that is mediated by interplay between intramolecular conformational changes in RT and intermolecular dynamics of the RT-template/primer-dNTP complex.

#### 2521-Pos Board B213

##### Tunable Regulation of the Serca/Phospholamban Complex by Single-Stranded DNA Sequences

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Heart disease is a leading cause of death in the Western world.<sup>1</sup> Failing hearts have been shown to exhibit abnormal calcium cycling within cardiomyocytes.<sup>2-5</sup> While there are several proteins that are involved with the calcium pathway in cardiomyocytes, the sarcoplasmic reticulum Ca<sup>2+</sup>-ATPase (SERCA) and phospholamban (PLN) function in the important role of calcium reuptake into the SR for storage. SERCA is inhibited by PLN, a 52 amino acid, single-pass membrane protein. Upon PLN phosphorylation at Ser16, this inhibition is relieved and SERCA's calcium affinity is restored to normal levels. It has been proposed that by reversing PLN's inhibition on SERCA in patients with heart failure proper Ca<sup>2+</sup> homeostasis and muscle contractility may be restored.<sup>6</sup> Using biophysical, biochemical, and analytical techniques including: enzyme coupled activity assays, solid and solution state nuclear magnetic resonance (NMR), fluorescence polarization, and affinity capillary electrophoresis we show that single stranded DNA sequences (ssDNA) target the SERCA/PLN complex and fully restore the ATPase function. Importantly, ssDNA sequences bind PLN at a nanomolar level of affinity. The restoration of SERCA activity by ssDNA is elicited in a length-dependent manner. Studying the unique interactions between the PLN and ssDNA will give us insight into this chemical and molecular mechanism that allows for restoration of SERCA activity and will aid in the development of small molecules able to interfere with the SERCA/PLN complex for possible therapeutic interventions.

#### 2522-Pos Board B214

##### Kinesin KIFC1 Actively Transports Double-Stranded DNA in Eukaryotic Cells

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We discovered that exogenous double-stranded DNA molecules are actively transported through the cytoplasm of eukaryotic cells. This transport is related to the activity of molecular motors belonging to the kinesin-14 family: KIFC1 and NCD.

This result was achieved through in cellula single molecule experiments, which allowed us observing the motion of the naked DNA molecules in HeLa cells, in real time. These experiments were complemented by mass spectroscopy measurements to isolate the motor(s) responsible for this active transport. Eventually, the NCD driven transport of dsDNA molecules was reproduced in an in-vitro minimal system.

#### 2523-Pos Board B215

##### DNA Binding of Porphyrin Conjugates: Characteristics and Consequences

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Recently cationic porphyrin-peptide conjugates were synthesized to enhance cellular uptake of porphyrins and to deliver peptide moieties to the close vicinity of nucleic acids.

The aim of our work was to characterize the binding of porphyrin-peptide conjugates to nucleic acids and nucleoprotein complexes (NP) and describe structural changes induced by the interaction.

Porphyrin-peptide conjugates were synthesized, linking tetra-peptides to tris(N-methylpyridyl)carboxyphenyl-porphyrin (TMPCP) and bis(methylpyridyl)carboxyphenyl-porphyrin (BMPCP). These structures can be considered as a typical monomer unit corresponding to the branches of porphyrin-branched chain polypeptide conjugates. DNA was isolated from T7 bacteriophage; as nucleoprotein complex the complete phage particle was used (NP).

Binding modes were studied by comprehensive spectroscopic methods such as absorption spectroscopy, CD spectroscopy and fluorescence lifetime measurements. To look for possible structural changes of NP and DNA caused by the porphyrin binding CD spectroscopy was used and the thermal stability of DNA/NP was detected by optical melting method.

Our results show that the interaction of porphyrin conjugates with DNA can be either intercalative or external binding. The dominant factor in the interaction with isolated DNA proved to be the charge distribution, with binding affinities higher for tri-cationic molecules than their di-cationic counterparts. Presence of protein capsid opposes but does not inhibit DNA-porphyrin interaction. In the case of NP, binding affinity of conjugates decreases and the mode of interaction is mostly external binding.

CD spectra recorded in 200-300 nm range revealed hypochromicity in the DNA bands and alteration of protein bands in the presence of conjugates that can be attributed to the distortion of helical structure and loosening of capsid structure, respectively. Absorption melting showed that the porphyrin binding stabilizes the helical structure, i.e. increases the strand separation temperature of DNA, but does not influence DNA - protein interaction in NP.

#### 2524-Pos Board B216

##### A Single Molecule Study of Gene Silencing by HNS Protein DNA Interactions

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Histone-like nucleoid structuring (H-NS) proteins are an important prokaryotic protein responsible for organizing and compacting chromosomal DNA. Recently, it was found that H-NS can identify foreign genes acquired by horizontal gene transfer (HGT), based on AT content, and selectively silence these genes. Single-molecule studies have shown that H-NS binding can affect DNA rigidity and sometimes loop the DNA. However, most of these studies are done with  $\lambda$  DNA, which is neither part of the bacterial genome nor a sequence commonly acquired by HGT. We have been using optical tweezers to study gene silencing by H-NS on a variety of Salmonella genes whose interaction with H-NS is well characterized in vivo. We have also been studying how the presence of various cofactors of H-NS, such as Hha, cooperatively affect these genes. These experiments greatly improve our understanding of the biophysical mechanism behind how H-NS protein selectively interacts with and silences foreign DNA.