3447-Pos
Cooperativity in Thyroid Hormone Receptor Binding to DNA Response Elements
Yi Chen, Matthew Young.
University of Michigan, Ann Arbor, MI, USA.
The human Thyroid Hormone Receptor (TR) is a member of the nuclear receptor (NR) protein family. Members of this family of proteins are responsible for propagating the signals from specific hormone ligands by binding to specific DNA response elements and regulating the transcription of target genes. Like many NRs, TR is composed of multiple domains. The N-terminal region contains two zinc-coordinating modules that can bind DNA. The C-terminal region contains a ligand binding domain that can bind thyroid hormones, co-regulator proteins, and is also able to dimerize in solution. Depending on the DNA sequence and ligation status, TR can bind different DNA response elements and function as either a heterodimer, a homodimer, or a monomer. The DNA associated dimerization capability has a major impact on the NR function as a transcription regulator. We have investigated the structural basis behind the cooperativity observed for TR binding to different DNA response elements. We have found specific interactions between the DNA binding domains are able to facilitate the cooperative binding at certain DNA response elements. The DNA mediated cooperative binding is observed even in the isolated DNA binding domains of TR and is thus not a result of the dimerization interface in the ligand binding domain.

3448-Pos
AFM Studies of DNA Loops Secured by Lambda Repressor Proteins
Haowei Wang[1], Laura Finzi[2], Dale A.E. Lewis[3], **David D. Dunlap[4]**.
[1]Emory University, Atlanta, GA, USA, [2]Emory University School, Atlanta, GA, USA, [3]NIH, Bethesda, MD, USA, [4]Emory University School of Medicine, Atlanta, GA, USA.
Large, cooperative assemblies of proteins that wrap and/or loop genomic DNA may shift configurational equilibria that determine developmental pathways. Such is the case of the lambda bacteriophage which may exhibit lytic or lysogenic growth. The lysogenic state of lambda prophages is maintained by the lambda repressor (CI), which binds to tripartite operator sites in each of the OL and OR control regions located about 2.3 kbp apart on the phage DNA and represses lytic promoters. Dodd and collaborators have suggested that an initial loop formed by interaction between CI bound at OR and OL provides the proper scaffold for additional CI binding to attenuate the PRM promoter and avoid over production of CI. The looping equilibrium as a function of CI concentration has been measured using tethered particle motion analysis, but the oligomerization of CI in looped states could not be determined. Scanning force microscopy has now been used to probe this directly. An equilibrium distribution of looped and unlooped molecules on planar poly-L-ornithine-coated mica was found to be commensurate to that for tethered molecules in solution, and the occupancies of specific operator sites for several looped and unlooped conformations were determined. Some loops appeared to be sealed by oligomers of 6-8, most by oligomers of 10-12, and a few by oligomers of 14-16.

3449-Pos
Self-Assembling Peptide Amphiphiles for DNA Binding and Nuclear Targeting
Rachel Marullo[1], Matthew Tirrell[2].
[1]University of California Santa Barbara, Santa Barbara, CA, USA, [2]University of California Berkeley, Berkeley, CA, USA.
Peptide amphiphiles can be used to create multivalent, multifunctional, self-assembled nanostructures for applications such as immunotherapy, cancer treatment, and in this case gene therapy. We have conjugated di-C16 hydrophobic tails to bZip, a thirty-eight amino acid sequence derived from the transcription factor GCN4, to promote the formation of extended micelles in solution. The peptide amphiphile headgroups adopt a high degree of alpha-helical content in the micelle corona compared to the free peptide chains as shown by circular dichroism. The hydrophobic moiety facilitates the DNA binding of bZip amphiphiles in an orthogonal arrangement similar to the native protein, in contrast to the unmodified peptide which binds via electrostatic collapse onto DNA. The peptide amphiphiles bind DNA in a cooperative fashion but do not recognize the AP 1 sequence that GCN4 binds specifically, although further modification of the monomers may enhance their biofunctionality. To implement the DNA binding peptide amphiphiles in gene therapy applications, a nuclear targeting platform is being developed by appending a hydrophobic tail to a nuclear localization signaling (NLS) peptide. The tail enhances cellular uptake of the peptide and delivery to the nucleus of HeLa cells as observed by fluorescence microscopy. Incorporating NLS peptide amphiphiles into a mixed micelle displaying DNA binding peptides or other therapeutic agents may aid in targeted delivery to the nucleus.

3450-Pos
Structure and Mechanism of Notch Transcription Complex Dimerization in Gene Regulation
Kelly L. Arnett[1], Debbie McArthur[1], Warren Pear[2], Jon Aster[1], Stephen Blacklow[1].
[1]Brigham and Women's Hospital, Boston, MA, USA, [2]University of Pennsylvania, Philidelphia, PA, USA.
Notch signaling triggers cell growth, differentiation, and death. Deficiency in Notch genes is embryonic lethal in mice, and aberrant Notch signals have been implicated in many human diseases. During activation, Notch is cleaved from the membrane and enters the nucleus where it cooperates with the DNA-binding transcription factor CSL and the transcriptional coactivator MAML, to form the Notch transciption complex (NTC). Recently, our lab observed dimerization of NTCs on a segment of DNA from the HES1 promoter region. This head-to-head pairing of CSL sites (called an SPS site for Su(H) paired site or sequence paired site) is found in the promoters of a number of well-characterized Notch targets in Drosophila, Xenopus, and in the Hes1 gene in mammals. The goal of this work is to determine the structural and energetic foundations underlying Notch mediated dimerization, and ultimately to understand how NTC dimerization influences the expression of different Notch targets. Toward this goal, the structure of a dimer of Notch transcription complex trimers on DNA has been determined to 3.5Å resolution. Each of the two NTC complexes superimposes well on an NTC bound to a single site and undergo little conformational change, but the two NTC are twisted relative to each other and the DNA untwists and bends away from the protein binding interface. Although HES1 is one of the most well characterized mammalian Notch target genes, the role of Notch dimerization in other contexts is unexplored. To begin to address this question, work has begun to identify other functional paired sites and to characterize the range of DNA duplexes able to cooperatively bind dimers of Notch Transcriptions complexes.

3451-Pos
Nucleic Acid Interaction Kinetics Modulate the Chaperone Activity of Retroviral Nucleocapsid and Gag Proteins
Fei Wang[1], Hao Wu[1], Jialin Li[1], Micah J. McCauley[1], Christopher Jones[2], Siddhartha A. Datta[3], Alan Rein[3], Robert J. Gorelick[4], Ioulia Rouzina[5], Karin Musier-Forsyth[2], **Mark C. Williams[1]**.
[1]Northeastern University, Boston, MA, USA, [2]The Ohio State University, Columbus, OH, USA, [3]NCI-Frederick, Frederick, MD, USA, [4]SAIC-Frederick, Inc., NCI-Frederick, Frederick, MD, USA, [5]University of Minnesota, Minneapolis, MN, USA.
Retroviral nucleocapsid (NC) proteins are essential for several viral replication processes including specific genomic RNA packaging and reverse transcription. The nucleic acid chaperone activity of NC facilitates the latter process. In this study, we use single molecule biophysical methods to quantify the DNA interactions of wild type and mutant human immunodeficiency virus type 1 (HIV-1) NC and Gag and human T-cell leukemia virus type 1 (HTLV-1) NC. We find that the nucleic acid interaction properties of these proteins differ significantly, with HIV-1 NC showing rapid protein binding kinetics, significant duplex destabilization, and strong DNA aggregation, all properties that are believed to be critical components of nucleic acid chaperone activity. In contrast, HTLV-1 NC exhibits significant destabilization activity but extremely slow DNA interaction kinetics and poor aggregating capability, which explains why HTLV-1 NC is a poor nucleic acid chaperone. To understand these results, we developed a new single molecule method for quantifying protein dissociation kinetics, and applied this method to probe the DNA interactions of wild type and mutant HIV-1 and HTLV-1 NC. We find that mutations to aromatic and charged residues strongly alter the proteins'nucleic acid interaction kinetics. Finally, in contrast to HIV-1 NC, HIV-1 Gag, the nucleic acid packaging protein that contains NC as a domain, exhibits relatively slow binding kinetics, which may negatively impact its ability to act as a nucleic acid chaperone. *This work was funded in part by Federal Funds from NCI, NIH under contract N01-CO-12400 (RJG).*

3452-Pos
Mechanisms of Force and Velocity Control in a Viral DNA Packaging Motor
James M. Tsay.
UCSD, San Diego, CA, USA.
dsDNA phages and viruses employ DNA packaging motors to translocate their genomes into small viral capsids against enormous internal pressures. Structural data, models, and sequence alignments have revealed the homology of critical putative functional domains of various nucleic acid translocases, including viral packaging motors, RNA helicases, and chromosome transporters. We used optical tweezers and mutational analysis to explore which functional