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

domains of viral packaging motors govern their force generation and determine the velocities of packaging. A Q motif mutant of the phage λ DNA packaging motor, Y46F, was shown to have a decreased velocity (~40% less than WT), increased slipping (~10X WT), and steeper force-velocity dependence (~6X WT), showing that the Q motif governs the force generation in translocation and DNA-motor interactions. In addition, we show that mutants with residue changes located in a previously undetermined domain of the motor, T194M and G212S, package dsDNA into viral capsids at ~8X and ~ 3X slower velocities than wild type (WT), respectively. Meanwhile a T194M pseudo-revertant (T194V) showed a near restoration of the WT velocity. The single molecule measurements of motor mutant translocation dynamics, genetic screening experiments, and structural modeling of ring ATPase dsDNA translocases suggest the location of a ''velocity controller'' domain within the phage λ packaging motor downstream the putative Walker B motif, which might be generalizable to other ring ATPase nucleic acid translocases. Importantly, this evidence may aid in explaining the different packaging rates of various dsDNA phages.

3453-Pos

Counting RAD51 Proteins Disassembling from Nucleoprotein Filaments Under Tension

Joost van Mameren[1], Mauro Modesti[2], Ronald Kanaar[3], Wyman Clair[3], Erwin J.G. Peterman[1], **Gijs J.L. Wuite[1]**.
[1]VU University Amsterdam, Amsterdam, Netherlands, [2]lUniversité d'Aix-Marseille, Marseille, France, [3]Erasmus MC, Rotterdam, Netherlands.

The central catalyst in eukaryotic ATP-dependent homologous recombination consists of RAD51 proteins, polymerized around single-stranded DNA. This nucleoprotein filament recognizes a homologous duplex DNA segment and invades it. After strand exchange, the nucleoprotein filament should disassemble in order for the recombination process to complete. The molecular mechanism of RAD51 filament disassembly is poorly understood. Here, we have combined optical tweezers with single-molecule fluorescence microscopy and microfluidics to reveal that disassembly results from the interplay between ATP hydrolysis and release of the tension stored in the nucleoprotein filament [1]. Applying external tension to the DNA, we found that disassembly slows down and can even be stalled. We quantified the fluorescence of RAD51 patches and found that disassembly occurs in bursts interspersed by long pauses. Upon relaxation of a stalled complex, pauses were suppressed resulting in a large burst. These results imply that tension-dependent disassembly takes place only from filament ends, after tension-independent ATP hydrolysis. This integrative single-molecule approach allowed us to dissect the mechanism of this key homologous recombination reaction step, which in turn clarifies how disassembly can be influenced by accessory proteins.

[1] van Mameren et al., Nature, 457, 745 - 748 (2009)

Membrane Dynamics & Bilayer Probes

3454-Pos

Revealing the Microstructural Changes in Tissues In-Situ with Positron Annihilation Spectroscopy

Petri Sane[1], Filip Tuomisto[1], Ilpo Vattulainen[1,2], Juha Holopainen[3].
[1]Aalto University School of Science and Technology, Department of Applied Physics, Helsinki, Finland, [2]Tampere University of Technology, Department of Physics, Tampere, Finland, [3]Department of Ophthalmology, University of Helsinki, Helsinki, Finland.

In this work we present a novel and promising tool for characterizing the microstructural changes in biomaterials, namely mammalian lens. Positron annihilation lifetime spectroscopy (PALS) is a widely used tool to study atomic scale defects in semiconductors and routinely used to study the voids in polymer materials [1]. Through the increased understanding of the biomolecular materials, results from PALS experiments can now be compared with simulations and further analysis of the results is possible. Recently we have showed that PALS can be applied to study and characterize free volume changes in lipid bilayers and the results are in full agreement with MD simulations [2]. In biomolecular material, a thermalized positron forms a meta-stable bound state, Positronium (Ps), with an electron from the material. This o-Ps-atom can be used as a probe, due to the Ps lifetime in the material being strongly affected by the free volume characteristics of the probed material.

Here we present results obtained by studying the temperature dependent changes in free volume parameters in mammalian lenses, lipids separated from the lenses and controlled Spingomyelin-cholesterol mixtures [3]. All the measurements provide strong evidence towards a minor structural reorganization near 35°C, far below strict phase transition temperatures. This change indicates a transition to liquid order phase and is not visible with conventional experimental methods used in the study, probably due to the microstructural scope of the change which does not provide strong enough signal for e.g. DSC.

[1] O. E. Mogensen, *Positron annihilation in Chemistry* (Springer-Verlag, Heidelberg, 1995)
[2] P. Sane et al, J. Phys. Chem. B: **113**:1810-12 (2009)
[3] P.Sane et al, ''*Temperature Induced Structural Transition in-situ in Porcine Lens - Changes Observed in Void Size Distribution",* submitted to BioPhys. J. on 7.9.2009

3455-Pos

The Use of Time-Resolved Fluorescence Anisotropy to Reveal Domain Structures in Model Membrane Vesicles: Prospects for Applications to Cell Membranes

Christopher D. Stubbs, Stanley B. Botchway.
Rutherford Appleton Laboratory, Didcot, United Kingdom.

Cell membranes are thought to consist of structurally distinct regions within which reside functionally-interacting cell membrane components. Although much research has been directed toward the detection of these regions, variously known as domains or rafts, they still remain elusive entities. A number of techniques have used probes that tend to reside in these regions and using this approach structures have been identified using florescence spectroscopic approaches. Here we have investigated the motion of the probes rather that their presence to identify the presence of domain regions using giant unilamellar vesicles (GUV) to model cell membranes. Using the electroformation method, GUV were made using 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine, sphingomyelin and cholesterol in various proportions. The fluorescent probes, diphenyhexatriene (DPH), 1-acyl-2-{6-[(7-nitro-2-1,3-benzoxadiazol-4-yl) amino]hexanoyl}-sn-glycero-3-phosphocholine (C6-NBD-PC), 1,2-dipalmitoyl-sn-glycero-3-phosphoethanolamine-N-(7-nitro-2-1,3-benzoxadiazol-4-yl) (NBD-PE) were pre-incorporated in the GUV. The time-resolved anisotropy of the probes was determined using 2-photon excitation from a Ti-sapphire laser (180 fs pulses at 75 MHz and 750 nm) coupled to a microscope. Excitation was performed with an incident vertically polarized light and a Y-output acquisition, generating simultaneous vertical and horizontal detection, using a multichannel photon multiplier tube. The output signal was fed through a Becker and Hickl SPC-830 module and anisotropy analysis software. The set up allows for picosecond time resolution as well as diffraction limited image resolution needed for the domain identification. It was found that each probe had two distinct motions corresponding to different orientations and locations in single-component vesicles, as known for DPH, but not previously observed for the NBD probes. Using polarization direction selectivity these two locations could be separately examined using the current development. The data shows that NBD selectively locates into domain regions in three-component vesicle systems.

3456-Pos

Protein-Induced Shape Changes in Phase-Separated Vesicles

Jaime B. Hutchison, Aruni Karunanayake, Bob Weis, Tony Dinsmore.
University of Massachusetts Amherst, Amherst, MA, USA.

We explore changes in curvature in vesicles composed of a mixture of phospholipids and cholesterol upon the binding of protein to the membrane. Experiments were done with model peptides and with members of the BAR family of proteins. Vesicles were prepared using electroformation with a mix of phosphatidylcholine, cholesterol, and sphingomyelin. As a sample is lowered through temperature Tmix, a homogeneous vesicle phase separates into two fluid phases with distinct compositions. A line tension at the boundary between the phases deforms the membrane, producing buds with a shape determined by a balance of membrane and line tension. Green fluorescent protein (GFP) with a 6X histidine tag was added to the solution, where it bound to Ni-chelating phospholipids present in the membrane at concentrations ranging from 0.1 to 5 mol%. Domain budding was recorded before and after GFP addition using both differential interference and fluorescence microscopy. Analysis of the shape of the vesicle before and after addition of GFP allows for the determination of the change in line tension owing to the GFP binding. Similar results were obtained with vesicles introduced to poly-L-histidine. A possible mechanism for the change in shape seen with both GFP and poly-L-histidine binding is formation of protein-Ni-chelating lipid complexes that sit at the boundary between phases and lower the line tension. Preliminary investigations into shape changes caused by BAR domains of Drosophila amphiphysin revealed fine vesicle tubulation that initiated at the boundary between the two lipid phases. The addition of both model proteins (GFP and poly-L-histidine) and biologically relevant protein (BAR domains) into our system allows for a broader understanding of the effect of protein, which are ubiquitous in cell membranes, on phase separation and budding.

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