**373-Pos Board B159**
**The Longitudinal Correlation Length Controls the Range of Interactions Between DNA-Bending Proteins**
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In statistical mechanics, the correlation length measures the distance over which the microscopic variables at different positions are correlated. When we stretch a DNA polymer by using uniform tension, we find that the zero-force correlation length (the persistence length $A$) along the DNA polymer will bifurcate into two different correlation lengths – the shorter longitudinal correlation length $\xi_{\|}(f)$ and the longer transverse correlation length $\xi_{\perp}(f)$. In the high-force limit, $\xi_{\|}(f) = \xi_{\perp}(f)/2 = \sqrt{k_B TA/f}/2$. We also find that when the DNA-bending proteins bind to the DNA molecule, both of the two correlation lengths are suppressed slightly with the increase of the concentration of the proteins. Furthermore, the interaction range between DNA-bending proteins that bind to DNA is controlled by the shorter longitudinal correlation length $\xi_{\|}(f)$. The origin of this effect is that the part of the energy describing binding of the proteins has the same rotation symmetry as the $z$-direction tangent component.

**374-Pos Board B160**
**ZBP1 and Ribosome Association with β Actin mRNA Measured by Fluorescence Fluctuation Spectroscopy**
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Intracellular mRNA localization plays an important role in post transcriptional gene regulation by targeting protein synthesis to a specific cellular compartment. mRNA localization has been extensively investigated with fluorescence in situ hybridization and live cell imaging in various model organisms. However, quantitative measurement of the translation of localizing mRNA with high spatiotemporal precision remains challenging. β-actin mRNA has been shown to localize to the leading edge of primary fibroblasts and provides an excellent model system to address this problem. The localization involves a cis-acting zipcode sequence and a trans-acting zipcode binding protein (ZBP1). Here we employ fluorescence fluctuation spectroscopy (FFS) to investigate the localization of β-actin mRNA and its association with ribosome subunits in live cell. Two technical advances make this experiment possible. First, we have developed a mouse model in which the β-actin gene has 24XMS2 sequences inserted. Furthermore, this mouse has been crossed with a ZBP1 knock out mouse. We generate primary mouse embryonic fibroblasts from these mice. By expressing GFP-MS2 capsid protein in the cell, all endogenous β-actin mRNA are fluorescently labeled. In addition, mCherry labeled ZBP1 or ribosome subunit are introduced into the cells to visualize their interaction with the β-actin mRNA by FFS. Second, we have developed a dual-color brightness analysis tool, specifically hetero-species partition analysis, to measure the interaction between the GFP-MS2 labeled mRNA and another mCherry labeled factor. We systematically measured the interaction between the β-actin mRNA and the ZBP1/ribosome in the perinuclear region and leading edge. Our data shows that β-actin mRNA binds more ZBP1 but less ribosome in the perinuclear region than in the leading edge, which supports the model that ZBP1 represses β-actin mRNA association with ribosome in the nuclear periphery until it reaches the leading edge.

**375-Pos Board B161**
**Sequence-Specific Control of ETS Transcription Factors**
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The ETS family of transcription factors participate in a broad array of developmental and disease processes. ETS proteins are united by a conserved DNA-binding domain that targets 10-bp sequences harboring a central 5'-GGAA/T-3' consensus. A primary recognition helix confers specificity for the consensus by direct readout of the major groove while adjoining mobile elements contact backbone contacts of flanking 5' and 3' sequences. Although flanking contacts play a secondary role in specifying the ETS binding site, they profoundly influence its affinity and transactivational activity. Elucidating the biophysical basis for the intrinsic selectivity of the ETS domain is therefore essential to understanding other interactions within the transcription regulatory complex. We have characterized the structure and thermodynamics of the ETS complexes with a high-, low-affinity, and nonspecific sequence. Calorimetric measurements reveal that the unbound ETS domain is a noncovalent, entropically-driven dimer. DNA binding induces ETS dissociation with sequence-dependent thermodynamics, suggesting distinct conformational arrangements accompanying the binding of each sequence variant. Footprinting titrations show that hypersensitivity to DNase I at the consensus is an experimental hallmark of sequence-specific ETS binding, regardless of affinity, indicating the importance of DNA distortion triggered by indirect readout. High- and low-affinity ETS cognates can be readily differentiated in terms of sensitivity to methylation in the latter. Based on this data, we present a model for ETS-DNA interactions in which indirect and direct readout are structurally linked to achieve sequence-specific binding. Complex formation in turn induces rheostatic rearrangements of ETS conformation (as manifest in terms of complex stability) that represent specific permissive or inhibitory states for protein-protein interactions (as manifest in ETS dimerization). Thus, the sequence identity of an ETS binding site may serve a functional role in initiating regulatory functions as well as an address for localization in the genome.

**376-Pos Board B162**
**Engineering Zinc Finger Proteins using Accessory Binding Modules**
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We report on the engineering of zinc finger proteins (ZFPs) as synthetic transcription factors by appending accessory modules to enhance protein binding affinity to target DNA. Over the last few years, transcription factor engineering has provided a valuable approach to genetically engineer a wide variety of cell types. ZFP technology is now being used to directly edit chromosomal DNA by creating synthetic nucleases. Recently, this approach has been used to produce genetically enhanced crops and improved biophama products and to cure genetic disease. The central feature of this technology is generation of synthetic transcription factors that bind to "target" DNA sequences with high specificity. In this way, modular assembly provides a convenient approach to synthesize target-specific ZFPs by generating modular proteins consisting of tandem repeats of well-characterized zinc fingers through standard molecular cloning. Oligomerized pool engineering (OPEN) and context-dependent assembly (CoDA) are alternative, albeit more complex protocols for creating ZFPs.
In this work, we describe the direct engineering of two- and three-finger ZFPs to improve DNA binding site recognition specificity and affinity. We generated chimeric ZFPs containing the accessory module PAR from the yeast transcriptional activator ADR1, a zinc finger transcription factor from Saccharomyces cerevisiae. We observed that addition of the PAR binding module increased the binding affinity for chimeric ZFPs to target DNA sequences without affecting the selectivity for two-finger ZFPs. Two-finger ZFPs are generally not used for targeted DNA binding, because the functionality of ZFPs containing only two modules results in a loss in binding affinity and selectivity. Here, we show that ZFPs consisting of only two-finger modules can be successfully engineered to target specific DNA sequences by appending accessory binding modules. Based on this work, we anticipate that these chimeric ZFPs will enable new applications involving genetic modification.

**377-Pos Board B163**
**Agonist AMD Antagonist Activity in a GFP Yeast Based Estrogen Receptor Functional Assay**
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Estrogen receptors (ERα and ER β) are ligand-binding transcription factors activated by the hormone 17-β estradiol. Ligand binding triggers ER dimerization, translocation of the receptor from the cytoplasm into the nucleus and eventually activation of the genes under control of ER. Studies have revealed a role for estrogen receptors in male and female sexual development, reproductive functions, bone metabolism and regulation of neuroendocrine and cardiovascular systems. ER is also known to bind to other non-native ligands known in pharmacology as receptor agonists or antagonists. Agonists provoke a biological response when bound to the receptor; antagonists inhibit a biological response when bound. Our lab is interested in the promiscuous binding of the estrogen receptor and its ability to activate different genes in different tissues. Fluorescence Resonance Energy Transfer (FRET) assays have previously been performed using ER to study ligand binding affinities for the receptor. However, this technique is unable to determine whether these ligands are agonists or antagonists and allow ER dimerization and gene activation. To investigate these phenomena, an activity assay that measures ER controlled gene expression has been developed which provides the opportunity to gain further insight into the functional activity in living systems. Recombinant yeast cells that express ERα use the green fluorescent protein (GFP) reporter to determine whether ER α, in the presence of a particular ligand, has activated gene of expression. We have correlated the binding to agonist and antagonist behavior of several xeno and phyto estrgens.