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Erratum: Characterizing the biomolecular interactions between insulin and G-QuadruplexDNA (Biophysical Journal (2013) 104:2 (419a))

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2150-Pos Board B169
Analyzing the Effect of Phosphorylation on the Translation Regulator Function of the Fragile X Mental Retardation Protein
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Fragile X Syndrome (FXS) is the most common form of inherited mental retardation affecting approximately 1 in 4,000 males and 1 in 8,000 females. FXS is linked to the expansion of cytosine-guanine-guanine trinucleotide repeats in the fragile X mental retardation 1 (fmr1) gene. This expansion causes hypermethylation of the cytosines, transcriptional silencing of fmr1, and loss of the fragile X mental retardation protein (FMRP). Normally, FMRP regulates the translation of a class of mRNAs, which adopt the G quadruplex structure, at neuronal dendrites. One mechanism by which the protein might perform its translation regulator function is the reversible phosphorylation of FMRP. In human FMRP, the highly conserved Serine 500 is the major phosphorylation site. This site is directly N-terminal of the FMRP arginine-glycine-glycine box, which specifically binds to the mRNA G quadruplex structure. In this study, we utilized different biochemical and biophysical methods to analyze the translation regulator function of both phosphorylated and unphosphorylated FMRP on the G quadruplex forming Microtubule Associated Protein 1B mRNA.

2151-Pos Board B170
Characterizing the Biomolecular Interactions between Insulin and G-Quadruplex DNA
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The formation of guanine (G)-quadruplex structures in the guanine-rich tandem repeats of the insulin-linked polymorphic region (ILPR) is linked to transcriptional effects on the insulin gene. Recent studies demonstrate that the ILPR G-quadruplexes can bind to insulin while the energetics of this interaction with the most common ILPR repeat sequences have been characterized. Studies have also measured the transcriptional activity of less common ILPR repeats. Transcriptional activity for these repeats is significantly lower than that of the consensus sequence but can be increased substantially by varying only one or two nucleotides. To determine the potential role of G-quadruplex formation and stability in regulating transcription, we have studied the second and third most common ILPR repeats as well as their variant sequences that exhibit increased transcriptional activity. Circular dichroism (CD) spectroscopy, differential scanning calorimetry (DSC), and isothermal titration calorimetry (ITC) have been used to characterize the binding interaction between insulin and each of the four ILPR repeat sequences. The bulk thermodynamic measurements performed at various temperatures from 20 - 37 degrees Celsius provide insight into these biomolecular interactions.

2152-Pos Board B171
Activated GTPase Movement on SRP RNA Drives Cotranslational Protein Targeting
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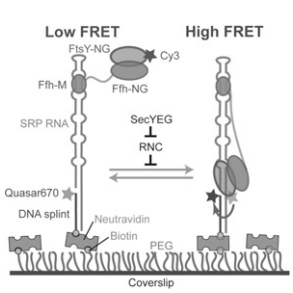
Signal Recognition Particle (SRP) and its receptor (SR) are co-translational protein targeting machineries responsible for delivering ribosome-nascent chain-complexes (RNCs) from cytosol to cellular membranes. Loaded with its cargo (RNC), SRP forms a complex with SR and bring the RNC to the membrane. Extensive rearrangements in this complex activate GTP hydrolysis and unload the RNC onto the translocon. During this process, the SRP RNA plays an essential role in accelerating both SRP-SR complex formation and GTP hydrolysis step. Here we show that the SRP RNA is a bi-functional molecule with its two ends, the tetraloop end and the distal end, stimulating different stages of SRP-SR interaction. using single molecule techniques to direct visualize the global relocalization along the SRP RNA, we demonstrate that the SRP-SR GTPase complex travels over 100A in the targeting reaction, from the RNA's tetraloop end during initial complex assembly to the distal end during GTPase activation. Moreover, this rearrangement is tightly regulated by the RNC and the translocon. The large-scale movement of the GTPase complex provides an attractive mechanism for coupling GTPase activation to the transfer of RNC from SRP to translocon, thereby ensuring productive protein targeting.



2153-Pos Board B172
Bioinformatic Analysis of Protein/DNA Interactions
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To analyze the geometry of the protein/DNA interface we built a database of more than 50 thousand protein/DNA contacts calculated from more than thousand crystal structures of their complexes. Various features of the interface may be analyzed by sorting the contacts by a wide range of criteria from the identity of the interacting atoms and residues to protein Pfam classification.
Novelty of the analysis lies in our fine-grained categorization of protein and DNA local conformations. Protein structures were sorted into sixteen pentapeptide units called ''peptide blocks'' [de Brevern et al. Proteins 41, 271 (2000)] and DNA into 14 dinucleotide conformers (plus three Z-DNA forms that were not analyzed) derived from the original dictionary of conformers [Svozil et al. NAR 36, 3690 (2008)]. We determined how distributions of peptide blocks and dinucleotide conformers differ at and outside the protein/DNA interface and discussed variability of the distributions between various groups of structures (DNA complexes of enzymes, transcription factors, structural proteins) overall and in the DNA minor and major grooves and phosphates. We examined how different are occurrences and logodds of peptide blocks and dinucleotide conformers for direct polar contacts (mostly hydrogen bonds) and water-mediated contacts and observed e.g. a unique behavior of the water-mediated contacts in the DNA complexes of transcription complexes. Analysis of temperature displacement factors (''B-factors'') of the analyzed complexes showed the fundamental difference between behavior of proteins and DNA at the interface.
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2154-Pos Board B173
Real-Time Single-Molecule Tethered Particle Motion Experiments Reveal the Requirements for Catalysis in the Tyrosine-Family Recombinase Active Site
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Flp recombinase, a member of tyrosine family site-specific recombinases, is important to maintain the copy number in *Saccharomyces cerevisiae* via the DNA rearrangements, including insertions, deletions and inversions. It has been known that its catalytic activity is regulated by the complicated conformation change in the the protein-protein contact. Even though detailed structural analysis and kinetic measurements have been done, more evidences indicated there is significant difference among tyrosine family recombinases, Cre and Flp. Here, the reaction process catalyzed by Flpe, a variant of Flp known to be more active at 37 °C than wild type, was investigated by single-molecule tethered particle motion (TPM) from start to the end. There are early formation of abortive complexes and the presence of wayward complexes in the Flpe-FRT system, similar to the Δ Int and Cre recombinases. Moreover, the presence of a Holliday junction intermediate served as a rate-limited barrier was found. Different from the Cre recombinase, the kinetic analyses on the synapse state duration indicated the irreversibility in the strand cleavage/ligation process. The new information offered here demonstrates that single-molecule approaches can be the potential methodologies to explore reaction mechanisms in different perspectives.

2155-Pos Board B174
Architectural Role of HMO1 in Bending, Bridging and Compacting DNA
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HMO1 proteins are abundant *Saccharomyces cerevisiae* (yeast) High Mobility Group Box (HMGB) proteins. HMGB proteins are nuclear proteins which are known to have an architectural function. HMO1 possesses two HMGB box domains. It has been reported that double box HMGB proteins induce strong bends upon binding to DNA. It is also believed that they play an essential role in reorganizing chromatin and therefore are likely to be involved in gene activation. To characterize DNA binding we combine single molecule stretching experiments and AFM imaging of HMO1 proteins bound to DNA. By stretching DNA bound to HMO1, we determine the dissociation constant and measure protein induced average DNA bending angles. To