Tracking double-stranded DNA extension with nanometer precision allows a direct observation of plectonemic coils formed during positive or negative supercoiling. Here, we use magnetic tweezers to set defined states of negative superhelicity with externally applied force. We can thus control the onset and behavior of DNA double strand separation (melting) and then vary parameters surrounding the formation of DNA melting bubbles. We find a strong SSB interaction with transient DNA bubbles even at low forces and superhelicities. SSB DNA bubble interaction is strongly force-dependent and additional superhelicity can displace SSB.

2014-Pos Board B151
Investigation of the Role Played by the RNA G-Quadruplex Structure in ALS/FTD Pathology
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Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disorder resulting in motor neuron loss in brain and spinal cord. Frontotemporal dementia (FTD) is one of the most common forms of young onset dementia and second most common form of dementia overall, after Alzheimer's, resulting in degeneration of temporal lobes along with personality changes and language impairment. ALS and FTD are now recognized as members of a broad continuum of neurodegenerative disorders, linked by similar pathology, mechanisms, and overlapping clinical symptoms. Two RNA-binding proteins of interest that link the two diseases are TAR DNA-binding protein 43 (TDP-43) and the fused in sarcoma/translocated in liposarcoma protein (FUS), which are the major protein components in over 90% of ALS and over 50% of FTD inclusions. We hypothesize that the G-quadruplex RNA structure might play an essential role in the pathogenic mechanisms of FUS in ALS and FTD. In this study, the G-quadruplex RNA binding properties of the wild type and C-terminal NLS mutant FUS protein implicated in ALS/FTD will be analyzed.

2015-Pos Board B152
Characterization of AIM2 DNA-Binding Properties and Filament Formation
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AIM2-Like Receptors (ALRs) are a class of innate immune receptors for foreign DNA, members of which exist in both the cytosol and nucleus. The eponymous member of this family, AIM2, is a cytosolic DNA sensor composed of an N-terminal pyrin domain (PYD) and a C-terminal HIN-200 domain (HIN). Previous studies of this protein has been limited to an N-terminal tag-protected version of the full-length or a truncated version of the protein. From these studies it has been concluded that AIM2 exists in a resting, autoinhibited form in which the PYD is bound to the HIN. Upon DNA binding, the PYD is released and forms a larger complex with its downstream partners to initiate the inflammation process. Recently, we have been successful in purifying the native (tag-less) full length protein. Using a combination of fluorescence, electron microscopy, and gel-shift assays, coupled with mutagenesis studies, we have characterized the DNA-binding properties of AIM2 and its ability to form filaments in solution.

2016-Pos Board B153
Characterization of IHF Binding to DNA Four-Way Junctions and Forks
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Integration Host Factor (IHF) is an architectural protein that binds and bends DNA, facilitating the formation of protein-DNA complexes important for gene regulation. IHF binds with high affinity to a specific consensus sequence and induces a 160° bend upon binding. We have shown that IHF binds DNA four way junctions (4WJ) that do not contain the consensus sequence with nanomolar affinity and 1:1 stoichiometry for the specific interaction. We have also observed that IHF binds DNA forks with nanomolar affinity. The binding to junctions and forks is in direct contrast to IHF binding to linear duplex DNA, which is typically 1000-fold weaker. In this study we investigate whether the presence of the IHF consensus sequence influences IHF binding to DNA junctions and forks. We utilized gel shift and fluorescence binding assays to measure affinity and have observed that the high affinity for these non-native structures is independent of the presence of the consensus sequence. We are further exploring how IHF binding influences junction conformation. Junction conformation is modulated by ion concentration where high concentrations of ions induces pairwise stacking of the helical arms resulting in quasi-continuous helices. In our investigations IHF binding to the non-consensus junction induces an open conformation. We are specifically examining the distortion of the junction and DNA fork substrates upon IHF binding using Förster Resonance Energy Transfer. Through these measurements, we are also exploring whether the mechanism of recognition differs between junctions and forks.

2017-Pos Board B154
Dynamics of Glyceraldehyde-3-Phosphate Dehydrogenase Interfacial Regions Affect Binding to AU-Rich RNA
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The homotetrameric protein glyceraldehyde-3-phosphate dehydrogenase (GAPDH) has been shown to possess many functions aside from its role in glycolysis. Of particular interest is its role in post-transcriptional regulation. Despite lacking a canonical RNA binding motif, GAPDH has been shown to bind to many mRNAs and subsequently alter their translation. Most of these protein-nucleic acid interactions have been shown to occur by GAPDH binding to Adenine-Uridine Rich Elements (AREs) within the 3' untranslated regions (UTRs) of specific mRNAs. While much evidence has been gathered in determining the means of RNA binding, the exact site and mechanism of binding still remain elusive. Variables that may be key to elucidating these two facets of RNA binding include the effects of posttranslational modifications, oligomerization, cofactor binding, and structural dynamics, of GAPDH. Herein, it is demonstrated for the first time that GAPDH binds to the core AREs of the tumor necrosis factor-α mRNA 3′UTR via a sequential two-step mechanism. As well, a single point mutation at the GAPDH dimer interface results in a reduction in binding affinity in the second step and an alteration in the bound RNA structure. In contrast to previous studies, it is shown here that this mutation does not affect protein oligomerization, but induces dynamic changes in protein regions localized along the P axis of the GAPDH tetramer. Based on our results, we propose a novel model for GAPDH binding to ARE-containing RNA that may be regulated by GAPDH post-translational modifications.

Membrane Physical Chemistry II

2018-Pos Board B155
Calcium Effect on Directed Lipid Flow in Membrane: Improving Knowledge about Directed Cell Processes in Biological Cells
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Observing the active role of lipids in response to chemical cues in artificial cell membranes could increase our understanding of directed cell transport phenomena in biological cells. Directed cell migration is essential in many biological processes including embryogenesis, wound healing, chronic inflammatory diseases, as well as cancer metastasis. Using biomimetic cell model systems makes it possible to use a minimal set of components for understanding directed cell movement and in-cell transport phenomena in regard to lipid sorting, formation of tubular protrusions and lipid movement. At present, we study directed lipid transport in artificial membranes by local biochemical gradient, calcium. We demonstrate that membrane tubulation and the flow of lipids in the membrane can be triggered and controlled by the chemical gradient applied along the lipid bilayer. This sheds light on interplay between membrane properties and chemical stimulation.

2019-Pos Board B156
Direct Measurement of Dipole Electric Field in Model Membranes using Vibrtaional Shifts of P-Cyanophenylalanine and Coupled with Molecular Dynamics Simulations
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The composition of biological lipid bilayer membrane creates a complex structural and electrostatic environment that regulates important membrane functions. The alignment of molecular dipole moments from lipid head groups and water molecules located at membrane-water interface creates the dipole potential (V_d). The dipole field (F_d) generated from this potential is the largest and about 1-10 MV/cm in magnitude, determined by indirect measurement techniques. It is located entirely within the membrane interior, and therefore, direct