processes work, but it will also open new venues in biomimetic applications. We focus on a biomimetic approach by which we learn the rules of protein sliding on DNA to be implemented in designing nanoparticles with the ability to slide on DNA as a means to perform directional transport for cellular delivery. Our models for proteins are poly(amido)amine (PAMAM) dendrimers, highly charged functional nanoparticles with significant promise as "artificial proteins" for targeted delivery of drugs and genetic material into cells. These dendrimers have similar composition to known DNA-binding protein, and their size, shape, and surface properties can be easily controlled synthetically. Our preliminary simulations with third generation (G3) PAMAM dendrimers suggest the possibility of following a helical path along the DNA phosphate backbone provided that the dendrimer charge distribution is optimized. In this respect, dendrimers can imitate the search mechanism of some sliding proteins that maintain a constant contact with the DNA backbone during their nonspecific target search mode such as Msh2-Msh6, T7 RNAP and hOgg1.

1312-Pos Board B204
Crystal Structure of a MepR-DNA Complex Reveals the Mechanism of Transcription Repression of S. Aureus Multidrug Efflux Pump mepA
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MepR is a multidrug binding transcription regulator, which represses the transcription of the S. aureus multidrug efflux pump gene mepA as well as its own gene. Repression is relieved by MepR binding to cationic lipopolipheic “drugs”, which are typical substrates of MepA. Dimeric MepR binds to inverted repeats of conserved pentad sequence GTAGG using winged helix-turn-helix (wHTH) motif. The mepA operator contains one inverted repeat, whilst the mepA operator has two MepR binding sites. Here, we report the crystal structure of MepR bound to an oligonucleotide containing a single MepR binding site as well as the structures of clinically relevant, multidrug-resistant MepR mutants, F27L and A103V.

MepR interacts with consecutive DNA major grooves via van der Waals interactions between residue P62 and the TTA bases of GTTAG motif. Residue R87 of each “wing” interacts specifically with the minor groove by making a hydrogen bond to O2 atom of T4 base and is buttressed by a hydrogen bond network involving D85 and R88. In addition, a multitude of non-specific electrostatic interactions are made between the protein and DNA. No significant binding of DNA occurs upon MepR binding. Modelling of MepR-mepA operator complex suggests no significant interactions between the two MepR dimers. Structural alignment of apoMepR and the MepR-DNA complex demonstrates that significant rotation of the DNA-binding domain is required for specific DNA binding. Electrophoretic mobility shift assays reveal that mutations A103V and F27L affect MepR repressor function by markedly diminishing its DNA-binding activity. These mutations are located at the link between DNA-binding and dimerization domains and, likely, affect the ability of the wHTH motif to adopt the orientation necessary for specific DNA binding.

1313-Pos Board B205
Characterization of the Assembly and Function of E. Coli RNA Polymerase-Promoter DNA Open Complexes in Transcription Initiation
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Transcription of RNA is a highly regulated process, as it is a central component of cellular gene expression. Differences in biochemical rate constants and ligand affinities (promoter DNA, NTP, protein regulators) during isomerization steps of RNA polymerase (RNAP) prior to the transition from initiation to elongation are key regulators of gene expression and overall cellular activity. In order to develop predictive models for regulation of transcription initiation, quantitative characterization of the isomerization steps of open complex formation after recruitment of RNAP to promoter DNA are required. However, the transient nature (1 ms - 1s) of these intermediates has historically precluded characterization of the biologically relevant closed and open intermediates. We have developed methods to obtain near homogenous populations of two of these transient intermediates using high concentrations of RNAP and sulfate/salt upshifts that allows for the detection of differences in their structures and biochemical activity. We now propose to use bulk real-time fluorescence measurements to monitor DNA bending and to characterize the kinetics of DNA opening, to define the large-scale conformational changes that convert the initial closed complex to the open complex and to investigate the functional roles of the different open intermediates. This structural information will be used to gain insight into the type and extent of conformation changes in the RNAP machinery and to make testable predictions about the function and regulation of initiation intermediates.

1314-Pos Board B206
Dissolution of DNA and F-Actin Bundles Stabilized by Polyelectrolyte Effects
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When intracellular anionic polyelectrolyte filaments are released into the extracellular space due to cell damage, they are exposed to polyanential cations such as antimicrobial peptides and other multations present in extracellular fluids. As a result, these filaments form large bundles stabilized by electrostatic interactions that cause a variety of pathologic states, including an abnormal increase in viscosity and elastic moduli in airway fluids and sputum from patients with respiratory disease.

DNase has long been used as a therapeutic treatment to fluidize sputum in cystic fibrosis patients, but its effectiveness in some cases is limited by the presence of other polyelectrolytes such as F-actin and the inability to access large dense DNA bundles before the enzyme is inactivated by proteases. Polyelectrolyte theories suggest that DNA and F-actin bundles can also be destabilized by addition of small soluble co-ions such as oligo-aspartate that potentially could act additively or synergistically to promote the depolymerizing effects of DNase on DNA bundles or gelsolin on F-actin bundles. We have analyzed approximately 100 sputum samples from cystic fibrosis patients to document their DNA and F-actin content, their elastic and viscous parameters and their susceptibility to fluidization by DNase, gelsolin, and oligo-aspartate. We show a significant benefit of adding oligo-aspartate on the ability of DNase-1 to reduce the abnormally high shear modulus of CF sputum. The ability of oligo-aspartate and DNase-1 to reduce sputum stiffness strongly correlates with the amount of DNA present in the sputum and is further modulated by the co-mingling of DNA and F-actin. These studies suggest that the principles of polyelectrolyte theory are relevant to the formation of filament bundles in a complex biological fluid and that they can guide production of more effective methods to disrupt the formation of abnormal biopolymer assemblies.

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Progress towards Chemical Cross-Linking of Sequence-Specific Proteins to DNA Templates with Single-Molecule Sensitivity
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Detecting the location of sequence-specific proteins along genetic DNA holds great promise for biomedical applications. For a number of single-molecule techniques, however, the relatively fast detachment rate (k detached) of bound proteins is a limiting factor. In this study, we describe efforts to cross-link a sequence-specific protein covalently to its DNA template in order to form a stable construct for further analysis. As a model system, we study the common restriction enzyme EcoRI under non-cutting conditions. Fragments of DNA containing the recognition sequence are measured in the presence and absence of the protein under varying concentrations of chemical crosslinkers. We monitor ensemble binding stability using electrophoretic mobility shift assays (EMSA) and at the single-molecule level with atomic force microscopy (AFM).