mass transfer and other issues that have limited the use of SPR in protein-DNA interactions under physiologically relevant conditions. We found that distamycin potently inhibits PU.1 ETS binding with an IC50 of 2.5 nM under physiologic conditions. Since distamycin binds in the minor groove and ETS domains recognize their consensus motif in the major groove, this is an example of allosteric inhibition of a DNA-binding protein. We are now extending our studies to explore the potential of inhibiting the *ptgs2* promoter, a native ETS-dependent promoter sequence, in live cells. We have engineered a reporter system based on a bright but destabilized green fluorescent protein (GFP) with a 2-hour biological half-life. We are characterizing this system in various cell lines that respond to various stimuli by inducing expression of the *ptgs2* gene. We expect the combination of SPR and GFP reporter to serve as a useful platform for screening and developing ETS-targeting drug candidates.

1307-Pos Board B199

Characterization of SRA-Methylated DNA Complexes Dynamics Related to Chromatin Structure Regulation

Yong-Woon Han[1], Hiroaki Yokota[1], Mariko Ariyoshi[1,2], Yasuo Tsunaka[1,2], Takuma Iwasa[1], Ryuji Yokokawa[2,3], Ryo Hiramatsu[4], Daichi Chiba[4], Teruo Ono[4], Yoshie Harada[1].
[1]Institute for Integrated Cell-Material Sciences, Kyoto University, Kyoto, Japan, [2]PRESTO, Kyoto, Japan, [3]Department of Technology, Kyoto University, Kyoto, Japan, [4]Institute for Chemical Research, Kyoto University, Kyoto, Japan.

Eukaryotic gene expression is regulated by chromatin structures and/or DNA modification such as CpG methylation. The basic unit of eukaryotic chromatin structure is a nucleosome consisting of approximately 150 bp DNA wrapped in 1.7 superhelical turns around a histone octamer. The histone octamer consists of two copies each of H2A, H2B, H3 and H4. Posttranslational histone modifications such as acetylation, methylation, phosphorylation and ubiquitylation regulate chromatin structure, resulting in activation or repression of gene expression. On the other hand, CpG methylation represses gene expression and is essential for silencing of parasitic DNA, genomic imprinting and embryogenesis. During DNA replication, methylated CpGs are converted into hemi-methylated CpGs and newly replicated CpGs should be methylated to inherit methylation pattern. DNA methyltransferase 1 (Dnmt1) is the enzyme to methylate hemi-methylated CpG regions. Uhrf1 is methylated CpG binding protein and interacts with Dnmt1, followed by recruitment of Dnmt1 to hemi-methylated CpG regions. SRA domain of Uhrf1 is responsible for hemi-methylated CpG binding activity. We characterize the process of hemi-methylated CpG recognition by SRA domain using Single-Molecule technique, and in this symposium, we will show our present data.

1308-Pos Board B200

Molecular Recognition in Complexes of Telomeric DNA with TRF Proteins as Studied by Molecular Dynamics Simulations

Milosz Wieczor, Adrian Tobiszewski, Pawel Wityk, **Jacek Czub**.
Gdansk University of Technology, Gdansk, Poland.

Telomeres are nucleoprotein heterochromatic structures that protect and stabilize the ends of eukaryotic chromosomes. In humans, telomeric DNA contains 2-20 thousand base pairs (bp) of double-stranded tandem repeats of a small motif 5'-TTAGGG-3' with 50-500 bp of single-stranded overhang. Telomeric DNA is capped by a number of structural proteins, including telomeric-repeat binding factor 1 and 2 (TRF1 and TRF2) that bind in a sequence-specific manner to double-stranded telomeric regions. TRFs recruit other proteins to telomere to form a complex known as shelterin that maintains genome integrity through preventing activation of DNA damage response by the ends of linear chromosomes. For cancer cells, loss of shelterin components has been shown to trigger a rapid DNA damage response leading to apoptosis and decreased tumorigenic potential. Therefore, the complexes of telomeric DNA with TRF proteins have been investigated as potential target for new anticancer strategies. To identify ways of inducing rapid telomere deprotection through destabilization of DNA-TRF complexes, the molecular details of the recognition in these structures are necessary. Here, using equilibrium molecular dynamics (MD), steered MD and free energy simulations, we examine the mechanism by which telomeric DNA sequences are recognized and bound by the TRF DNA-binding domains. To further clarify this mechanism, we analyze how DNA-TRF binding is affected by the presence of C-1305, a triazoloacridone derivative that was previously shown in our group to selectively cause dissociation of TRF proteins from telomeric DNA and to induce fast-pathway response via DNA-repair machinery. To our knowledge, this is the first report in which molecular details of small molecule-induced destabilization of DNA-protein complexes are presented.

1309-Pos Board B201

Fret-Based Approach to Probe Domain Motions upon ProRS/YbaK/tRNA_Pro Ternary Complex Formation

Chamaree de Silva[1], Marina Bakhtina[2], Karin Musier-Forsyth[2].
[1]Mercer University, Macon, GA, USA, [2]The Ohio State University, Columbus, OH, USA.

To obtain a high level of accuracy during protein synthesis, several different quality control steps are employed by the cellular machinery. The aminoacyl-tRNA synthetases (aaRS) play a critical role in identifying amino acids and pairing them with their cognate tRNAs. Prolyl-tRNA synthetase (ProRS) from all three domains of life has been shown to mischarge alanine and cysteine onto tRNAPro. Most bacterial ProRSs have an editing domain that deacylates mischarged Ala-tRNAPro. However, this double-sieve editing mechanism is not sufficient to clear Cys-tRNAPro. Instead, a free-standing homolog of the ProRS editing domain called YbaK deacylates mischarged Cys-tRNAPro species.
We have demonstrated that tRNAPro, ProRS and YbaK form a ternary complex in vitro and in vivo, but the details of this complex are not known.
Based on preliminary computational studies, we hypothesize that the alanine editing domain of ProRS undergoes a conformational change to facilitate YbaK binding. In addition, the CCA-3′ end of the tRNA must also be involved in significant conformational changes, translocating between the synthetic active site, the ProRS editing domain active site located 35 Å away, and YbaK. To probe these protein and RNA domain movements, we devised a fluorescence resonance energy transfer (FRET)-based approach. To date, using ensemble time-resolved FRET, we have measured an ~20 Å conformational change in the ProRS editing domain upon YbaK binding, confirming our hypothesis of a large conformational change to accommodate YbaK. Moreover, the distance between tRNA and YbaK differ by ~15 Å in the presence and absence of ProRS, further verifying a conformational change. Current studies are aimed at obtaining additional distance constraints between components of the ternary complex.

1310-Pos Board B202

Proteins Searching for their Target on DNA by One-Dimensional Diffusion: Overcoming the "Speed-Stability" Paradox

Shi Yu, Shihu Wang, Ronald G. Larson.
University of Michigan, Ann Arbor, MI, USA.

The sequence dependence of DNA-protein interactions that allows proteins to find the correct reaction site also slows down the 1D diffusion of the protein along the DNA molecule, leading to the so-called ''speed-stability paradox,'' wherein fast diffusion along the DNA molecule is seemingly incompatible with stable targeting of the reaction site. Here, we develop diffusion-reaction models that use discrete and continuous Gaussian random 1D diffusion landscapes with or without a high-energy cut-off, and two-state models with a transition to and from a ''searching'' mode in which the protein diffuses rapidly without recognizing the target. We show the conditions under which such considerations lead to a predicted speed-up of the targeting process, and under which the presence of a ''searching'' mode in a two-state is nearly equivalent to the existence of a high-energy cut-off in a one-state model. We also determine the conditions under which the search is either diffusion-limited or reaction-limited, and develop quantitative expressions for the rate of successful targeting as a function of the site-specific reaction rate, the roughness of the DNA-protein interaction potential, and the presence of a ''searching'' mode. In general, we find that a rough landscape is compatible with a fast search if the highest energy barriers can be avoided by ''hopping'' or by the protein transitioning to a lower-energy ''searching'' mode. We validate these predictions with the results of Brownian dynamics, kinetic Metropolis, and Kinetic Monte Carlo simulations of the diffusion and targeting process, and apply these concepts to the case of T7 RNA polymerase searching for its target site on T7 DNA.

1311-Pos Board B203

Biomimetic Strategy for Nanoparticle Sliding on DNA Tracks

Emel Ficici, Ioan Andricioaei.
University of California Irvine, Irvine, CA, USA.

Many vital biological processes rely on the fast searching of DNA by proteins with an average scanning rate of about 10 microseconds per base pair. The underlying mechanism involves the ability of the protein to first bind nonspecifically and then to move along DNA with ''facilitated diffusion'' involving four processes: sliding, hopping, jumping, and intersegmental transfer. In order to fully explain sliding dynamics of proteins along DNA an atomic representation of the system is needed. Studying the dynamics of the nonspecific binding and facilitated search mechanisms on DNA strands in atomic detail will not only provide an understanding of how one of the most important cellular regulatory

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, charge, 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

Ivan Birukou[1], Glenn W. Kaatz[2], Richard G. Brennan[1].
[1]Duke University, Durham, NC, USA, [2]Wayne State University School of Medicine and the John D. Dingell Veterans Administration Medical Center, Detroit, MI, USA.

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 lipophilic ''drugs'', which are typical substrates of MepA. Dimeric MepR binds to inverted repeats of conserved pentad sequence GTTAG using winged helix-turn-helix (wHTH) motif. The *mepR* 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 bending 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

Raashi Sreenivasan, Sara E. Heitkamp, Thomas M. Record.
University of Wisconsin-Madison, Madison, WI, USA.

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 solute/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 initation intermediates.

1314-Pos Board B206

Dissolution of DNA and F-Actin Bundles Stabilized by Polyelectrolyte Effects

Robert Bucki, Katrina Cruz, Paul Janmey.
University of Pennsylvania, Philadelphia, PA, USA.

When intracellular anionic polyelectrolyte filaments are released into the extracellular space due to cell damage, they are exposed to polyvalent cations such as antimicrobial peptides and other multications 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-anions 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.

1315-Pos Board B207

Progress towards Chemical Cross-Linking of Sequence-Specific Proteins to DNA Templates with Single-Molecule Sensitivity

Mehrdad Tajkarimi, Autumn Carlsen, Jan Ruzicka, Adam R. Hall.
Joint School of Nanoscience and Nanoengineering, Greensboro, NC, USA.

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_{off}) 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 *Eco*RI 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 cross-linkers. We monitor ensemble binding stability using electophoretic mobility shift assays (EMSA) and at the single-molecule level with atomic force microscopy (AFM).