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.

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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.

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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