1478-Pos Board B322
Use Of Single-Molecule Imaging To Analyze The Distribution Of Binding Ability In RNA Aptamer Populations
Mark Elenko[1], Jack W. Szostak[1,2], Antoine M. van Oijen[1].
[1]Harvard Medical School, Boston, MA, USA, [2]Howard Hughes Medical Institute, Massachusetts General Hospital, Boston, MA, USA.
The number of possible sequences for nucleic acid and protein biopolymers of functionally plausible lengths is literally beyond astronomical, exceeding the probable number of atoms in the universe. The nature of the functionality landscape across sequence space for such molecules is of great interest to the origins of life field, and, more pragmatically, to those interested in the design, screening, or *in vitro* evolution of functionally useful molecules. This encompasses many questions relating characteristics of such molecules, such as stability or structural motifs, to the distribution of ability, as measured by kinetic rates. A key question concerns the likelihood of finding particular functional abilities (binding or catalysis) in a pool of sequences with a given length and/or other complexity-determining attribute.
Investigating the ''kinetic structure'' of a population is not possible with conventional bulk methods as subpopulations are simply averaged together. Such fine structure can only be approached using single-molecule techniques. This project uses a single-molecule fluorescence microscopy technique (Total Internal Reflection, or TIR) to analyze binding kinetics in populations of RNA aptamers. RNA is a particularly good candidate for exploration, owing both to its centrality in the RNA world hypothesis and the current interest in developing RNA aptamer based drugs.
Experiments with known GTP aptamers yield on and off rates that differ by species, are comparable to bulk results, and enable species separability in kinetic space. The goal is to enable quantification of the distribution of binding ability in heterogeneous, high complexity pools. In addition to addressing questions related to functional RNA, this is useful for designing and understanding *in vitro* selection experiments, a key tool for the origins of life field and the expanding field of applied molecular evolution.

1479-Pos Board B323
Sensitivity Of Dna-hairpins Dynamics To The Mechanism Of Force Feedback Probed Using A Surface-coupled Passive Force Clamp
Yeonee Seol[1,2], Thomas Perkins[2,3].
[1]National Institute of Health, Bethesda, MD, USA, [2]JILA, National Institute of Standards and Technology and University of Colorado, Boulder, CO, USA, [3]Department of Molecular, Cellular and Developmental Biology, University of Colorado, Boulder, CO, USA.
Optical-trapping experiments have yielded new insight into the mechanical behavior of individual biomolecules. A common experimental assay consists of an enzyme or nucleic acid molecule attached to a cover slip at one end and to a small polystyrene bead at the other. The bead is captured and held under tension with an optical trap. Active feedback maintains constant force, often called a force clamp, to increase measurement precision. Yet, active feedback is inherently bandwidth limited. This limited bandwidth leads to significant fluctuations in force that are particularly pronounced for rapid, large changes in molecule extension (e.g. DNA hairpin unfolding). A novel, passive force clamp circumvents this limitation by pulling the bead to a non-linear region of the trap where $k_{trap} = 0$. To date, this passive force clamp has required a specialized dual optical trapping apparatus where one trap measures position and the other measures force. Here, we demonstrate a passive force clamp achieved with a single trap in a surface-coupled assay using a previously characterized DNA hairpin. In an active force clamp, rapid back-and-forth transition between open and closed hairpins states were observed within the update period of the active force clamp (2 or 10 ms) as well as a change in the long term dynamics. By using a passive force clamp, these spurious transitions were eliminated and the correct dynamics measured. By analyzing the fluctuations in the bead position in conjunction with the known elasticity of DNA, we simultaneously measured force and position in a single-beam, passive force clamp. Thus, the benefits of the passive force clamp are now available to the widely used surface-coupled optical trapping assays.

1480-Pos Board B324
Proof of Principle for Shotgun DNA Mapping by Unzipping
Lawrence J. Herskowitz, Anthony L. Salvagno, Linh Le, Steven J. Koch.
University of New Mexico and Center for High Technology Materials, Albuquerque, NM, USA.
We are developing single-molecule methods for mapping protein-DNA interactions inside living cells by unzipping single chromatin fragments isolated from living cells. One avenue towards this capability involves unzipping random fragments that have been generated by site-specific restriction endonuclease digestion of whole genomic DNA or chromatin, a process we are calling shotgun DNA mapping or shotgun chromatin mapping. A key enabler of shotgun DNA mapping (SDM) will be the ability to assign the individual fragments to their specific sites in the genome, based on the sequence-dependent unzipping force of the underlying naked DNA sequence. We will present proof-of-principle results demonstrating the ability to match experimental data sets for pBR322 unzipping to the correct pBR322 sequence hidden in a library of approximately 3,000 yeast genome sequences arising from the known locations of XhoI recognition sites. We do so via an algorithm that scores the experimental data against simulated unzipping forces from a quasi-equilibrium model (Bockelmann, Essevaz-Roulet, & Heslot, 1997). Our next step is to perform SDM on yeast genomic DNA fragments produced by ligation of XhoI-digested DNA to unzipping constructs. Enhancements of the matching algorithm, data processing, and unzipping simulation will be discussed, along with studies of the robustness of the SDM method as a function of number of sites in genome and other parameters. In addition to the impact on our goal of single-molecule mapping of chromatin from living cells, SDM may have important applications in other areas of genomics, including high-throughput structural DNA mapping and genome-wide mapping of sequence-specific DNA binding proteins.

1481-Pos Board B325
Direct Observation of DNA Untangling Magic by a Type-II DNA Topoisomerase
Katsunori Yogo, Taisaku Ogawa, Saki Obata, Gen Nakajima, Junpei Suzuki, Kazuhiko Kinosita, Jr.
Waseda University, Tokyo, Japan.
Type-II DNA topoisomerases(topoII) are ubiquitous enzymes that play key roles in the maintenance of DNA topology in cells. They control the degree of supercoiling of DNA and untangle the catenanes that arise during replication or recombination. Lack of their activities during cell division ultimately causes cell death. TopoII untangles DNA catenanes in an ATP-dependent manner, by catalyzing the transport of one DNA segment to the other side of a second DNA segment through a transient double-stranded break in the second segment. The work of topoII would seem like that of a magician who fascinates the audience, by solving a knot of rope without touching the knot.
Here, we show movies of this unlinking magic taken in real time under an optical microscope.

1482-Pos Board B326
Real-time Observation of Positive Supercoiling by Reverse Gyrase
Taisaku Ogawa.
Waseda University, Tokyo, Japan.
The degree of DNA supercoiling in living cells is an important factor that affects diverse processes such as DNA replication, transcription, and recombination. Supercoils are under control of DNA topoisomerases which either increase or decrease the linking number (Lk) of DNA. Type I topoisomerases cut one of the two strands of DNA, rotate and reseal the nicked strand, whereas type II cuts both strands to pass another DNA segment and then reseals. Although most of topoisomerases only relax DNA supercoils, DNA gyrase and reverse gyrase actively introduce negative and positive supercoils, respectively, into a substrate DNA. The reverse gyrase, found in thermophilic archea, is unusual because positive supercoils are normally harmful. Thermophiles are thought to exploit the reverse gyrase to overwind DNA to prevent denaturation at high temperatures. Bulk studies have shown that the reverse gyrase is an ATP-dependent type I topoisomerase active only at temperatures above 50°C. Here we show the action of reverse gyrase from *Sulfolobu*s in real time, tracking the rotation of DNA under a temperature-controlled microscope (50~70°C).

1483-Pos Board B327
Effect of Single-Strand Break on Holliday Junction Migration Dynamics: A Single-Molecule Fluorescence Study
Mikhail A. Karymov, Dmytro Palets, Yuri L. Lyubchenko.
University of Nebraska Medical Center, Omaha, NE, USA.
Single-strand breaks (SSBs) are the most common DNA lesions in living cells. SSBs occur three orders of magnitude more frequently than double-strand breaks. Unrepaired SSBs lead to blockage or collapse of DNA replication forks, possibly causing formation of double-strand breaks. Holliday junctions (HJ) play central role in various DNA functions including repair of lesions, replication, homologous and site-specific recombination. Branch migration, either spontaneous or protein-mediated, is among widely employed mechanisms in these functions. Therefore, elucidation of the SSB effect on spontaneous branch migration of HJs is a problem of great importance. To accomplish this task, we employed single-molecule FRET approach developed before, allowing us to follow spontaneous branch migration of one HJ at a single base pair level in real time. One SSB was incorporated in the middle of the homologous region of mobile HJ with the donor and acceptor dyes placed on its opposite arms. The data showed that branch migration does not stop at these lesions or reflects from them. Our previous results showed that branch migration is a step-wise process and one step can cover entire homology region. The analysis of the time trajectories showed that such

long steps are not prevented by SSBs. Step size distributions for HJs containing SSB were obtained and such characteristics of these distributions as the mean hop size and the distributions width remain virtually the same as in the designs without a break. Lifetimes of folded states were found to be very similar to that in control, just slightly increasing around the SSB in the AT-rich design. We also studied an immobile HJ with a SSB at the junction. Its arms remain in antiparallel orientation, very similarly to our previous studies.
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1484-Pos Board B328
Nucleocapsid Protein Locally Melts The Iib Region Of Hiv-1 Rre Rna, But Rev Protein Does Not: A Single-molecule Spectroscopic Study
Yu-shan Yeh[1], Hui Wang[1], Paul F. Barbara[1], Matthew D. Daugherty[2], Alan D. Frankel[2], Karin Musier-Forsyth[3].
[1]The University of Texas at Austin, Austin, TX, USA, [2]University of California, San Francisco, San Francisco, CA, USA, [3]The Ohio State University, Columbus, OH, USA.
The focus of this work is to develop a single molecular-level understanding of how the binding of two important viral proteins of human immunodeficiency virus type-1 (HIV-1), Rev and nucleocapsid (NC) proteins, locally change the secondary structures of the IIB stem-loop region of the Rev-Responsive Element (RRE) of the HIV-1 viral genomic RNA. Rev and NC represent two types of nucleic acid-binding proteins in HIV with distinct structures, behaviors and functionalities. Rev is a sequence-specific RNA-binding protein that binds to stem IIB and other regions of the RRE, facilitating nuclear export of unspliced HIV RNAs. In contrast to Rev, NC is a multifunctional protein that plays a role in almost every step of the retroviral life-cycle. NC can bind both DNA and RNA hairpin structures using its CCHC-type zinc fingers and basic domains. Here we systematically investigate how the binding of Rev and NC on the 42-nt IIB RNA hairpin locally changes the RNA secondary structures using a single-molecule fluorescence resonance energy transfer (SM-FRET) approach. A series of RNA and DNA oligonucleotides containing appropriate dyes have been designed to probe the local melting of the IIB RNA hairpin using a single-molecule oligonucleotide annealing assay. Our study shows that NC locally melts the IIB RNA hairpin but Rev does not, illustrating how two proteins that use dramatically different nucleic acid recognition motifs can give rise to very different secondary structural changes upon RNA binding. This SM-FRET-based approach provides a unique way to gain insight into the secondary structural change of HIV viral RNAs induced by protein binding with minimal interference from protein aggregation.

1485-Pos Board B329
Single-pair Fluorescence Resonance Energy Transfer study of mononucleosomes dynamics
Barbara Treutlein[1], Josef Exler[2], Gernot Längst[2], Jens Michaelis[1,3].
[1]Department of Chemistry and Biochemistry, Ludwig-Maximilians-Universität (LMU), München, Germany, [2]Department of Biochemistry III, University of Regensburg, Regensburg, Germany, [3]Center for Integrated Protein Science (CIPSM), München, Germany.
Nucleosomes, the primary repeating unit of chromatin, package DNA by wrapping 147 base pairs tightly around an octamer of histone proteins in approximately 1.7 helical turns. In order to allow central nuclear processes to happen, such as DNA replication, recombination, repair and transcription, regulated exposure of the template DNA is required. Therefore, the nucleosome has to undergo certain conformational changes. One possible strategy for such a regulated exposure is the translocation or 'sliding' of nucleosomes along the DNA by the class of ATP-hydrolyzing enzymatic machines called chromatin remodellers.
We apply single pair-FRET for direct observation of intrinsic nucleosome dynamics as well as conformational changes of mononucleosomes induced by chromatin-remodeling complexes. The movement of DNA around the nucleosome surface is tracked in the presence and absence of remodeller by using mononucleosomes, which are reconstituted with a 200 bp long DNA containing the nucleosome positioning sequence 601 and a donor- and acceptor-dye pair at well-defined positions. Mononucleosomes are immobilized on DOPC-bilayer functionalized quartz slides and visualized by Total Internal Reflection Microscopy.

1486-Pos Board B330
Unraveling Nucleosome Dynamics Using Fluorescence Fluctuation Analysis
Kaushik Gurunathan, Marcia Levitus.
Arizona State University, Tempe, AZ, USA.
Nucleosomes are the smallest repeating units of eukaryotic chromatin. Target DNA sites for various biological processes are often buried inside these nucleosomes. There are various mechanisms by which DNA accessibility is increased. Recent studies show that DNA spontaneously and transiently unwraps from the histone core, presenting itself to the protein machineries. We have studied this dynamics using fluorescence correlation analysis. Our approach is to use a FRET construct where the acceptor (Cy5) is on the histone while the donor (Cy3) is moved along the length of the DNA, starting from the 5′ terminus all the way to the center of the dyad axis. We have used a combination of experimental FCS techniques and Monte Carlo simulations to determine the timescales of the nucleosome wrapping-unwrapping process. Conventional FCS methods face the challenge of separating the kinetic contributions from the diffusion contributions to the autocorrelation functions. Here, we will present and discuss a variety of approaches aimed to overcome these difficulties, including the analysis of the donor-acceptor cross-correlation and correlation of the generalized polarization function.

1487-Pos Board B331
A Polarized View Of DNA Under Tension
Joost van Mameren, Gijs J. Wuite, **Erwin J.G. Peterman**.
VU University, Amsterdam, Netherlands.
Polarized fluorescence measurements are valuable tools for determination of local structure and dynamics of biomolecules. In many applications, a great difficulty is to orient the biomolecules in a fixed and well-defined orientation, such that absolute measures of the directions of the absorption and emission dipole moments are attainable. Often alignment in flow or in a compressed gel is used, but this yields heterogeneity in the orientations. Here we present a novel and different approach: we use two optical tweezers to extend and align a piece of DNA and measure, at the same time, the polarization of the fluorescence of intercalating dye molecules (YOYO). This approach not only provides superior control of the orientation of the DNA but also of tension and consequently its conformation. By using this approach, we resolve an inconsistency in reports on the tilt angle of intercalated dyes with respect to the DNA long axis. We find that intercalated dyes are on average oriented perpendicular to the DNA, yet undergo fast dynamics on the fluorescence time scale. In addition, we assess the structural changes occurring in and beyond the overstretching transition of double-stranded DNA, during which the base pairs gradually melt. We observe that at low forces, when the DNA is shorter than its contour length, the alignment of the dyes increases with force, which can be well-described with a simple Monte-Carlo model of DNA flexibility. During the overstretching transition, the orientation of the dipoles does not change, which we attribute to the intercalating dyes acting as local stabilizers of the helical DNA structure. Beyond the overstretching transition, the dipole moments rotate towards the DNA's long axis, which we attribute to shearing of the locally stabilized double-stranded structure, leading to tilted intercalators and bases.

1488-Pos Board B332
A Novel Approach to the Detection of DNA-Enzyme Interaction Processes at a Single-Molecule Level
Daisuke Onoshima[1], Noritada Kaji[2], Manabu Tokeshi[2], Yoshinobu Baba[2].
[1]MEXT Innovative Research Center for Preventive Medical Engineering, Nagoya University, Nagoya, Japan, [2]Department of Applied Chemistry, Graduate School of Engineering, Nagoya University, Nagoya, Japan.
A fundamental problem in both molecular biology and biophysics is how DNA-binding proteins find specific sites among huge amounts of non-specific (chromosomal) DNA. This in turn requires experimental procedures to elucidate the mechanisms of both non-specific and sequence-specific interactions of proteins with DNA. Current techniques are incapable of directly observing these interaction processes. We recently constructed a setup (See Image) for tracking trajectory of single reactant DNA molecules during enzymatic reaction. It uses a microfluidic flow system to pick reacting DNA molecules (previously tagged at one end by a quantum dot) out of the crowd of molecules. Restriction enzymes Not I, Apa I and EcoR I with their target and non-target DNA were analyzed. Quantitative assessments of their interaction time were achieved by