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
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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
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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
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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
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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
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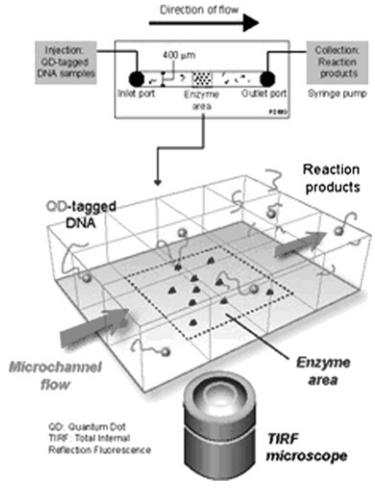
A fundamental problem in both molecular biology and biophysics is how DNA-binding proteins find specific sites among huge amounts of non-specific (chromosomal) DNA. This in turn requires experimental procedures to elucidate the mechanisms of both non-specific and sequence-specific interactions of proteins 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



sampling surveys of the single-molecule trajectories. With this system, we measured the duration difference of restriction-site-searching by these enzymes. The simplicity and versatility of the method suggest the possibility of more practical process analyses in living organisms.

Imaging & Optical Microscopy II

1489-Pos Board B333

Dronpa-1, Dronpa-2 And Dronpa-3 Separation Using Phase Resolved Optical Lock-in Detection (pholid) Microscopy

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In living cells, protein complexes are dynamic structures that control many distinct aspects of cell behavior. Addressing the complexity of protein function requires a comprehensive understanding of subcellular localization and protein stoichiometry. Fluorescence microscopy provides a high contrast method to determine specific localizations of individual proteins contained within living cells. Determining localizations of protein pairs is made difficult by inherent photobleaching, cross-talk, and autofluorescence contained within the sample being imaged. Traditionally, to separate fluorescent proteins (FPs) with overlapping spectra, techniques such as linear unmixing and fluorescence lifetime imaging (FLIM) have been used. Such techniques rely on complex microscope configurations and often require a compromise in signal to noise ratios. In these studies we use phase information derived from optical lock in detection (OLID) (Mao et al. Biophys J. 2008 Jun;94(11):4515-2) to separate the photoswitchable FPs Dronpa-1, Dronpa-2, and Dronpa-3 from GFP and autofluorescence with no effect in signal to noise. Additionally, relative stochiometric ratios between each of the Dronpa variants contained within microinjected zebrafish embryos were obtained with a standard confocal microscope.

1490-Pos Board B334

Near Infrared Emitting Dye Di-4-ANBDQBS for Recording Action Potentials in Isolated Cardiomyocytes

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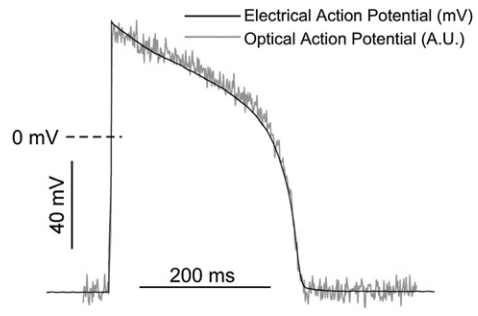
The use of voltage-sensitive dyes (VSDs) for noninvasive measurement of action potentials (AP) in isolated cells is limited by photodynamic damage. Here we tested a new red-shifted VSD Di-4-ANBDQBS as an optical AP (OAP) reporter. **Methods**: Guinea-pig ventricular myocytes were loaded with Di-4-ANBDQBS (18.4-73.6 μM), paced (CL=1-2s) and imaged (excitation, 660 nm laser, emission, >750 nm) in an inverted microscope (40x objective) using an EMCCD camera at 860 frames/s. The VSD signal was integrated over the cell image. **Results**: Limited exposure to laser (700 ms every 5 min) yielded OAPs with fast upstrokes (2-3 ms), stable duration (APD) and signal-to-noise (SNR) exceeding 20 for at least 30 min. The OAP faithfully followed the electrical AP obtained via patch pipette (Figure) yielding a high correlation between the respective APD measurements (R^2=0.985). Longer laser exposures (10 s on/50 s off for 5 minutes) caused APD prolongation which could be alleviated by reducing laser power and/or dye concentration at the expense of reduced SNR. **Conclusion**: With careful consideration of laser exposure, Di-4-ANBDQBS shows significant promise for noninvasive AP recordings in cardiomyocytes with an acceptable SNR.



1491-Pos Board B335

Optical Switchable Spironaphthoxazine (NISO)-derived Probes for Optical Lock-in Detection (OLID) Imaging Microscopy and OLID-FRET

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An optical switch probe based on spironaphthoxazine (NISO) harboring a O^6-benzylguanine (BG) functionality was synthesized and characterized for applications in optical lock-in detection (OLID) Image microscopy. NISO undergoes rapid and reversible, high-fidelity transitions between a colorless spiro (SP) state and a blue colored MC-state that serves as an acceptor probe in Förster resonance energy transfer (FRET) with GFP, YFP and other green or red emitting donor probes. The transition from SP to MC is brought about with high quantum yield by exciting SP with 365 nm or 720 nm (2-photon) while the MC to SP transition is rapidly affected upon excitation of MC with 543~632 nm light. Thus a defined waveform of optical or opto-thermal manipulations of the NISO switch provides a simple means to modulate the intensity of a donor probe via FRET. NISO was linked to BG via a polyethylene glycol (PEG) linker and this substrate was shown to efficiently label O^6-benzylguanine-DNA alkyltransferase (AGT) and AGT-fusion proteins. The optical switching properties BG-PEG-NISO coupled to AGT in fusion proteins with GFP (GFP-AGT/PEG-NISO) and mCherry-(mCherry-AGT/PEG-NISO) were studied. *In vitro* OLID-FRET imaging studies showed the suitability of NISO as an acceptor probe for GFP and mcherry. The R_0 for FRET between GFP and MC-NISO is 4.7 nm and GFP-AGT fusion protein covalently labeled with BG-PEG-NISO exhibited a 55% decrease in GFP fluorescence intensity upon conversion of SP to MC. Other examples of *in vitro* and *in vivo* OLID-FRET using GFP-AGT and mcherry-AGT labeled with NISO-BG will be presented.

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rsCherryRev and NISO Red-shifted Optical Switch Probes for Optical Lock-in Detection (OLID) Imaging and 2-colour OLID-FRET

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Optical lock-in detection (OLID) microscopy using synthetic or genetically encoded optical switches is was developed by our group to generate high-contrast images of the distributions and interactions of proteins in the presence of high and time-varying background signals such as those found in living cells in culture and in live tissue. OLID requires an optical switch probe whose fluorescence intensity can be modulated through deterministic optical control of its fluorescent and non-fluorescent states. Our initial studies focused on Dronpa, while in this study we show how the genetically-encoded optical switch rsCherryRev can be used to extend the wavelength region of OLID to the red (>550 nm) and for 2-colour OLID imaging in combination with Dronpa.

Optical lock in detection (OLID) of Foerster resonance energy transfer, OLID-(FRET) using optical switches as acceptor probes can overcome several limitations of FRET imaging of protein interactions in living cells, including detecting low levels of protein complexes that result from endogenous unlabeled proteins, and non-stoichiometric formation of protein complexes between donor and acceptor probes. We have previously used NitroBIPS as an optically switchable acceptor probe for GFP, and in this study we show how the synthetic, red-shifted optical switch spironaphthoxazine (NISO) serves as a switchable acceptor for both GFP and mCherry in OLID-FRET. These new probes are used for 2-colour OLID-FRET of protein interactions in living cells.