of hybrid F_1 with various mutants and discuss candidate models for each elementary event while considering other recent reports.

References

[1]. Ariga, T., Muneyuki, E. & Yoshida, M. *Nat Struct. Mol. Biol.* ***14***, 841–846 (2007)

2507-Pos ATP-driven Rotation of F_oF_1-ATP Synthase Reconstituted into Supported Membrane

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Board B621

F_oF_1-ATP synthase (F_oF_1) is a rotary molecular motor that reversibly catalyzes ATP hydrolysis/synthesis reaction coupled with the proton translocation across the cell membrane. Rotation mechanism of F_oF_1 (or isolated F_1 part) driven by ATP hydrolysis has been extensively studied by single molecule techniques. However, rotation driven by protonmotive force, generated by the membrane potential and the difference in proton concentration across the membrane, has not been directly observed yet. Although we have addressed this issue using the planar membrane method (Ide and Yanagida, 1999), even the ATP-driven rotation of F_oF_1 embedded in planar membrane has been rarely observed.

In this study, we tried supported membrane method as an alternative. F_oF_1 from *Escherichia coli* was reconstituted into the large supported membrane (>10 μm in diameter) formed on the NiNTA-modified coverglass, and immobilized via histidine-tags introduced into c-ring of F_o. Rotation was observed by streptavidin-coated 200nm latex beads attached to the biotinylated β subunits of F_1. The number of rotating particles (~5) found in a single observation chamber increased significantly as compared with that found in the planar membrane (<0.1). Furthermore, the rotational speed (>10Hz) was much faster than that observed in the planar membrane (<1Hz) at high ATP concentration. These results indicate that planar membrane, formed in the presence of an organic solvent such as squalene, may interfere with F_oF_1 rotation, presumably due to its thickness. To drive the reverse rotation of F_oF_1, we are trying to apply protonmotive force across the supported membrane.

Single Molecule Biophysics - II

2508-Pos Single Molecule TIRF Imaging And Analysis Of Nonspecifically Labeled Fibrinogen - A Molecular Calibration

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Board B622

Single molecule imaging studies can in principle yield information on molecular processes not obtainable by any other method, but its usefulness depends on accurate quantitative characterization of the labeling. The size of fibrinogen and the complexity of its structure makes available on its surface a large number of amino groups to which fluorescent dyes can attach nonspecifically. The bleaching behavior of fluorescently labeled fibrinogen molecules observed in total internal fluorescence microscopy (TIRF) together with theoretical calculations of labeling probability were used to determine the number of active fluorophores attached to each fibrinogen molecule and characterize the uniformity of nonspecific labeling of this molecule. Two different dyes (Tetramethyl Rhodamine and Alexa 488) in bulk dye/fibrinogen ratios ranging from 0.3 to 4.2 were used with similar results. Whereas the predominant labeling was shown to be one active dye molecule per fibrinogen, with increasing bulk labeling ratios, two or more active dyes per fibrinogen start to be significant. From the intensity distribution of the bleaching steps and the probability of active labeling of fibrinogen molecules, a single molecule intensity calibration was obtained. Such calibration is necessary for further studies of fibrin fibers formed from fluorescent fibrinogen, to provide information at the molecular level on the structure of the fibers and their growth kinetics.

2509-Pos Cell-based Single-molecule Detection of a Fluorescent Unnatural Amino Acid Incorporated into the Nicotinic Receptor

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Board B623

Fluorescent unnatural amino acids (fUAAs) represent an attractive strategy for labeling ion channel proteins. Here we report the first successful incorporation of a fUAA, Lys(BODIPYFL), into the muscle nicotinic acetylcholine receptor (nAChR). *Xenopus* oocytes were injected with the frameshift suppressor tRNA aminoacylated with Lys(BODIPYFL) (YFaFS$_{ACCC}$-Lys(BODIPYFL)) and nAChR (α/β19′GGGU/δ/γ) mRNAs. Two-electrode voltage-clamp recordings confirmed the presence of functional surface-expressed nAChRs with respective ACh EC_{50} and Hill coefficient of 37.8 ± 1.84 μM and 1.13 ± 0.05 (n = 5). We measured fluorescence from oocytes expressing the nAChR β19′GGGULys(BODIPYFL) using time-lapse total internal reflection fluorescence (TIRF) microscopy. Under conditions of relatively low expression (<0.1 receptors/μm^2), puncta with discrete decrease in fluorescence intensity consistent with single-molecule photobleaching were detected. The puncta displayed a Gaussian distribution of intensities; the average single-molecule signal-to-background (ΔF/F ± SD) was 0.23 ± 0.01. Puncta densities were much lower in oocytes injected with YFaFS$_{ACCC}$-Lys(BODIPYFL) (~0.007 puncta/μm^2). To confirm that the puncta originated from Lys(BODIPYFL) incorporated into

a nAChR, we incubated oocytes with α-bungarotoxin mono-conjugated with Alexa-488 (α-Btx-Alexa488). The nAChR has two α-Btx binding sites; thus, three days after injection, 23% of the puncta containing the Lys(BODIPYFL) labeled with α-Btx-Alexa488 yielded three discrete photobleach steps. We also performed experiments with a nAChR mutant that contained eGFP in the γ subunit M3-M4 loop; as expected, these puncta were less intense ($\Delta F/F \pm SD = 0.14 \pm 0.04$) than the BODIPYFL or α-Btx-Alexa488 puncta ($\Delta F/F \pm SD = 0.27 \pm 0.13$) and displayed characteristic eGFP blinking. Thus, we report the cell-based single-molecule detection of nAChR β19′GGGULys(BODIPYFL), which is consistent with two different labels and labeling schemes.

Support: NS-11756, NS-24407, Ford Fellowship, APA-DPN Fellowship, NRSA to MD, NSF predoctoral fellowship to EAR.

2510-Pos Single Molecule Dynamics of The Epsilon Subunit in F_1 Forced-rotated by Magnetic Tweezers

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Board B624

F_1-ATPase (F_1) is a soluble part of the F_oF_1-ATP synthase, which reversibly catalyzes ATP synthesis/hydrolysis reaction. F_1 has a subunit structure of $\alpha_3\beta_3\gamma\delta\varepsilon$. It hydrolyzes ATP accompanied by counterclockwise rotation (when viewed from F_o) of the rotor part (consist of γε), and synthesizes ATP in clockwise rotation.

The ε is known as a regulatory subunit of this enzyme. Previous studies have shown that C-terminal α-helices of the ε extend into the $\alpha_3\beta_3$ ring when F_1 synthesizes ATP, while these parts are retracted in hairpin-folded form during ATP hydrolysis. However, the factors that keep the ε in different conformations in these reversible chemical reactions are not known.

To test the hypothesis that the direction and rate of the γ rotation determine conformations of the ε, we set up a microscope system that enabled us to manipulate the γ rotation and monitor the conformational states of the ε in single F_1 molecule. Rotation of F_1 was observed through magnetic beads attached to the γ, and manipulated by magnetic tweezers. Simultaneously, conformational states of the ε, which is labeled by single donor and accepter dyes, were probed by FRET.

Using this system, we found that forced-rotation of the γ in hydrolysis direction induces rapid conformational change of the ε from extended form to folded form. This suggests that F_oF_1 is regulated by a positive feed back mechanism in that mechanical rotation of the γ in hydrolysis direction shifts the ε to folded, ATP hydrolysis active form.

2511-Pos Internalization of functionalizable quantum dots by E.coli

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Board B625

Fluorescent semiconductor nanocrystals, or quantum dots (QDs), are rapidly emerging as superior labels for single-molecule fluorescence microscopy of biological systems. QDs are brighter and more photostable than conventional organic fluorophores, providing access to extended observation times (>10min) and higher signal-to-noise ratios. Despite the appeal of using quantum dots to probe intracellular dynamics, prokaryotes such as *Escherichia coli* and *Bacillus subtilis* are difficult to label internally with quantum dots, owing largely to their poor cell wall permeability, and incompatibility with microinjection techniques. Here we report the simple, rapid internalization of functionalizable, fatty acid-capped CdSe/ZnS quantum dots by live *E.coli*. Widefield fluorescence microscopy and Fluorescence Correlation Spectroscopy (FCS) of bacteria immobilized on glass slides show that internalized quantum dots diffuse within the cytoplasm, and remain highly resistant to photobleaching. We are developing enzymatic and electrostatic methods of coupling internalized QDs to plasmid-encoded proteins or nucleic acids, as a generalizable, genetic platform for tagging and extended visualization of single biological molecules in vivo.

2512-Pos Angle Dependence of Nucleotides Affinity in Rotary Motor F_1-ATPase

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Board B626

F_1-ATPase is a rotary molecular motor in which a central γ subunit rotates against hexagonally arranged subunits $\alpha_3\beta_3$. Three β-subunits, each hosting a catalytic site, hydrolyze ATP sequentially to power the rotation of γ. It is a reversible machine in that, when γ is rotated in reverse direction by an external force, ATP is synthesized in the catalytic sites from ADP and inorganic phosphate. The rotary angle of γ is expected to determine which chemical reaction is to occur in each catalytic site, binding/release and hydrolysis/synthesis of a nucleotide. To see how this γ-dictator mechanism operates, we have measured the angle dependence of nucleotide affinity at single-molecule level. The angle of γ was controlled by attaching magnetic beads (< 0.7 μm) to the γ and applying a rotary magnetic field. Simultaneously, binding/release of a fluorescent nucleotide (Cy3-

ATP or -ADP) in a single molecule of F_1 was observed with TIRF microscopy. With Cy3-ATP at 100 nM and unlabeled ATP at 100 nM, the angle between binding and release of Cy3-ATP during continuous hydrolysis or synthesis rotation was ~240° and ~70° on average, respectively. The probability of binding during hydrolysis rotation was maximal ~30° ahead of an ATP-waiting angle. During synthesis rotation, binding of Cy3-ATP was most frequent ~40° prior to an ATP-waiting angle, and release was observed after ~70° of rotation. Cy3-ATP alone, in the absence of unlabeled ATP, gave basically similar results. We are currently measuring the binding/unbinding kinetics of Cy3-ADP in the presence and absence of unlabeled ADP.

2513-Pos Zero-Mode Waveguides: A Powerful Tool for Single-Molecule Optical Studies

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Board B627

Single-molecule fluorescence studies of enzymes that incorporate fluorescently labeled substrate nucleotides typically operate at substrate concentrations well below their K_m values. While this is inevitable given the femtoliter observation volumes accessed via conventional fluorescence microscopy, the biological relevance of the insights gained into enzyme mechanism may be compromised. Zero-mode waveguides (ZMWs), sub-wavelength holes in a thin metal film, provide an excellent solution to this problem by greatly reducing the observation volume [Levene, M. J. *et al*. 2003. *Science*. 299:682–686].

We report on the nanofabrication, coating, and characterization of ZMWs for single-molecule studies of DNA polymerizing enzymes, such as DNA polymerase and telomerase. The aim is to monitor in real-time the incorporation of fluorescently-labeled nucleotides at biologically relevant concentrations and thus gain insight into the mechanisms and kinetics of these enzymes.

To guide the fabrication, numerical simulations of the optical properties of ZMWs have been utilized to optimize the design of the ZMW geometry. ZMWs of 100nm in width have been successfully fabricated and characterized. In addition, we have developed a PEG coating protocol for the surface treatment of ZMWs to make them biocompatible and to selectively tether DNA substrates used in polymerization assays. We will demonstrate our ability to fluorescently detect DNA polymerase activity within ZMWs.

2514-Pos Precision Steering of an Optical Trap Using Electro-optic Deflection

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Board B628

Optical trapping instruments facilitate precision application of piconewton-scale forces to single motor proteins while achieving nanometer-scale resolution at kilohertz bandwidths. The advent of feedback-based force clamps, where the separation between the bead and laser trap center is maintained at a predetermined value, has allowed controlled forces to be applied to biological specimens, thereby changing the energy landscape in a tractable manner. Acousto-optic deflectors (AODs) provide a convenient means to control both the optical trap position and intensity, but these often generate significant variation in transmission over the useful working range and, more importantly, exhibit systematic pointing errors that lead to fluctuations in the applied force and the measured position under force-clamped conditions. To improve the force and position control in our optical trapping measurements, we constructed a single-beam optical trapping instrument that incorporates two electro-optic deflectors (EODs) that independently steer the optical trap along orthogonal axes. We find that EODs are highly linear in their responses to an external driving voltage, and are ~90% transmissive, with $<0.5\%$ variation in throughput over the entire range of deflections. These attributes make EOD-based devices ideal for nanomechanical measurements of biological materials. Here, we present a detailed description of the optical design, calibration, and characterization of the instrument, comparing the properties of our EOD-driven optical trapping apparatus to those of more conventional AOD-based devices.

2515-Pos Photoactivation Yields and Bleaching Yield measurements for PA-FPs

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Board B629

Recent developments in single-molecule localization-based fluorescence methods are breaking the diffraction barrier of light microscopy. Hence a better understanding of photophysical parameters for cell-compatible photoactivatable/switchable fluorescent proteins (PA-FPs) is in demand. The photoactivation yield (probability of activation of such a molecule upon receiving an activation photon), readout laser photoactivation yield, and the photobleaching yield are important parameters of interest to optimize these microscopic techniques. A basic model for the dependence of the number of active molecules as a function of excitation time for two commonly used PA-FPs is derived using linear first-order kinetics. Single molecules were imaged by a high-sensitivity camera in a widefield fluorescence microscope under laser excitation. Analyzing measurements using the above model, the activation yields and the bleaching yield for PAGFP and Wild type EosFP were determined under different conditions. Here we present the data and compare results for each of the species. Practical implications of the measured yields will be discussed.

2516-Pos Replisome Protein Dynamics And Stoichiometry Using Moleculer Fluorescence Microscopy

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Board B630

The replisome is the complex formed by several key proteins at the DNA replication fork. Here we studied the dynamics and stoichiometry of several of these proteins using real-time high-contrast single-molecule fluorescence microscopy on genetic fusions of different mutants of green fluorescent protein to a variety of components of the replisome machinery in single, functional *Escherichia coli* cells at physiological levels of gene expression.

2517-Pos F_1-ATPase without a Rotary Shaft can Still Rotate in the Correct Direction

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Board B631

F_1-ATPase is a rotary molecular motor driven by ATP hydrolysis. Its minimal active complex is composed of a rotor subunit γ and a stator cylinder made of alternatively arranged by three α and three β subunits that surround the γ shaft. The rotor shaft, an anti-parallel α-helical coiled coil of the amino- and carboxyl-termini of the γ subunit, deeply penetrates the central cavity of the stator cylinder. To examine the roles of the γ shaft in rotation and ATP hydrolysis, we tried to make mutants by truncating both the amino- and carboxyl-termini of the γ subunit step by step until the remaining rotor head would be outside the cavity and simply sit on the concave entrance of the stator orifice. Although truncation mutants were obviously unstable relative to the wild type, we could purify them. All the mutants rotated in the correct direction, when observed by attaching a 40-nm gold bead(s) to the γ shaft, although some mutants showed moments of irregular motions. Even the shortest mutant rotated in the correct direction. Rotation and ATP hydrolysis rates gradually decreased with the degree of truncation.

2518-Pos Determining The Hydrodynamic Size Of Biomolecules By Probing Single Molecule Brownian Rotational Motion

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Board B632

By monitoring the Brownian motion of a fluorescent biomolecule of interest in free solution, information regarding its hydrodynamic shape and size is obtained. While the translational diffusion of a fluorescent biomolecule, typically occurring on the micro- to millisecond time scale, is conveniently obtained from a conventional fluorescence correlation spectroscopy experiment, the more sensitive rotational diffusion dynamics of the molecule, occurring on the pico- and nanosecond time scale, is generally obtained from the measurement of its time-resolved fluorescence anisotropy upon pulsed excitation. The application of the latter technique, however, is inherently limited to the measurement of rotational correlation times not exceeding the fluorescence lifetime of the fluorophore. Hence, the accurate measurement of the rotational diffusion of a biological macromolecule, which is typically in the order of tens of nanoseconds, is not feasible.

Here, we investigate the polarization-sensitive fluorescence of a single molecule by means of an experimental technique [S. Felekyan et al., Rev. Sci. Instrum., *76* (2005) 083104] registering distinct photon arrival times with picosecond time resolution followed by an exact theoretical analysis in terms of their second-order correlation function [S.R. Aragon and R. Pecora, Biopolymers, *14* (1975) 119]. This way, we probe the Brownian rotational diffusion of a biological macromolecule in free solution at time scales from a few picoseconds to seconds along with its translational diffusion without the need for pulsed excitation. We present this experimental technique and its application for the determination of the hydrodynamic shape and size of fluorescent proteins, i.e. GFP and GFP-tagged membrane proteins.

2519-Pos Orientation Dependence of Fluorescence Resonance Energy Transfer on Nucleic Acid Structures

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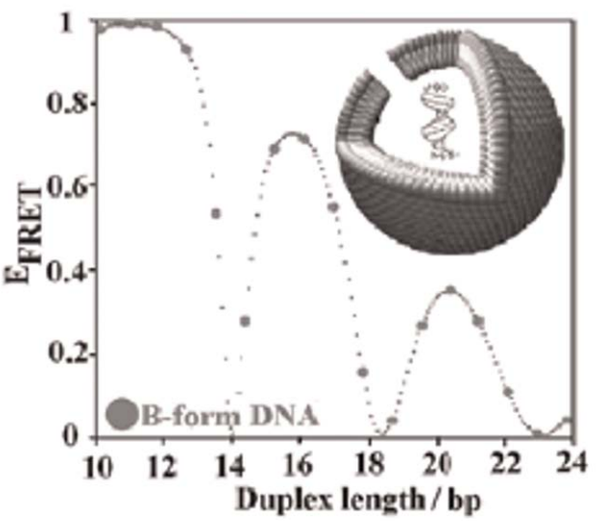
Board B633

Fluorescence resonance energy transfer (FRET) has become a nanoruler over macromolecular distances in ensemble and single-molecule experiments in biology. FRET efficiency (E_{FRET}) depends upon the sixth power of the distance between fluorophores as well as the relative orientation of the transition dipole moments, which has

not been experimentally demonstrated to date at single molecule level with immobilized fluorophores on DNA/RNA scaffold Our earlier NMR studies showed that fluorophores at the 5′-termini of helices are stacked with the bases. Taking advantage of this geometry, we studied s. vesicle encapsulated DNA-DNA and DNA-RNA duplexes using single molecule and bulk FRET. We found that E_{FRET} not only decreases with duplex length, but also exhibits a modulation with twice the periodicity of the helices. We also found that the relative orientation of the transition moments was determined by the geometry of the B- and A-form helices for the DNA-DNA and DNA-RNA duplexes, respectively.

Simulation of the data suggests that fluorophores undergo significant lateral motion at the end of helices which reduces but does not remove the orientation dependence of E_{FRET}. Relative orientation of fluorophores, therefore, is essential when interpreting FRET data in some circumstances.



2520-Pos Fluorescence Characterization of a Protease Inhibitor Using Single Molecules Confined in Optically Trapped Aqueous Nanodroplets

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Board B634

The serpin family of metastable serine protease inhibitors regulates physiologically important serine and cysteine proteases. Serpins have a solvent exposed reactive center loop (RCL) that is a substrate for serine protease molecules. Once the protease cleaves the RCL, the serpin acts like a spring-loaded mousetrap, undergoing a large conformational change, incorporating the RCL into a β sheet at the front of the serpin molecule and forming a metastable covalent complex with the inactivated protease. We use single molecular pair fluorescence resonance energy transfer (spFRET) to study the reaction between the serpin α_1 protease inhibitor (α_1-PI) and the protease rat trypsin in a confocal microscope. Isolated α_1-PI or trypsin molecules as well as the reacted complex are confined in optically trappable aqueous nanodroplets emulsified in an immiscible perfluorinated matrix with a lower index of refraction than water. A single nanodroplet is captured and positioned at the focus of a visible laser using optical tweezers, the molecules are excited, and the fluorescence is detected. In addition, the nanodroplets can be easily mixed to trigger the serpin-protease reaction allowing complex formation to be monitored in real time. We use piezoelectric actuated micropipettes to inject aqueous nanodroplets containing α_1-PI and trypsin into the matrix where each droplet is grabbed with separately controlled optical traps, then fused and immediately interrogated. Fluorophore positioning at appropriate amino acid sites are designed to provide dramatic changes in FRET intensity over the course of the reaction, yielding information about distances and dynamics during covalent complex formation. We demonstrate the feasibility of this approach and discuss spFRET results both from individual spFRET-labeled α_1-PI molecules and from the reacted complex.

2521-Pos Plasmonic Enhancement of Single Molecule Fluorescence for Real-time Optical Monitoring of Bundle Crossing Activity in KcsA Ion Channels

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Board B635

In KcsA, it has been previously shown that a fluorophore can be used to optically monitor the state of the lower second transmembrane segment (TM2) comprising the bundle crossing (Blunck et al, 2006). When KcsA is labeled at a cysteine-mutated position in the lower TM2 such as Q119C, tetramethylrhodamine-5-maleimide (TMR-5-M) fluorescence lifetime and intensity vary depending on the state of the bundle crossing. Based on this finding, we have developed a technique to optically monitor this signal by use of high-speed fluorescence imaging at the single molecule level (McGuire et al, this meeting; Blunck et al, Biophys. Soc. Meeting, 2007). KcsA cysteine mutants are purified, fluorescently labeled, and reconstituted in lipid vesicles. They are then seeded on a coverglass to form a supported bilayer for imaging using a high numerical aperture TIRFM objective. Our goal is to increase the signal-to-noise ratio (SNR) of the fluorescence signal using surface plasmon resonance (SPR) excitation and surface plasmon-coupled emission (SPCE) in an imaging configuration using “plasmon chips” (Hyde et al, Biophys. Soc. Meeting, 2007) as well as testing new fluorophores. We have scanned a variety of red ATTO fluorophores, which possess excellent photostability and more favorable conditions for SPR, to find those most sensitive to local environment, yet insensitive to pH. Among those tested, we have chosen two particular ATTO fluorophores that significantly improve the fluorescence SNR beyond that of TMR-5-M. Because they increase the SNR, these enhancements allow us to reduce the fluorescence excitation intensity and

thereby increase the observation time. Since this is the limiting factor in analysis of single molecule fluorescence, they will help us to better analyze gating of KcsA at the single molecule level.

Supported by NIH 1R21MH078822-01 & 1F31NS054532-02.

2522-Pos Performing Fluorescence Correlation Spectroscopy inside water-in-oil emulsions

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Board B636

Fluorescence Correlation Spectroscopy (FCS) is a quantitative technique used to analyze samples usually diffusing freely in dilute solutions. Using confocal or two-photon microscopy, light is focused on the sample and the fluctuations in fluorescence intensity (due to diffusion, FRET, chemical reactions, photophysical reactions, aggregation, etc.) are analyzed using temporal autocorrelation. This can yield kinetic information about the processes that contribute to fluctuations in the fluorescence intensity of free dyes and dyes attached to molecules of interest. When performing FCS using confocal microscopy, the short residence time of the molecules inside the confocal volume, which is of the order of a few milliseconds, limits the study of slow kinetic processes. Here, we investigated the use of water-in-oil emulsions with the goal of increasing the residence time in FCS experiments. Macromolecules were enclosed within the aqueous phase of the emulsion, and their diffusion properties were studied by FCS. We expect that the increase in the residence time of the macromolecule will allow the study of slower kinetic processes by this method.

2523-Pos Single Particle QD-FRET: Evaluation of the Stability and Composition of Nanocomplexes for Gene Delivery

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Board B637

The need to develop a safe and effective nonviral gene vector has increased with the growing promise of genetic medicine. Rational design of more efficient gene carriers will be possible only with sufficient insight into the physicochemical properties and stability of the DNA nanocomplexes, and their intracellular trafficking in the gene transfer process. To that end, we have adopted a sensitive single particle QD-FRET approach to investigate the DNA nanocomplexes through a combination of quantum dots (QD)-mediated fluorescence resonance energy transfer (FRET) and confocal fluorescence spectroscopy. Cationic polymer and plasmid DNA were labeled with a fluorescent organic dye and CdSe-ZnS quantum dots, respectively, generating a FRET pair within a nanocomplex. As an efficient FRET donor yielding a high signal-to-noise ratio, QDs serve as a sensitive probe for conformational changes in the nanocomplex state at the single-particle level and under different microenvironments. Characterization of batches of single nanocomplex provides valuable insight to the heterogeneity of nanocomplexes, a parameter difficult to study with other techniques and may be the source of many experimental discrepancies. Meanwhile, intracellular trafficking of the FRET-mediated signals would shed light on the unpacking behavior of the DNA nanocomplexes across cellular compartments. Integration of extracellular characterization and intracellular monitoring of QD-FRET nanocomplexes enable the correlation between structural properties of nanocomplexes and their intracellular kinetics, helping to unravel the mechanisms of nanocomplex unpacking and release of DNA in the delivery route. We have demonstrated in this study that single particle QD-FRET provides a sensitive and quantitative measure to evaluate the stability and composition of DNA nanocomplexes at different microenvironments, and is expected to facilitate the optimum design of gene carriers.

2524-Pos Modulation of Syntaxin's Spontaneous Conformational Dynamics by Syntaxin Binding Proteins

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Board B638

Syntaxin is a SNARE protein that is essential for membrane fusion at the synapse, which results in neurotransmitter release. Switching between an open and closed conformation is thought to be involved in regulating syntaxin's ability to enter into the full SNARE complex on the path to membrane fusion. We have used single molecule fluorescence resonance energy transfer (smFRET) to report in real-time the open-closed conformational switching of syntaxin molecules immobilized at a surface by incorporation inside tethered liposomes. The effects of several SNARE binding proteins on syntaxin's spontaneous open-closed conformational dynamics are reported.

2525-Pos Molecular Brightness Analysis Of Single Molecules By Combining 2-focus Fluorescence Confocal Detection With Microfluidics

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Board B639

In recent years, 2-focus confocal fluorescence microscopy has been proven to be a versatile tool for precision single molecule fluorescence spectroscopy, in particular for fluorescence correlation spec-

troscopy (2fFCS). Here, we present a new application of the 2f approach. By combining a 2fFCS system with a microfluidic device the 2f-setup is a promising tool for molecular brightness measurements on single molecule level. The two laterally shifted (~ 400 nm) but overlapping (alternating pulsed) laser foci are oriented orthogonally to a steady and laminar flow in x-direction. For each laser a separate time-trace of detected fluorescence will be recorded simultaneously. Molecules which pass both foci at the same distance (y = 0) away from their center of optical axis (y = ± 200 nm), will generate in each time-trace, bursts with approx. the same intensity. Molecules which flow above or below (z-direction) the laser beam waist (z = 0) of both laser foci will result in bursts which are broader with respect to molecules which flow along z = 0. Thus, by restricting the analysis to events which have the same intensity in both laser foci and also to events which have shortest burst duration, only molecules are taken into account which have flown right through the middle (y = 0, z = 0) of the overlapping laser foci. As a consequence we know that all sorted bursts originating from the same region of excitation and therefore the extracted brightness values are well comparable to each other. We will show recently achieved results of simulations based on full wave optical calculations. With this method it should be possible to resolve easily subpopulations having different molecular brightness values.

2526-Pos Label-free, Single-molecule Detection Of Cytokines In Serum

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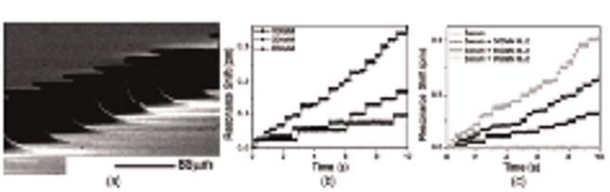
Board B640

Ultra-high-Q optical microresonators have demonstrated label-free, single-molecule detection of both antigens and antibodies. The sensitivity is derived from the photon lifetime within the microcavity and the specificity is achieved through surface functionalization.

In the present work, planar arrays of ultra-high-Q microtoroid resonators (Figure 1a) were used to perform label-free, single-molecule detection of Interleukin-2 (IL-2), a cytokine released in response to immune system activation. Additional solutions in serum were used to verify the sensor's application in a more realistic environment. Several IL-2 concentrations were used, ranging from 100aM to 900aM. The data acquisition rate, solution injection rate and IL-2 concentration were optimized to allow single molecule binding events to be resolved (Figure 1b,c).

Figure 1: a) SEM of microtoroid array. b) As molecules bind to the surface, the resonant wavelength position changes, resulting in steps. c) Experiments in fetal bovine serum. Also shown are pure serum results.



2527-Pos Probing Nucleosome-nucleosome Interactions In Single 30nm Chromatin Fibers Using Dynamic Force Spectroscopy

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In eukaryotic cells, genomic DNA, core histones and linker histones, form dense 30 nm chromatin fibers whose compaction is carefully regulated. We investigated reconstituted chromatin arrays containing 25 nucleosomes with dynamic force spectroscopy (DFS) under physiological conditions using magnetic tweezers. The observed DNA compaction agrees well with reported values for DNA condensation into 30nm fibers. The fibers ruptured elastically into 10nm fibers at 4pN. Linker histones prevented the rupture up to 6pN. Depletion of Mg^{2+} facilitated rupture resulting in non-elastic deformation of the fiber. The observed Force-Distance curve (F-D) of 30nm fibers fitted well to a Hookian spring in series with a Worm Like Chain (WLC), resolving the spring constant, number of nucleosomes, and condensation ratio in individual fibers. These parameters did not change by the presence of linker histones. After rupture the F-D data closely follow two WLC's in series, consistent with strong bends in the DNA trajectory that be expected in nucleosome arrays. The amount of wrapped DNA and the exit angle that were deduced from these fits strongly changed upon addition of linker histones. These experiments provide the first single molecule data of reconstituted 30nm fiber, whose structure is still unresolved.

2528-Pos Probing Structural Dynamics in the Multidomain Scaffold Protein PSD-95 with Single Molecule FRET

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The postsynaptic density is a signal processing machine, composed of thousands of proteins, that detects and responds to neurotransmitter signals. Scaffold proteins form a physical framework that links these proteins together insuring that signaling information is not lost through random diffusion. Because scaffold proteins and many of their ligands contain natively-unfolded polypeptide, conventional high-resolution structural biology has not provided much insight into molecular details of such signaling assemblies. New methods are needed to gain structural information on samples not amenable to classical approaches. Single molecule FRET can provide structural information on the entire distribution of possible conformations. Although there is uncertainty associated with

FRET-based distance constraints, with the proper controls this approach provides structural information about individual proteins and their complexes. The synaptic scaffold protein PSD-95 contains five protein binding domains arranged in tandem like beads on a string. High resolution structures have been solved for all the isolated domains, but this has not provided a picture of how the conjoined domains work together. By measuring intramolecular FRET efficiency for a series of PSD-95 constructs, each containing two unique cysteine residues in different positions, the relative position of domains in the full-length protein can be determined. Unlike beads on a string, PSD-95 appears to adopt a largely globular structure. Calculated distance constraints based on FRET efficiency place the N- and C-terminal domains only 5 nm apart. Stochastic changes in FRET efficiency suggest that large scale motions can bring these domains in closer proximity. These data agree favorably with the open and closed conformations observed in electron micrographs. Thus, single molecule FRET can be used as a structural biology tool to examine dynamic protein conformations.

2529-Pos An Experimental Investigation of the Dynamics of Single DNA Molecules Confined in Nanochannels and Slits

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The ability to fabricate devices with nanoscale dimensions has made it possible to manipulate single biomolecules. Designing these devices requires an understanding of the physics that governs the dynamics of confined polymers. We optically investigate the dynamics of single DNA molecules confined in nanochannels and slits and compare our results to theoretical predictions. We measure the DNA diffusivity and relaxation time as a function of confinement and DNA length. Additionally, we investigate instances when DNA molecules driven by an applied electric field enter a nanochannel with monomers near the middle of the molecule entering first, a conformation we refer to as folded. We study the dependence of the unfolding rate on the dimension of the nanochannel and relate it to the effective width of the molecule.

2530-Pos Dynamic DNA Looping by Lambda Repressor

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After infecting a host bacterium, bacteriophage lambda, may lie latent (lysogeny), or reproduce and lyse the host bacterium. Only one infected cell in 10 million lyses spontaneously, but the switch to lysis is extremely efficient if the bacterial host becomes endangered. The phage protein CI is responsible for maintaining lysogeny by regulating its own transcription and repressing that of genes responsible for lysis. It does so by occupying specific sites on the DNA close to the promoters of these genes and inducing the formation of a long DNA loop. Using the tethered particle motion technique we characterized the dynamic nature of the loop and we now propose a physico-chemical mechanism by which lysogeny maintenance is ensured, yet efficient switching to lysis is possible.

2531-Pos Sequence-directed DNA Export Guides Chromosomal Translocation During Sporulation In Bacillus subtilis

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In prokaryotes, the transfer of DNA between cellular compartments is essential for the segregation and exchange of genetic material. A dramatic example of intercompartmental DNA transfer occurs during sporulation in Bacillus subtilis, in which ~3Mbp of chromosomal DNA are transported across a division septum by the SpoIIIE ATPase. Previous work proposed that SpoIIIE is a unidirectional DNA transporter that exports DNA from the compartment in which it assembles. The mechanism regulating directional DNA transfer, however, has remained unclear. Here, we employ single-molecule, bioinformatics, and in vivo time-lapse fluorescence microscopy methods to show that SpoIIIE establishes DNA translocation directionality by interacting with short DNA sequences (SpoIIIE Recognition Sequences, or SRS) that are highly skewed along one strand of the chromosome from origin to terminus of replication. SpoIIIE interactions with SRS are specifically mediated by the C-terminal DNA binding domain of SpoIIIE (γ-domain) in vitro and in vivo. Our findings indicate that in vivo SpoIIIE forms a bidirectional motor complex that converts into a unidirectional exporter in response to SpoIIIE-γ /SRS interactions, leading to productive DNA transfer. This sequence-directed DNA exporter model reconciles previously proposed simple exporter and sequence-directed models for directional DNA transport by the SpoIIIE/FtsK family of DNA translocases.

2532-Pos Probing RNA Structure With Optical Tweezers And Nanopores

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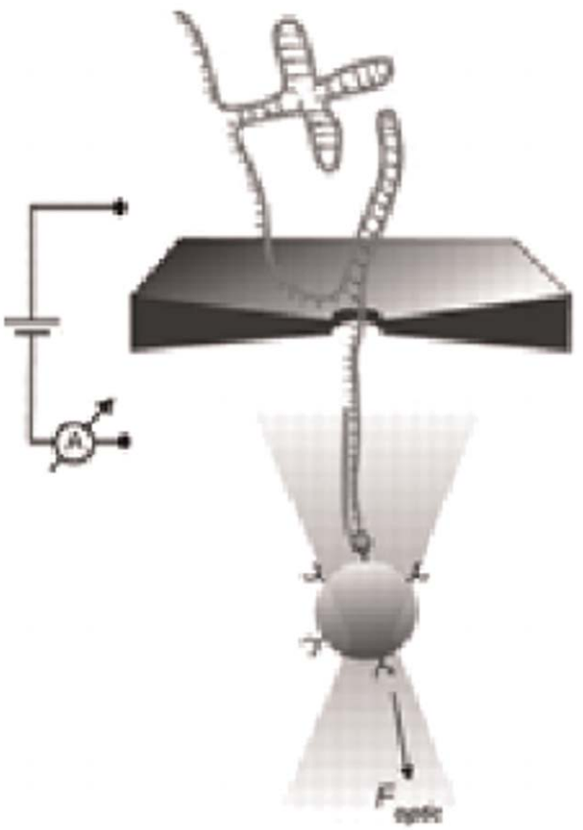
We report our experimental progress towards studying RNA molecules using a combination of optical tweezers and solid-state nanopores[1]. Combining these two powerful single-molecule techniques allows us to locally apply mechanical forces on single RNA molecules, which can be used to reduce their translocation velocity and probe their structure[2]. In addition, by using the optical tweezer to balance the force applied by the electrical field across the nanopore, we can calibrate the effective electrical force on the molecule. Here we show the intermediate steps towards this goal. We demonstrate a new and simple method for efficiently joining RNA molecules of arbitrary sequence to very long DNA handles that are held in the optical trap, based on the well-known biotin-streptavidin linkage. Furthermore, we present preliminary measurements of RNA molecules translocating through nanopores and the corresponding force involved.



References

1. U. Keyser, et al., Nature Physics ***2***, 473–477 (2006)
2. U. Gerland, et al., Phys. Biol. ***1*** 19–26 (2004)

Single Molecule Biophysics - III

2533-Pos Fitc-functionalized Peptide Quantum Dots and Anti-fitc Single-chain Fragment Antibody Protein Fusion as a New Tool for Single Membrane Protein Targeting and Tracking In Live Cells

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Single-molecule targeting and tracking in live cell requires small probes with high specificity and affinity to their targets. For fluorescence microscopy, quantum dots have proven to be ideal probes due to their photostability and small size, provided the solubilization and functionalization steps preserve these properties. We have previously demonstrated such an approach, using biotinylated peptides for quantum dot functionalization, and demonstrating efficient targeting to single fusion proteins containing an avidin moiety (Pinaud, King et al. 2004; Michalet, Pinaud et al. 2005). In order to specifically target different proteins with different color quantum dots, several orthogonal high affinity pairs such as the biotin-avidin one are needed. Here we describe the functionalization of quantum dots with FITC-peptides, and specific targeting of these quantum dots to fusion proteins containing a single-chain fragment antibody (scFv) against FITC. We report preliminary results of single-molecule tracking in live cells using this approach.

References

Pinaud, F., D. King, et al. (2004). "Bioactivation and cell targeting of semiconductor CdSe/ZnS nanocrystals with phytochelatin-related peptides." Journal of the American Chemical Society *126*(19): 6115–6123. Michalet, X., F. F. Pinaud, et al. (2005). "Quantum dots for live cells, in vivo imaging, and diagnostics." Science *307*(5709): 538–544.

2534-Pos Resolving Entropic Recoil Trajectories in the First Stage of the Individual Folding Pathway of Ubiquitin

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We use a new fast AFM to study the first stage in the folding pathway of a single protein by capturing the individual recoil trajectories of an unfolded polyubiquitin. A single polyprotein is first unfolded to 89% of its contour length under a constant high force. The stretching