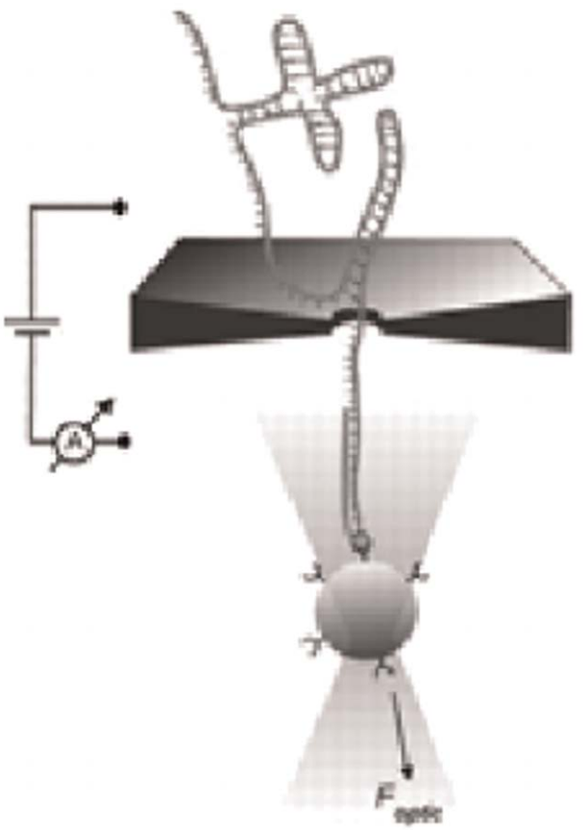
Board B646

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.



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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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Board B647

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.

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

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Board B648

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

force is then quickly relaxed (< 50 μs) to a lower value that still keeps the protein in a highly stretched conformation (> 50% contour length) and prevents the protein from collapsing to its folded length. The recoil trajectory between both stretched states shows an exponential evolution with a decay constant of 100–800 μs. We experimentally measure a linear relationship of the recoil time, τ_{recoil}, with the number of residues in the chain, *N*. Previous theoretical studies on polymers have predicted that the expected collapse time, $\tau_{col,}$ correlates with *N* as $\tau_{col} \sim N^{\gamma}$, with $2<\gamma<3$. These models apply to initial conformations exhibiting a radius of gyration of the order of $R_g \sim N^{3/5}$, characteristic of a solvated random coil. In contrast, our experiments probe stretched conformations with lengths in the order of $R_g \sim N$, which have not been fully explored. Interestingly, a significant variability in both the recoil length and rate is measured within individual trajectories. We hypothesize that such variability may be explained in terms of the different orientations of the side chains that are sampled in each individual trajectory. This work sets the stage to experimentally test the adequacy of existing statistical theories describing the early stages in protein folding and polymer collapse when the initial states involve highly stretched conformations. Our new AFM experimental design allows us to unveil unprecedented new information about relaxation processes at the single molecule level occurring on the microsecond timescale.

2535-Pos Multiple Interactions of Glycine-rich RNA-binding Proteins with Target Sequences Revealed by Single Molecule Force Spectroscopy

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Bielefeld University, Bielefeld, Germany.

Board B649

Post-transcriptional regulation represents an important mechanism to control gene expression in higher plants. It implies processes at various hierarchic levels including pre-mRNA maturation, mRNA transport, translation and breakdown. The major players are RNA-binding proteins that, by binding to defined RNA sequences, influence and control the fate of an mRNA molecule either directly or indirectly through protein-protein interaction.

We are using atomic force microscopy (AFM) based force spectroscopy to investigate the interaction of the Arabidopsis thaliana glycine rich RNA binding protein ATGRP8 with RNA target sequences at the single molecule level. By introducing mutations in the protein as well as in the RNA sequences in combination with improvements of the analysis technique for single molecule force spectroscopy data, we are able to discriminate sequence-specific from nonspecific interactions. This enables us to gain insights into the molecular binding mechanism.

2536-Pos Direct detection of UVA damage to DNA by Atomic Force Microscopy

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Board B650

Even though, UVA (320–400 nm) is the major component of solar UV light its genotoxic role in skin cancer has remained controversial and warrants further studies. Two models have been proposed to explain DNA lesions induced by UVA: the first model postulates that as yet an unidentified photosensitizer(s) (chromophore) is necessary to transfer light energy to DNA, while the second model proposes that DNA damage is directly caused by a weak (but non-zero) absorption of UVA by nucleobases.

To verify which of these models is correct, we examined UVA-induced DNA damage by implementing on the atomic force microscopy platform the classical supercoiled plasmid relaxation assay. We irradiated pUC18 supercoiled plasmids in physiological buffer (TE) and in pure water after dialyzing the DNA to remove putative chromophores that could have been co-extracted from E. coli cells. UVA-induced DNA damage sites such as pyrimidine dimers (CPD) and oxidized bases were converted into single strand breaks (SSB) by incubating pUC18 with appropriate DNA repair enzymes, such as T4 Endonuclease V (detects CPD), Fpg (detects oxidized purines) and endonuclease III (detects oxidized pyrimidines). These induced SSBs relax supercoiled plasmids and this drastic topological change was easily detected by AFM imaging of individual molecules. In this way we accurately determined fractions of plasmids relaxed by a given enzyme as a measure of enzyme-specific DNA damage. Importantly, we found that the purified and dialyzed DNA that was irradiated in distilled water developed a significant number of CPDs in the absence of other molecules that could play the role of the putative chromophore. Thus, our results seem to support the model that suggests that UVA may induce CPDs directly, without mediation of any photosensitizers.

2537-Pos A Bayesian Approach to 3D Position Determination on the Nanometer Scale by Single-Pair FRET Experiments

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Board B651

It is often desired to infer the relative position of a domain of a protein / nucleic acid complex that can't be localized by crystallographic means due to its high flexibility. Measurements of distances between two fluorescent dyes based on Fluorescence Resonant Energy Transfer (FRET) can yield this information if one dye molecule (target) is attached to the domain of interest and at least three distance measurements to three other dye molecules (anchors) sitting in different but well known positions are carried out. Since the domain of interest is highly flexible, single-molecule measurements have to be carried out in order to understand the underlying dynamics.

We present a new data analysis method for such experiments that is based on a Bayesian approach and capable of inferring the target dye position and its corresponding uncertainty.

We calculate the probability density distribution of the target dye position based on our measured data and display confidence volumes in the context of crystallographic structure. In contrast to ordinary trilateration this method is able to manage an arbitrarily large number of measurements and accounts for uncertainties in the specification of the anchor dye positions, the corresponding Förster radii and the FRET efficiency measurements itself. We apply this method to the study of yeast RNA polymerase II elongation complexes.

2538-Pos Single-Molecule Detection Of Folding And Unfolding Of The G-Quadruplex Using A Protein Nanocavity

Ji Wook Shim, Li-Qun Gu

University of Missouri, Columbia, MO, USA.

Board B652

Guanine-rich nucleic acids can form four-stranded, cation-stabilized quadruplexes that are useful in gene regulation, pharmaceuticals, biosensors and nanobiotechnology. Understanding quadruplex folding pathways is important in molecular recognition and rational design. We have proposed a supramolecular system in which a single G-quadruplex is encapsulated in the nanocavity of the α-hemolysin protein pore for single-molecule manipulation (Shim et al, 9F Micro- and Nanotechnology; Nanopores). Here, we report using this system to electrically observe how cations regulate quadruplex folding and unfolding reactions. Our target was the thrombin-binding aptamer (TBA), a 15-base DNA (GGTTGGTGTGGTTGG) that must fold into a two-tetrad, one-cation G-quadruplex to facilitate its function as an inhibitor to thrombin clotting and as a biosensor for protein detection. We established an analytical method to discriminate single TBA molecules in the folded and unfolded forms and to calculate the rate (k_f and k_u) and equilibrium (K_f) constants. Our single-molecule detection revealed that: (1) K_f values confirm an empirical ionic radii law that K^+, Ba^{2+} and NH_4^+, which have close ionic radii between 1.3–1.5 Å, are more efficient than other ions (Li^+, Na^+, Cs^+) to form a quadruplex with TBA. However, the specific folding and unfolding reactions are not determined by the ion geometry; (2) According to k_f and k_u data, high K_f values for K^+-, Ba^{2+}- and NH_4^+-quadruplexes are achieved through slow unfolding reactions; (3) Although Na^+- and Li^+-quadruplexes feature similar K_f values, they undergo different pathways: the Na^+-quadruplex folds and unfolds at the fastest rates, whereas the Li^+-quadruplex performs both reactions at the slowest rates; and (4) Divalent cation interactions with DNA can destabilize G-quadruplexes. This understanding of the ion regulation of G-quadruplex structures and processes could be significant in fine-tuning molecular properties for new nano-construction and biosensors.

2539-Pos Single Molecule Characterization of Bovine α-Chymotrypsin Using Microfabricated Cylindrical Chambers

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Board B653

Single molecule studies of α-chymotrypsin provide kinetics unique to individual molecules that bulk studies are unable to show. The isolation of α-chymotrypsin into single molecules was achieved by using a microfabricated array of cylindrical chambers designed 2um in diameter and 1.35um in height. A silicon wafer with cylindrical pillars served as the mold, and PDMS femtoliter chambers were created by filling the cavities of the mold. The concentration of α-chymotrypsin was held at 0.31nM to ensure that each 2um chamber enclosed 0.8 molecules of α-chymotrypsin on average. By performing a protease assay with casein conjugated to BODIPY Texas Red as the substrate and α-chymotrypsin as the enzyme, the fluorescence of each 2um chamber was captured by a charge-coupled device camera, recording an image every 15 minutes after one hour of incubation over a period of 5 hours. The turn-over rate of substrate to product in bulk was determined by performing the protease assay with 96-well microplates in the fluorometer, and results showed that 3.8×10^{10} molecules of enzyme turned over 6.0×10^{13} molecules of substrate per minute. By monitoring single molecules of α-chymotrypsin in microfabricated cylindrical chambers over time, the turnover rate of individual α-chymotrypsin could be determined.

2540-Pos Microfluidic Parallel Analysis of Low-Copy-Number Proteins in Single Cells

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Board B654

We have developed a microfluidic device that allows quantitative analysis of proteins from a single cell in a parallel fashion. Our device utilizes laser-induced fluorescence based on cylindrical optics as the detection modality, which enables highly efficient single-molecule detection of rare protein species. Lysis of individ-

ual cells with minimal sample dilution is achieved by creating picoliter-sized reaction chambers using pneumatically controlled microvalves. A new generation of this microchip is designed to enable simultaneous analysis of multiple lysates from single cells for higher throughput. The improvement of the fluorescence detection scheme for parallel screening is also discussed. As a demonstration, the phycobiliprotein profiles of laboratory-cultured cyanobacteria cells are analyzed. When extended to environmental samples that are difficult to culture, we expect this method to become a useful tool for studying biodiversity in microbial communities.

2541-Pos Single-Molecule Spectroscopy in Nano-fluidic Devices

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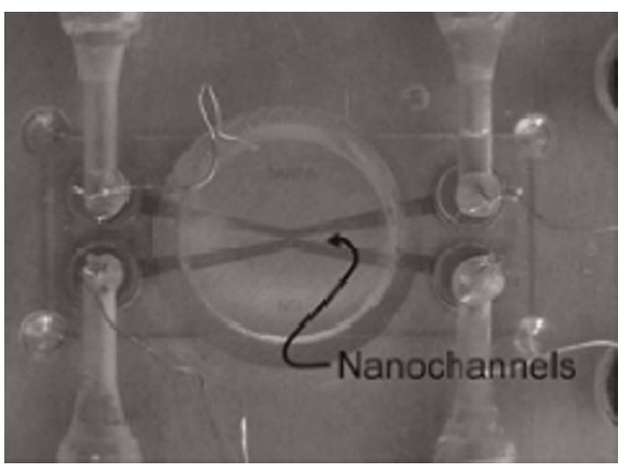
University of Tennessee Space Institute, Tullahoma, TN, USA.

Board B655

Single molecule detection by confocal fluorescence microscopy has become a powerful tool for elucidating complex biophysical processes in solutions of bio-molecules, such as proteins and nucleic acids. However, the limited diffusional residence time of a molecule within the femtoliter-sized probe volume presents a key constraint in many studies. We discuss single-molecule measurements within nanometer-sized channels fabricated between two microchannels within a fluidic device made of fused silica, as seen in the Figure. The nanochannel constrains the molecule to move along one dimension and the rate of diffusion is observed to be considerably slowed compared to that in bulk solution. Electrodes have been placed within the microchannels for electrokinetic transport of molecules along the nanochannels. We discuss experiments in which the single molecule is excited to levels approaching saturation in order to extract higher photon count rates for faster time resolution of molecular dynamics. For intense laser irradiation, background due to autofluorescence from the surrounding material becomes important and hence the device is fabricated from fused silica, which presents a lower autofluorescence than glass.



2542-Pos A Novel Nanosource For Fast Localised Dosing

CHAO LI[1], Joe D. Piper[1], Yuri Korchev[2], Liming Ying[2], David Klenerman[1]

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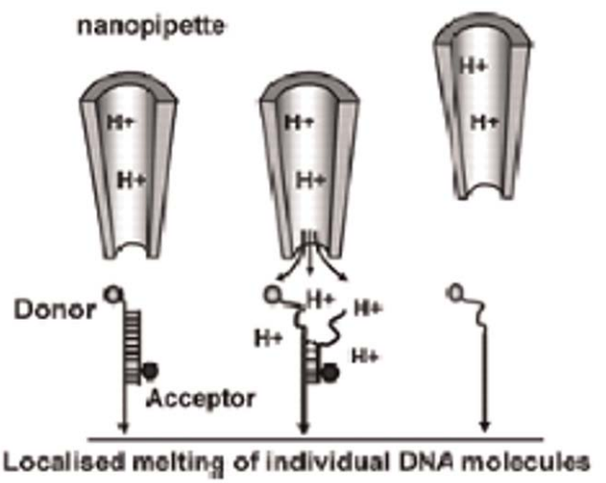
Board B656

There is a general need for extremely local and fast changes in concentration to trigger biological or chemical processes at a surface. In the Single Molecule field this would allow the study of catalysis and folding under non-equilibrium conditions. Here we present a simple and general method that is based on the use of a nanopipette to locally dose an area of a few microns, using distance feedback control based on the ion current through the tip.

Using Total Internal Reflectance Fluorescence (TIRF) and confocal imaging, we have quantified the performance of nanopipette dosing for writing with biotinylated DNA and for triggering local reactions. We achieved 100 times faster writing by pulsed dosing than previous continuous delivery[1]. Furthermore, we characterised the dosing of Na^+ and H^+ with time, achieving a fine control of the delivery area from 1 to 50 μm within 10ms.

Such fast localised dosing is especially useful for triggering individual molecules without flooding the sample surface. To demonstrate this, acid was applied to locally melt individual DNA molecules, which was traced by changes in fluorescence resonance energy transfer.



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2543-Pos Imaging Axonal Transport In A Microfluidic Device

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Board B657

Nerve growth factor (NGF) signaling begins at the nerve terminal, where it binds and activates the receptor tyrosine kinase TrkA; the NGF signal is then subsequently transmitted retrogradely to the cell body through the axon. Here we observed the direct real-time observation of NGF retrograde transport in live neurons. Transport of individual NGF-containing endosomes was imaged using quantum dots conjugated NGF in combination with pseudo total-internal-reflection-fluorescence microscopy. Our results revealed that the active transport of NGF was comprised of rapid, directional movements interrupted by frequent pauses. The net movement was exclusively retrograde, but short-distance antegrade movements were observed occasionally. Furthermore, quantitative analysis at the single molecular level demonstrated that the majority of endosomes contained only a single NGF dimer, raising the possibility that a single NGF dimer is sufficient to sustain signaling from an endosome during transport to the cell body.

2544-Pos Porous Biomimetic Nanocontainer for Single Molecule Fluorescence Study

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Board B658

Lipid vesicle can serve as a nanocontainer allowing single molecule fluorescence measurements of freely diffusing fluorescently labeled bio-macromolecules over time range of seconds to minutes in a biologically relevant environment. One serious limitation of vesicle encapsulation method is that the lipid membrane is practically impermeable to most of the ions and small molecules, limiting its application to studying single molecule dynamics in equilibrium. Recently, we developed a porous nanocontainers for single molecule fluorescent study by using Staphylococcal toxin α-hemolysin pores. α-hemolysin is a water soluble protein which can spontaneously insert into the membrane and oligomerize to form pores. Transmembrane permeability of ions (magnesium and potassium) and small molecule (ATP) through α-hemolysin pores have been demonstrated with three different types of biological systems: RNA: hairpin ribozyme; DNA: G-quadruplex; DNA-protein: Rep helicase. Upon exchange of ligand concentration, synchronous dynamic change has been observed using single molecule FRET measurement at room temperature and at 37 °C, while molecules in vesicles without α-hemolysin remained unaffected. This temperature independent porous container is an ideal tool for studying transient and weakly interacting biological systems under various solution conditions.

2545-Pos Single Molecule Coiled Coil Unzipping

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Board B659

Coiled coils play an important role in many mechanical processes in the cell. Examples are ranging from dimerisation of double headed motor proteins to fusion of synaptic vesicles. Yet few is known about the mechanical forces associated with coiled coil formation or dissociation.

We use atomic force microscopy to mechanically unzip and rezip single coiled coil molecules based on dimerisation motifs from three different molecular motors and from the yeast transcriptional activator (GCN4). In particular we examined the neck regions of kinesin from neurospora crassa and drosophila melanogaster as well as the proximal tail from myosin VI. The observed unzipping forces for all constructs occur in a force regime below 16 pN and are very specific. While unzipping of the GCN4 zipper occurs close to thermal equilibrium, unzipping of all other constructs is shifted towards non-equilibrium. By determining the equilibrium free energies for all constructs we identify the myosin VI sequence as the least stable one, followed by both kinesin coiled coils and GCN4 as the most stable construct.

2546-Pos A New Strategy To Measure Torque On Isolated DNA

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Board B660

Measurement of torque on isolated DNA molecules is essential to complete the knowledge accumulated in the past 20 years through single DNA force experiments. The behaviour of DNA under an applied external force has been extensively characterized and many are the single molecules studies on its interaction with protein motors such as topoisomerases, helicases, chromatin remodelling factors, polymerases etc.

Independently of the technique used, the observable quantities in most of these studies are the force applied to the molecule and its extension. Bending deformations can thus be studied in a direct way through the observation of length variations. A further characterization is possible through the use of magnetic tweezers, that allow to indirectly monitor the variations in the torsional state of a torsionally constrained DNA molecule.

DNA is a thin helical molecule and proteins acting on it are very likely to apply torque in order to deform it. It would thus be natural to study the torques involved in DNA-protein interactions in conditions similar to the force measurement mentioned above. Until very recent, direct torque measurement on DNA was not possible due to technical limitations of most micro-manipulation techniques.

We have developed a new strategy, based on magnetic tweezers, to measure the torque and the force applied on a DNA molecule and we will discuss its application to supercoiled DNA molecules.

2547-Pos Dwell Time Analysis Of A Single Molecule Mechanochemical Reaction

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Board B661

Force-clamp spectroscopy is a novel technique to study mechanochemistry at the single bond level. Single disulfide bond reduction events are accurately detected as stepwise increases in the length of polyproteins that contain disulfide bonds and that are stretched at a constant force with the cantilever of an atomic force microscope (AFM).

To examine the kinetics of reduction of disulfide bonds measured from single molecule force-clamp spectroscopy traces we use dwell time analysis. Exponentially distributed dwell time data is plotted as a histogram with a logarithmic time scale and a square root ordinate. The advantage of logarithmic histograms is that exponentially distributed dwell times appear as well defined peaks in the distribution, greatly enhancing our ability to detect multiple kinetic pathways.

We apply this technique to examine the distribution of dwell times of a few thousands single disulfide bond reduction events measured in the presence of two very different kinds of reducing agents: tris-(2-carboxyethyl)phosphine hydrochloride (TCEP) and the enzyme thioredoxin (TRX) from *E.Coli*, or human TRX (hTRX). In the case of TCEP, the logarithmic histogram of dwell times showed a single peak, corresponding to a single reaction mechanism. By contrast, similar experiments done with TRX showed two well separated peaks, marking two distinct modes of chemical reduction operating simultaneously[1]. Logarithmic histograms for different clamping forces in the case of hTRX allowed direct decoupling of the previously postulated[2] force-dependent and the force-independent disulfide reduction mechanisms.

References

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2548-Pos Geometry Of Mediating Protein Affects The Probability Of Loop Formation In DNA

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Board B662

Recent single molecule experiments have determined the probability of loop formation in DNA as a function of the DNA contour length for different types of looping proteins. The optimal contour length for loop formation as well as the probability density functions have been found to be strongly dependent on the type of looping protein used. We show using Monte Carlo simulations and analytical calculations that these observations can be replicated using the worm-like-chain model for double stranded DNA if we account for the non-zero size of the looping protein. The simulations have been performed in two dimensions so that bending is the only mode of deformation available to the DNA while the geometry of the looping protein enters through a single variable which is representative of its size. We observe two important effects that seem to directly depend on the size of the enzyme:

(i) the overall propensity of loop formation at any given value of the DNA contour length increases with the size of the enzyme, and
(ii) the contour length corresponding to the first peak as well as the first well in the probability density functions increases with the size of the enzyme.

Additionally, the eigenmodes of the fluctuating shape of the looped DNA calculated from simulations and theory are in excellent agreement and reveal that most of the fluctuations in the DNA occur in regions of low curvature.

2549-Pos Lifetimes of Multiple P-selectin/PSGL-1 Bonds Suggest Force History-Independence

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Board B663

It has been suggested by us and others that the lifetimes of receptor-ligand complexes may depend on both the instantaneous force experienced by the bond and the history of force application, an idea known as force history-dependence. We tested this hypothesis by using the distribution of load between multiple receptor-ligand bonds to create a complex loading history. Briefly, chimeric P-selectin was covalently coupled to polystyrene beads. Human P-selectin glycoprotein ligand-1 (PSGL-1) was adsorbed to nitrocellulose-coated glass beads on a coverslip. A P-selectin bead was held in a laser trap transducer and touched to the vertical surface of a glass bead, allowing one or more bonds to form with the ligand-coated surface. The laser trap was deflected away from the microsphere, applying a nearly instantaneous load. When multiple bonds were present, we were able to discern the ruptures of each bond as a step displacement of the trapped bead away from the stationary bead. In this way we were able to monitor both the number of bonds and the lifetime of each bond. When more than one bond was present we assumed that the applied load was evenly distributed between them; we were thus able to monitor bond lifetimes across complex loading histories as the bonds ruptured asynchronously. Bonds displayed

catch-slip behavior (first rising, then falling lifetimes with increasing force) regardless of whether they were single or multiple bonds. Thus catch bond behavior cannot be explained simply by the presence of multiple bonds. Further, the lifetime of single bonds was similar to the lifetime of bonds that had previously shared load with others. These data suggest that P-selectin/PSGL-1 bonds do not display strong force history-dependence.

2550-Pos DNA Torsional Properties

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Board B664

DNA torsional properties determines the energetics of enzymes and DNA interactions. We develop a new magnetic tweezers instrument that uses a magnetic nanorod instead of a bead to pull a single DNA molecule. We measure torsional stress of the DNA molecule from the distribution of the nanorod angular thermal fluctuations. Two kinds of experiments can be performed according to the applied magnetic field:

1. Free angular fluctuations mode, in which the twisting rigidity of the molecule without torque is obtained
2. Restricted angular fluctuation mode, in which a known torque is applied to the molecule.

We attach a DNA molecule to a magnetic nanorod 200 nm diameter and 5 micron in length. Magnetic nanorods are custom made with segments of Pt and Ni to obtain specific magnetic properties. Pulling forces in the range of 0.01 to 1 pN and torques between 0 and 100 pN nm are applied. Using this instrument, we measure the elastic energy of DNA as a function of DNA length and pulling forces. Torques required to twist buckle DNA are obtained.

2551-Pos Kinetics of Binuclear Ruthenium Complex Threading Through DNA Bases

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[2] *Nano-Science Center, University of Copenhagen, Copenhagen, Denmark,*
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Board B665

The binuclear ruthenium complex $\Delta\Delta$-[μ-(bidppz)(phen)$_4$Ru$_2$]$^{4+}$ has been shown to intercalate DNA with high affinity and has slow dissociation, which is an important characteristic in therapeutic application for cancer. Due to steric constraints, this complex can only intercalate DNA by threading through its bases, which requires local melting of the DNA to occur. In bulk experiments, such threading intercalation can be monitored spectroscopically, but it occurs extremely slowly despite the high binding affinity that this molecule exhibits to DNA. By stretching single DNA molecule with optical tweezers in the presence of the binuclear intercalator, we can directly monitor DNA intercalation as the DNA length increases upon intercalative binding. In addition, the application of force causes intercalation to occur at higher rates as the DNA is destabilized by force and intercalation is favored by force. By observing the lengthening of DNA held at a constant force in the presence of different concentration of these molecules, we directly determine the rate of intercalation and the equilibrium fractional ligand binding. The intercalation rate and amount of intercalator bound at a given ligand concentration is observed to depend strongly on force. The results yield quantitative insights in our understanding of the thermodynamics and kinetics of this complex binding process.

2552-Pos Single-Molecule Force Spectroscopy Studies of Protein Folding Under Denaturing Environment

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Rice University, Houston, TX, USA.

Board B666

Single molecule force spectroscopy has given us insight into molecular systems that were previously considered unobservable. By use of the Atomic Force Microscope, we can measure forces exerted on molecules when they are being stretched. Recently, a method has been pioneered in our lab that allows us to reconstruct the free energy surface of a single domain of the human cardiac muscle protein, titin. Using this surface, we can determine the free energy unfolding barrier, ΔG_u , at a specific environment. In our experiment, we used the AFM to stretch a polyprotein consisting of eight serially linked domains of the I27 domain of titin under various concentrations of Guanidine Hydrochloride. We measured the force at which the domains unfolded and calculated ΔG_u for each denaturant concentration. We then observed the trend in the most probable unfolding forces and ΔG_u as a function of denaturant concentrations. Our results show that the average unfolding forces and ΔG_u decrease as a function of denaturant. When compared to bulk solution denaturant studies, the obtained values compare favorably.

2553-Pos Comparison Between Calculated and Experimental Pulling Forces on Single Molecules of dsDNA in a Magnetic Tweezers Instrument

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Board B667

Magnetic tweezers are used in single-molecule manipulation experiments to probe systems such as supercoiled DNA and the mechanism of topoisomerases. In this technique, an external magnetic field interacts with a superparamagnetic bead to exert sub-pN to pN forces and torque on a tethered molecule. In this study, we compare expected forces on a bead tethered by dsDNA to empirically determined forces in DNA pulling experiments. Magnetic fields are calculated and experimentally characterized for a pair of rare earth magnets. From knowledge of the field strengths and the magnetization properties of the bead, the expected force on a bead is calculated. By comparing these forces with those obtained by the traditional analysis of the variance of a tethered bead, we find important experimental factors to consider. We present key corrections involved in the analysis of the experimental data, which account for instrumental drift and spherical aberration when using an oil-immersion objective. Also, we describe the difference in Brownian motion of the bead when viewed parallel and perpendicular to the direction of the magnetic field.

2554-Pos Photobleaching Effects Of Optical Trap Radiation On Fluorescent Dyes

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Board B668

The direct combination of optical tweezers force microscopy with single molecule fluorescence detection has long been complicated by accelerated photobleaching effects. This phenomenon, which significantly reduces fluorescence emission time, is thought to be due to the population of photoreactive triplet states by the high photon flux found in an optical trap. However, the excitation of these states can be avoided through the use of Interlaced Optical Force-Fluorescence Spectroscopy (IOFF), which alternately modulates optical trapping and fluorescence excitation lasers. The effectiveness of this approach, which has been previously demonstrated for the single molecule dye Cy3, is directly influenced by the different electronic structures of popular fluorescence dyes. In this work, we present a systematic study of several popular fluorescence dyes to quantify those that are most effected by trap induced photobleaching, such as Cy3, and those that are nearly unaffected, such as fluorescein. In addition to these results, we show new evidence of the photoelectronic mechanism of trap induced photobleaching, which is characterized by higher rates of reverse intersystem crossing between fluorescent singlet states and dark, photoreactive triplet states. These results will facilitate more effective application of IOFF and better design of single molecule experiments.

2555-Pos Extending The Possibilities Of Optical Manipulation With Microstructures Of Special Shape

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Board B669

Laser tweezers are used to trap micrometer sized particles. In the basic case, the location of the particle is determined but not the orientation. Often it is desirable to have additional control: it is advantageous if the orientation of the grabbed particle is also determined. There are several ways to achieve orientational control.

It is an efficient possibility to use non spherical particles as test object. The anisotropy of the light scattering by the non spherical object will have an orientational effect upon the trapped particle. In addition, if the light forming the tweezers is also polarized, additional polarization effect can be achieved. We generated by photopolymerisation micrometer sized objects of different well defined shapes to study the effect in detail.

We investigated two basic approaches: If we generate a helical shape, it will rotate in the optical traps. Such rotors, propellers can utilize different components of the momentum of light. They can be even used to drive optomechanical systems. If the optical tweezers are formed by linearly polarized light, a flat object will be oriented in the trap. This effect can be used to order, orient particles. Torque can be measured and exerted by this system, giving a versatile tool for single particle manipulation. In addition, the torque exerted can be adjusted largely independently from the trapping force We used this method to determine torsional properties of biopolymers like actin, dsDNA. We determined the torsional elasticity of single DNA molecules at different extensions and tested different models of twist storing polymers used to characterize the molecule.

2556-Pos Experimental Estimation of the Free Energy Landscape Roughness of Protein Molecules

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Board B670

A protein's folding behavior is determined by its free energy landscape. Characterizing these landscapes experimentally is difficult due to their high dimensionality and irregularity. The polymeric nature of the polypeptides and the heterogeneity of the amino acid sequence cause frustration during folding and make the free energy landscape rugged. Mechanical unfolding and refolding experiments probe the structural and energetic changes during folding and unfolding of individual protein molecules along a well-defined reaction coordinate, providing direct experimental evidence of the shape and roughness of the free energy landscape. According to a theoretical model developed by Hyeon and Thirumalai (PNAS, 2003), the roughness of the free energy landscape of a protein

molecule can be determined from unfolding forces measured at different temperatures and force loading rates. We have measured the unfolding forces of tandem repeats of Ubiquitin and Titin I27 as a function of temperatures from 5 to 45 degrees C and force loading rates from 2.5 to 250 nanonewtons per second using the atomic force microscope. At the same force loading rate, the unfolding forces were observed to increase as the temperature decreases. These data allow us to estimate the roughness of the free energy landscape of the protein molecules.

2557-Pos Energy Landscapes For Folding And Signaling In A Photoreceptor By Single Molecule Measurements And Coarse-grained Calculations

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Board B671

Receptor activation is a fundamental process in biological signaling. We study the structural changes during activation of photoactive yellow protein (PYP). This is triggered by photoisomerization of the *p*-coumaric acid (pCA) chromophore of PYP, which converts the initial pG state into the activated pB state. Mechanical unfolding of Cys-linked PYP multimers probed by atomic force microscopy (AFM) in the presence and absence of illumination reveals that the core of the protein is extended by ~3 nm and destabilized by ~30% in pB. These results establish a generally applicable single molecule approach for mapping functional conformational changes to selected regions of a protein and indicate that stimulus-induced partial protein unfolding can be employed as a signaling mechanism. Comparative measurements, Jarzynski-Hummer-Szabo analysis of the data, and steered MD simulations of two double-Cys PYP mutants reveal strong anisotropy in the unfolding mechanism along the two axes defined by the Cys residues. Unfolding along one axis exhibits a transition-state-like feature where six hydrogen bonds break simultaneously. The other axis displays an unpeaked force profile reflecting a non-cooperative transition, challenging the notion that cooperative unfolding is a universal feature in protein stability. Coarse-grained protein model calculations show that the folding of pG is two-state, consistent with experimental observations. In contrast, the folding free energy surface of pB involves an on-pathway partially unfolded intermediate that closely matches experimental data. The results reveal that interactions between the pCA and its binding pocket can switch the energy landscape for PYP from two- to three-state folding, and show how this can be exploited to trigger large functionally important protein conformational changes.

2558-Pos F_1-ATPase with Fused γ and β Subunits

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Board B672

F_1-ATPase is an ATP-driven rotary motor in which the central γ-subunit rotates inside a cylinder made of $\alpha_3\beta_3$-subunits. Crystal structures of Mitochondrial F_1 indicate that the C- and N-termini of the γ-subunit form a coiled-coil structure which contacts with the αβ hexamer. In several studies, the γ-subunit has been truncated at its C-terminus, and the results indicate that the C-terminal tip is not essential for rotation and ATP hydrolysis. To learn whether the N-terminal residues of the γ-subunit are necessary for driving rotation, we deleted N-terminal residues of the γ-subunit of F_1 derived from thermophilic Bacillus PS3. However, these mutants tended to lose the truncated γ-subunit during purification. To obtain stable mutants, we connected genetically the C-terminus of the γ-subunit to the N-terminus of the β-subunit with a peptide linker and expressed this $\alpha_3\beta_3\gamma_3$ complex. This γ-β fusion mutant showed an almost the same ATP hydrolysis activity as with wild type and its rotational speed, monitored by attaching a duplex of 0.29-μm beads to the γ subunit, was comparable to that of the wild type. We are now deleting N-terminal residues of the γ-subunit in this γ-β fusion mutant and will measure the ATP hydrolysis activity and the rotational torque. We will discuss the role of N-terminus of the γ-subunit in rotation.

2559-Pos Probing Single Molecule Dynamics with Molecular Dynamics Simulation and Single Molecule Tracking

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Board B673

Research on cell and artificial membranes has revealed heterogeneous dynamics of phospholipid and protein components. Biophysical research has shown that phospholipids and membrane proteins diffuse not only under normal Brownian diffusion, but with confined and anomalous behavior. We are motivated to understand this anomalous diffusion because cells may use it to control signaling. We use single molecule tracking on phase separated, supported lipid bilayers to investigate the origin of this unusual diffusion and how it is affected by bilayer composition. Most fluorescence microscopy techniques, including single molecule tracking, assume that the fluorescent molecule being observed mimics the behavior of the molecules of interest. There is some question about whether this assumption is accurate, especially for large, charged fluorescent molecules, such as those used in single molecule tracking. With molecular dynamics simulations of Texas Red molecules in a DPPC

bilayer, we examine how the dynamics of the Texas Red molecule compare to those of the lipids and how the large fluorescent molecule may affect the structure of the bilayer. Together, single molecule tracking and molecular dynamics simulations will allow us to develop a clearer picture of the dynamics taking place at the single molecule level in model membrane systems.

2560-Pos Live Tracking Of Single Cellular Prion Protein Prp Using Peptide-coated Quantum

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Board B674

Prp, a glycosylphosphatidylinositol (GPI) anchored membrane protein, is believed to play a central role in prion diseases. The GPI anchor of prp is important for its membrane association and endocytic trafficking, both of which are crucial for the interactions between cellular prp and pathologically altered isoform, prion amyloids. To gain insights into the details of prp diffusion and trafficking, we use FITC-functionalized peptide-coated quantum dots to track the movement of prp fused to a single chain antibody against FITC. Here we present preliminary results of real time single molecule tracking of prp in live neuronal cells.

2561-Pos Effect Of Zipcode Sequence And Molecular Weight Of mRNA On Its Subcellular Localization Revealed By Single Molecule Imaging

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Board B675

In chicken embryo fibroblasts (CEFs), β-actin mRNA localizes at the leading edge, which is essential for cell motility. It was reported that mRNA localization requires a zipcode sequence in its 3′ untranslated region (3′ UTR). However, the localization of β-actin mRNA without the zipcode has never been observed in CEFs. In this study, we observed the localization of β-actin mRNA in CEFs using single molecule fluorescence microscopy to evaluate the influence of the zipcode and molecular weight on the localization of mRNA. To visualize β-actin mRNA, we employed a method involving a fusion protein consisting of GFP linked to the RNA binding coat protein of the phage MS2. We used the following three mRNAs written from 5′ end:

(i) 5′ UTR, ORF, 3′ UTR and MS2 tag (1,116 kDa),
(ii) 5′ UTR, ORF and MS2 tag (945 kDa),
(iii) 5′ UTR, 3′ UTR and MS2 tag (750 kDa).

Unexpectedly, the mRNAs localized regardless of the presence of 3′ UTR. Larger mRNAs were more accumulated at the leading edge than smaller mRNAs, indicating that higher molecular weight induces more localization. The same phenomenon was also observed with fluorescently labeled dextrans (2,000 kDa and 70 kDa). This may be the result of the fact that larger molecules are excluded from some small cavities, known as molecular sieving effect. To understand the mechanism of this effect, we measured the diffusion coefficients (D_{MACRO} ($\tau \sim 0.3$ sec) and D_{micro} ($\tau \sim 0$ sec)) of mRNA located on and off the leading edge. The ratios between the coefficients on and off the edge were 99 for D_{MACRO} and 1.6 for D_{micro}, respectively, implying the existence of a microarchitecture which can contribute to the molecular sieving effect.

2562-Pos Single Molecule Force-clamp Spectroscopy Reveals Evolutionary Signatures Of Thioredoxin Activity

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Board B676

Predicting functional aspects of enzymatic catalysis a priori, solely with knowledge of changes in protein sequence through evolution, remains a major challenge. We have recently demonstrated single molecule force clamp spectroscopy as a novel method to study the catalytic mechanism of the enzyme thioredoxin, a ubiquitous disulfide bond reductase. By monitoring the effect of a range of forces on the catalytic rate, we can obtain insight into both the catalytic mechanism of thioredoxin as well as dynamic, sub-Å ngström conformational changes which occur during catalysis [1]. Here we use force-clamp spectroscopy to probe variations in enzymatic catalysis resulting from correlated mutations in E. coli thioredoxin evolution, as identified through a statistical tool for sequence analysis. Using this single molecule method, we find that mutations in coupled residues which are either positively (D26/P34) or negatively (P34/G74) correlated result in substantial changes in the force-dependency of the catalytic rate. Significantly, these changes in rate agree with the sign of the correlation. These effects can not be identified in a typical bulk biochemical assay of thioredoxin activity. We interpret these results in terms of alterations in the protein-protein interaction between enzyme and substrate, as well as changes occurring in the chemistry of the enzymatic active site. These results demonstrate a significant advance in combining the prediction and exploration of the catalytic activity of an enzyme solely using knowledge of protein sequence. With these experiments we further show the potential of force clamp spectroscopy as a sensitive new tool to probe the effects of variations in enzymatic chemistry on the sub-Ångström scale.

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Single Molecule Biophysics - IV

2563-Pos Direct Imaging Of Human Rad51 Nucleoprotein Dynamics On Individual Double-Stranded DNA Molecules

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Board B677

Human Rad51 protein (Rad51) is central to recombinational repair of double-strand DNA breaks. It functions by polymerizing onto DNA and promoting the pairing and exchange of DNA strands between the damaged and intact homologous chromosomes. We visualized the real-time assembly and disassembly of Rad51 nucleoprotein filaments on individual double-stranded DNA molecules by single-molecule fluorescence microscopy. Rad51 assembly extends the DNA by 65%. Nucleoprotein filament formation occurs via rapid nucleation followed by growth from these nuclei. However, growth does not continue indefinitely, and nucleoprotein filaments terminate when 1–2 kb in length. The dependence of nucleation on of Rad51 concentration, suggests that ~3 Rad51 monomers are involved in the initial binding events. Rad51 nucleoprotein filaments are stable and remain extended when ATP hydrolysis is prevented; however, when ATP hydrolysis is permitted, filaments decrease in length as a result of protein dissociation and/or conversion to ADP-bound nucleoprotein complexes. Dissociation of Rad51 from dsDNA is slow and incomplete, demonstrating the necessity for a dsDNA translocating enzyme to accelerate disassembly.

2564-Pos Observing Prokaryotic Nucleotide Excision Repair: one molecule at a time

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Board B678

Nucleotide excision repair (NER) corrects DNA damage induced by a range of insults, from UV-induced thymine dimers to protein-DNA crosslinks. Here, we study the prokaryotic NER system from B. caldotenax which is comprised of three proteins: UvrA, UvrB, and UvrC. These proteins bind and operate sequentially to remove a 12–13nt oligonucleotide containing the DNA lesion. By using fluidics to suspend DNA strands between surface immobilized beads, we create tightropes upon which Qdot-labeled expressed Uvr proteins are seen to bind and slide. Using this assay we probe the interaction between DNA and the first two enzymes in the repair process: UvrA and UvrB. We have observed two modes of DNA interaction for UvrA: a transient static mode and a diffusive mode. The static interactions have an average lifetime of ~10s, and the diffusive interactions are slower than the predicted barrier-less diffusion constant on DNA. At 1M salt, the diffusion constant increases towards barrier-less diffusion suggesting electrostatic interactions are important for its physiological interaction with DNA. In agreement with solution studies we observe that UvrB requires UvrA to bind DNA. However, we find UvrB does not stably bind DNA, instead UvrB too shows two modes of interaction: transient static, and diffusion between defined regions on the DNA. Using two color differential labeling of UvrA and UvrB, we have observed rapid coincident diffusion under high salt conditions, mirroring UvrA alone. At physiological ionic strength, whether UvrA assists in UvrB's diffusive motion remains to be established. These experiments mark the beginning of a new single molecule approach to understanding the kinetics and order of processes that underlie prokaryotic NER. To our knowledge this is the first time a multi-protein complex has been visualized at the single complex level using differentially Q-Dot labeled constituent proteins.

2565-Pos Single Molecule Study Of Addab Helicase

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Board B679

DNA helicases are multi-functional motor proteins which translocate along DNA using the energy released from ATP hydrolysis. Translocation is coupled to diverse enzymatic activities, such as duplex unwinding, strand degradation and protein displacement. Resolving such complex biochemical properties requires a sensitivity and time resolution high enough for detecting rare and short-lived stochastic events. Unlike bulk measurements, single molecule approaches fulfil this requirement. We have developed a novel fluorescence-based assay, which allows us to monitor DNA unwinding by single helicases *in vitro*. Biotinylated double-stranded DNA (dsDNA) fragments are specifically immobilised on poly-ethylene glycol-coated surfaces through biotin-streptavidin interaction. Total internal reflection fluorescence (TIRF) microscopy is used to achieve high signal-to-noise ratio. The helicase activity is probed by a fluorescently labelled version of the *E.Coli* single-stranded DNA-binding protein (SSB), which preferentially binds single-stranded DNA (ssDNA). Single, helicase-mediated DNA unwind-