956-Pos

Fluorescence Cross-Correlation Spectroscopy as a Universal Method for Protein Detection with Low False Positives

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Specific, quantitative and sensitive protein detection with minimal sample preparation is an enduring need in biology and medicine. Protein detection assays ideally provide quick, definitive measurements that use only small amounts of material. Fluorescence cross-correlation spectroscopy (FCCS) has been proposed and developed as a protein detection assay for several years. Here, we combine several recent advances in FCCS apparatus and analysis to demonstrate it as an important method for sensitive, quantitative, information-rich protein detection with low false positives. The addition of alternating laser excitation (ALEX) to FCCS along with a method to exclude signals from occasional aggregates leads to a very low rate of false positives, allowing the detection and quantification of the concentrations of a wide variety of proteins. We detect human chorionic gonadotropin (hCG) using an antibody-based sandwich assay, and quantitatively compare our results with calculations based on binding equilibrium equations. Furthermore, using our aggregate exclusion method, we detect smaller oligomers of the prion protein PrP by excluding bright signals from large aggregates.

957-Pos

Direct Measurement of Heating by Optically Trapped Gold Nanoparticles Using Molecular Sorting in a Lipid Bilayer

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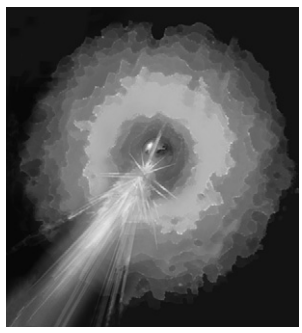
Gold nanoparticles have are extremely useful as multi-functional and sensitive probes for investigation and manipulation of biological systems. Particles, as small as tenths of nanometers, can be visualized, optically manipulated, and used as controlled force transducers. Their high absorbance also makes them excellent converters of electromagnetic radiation into thermal energy. If the heating can be quantified it can also be advantageously used to perform controlled thermal treatment. We performed a direct measurement of the heating associated with optical trapping of individual gold nanospheres. A trapped gold nanosphere was embedded in a two dimensional supported gel phase lipid bilayer with incorporated fluorescent molecules which preferentially located, e.g., in the gel phase. Visualization of the melted region gave direct information about the temperature profile around the irradiated particle. The heating is highly dependent on particle size and laser power, with surface temperatures increasing from a few to hundreds of degrees Celcius. This quantification allows for creating controlled and localized temperature gradients which can be utilized for destruction of unwanted biological material such as cancer cells, to create local temperature gradients in lipid bilayers, or for nano-engineering purposes.



958-Pos

Covalent-Bond-Based Immobilization Approaches for Single-Molecule Fluorescence

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The streptavidin-biotin bridge is commonly used in single-molecule studies to surface immobilize biomolecules onto microscope slides. However, the presence of tryptophanes impedes utilization of UV light and numerous fluorescent nucleotide analogs, such as 2-aminopurine. We present two new approaches to surface-immobilize nucleic acids for single molecule fluorescence experiments using covalent bonds and self-assembled monolayers instead of the traditional avidin-biotin linkage. The first approach takes advantage of a click-chemistry reaction between an azide and an alkyne to surface-immobilize nucleic acids through the resulting triazole linkage. The second approach uses disulfide bond bridges for immobilization. We have characterized the properties of the resulting surface-immobilized fluorophore-labeled DNA molecules and single-molecule fluorescence detection. We find that both approaches are specific and yield comparable surface densities and low fluorescence background to the avidin-biotin linkage, but offer new surface chemical properties that might be advantageous to prevent non-specific binding of biopolymers to the surface and to expand the range of fluorescent probes that can be employed in single molecule studies.

959-Pos

Combined Fluorescence and Force Microscopy to Study Lipid Transfer from Lipoproteins to the Supported Lipid Bilayers

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Advances in nanobiotechnology have been driven by continuous refinement of observation and manipulation technologies, enabling the study even of single biomolecules. From the advent of ultra-sensitive microscopy, the combination of force and fluorescence techniques was pursued as a way to gain additional insights into protein mechanisms. The combination of high spatial and topographical resolution of the AFM with chemical contrast and high time resolution achieved in fluorescence microscopy rendered such an instrument in particular attractive for membrane biophysics to identify and characterize the properties of nanoscopic structures in supported lipid bilayers, or the cellular plasma membrane. By utilizing the AFM tip as a nanopipette, a single bioparticle can be delivered in a controlled way to its receptor directly in the cellular plasma membrane. Upon specific delivery of one particle attached to the AFM tip per time, the flux of fluorescently labeled molecules out of the particle into a supported lipid bilayer (SLB) or the plasma membrane of various cell lines was measured. In particular, the transfer of the fluorescently labeled lipids DiI and Bodipy-Cholesterol from HDL particles to a supported lipid bilayer or the cellular plasma membrane is addressed. In order to characterize the influence of the lipid and protein environment, particles are brought into contact with different regions of a phase separated bilayer or the plasma membrane of living cells by performing force distance cycles. The measurement results clearly indicate that a transfer can only be detected when a HDL gets in contact with a SLB. The devised measurement mode is envisioned to enable analogues experiments for similar bioparticles such as viruses or other lipoproteins; even the interaction between cytoplasmic vesicles and the cytosolic leaflet of the plasma membrane may become addressable.

960-Pos

Unraveling the Dynamics of TBP-NC2 with Hidden Markov Modeling

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One of the early steps in initiation of transcription is the binding of the TATA box Binding Protein (TBP) to a core-promoter TATA Box. After binding, the transcription complex is assembled in cooperation with our general transcription factors. The binding of TBP to DNA is also a prime target for regulating gene transcription and many cofactors can interact with TBP at this initial stage.
In a recent publication (Schluesche et al), we showed that the binding of the Negative Cofactor 2 (NC2) leads to dynamic behavior of the TBP-NC2 complex along the DNA using single-pair Förster Resonance Energy Transfer (spFRET). To extract detailed kinetic information from the single molecule experiments, we have adapted a Hidden Markov Model to analyze the spFRET data collected using a EMCCD camera. With add of the HMM analysis, it is possible to determine how many states are available which transitions are possible, providing new insights into the origins of these different states.
We performed measurements on four DNA strands, two containing the AdML promoter with identical labeling but different DNA lengths and two containing the H2B promoter with labels at two different positions on the DNA. Results of the AdML promoter showed the high reproducibility of the analysis method. Four FRET states were clearly observable. For the H2B promoter, many more states were observable in the HMM analysis showing a high complexity of dynamics but yet very similar patterns as observed for the AdML promoter.
Schluesche et al 2007 NSMB 14:1196-1201

961-Pos

Visual Biochemistry: High Throughput Single Molecule Imaging of Protein DNA Interactions

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Our group uses single-molecule optical microscopy to study fundamental interactions between proteins and nucleic acids - we literally watch individual protein molecules or protein complexes as they interact with their DNA substrates. Our overall goal is to reveal the molecular mechanisms that cells use to repair, maintain, and decode their genetic information. This research combines aspects of biochemistry, physics, and nanoscale technology to answer questions about

complex biological problems that simply can not be addressed through traditional biochemical approaches. The primary advantages of our approaches are that we can actually see what proteins are bound to DNA, where they are bound, how they move, and how they influence other components of the system - all in real-time, at the level of a single reaction. Our research program is focused on studying the regulation and activity of proteins that are involved in repairing damaged chromosomes. We are particularly interested in determining the physical basis for the mechanisms that proteins use to survey DNA molecules for damage and initiate repair processes, and how these initial steps are coordinated with downstream events that lead to completion of repair. As part of our work, we are also actively pursuing the development of novel experimental tools that can be used to facilitate the study of single biochemical reactions. In particular, we are applying techniques derived from nanotechnology to our biological research, and using nano- and micro-scale engineering to facilitate the development of new, robust experimental platforms that enable ''high throughput'' single molecule imaging.

962-Pos

Watching Conformational and Photo-Dynamics of Single Fluorescent Proteins in Solution

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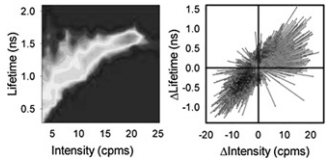
Observation of dynamics of single biomolecules over a prolonged time period without significantly altering the biomolecule via immobilization is a difficult challenge. This result is achieved with the Anti-Brownian ELectrokinetic (ABEL) Trap, which allows extended investigation of solution-phase biomolecules without immobilization via real-time electrokinetic feedback. We apply the ABEL trap to study an important photosynthetic antenna protein, Allophycocyanin (APC). Single molecules of solutionphase APC can often be studied for more than one second. We observe a complex relationship between fluorescence intensity and lifetime that cannot be explained by simple static kinetic models. Light-induced conformational changes are shown to occur. Further, evidence is obtained for fluctuations in the spontaneous emission lifetime, which is typically assumed to be constant. Our observations provide a new window into the dynamics of fluorescent proteins and are relevant for interpretation of in vivo singlemolecule imaging experiments, bacterial photosynthetic regulation, and biomaterials for solar energy harvesting.



963-Pos

What Can We Learn From Single-Molecule Diffusion

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There is increasing interest in a detailed understanding of the structure and dynamics of the cellular plasma membrane, primarily based on recognizing its essential role for controlling cellular signaling processes. Various pictures emerged, which ascribe the plasma membrane a high degree of organization at very short length scales of tens of nanometers. We employed single molecule fluorescence microscopy to study diffusion of CD59, a GPI-anchored protein, in the plasma membrane of living T24 cells at sub-wavelength resolution, both on the cell body and on tunneling nanotubules connecting cells. By separating longitudinal and transversal mobility, we found isotropic diffusion behavior on the surface of tunneling nanotubules, rendering direct influences of the membrane skeleton unlikely.

In both studies we analyzed the mean square displacement as a function of the time-lag and the distribution of displacement steps. However, a closed analytical theory for these analysis is only available for the simplest models. To address a suspected diffusion process we reasoned that a full analytical description may not be required; it may well be sufficient to compare the experimental data with Monte Carlo simulations of the process. We demonstrated the working principle for the analysis of free diffusion, hop diffusion and transient binding of the tracer molecule to slowly moving receptors.

In the recent years increasing evidence was reported for an inherent heterogeneity of cell populations. Our reasoning was that mobility probes nanometer-sized properties of the moving protein and its local environment. Automated and tailored data analysis routines allowed for the analysis of the required large data sets: ~200.000 trajectories obtained on ~350 cells were analyzed in total. We found up to five-fold higher variability of the diffusion constant between cells compared to the uncertainty for the determination of the diffusion constant on a single cell.

964-Pos

Electrostatic Switching of Polysaccharide Conformation Probed at the Single Molecule Level

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Polysaccharides play a key mechanical role in maintaining cell integrity and in cell-cell recognition. Single molecule AFM stretching measurements have revealed that upon loading, the backbone of polysaccharide molecules change their conformation and these conformational changes depend on the linkages between the sugar rings. It has been proposed that these force-induced conformational transitions may play an important role in biological systems. However a mechanism to switch on/off these conformational transitions and control the nanomechanical properties of carbohydrates has not yet been shown.

Here we demonstrate an electrostatic switch that can be used to toggle the force dependent conformational transition in acidic polysaccharides. Using single molecule AFM force spectroscopy we show that the tension dependent conformation of the polysaccharide molecules can be controlled by varying the backbone charge density and solution electrostatics.

965-Pos

An Optical Conveyor for Molecules

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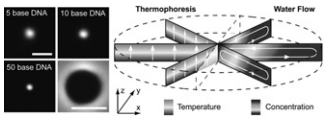
We optically trap molecules in free solution, which allows to accumulate 5-base DNA to a hundredfold excess within seconds [1]. The concentration of the trapped DNA scales exponentially with length, reaching trapping potential depths of 14kT for 50 bases. This novel way to trap molecules could be used to enhance diffusion-limited surface reactions, redirect cellular signaling, observe individual biomolecules over a prolonged time or separate small molecules in solution by their diffusion constant.

The mechanism is based on the microscale analog of a conveyor belt: a bidirectional flow, driven optically by the recently shown thermo-viscous fluid pump [2,3], is combined with a perpendicular thermophoretic molecule drift. Arranged in a toroidal geometry, no microfluidics, electrodes or surface modifications are required. As a result, the trap can be dynamically relocated.

[1] Weinert and Braun, Nano Letters, accepted
[2] Weinert, Kraus, Franosch and Braun, PRL 100, 164501 (2008)
[3] Weinert and Braun, JAP 104, 104701 (2008)



966-Pos

Recovering Absolute Fret Efficiency from Single Molecules: Comparing Methods of Gamma Correction

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Fluorescence resonance energy transfer is widely thought of as a ''spectroscopic ruler.'' Because biological processes and cellular assemblies occur on the nanometer scale, FRET is a popular tool for structural biology. In contrast to ensemble solution FRET measurements which record the entire emission spectrum, microscopy- based FRET experiments separate donor and acceptor intensity by passing the emission through a series of optical elements. Observed FRET efficiency, determined from the uncorrected donor and acceptor intensities, has been called a relative proximity ratio, which is internally consistent *only* if the photophysical properties and instrument remain unchanged. However, it is desirable to measure absolute distances using FRET, which requires that FRET efficiency be corrected for both instrument response and fluorophore properties. Thus, ''gamma'' correction adjusts for differences between the donor and acceptor dyes in their probability of photon emission upon excitation and the probability that emitted photons will be detected. Methods of gamma correction vary depending on the single molecule methodology. To test different methods for correcting FRET efficiency, we recorded smFRET distributions for protein and DNA on different instruments and with different filter sets which altered the observed FRET efficiency. Knowledge of filter set transmission allows for comparison of results between groups using different instruments. Applying empirically-derived corrections for instrument response and quantum yield was only slightly better than corrections based solely on filter set transmission data. We found that gamma correction based on single molecule photobleaching was the most effective particularly when gamma was determined for each sample or even each molecule. Variations in focus of the two colors and sub-pixel errors