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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.

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

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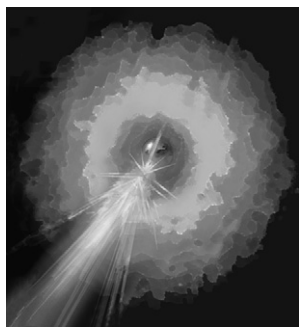
Gold nanoparticles have are extremely useful as multi-functional and sensitive probes for investigation and manipulation of biological systems. Particles, as small as tenths of nanometers, can be visualized, optically manipulated, and used as controlled force transducers. Their high absorbance also makes 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.



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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.

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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.

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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

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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