approach will be broadly applicable for studies of other macromolecular machines.

989-Pos Board B744

Dual Focus Fluorescence Cross-Correlation Spectroscopy for the Investigation of Biomolecule Folding and Binding in Flowing Liquids

Alan K. Van Orden[1], Farshad Abdollah-Nia[2], Martin P. Gelfand[2], Kevin J. Whitcomb[1].
[1]Department of Chemistry, Colorado State University, Fort Collins, CO, USA, [2]Department of Physics, Colorado State University, Fort Collins, CO, USA.

Fluorescence correlation spectroscopy measures the time constants for rate processes giving rise to fluorescence intensity fluctuations observed from one or more microscopic sub-volumes of a nanomolar aqueous solution containing the biomolecules of interest. In our approach, the analyte molecules flow through an electrophoresis capillary under the influence of pressure driven flow or electrophoretic flow and are probed by two spatially offset probe volumes in such a way that the molecules flow sequentially from one probe volume to the next. Fluorescence fluctuations are analyzed from each probe volume independently using autocorrelation analysis, and from the two spatially offset probe volumes using cross-correlation analysis. In this way, we are able to resolve fluorescence fluctuation time constants arising from diffusion, flow, triplet blinking, and conformational fluctuations. Conformational fluctuations are monitored by quenching and unquenching of dye-quencher molecules attached to DNA or RNA hairpin structures and probe the folding and unfolding kinetics of the hairpins. In this presentation, we will discuss recent results that show how base stacking within the loop region of the DNA and RNA hairpins alters the kinetics and thermodynamic stability of the hairpins. We will also discuss binding and unbinding of counterions to individual nucleotides as they flow through the capillary under the influence of an applied electric field. Emphasis will be placed on how the desired information can be extracted using our unique approach to fluorescence correlation spectroscopy.

990-Pos Board B745

A Single-Molecule Study of Toll-Like Receptor 4 Structure and Signalling

Sarah L. Latty[1], Kristina A. Ganzinger[1], Lee J. Hopkins[2], Clare Bryant[2], David Klenerman[1].
[1]Department of Chemistry, University of Cambridge, Cambridge, United Kingdom, [2]Department of Veterinary Medicine, University of Cambridge, Cambridge, United Kingdom.

In this study, we use a single-molecule fluorescence approach to image the reorganisation on the surface of live cells of individual fluorescently labelled Toll-like Receptor 4 (TLR4) molecules during signalling. TLR4, a key membrane protein in the innate immune system, is involved in the recognition of microbial pathogens, by detecting the presence of the lipopolysaccharide (LPS) component of exogenous Gram-negative bacteria.
Single-molecule tracking experiments will be described that allow us to follow changes in the diffusion of TLR4 and its oligomerisation state over a period of 30 minutes following addition of LPS. These studies provide new insights into how the TLR4 receptor is organised on the cell surface and cooperatively reorganises on binding LPS to trigger downstream signalling and modulate the immune response.

991-Pos Board B746

Maximizing the Fluorescence Signal and Photostability of Fluorophores by Quenching Dark-States

Denis Doerr[1], Deborah Sandrin[1], Stanislav Kalinin[1], Ralf Kuehnemuth[1], Sebastian Overmann[1], Daniela Pfiffi[1], Klaus Schaper[2], Claus A.M. Seidel[1], Thomas J.J. Mueller[2], Andriy Chmyrov[3], Jerker Widengren[4], Brigitte A. Bier[2].
[1]Institute of Physical Chemistry II, Heinrich-Heine-University, Duessldorf, Germany, [2]Institute of Organic Chemistry, Heinrich-Heine-University, Duessldorf, Germany, [3]Department of NanoBiophotonics, Max Planck Institute for Biophysical Chemistry, Goettingen, Germany, [4]Department of Applied Physics, Royal Institute of Technology, Stockholm, Sweden.

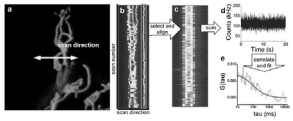
Due to its easy detectability fluorescence is widely used in spectroscopy to investigate a variety of chemical and biochemical samples. The characteristics of fluorescence like intensity, lifetime, anisotropy and quantum yield contain information about electronic structure, mobility and orientation of fluorophors. The precision of fluorescence signal is limited by the number of detected fluorescence photons. Furthermore, experiments that require high time resolution for investigations of protein folding and dynamics are generally limited by the photon flux. Hence it is important to investigate and extend the fluorescence photon emission capabilities of fluorophors. In this study, three different additives which enhance the fluorescence signal were investigated as selective quenchers for triplet or radical states of Rhodamine 110 (Rh 110).
Fluorescence correlation spectroscopy (FCS) in combination with power plot analysis was used to describe the entire fluorescence output according to a derived kinetics model for excitation and fluorescence of Rh 110. The application of additives effectively prevented the triplet and radical formation of Rh 110 even at high excitation irradiance in the range of MW/cm^2 leading to more than ten times increased fluorescence countrate. Furthermore we demonstrate that additives also increase the fluorescence signal of labeled biomolecule.

992-Pos Board B747

Scanning Fluorescence Correlation Spectroscopy in Mitochondria of Living Cells

Joseph D. Unsay[1,2], Ana J. Garcia-Saez[1,3].
[1]Max Planck Institute for Intelligent Systems, Stuttgart, Germany, [2]German Cancer Research Center, Heidelberg, Germany, [3]Interfaculty Institute of Biochemistry, Universität Tübingen, Tübingen, Germany.

New methods to quantify dynamics and interactions of intracellular species provide key insights in cell biology. Fluorescence Correlation Spectroscopy (FCS) utilizes temporal autocorrelation of fluorescence fluctuations to study the dynamic properties of labeled molecules. Previous studies characterize diffusion and interaction of proteins in the nucleus and cytosol using FCS. However, only few deal with tubular organelles like the endoplasmic reticulum and mitochondria. The ability to accurately place the confocal volume in these dynamic organelles limits point FCS in vivo. Originally applied in membranes, scanning FCS (SFCS) addresses these challenges.
We applied SFCS to measure concentration and dynamic properties fluorescently-labeled molecules in mitochondria. SFCS accurately positions the confocal volume (in the x-y plane) by moving it along a linear scanning path. At a scanning orientation perpendicular to the mitochondria, we can also reduce photobleaching due to the brief residence times in the confocal volume.



General scheme of SFCS. (a) shows a fluorescence image of a mitochondria. The scan direction is oriented perpendicular to the mitochondria. (b) shows the acquired data before selection of mitochondria(orange box) and alignment(c). (d) represents the fluctuation trace, which is correlated and fitted to diffusion models (e).

993-Pos Board B748

Parallel Single-Molecule Excitation Spectroscopy of Gold Nanorods

Sara Carozza, John Van Noort.
Leiden University, Leiden, Netherlands.

Gold nanorods show intense two photon luminescence arising from surface plasmon resonances (SPRs). Such SPRs exhibit a strong dependence on the environmental conditions and can be used to detect interactions between a nanorod and single biomolecules. Here we report a novel technique to acquire excitation spectra of multiple single gold nanorods in parallel, within a few seconds. We acquired two-photon excitation spectra of tens of single gold nanorods, and analyzed individual gold nanorod features. 3-dimensional fitting yields a spectral resolution of 1 nm. This allows for discriminating between single and multiple gold nanorods, as well as analysis of spectral changes in time. Using this technique we aim to detect single protein-gold nanorod interactions within a living cell.

994-Pos Board B749

Single-Cell Single-Molecule Co-IP Analysis

Ji Young Ryu, Jihye Kim, jiseop Bae, WonHee Lee, Tae-Young yoon.
KAIST, Daejeon, Korea, Republic of.

Recent advances in single molecule imaging technique, such as single molecule Co-IP, have allowed us to probe interactions between weakly binding