

with quantum dots are hydrodynamically stretched and imaged with a TIRF microscope. Activity of the replisome is observed as a change in the DNA length due to the differing force-dependent extension of single- and double-stranded DNA at low pico-Newton forces. We employ a two-color imaging scheme to monitor DNA length in real-time and to stroboscopically image fluorescently labeled single proteins interacting with DNA. Observation of labeled proteins in an ongoing replication reaction allows us to pose structural questions about the stoichiometry and exchange of proteins at the prokaryotic replication fork. We will discuss preliminary results on primer extension by the T7 DNA polymerase and strand-displacement synthesis by the coupled activity of the T7 helicase and polymerase.

2865-Plat

Real-time DNA Synthesis Dynamics Of Single f29 DNA Polymerase Molecules With Base Pair Resolution

Jonas Korlach.

Pacific Biosciences, Menlo Park, CA, USA.

Real-time observation of uninterrupted DNA synthesis by individual f29 DNA polymerase molecules is presented. Four spectrally distinct fluorophores were used to label the four nucleotides via the terminal phosphate moiety, enabling identification of nucleotide incorporation events into the growing DNA strand with base-pair resolution, and correlating these events to the DNA template sequence. Immobilization of polymerase molecules inside zero-mode waveguide nanostructures allowed detection of incorporation events at 100-500 nM nucleotide concentrations, resulting in polymerization rates of ~1-5 bases/s, and lasting for thousands of bases synthesized. Many aspects of the underlying DNA polymerase dynamics are directly observable, such as nucleotide binding, catalysis, pausing, persistence of distinct kinetic states, DNA template sequence context effects, and switching between polymerization and exonuclease activities.

2866-Plat

Anchoring, Sliding, And Rolling: Visualizing The Three-dimensional Nano-motion And Orientation Of A Single Virus As It Diffuses On A Flat Membrane

Philipp Kukura, Helge Ewers, Alois Renn, Ari Helenius, Vahid Sandoghdar.

ETH Zurich, Zurich, Switzerland.

The localization of objects within the cell and the accurate measurement of relative positions on the molecular level are essential to an understanding of the function of macromolecular complexes. As a consequence, much effort has been directed towards developing imaging techniques that allow the temporal and spatial resolution of events on the nanoscopic scale. Due to their non-invasive nature, optical techniques are particularly suited for studying live samples and several methods have recently demonstrated subdiffraction resolution. However, all these novel methods are based on detecting fluorescence and thereby face strict limitations in accuracy, time-resolution and dimensionality. Here, we show how the combination of label-free detection of nano-objects and single molecule fluorescence detection allows one to map the center of mass motion and the absolute orientation of a single virus with nanometer resolution in real time. We use interferometric scattering detection to resolve the position of individual virions of Simian Virus 40 (a 45 nm DNA tumor virus) with 2 nm accuracy while bound to its cellular receptor GM1 in supported membrane bilayers. At the same time, we detect the fluorescence of a single fluorescent quantum dot attached to the virus via streptavidin-biotin linkage and determine its position with 4 nm accuracy. By overlapping the fluorescence and scattering trajectories, we can resolve the absolute three-dimensional nano-motion of the virus as it diffuses on a two-dimensional membrane. We find that membrane-bound virions exhibit different modes of motion that are strongly influenced by the concentration of the GM1 receptor in the membrane. Besides Brownian motion in the plane of the membrane, we also observe rolling motion on the sub-20 nm scale and periods of apparent standstill in both two and three dimensions.

Platform AZ: Heme Proteins

2867-Plat

The Single Domain Hemoglobin From *Campylobacter* Jejuni: The Unique Structural Features Underlying Its NO Dioxygenase Activity

Syun-Ru Yeh¹, Changyuan Lu¹, Tsuyoshi Egawa¹, Yu Lin¹, Masahiro Mukai², Guanghui Wu³, Mark Shepherd³, Robert K. Poole³.

¹Albert Einstein College of Medicine, Bronx, NY, USA, ²Mitsubishi Kagaku Institute of Life Sciences, Tokyo, Japan, ³University of Sheffield, Sheffield, United Kingdom.

Recently three groups of hemoglobins (Hbs) have been identified in unicellular organisms: (1) the truncated Hbs (trHb) that display a novel two-over-two

alpha-helical structure, (2) the flavohemoglobins (FHB) that comprise a Hb domain with a classical 3-over-3 alpha-helical structure and a covalently attached flavin-containing reductase domain, and (3) the single-domain Hbs (sdHb) that exhibit high sequence homology and structural similarity to the Hb domain of FHB. On the basis of phylogenetic analysis, the trHbs can be further divided into three subgroups: TrHb-I, TrHb-II, and TrHb-III. *C. jejuni* contains two globins, a single domain hemoglobin, Cgb, and a truncated hemoglobin, Ctb. Neither Cgb nor Ctb are required for the survival of the bacterium in air. Cgb knock-out mutant cells are hypersensitive to reactive nitrogen species, while Ctb knock-out cells do not display any sensitivity to nitrosative stress. As the expression of Cgb is induced by nitrosative stress, it is believed to function as a NO dioxygenase to protect *C. jejuni* against the toxic effects of NO. In contrast, Ctb is thought to be involved in regulating O₂ flux into and within the cell. To study the structural and functional properties of Cgb, we have purified the recombinant Cgb protein expressed in *E. coli*. Due to its high affinity towards cyanide, Cgb is isolated in the cyanide-bound ferric state. By using resonance Raman scattering and fast kinetic techniques, we have studied the structural, functional and ligand binding properties of Cgb, with respect to Ctb as well as other globins. The implications of these data will be discussed in the context of the NO dioxygenase chemistry carried out by this fascinating globin.

2868-Plat

Two distinct functional globin classes in *Caenorhabditis elegans*

Eva Geuens¹, David Hoogewijs², Evi Vinck¹, Sabine Van Doorslaer¹, Jacques Vanfleteren², Luc Moens¹, Sylvia Dewilde¹.

¹University of Antwerp, Wilrijk, Belgium, ²Ghent University, Ghent, Belgium..

The nematode *Caenorhabditis elegans* expresses 33 different globin genes. We studied the expression of two of them, *glb-1* and *glb-26* in more detail using green fluorescence protein technology and found that both are expressed in distinct subsets of cells. GLB-1::GFP is mainly observed in head and tail muscular or hypodermal tissue and in a subset of nerve cells. GLB-26::GFP is seen in the head mesodermal cell and in stomato-intestinal muscle. Hypoxia causes upregulation of the expression of *glb-1*, but not *glb-26*. After expression in an *E. coli* system purified GLB-1 and GLB-26 were spectroscopically (UV/VIS, resonance Raman, electron paramagnetic resonance) and kinetically characterized. The 3D structure of GLB-1 was determined.

GLB-1 is pentacoordinated and exhibits high affinities for O₂ and CO. The bound O₂ is stabilized through hydrogen bonding interactions involving a B10 tyrosine and an E7 glutamine. In contrast, in absence of ligands, GLB-26 is strongly hexacoordinated with E7 distal histidine as sixth ligand. In the presence of O₂, this globin is instantly oxidized to the ferric form and is therefore incapable of reversible oxygen binding. Most likely this molecule will function as an electron transfer protein.

Taken together these data strongly suggest that GLB-1 and GLB-26 belong to two globin classes with totally different functions. Potential functions will be discussed.

2869-Plat

Peroxidase Activity Of Respiratory Proteins. The Role Of Protein Bound Free Radicals

Dimitri Svistunenko.

University of Essex, Colchester, United Kingdom.

Peroxidase activity of respiratory haem proteins is associated with formation of two active species on the protein when it reacts with peroxides: the oxoferryl state of the haem and a protein bound free radical. Both the free radical and the oxoferryl haem are capable of oxidising a range of substrates. General principles of formation of these two species and of their further reactions will be considered. Experimental confirmation of the proposed view involves a complex analysis of diverse pathways by which electrons can be passed between different parts of the protein. Associated with this electron transfer, the process of free radical character transfer around the protein as well as between different protein molecules can be monitored by the EPR spectroscopy. If a protein contains tyrosine residues (which is often but not always the case), the chance of observing a tyrosyl radical is high. EPR data will be presented on formation, transformation and transfer of protein bound radicals in different respiratory proteins. A method will be described that allows very accurate determination of the tyrosyl radical parameters. By using the method, it is possible to determine the three principal g-values of the radical solely from an X-band EPR spectrum. (And if a high field EPR spectrum of the radical is available, and the g-values are measured directly, the other radical parameters, e.g. the hyperfine interaction constants, can be determined much more accurately.) The radicals parameters extracted from experimental EPR spectra are then compared with either some