Posters

– 31. Imaging molecules of life –

P-959

Revealing protein oligomeric states in solution at the single molecule level with iSCAT

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Protein oligomerisation plays an essential role in many biological processes from formation of filaments to protein-ligand interactions. By elucidating the oligomeric populations of a protein, it is possible to gain mechanistic insights into its function in native and disease-related processes. Here, we show that interferometric scattering microscopy (iSCAT) can be used to directly determine the oligomeric state of proteins in solution without the use of any labels, one molecule at a time. Since the scattering intensity of a protein is proportional to its mass, we used proteins of known molecular weight to calibrate our mass measurements. We demonstrate the capabilities of single molecule solution mass spectrometry based on iSCAT to probe the nucleation of tubulin heterodimers leading to microtubule formation and growth.

P-960

3D millisecond tracking of single-molecule fluorescent protein translocation in eukaryotic cells

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Metabolic processes are the basis of all life. An organism must be able to utilise chemical energy to stay alive and eventually reproduce. These are the central features of life, regardless of organism length scale. To achieve this, an organism must be able to adapt to varying surrounding environmental conditions. Cells respond to external stimuli by releasing kinase cascades along often intricate signalling pathways which regulate cellular function. These chemical signals eventually bring about some cell level response.
In our research on transcription factor dynamics in Brewer's yeast, *Saccharomyces cerevisiæ,* we have found that a key transcription factor, Mig1, forms functional clusters that translocate between nucleus and cytosol as a response to environmental glucose fluctuations (Wollman et al. 2017). The bulk behaviour of Mig1 glucose sensing in yeast has recently been well characterised (Bendrioua et al. 2014). However, the dynamics and interactions of individual molecules and clusters in the pathway have not.
By using astigmatic imaging at high speed, we can track fluorescently tagged proteins translocating in living cells over several tens of milliseconds. Using mutant yeast strains with fluorescent protein tags attached to the transcription factors Mig1. Furthermore, a microfluidic flow channel provides a consistent environment during an experiment.

P-961

Label-free visualisation of actin polymerisation using interferometric scattering microscopy

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Actin filaments are a major component of the cytoskeleton and ubiquitously found in all cell types. Actin is involved in many basic processes such as cell migration, cell adhesion, cell division and muscle contraction. The most important property of actin is that it forms filaments (F-actin) out of globular subunits (G-actin). In the 1960s, Oosawa and coworkers developed a model for the G- to F-actin transition. The rate-limiting step of this transition is the formation of a stable actin nucleus, which was later determined to consist of 2-4 actin monomers. The nuclei are elongated by the addition of monomers to the filament ends. This model is widely accepted, although the molecular details could never be confirmed by direct observation.
Here, we used iSCAT to visualise the growth of unlabelled actin filaments. By applying a Gaussian worm model to fit the filament PSF we could follow the position of the filament ends. The advanced spatiotemporal precision of iSCAT allowed us to observe end displacements of ∼2.7 nm, which correspond to the addition of single actin subunits. With these results we demonstrate the potential of iSCAT for label-free single-molecule imaging and for investigating the mechanisms of actin dynamics and their regulation.

P-962

Time-resolved imaging of oxidative stress and cell/tissue oxygenation

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Cell metabolism is often altered in the process of cancerogenesis. O_2 imbalance can be measured by several techniques. Fluorescence lifetime spectroscopy is one interesting noninvasive approach to visualized malfunctions in cell metabolism. Unfortunately, many O_2 sensors such as porphyrins are used for these visualizations and have different levels of phototoxicity. We have compared different O_2 sensors phototoxicity, O_2 sensitivity, and ability to be used as a photosensitizer in photodynamic therapy (PDT). We have focused our works on mitochondria diseased cells with imbalanced respiration and metabolism. Hypoxic tissues exhibited dramatically decreased responses to PDT due to O_2 deprivation. We have also assessed the level of reactive O_2 species (ROS) by time-resolved fluorescence microscopy (FLIM) in cancer cells treated with regulators of apoptotic pathways affecting the mitochondrial network. The results were compared and analyzed by badging and boosting the advanced statistical methods that reduce non-Gaussian effects. Acknowledgement: the Slovak grants APVV 15-0485 and VEGA 1/0425/15 and by the Swiss NSF (project N° CR32I3_159746).

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