Title

Multiparametric fluorescence imaging approaches to investigate metabolic signature, lipid droplets, and collagen remodeling in breast cancer spheroids

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temperature shows a first peak in the range 60-75°C, attributed to the loss of the C_H2 native domain structure. SANS data collected near the lowest melting temperature is consistent with this result. Complementary ex-situ methods are often based on higher temperature data with subsequent extrapolation of interpretations to the cold temperature regime. The in-situ approaches described in this work offer a means to characterize conformational stability of biopharmaceuticals under e.g. cold temperature stress by assessment of structural alteration, self-association, and reversibility of each process.

Posters: Optical Microscopy and Superresolution Imaging III

1978-Pos

Nanoscale dynamics of cellulose digestion by the cellobiohydrolase *Tr*Cel7A

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Understanding the mechanism by which cellulases from bacteria, fungi, and protozoans catalyze the digestion of lignocellulose is important for developing cost-effective strategies for bioethanol production. Cel7A from the fungus *Trichoderma reesei* is a model exoglucanase that degrades cellulose strands from their reducing ends by processively cleaving individual cellobiose units. Despite being one of the most studied cellulases, the binding and hydrolysis mechanisms of Cel7A are still debated. Here, we used single-molecule tracking to analyze the dynamics of 11,116 quantum dot-labeled *Tr*Cel7A molecules binding to and moving processively along immobilized cellulose. Individual enzyme molecules were localized with a spatial precision of a few nanometers and followed for hundreds of seconds. Most enzyme molecules bound to cellulose in a static state and dissociated without detectable movement, whereas a minority of molecules moved processively for an average distance of 39 nm at an average speed of 3.2 nm/s. These data were integrated into a three-state model in which *Tr*Cel7A molecules can bind from solution into either static or processive states and can reversibly switch between states before dissociating. From these results, we conclude that the rate-limiting step for cellulose degradation by Cel7A is the transition out of the static state, either by dissociation from the cellulose surface or by initiation of a processive run. Thus, accelerating the transition of Cel7A out of its static state is a potential avenue for improving cellulase efficiency.

1979-Pos

SOS response dynamics during sub-lethal antibiotic treatment

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In bacteria, the key mechanism facilitating survival and adaptation upon DNA damage is the SOS response. Antibiotics cause DNA damage and trigger autoproteolytic cleavage of the transcriptional repressor LexA, which controls over 50 SOS genes including drivers of mutation. Efforts to inhibit this response and thereby combat antibiotic resistance rely on a broad understanding of its behavior *in vivo*. Here, we use a single-molecule localization microscopy assay to directly track LexA dynamics in *Escherichia coli* under low antibiotic stress. LexA exhibits a great mobility landscape with four diffusive states that can be assigned to operator bound, non-specifically DNA bound, freely diffusing, and cleaved repressor. By measuring the time-evolution of these populations, the SOS response can be monitored on the level of transcription factor activity. The SOS response strength is dependent on antibiotic dosage and induction was observed at sub-lethal antibiotic stress. We further visualize SOS gene products and found an increased expression even without a strong SOS induction. Protein abundance showed substantial cell-to-cell heterogeneity during the late SOS response, suggesting a bacterial survival strategy.

1980-Pos

Multiparametric fluorescence imaging approaches to investigate metabolic signature, lipid droplets, and collagen remodeling in breast cancer spheroids

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Tumor spheroids are 3D cell cultures models to study the behavior of avascular solid tumors in a controlled environment. Cellular metabolism, lipid storage and their relationship with the extracellular matrix (ECM) have been shown to be important elements in the characterization of tumor cell proliferation and metastatic invasion, but their simultaneous assessment remains challenging. In order to obtain all the information simultaneously, we imaged tumor spheroid with the following three imaging approaches over a 24 hours period. The metabolic signature at the single cell level was obtained by analyzing NADH lifetime, using the Phasor Fluorescence Lifetime Imaging Microscopy (FLIM) approach. Differences in lipid droplets content were obtained by analyzing the emission signal of an environmental-sensitive (solvatochromic) dye, Nile Red, with Spectral Phasors. Finally, we evaluated the spheroids-ECM interaction with Second Harmonic Generation (SHG) with PLICS (Phasor analysis of Local Image Correlation Spectroscopy). The main advantage of the proposed techniques is the minimally invasive approach, since NADH Phasor-FLIM and SHG are label-free techniques and Nile Red does not interfere with cellular physiology. Furthermore, we used 2-photon microscope excitation which allows for deep sample penetration and reduced phototoxicity. Spheroids were obtained from two different human breast adenocarcinoma cell lines (MCF7 and MDA-MB231) and a non-malignant breast epithelial cell line (MCF10A) and embedded them in a collagen Type-1 matrix to mimic the ECM. With this framework, we could determine a significant difference in the NADH and lipid droplet signatures between the spheroids grown from the three cell lines, between different areas of the spheroids themselves as well as changes over time. Additionally, we could quantify the remodeling of the surrounding ECM upon MDA-MB231 cell invasion.

1981-Pos

Characterizing the distribution of myosin H in the apical complex of conoid protruded and conoid retracted *Toxoplasma gondii*

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An estimated 1 billion people globally are infected by the parasite *Toxoplasma gondii*. While most infections are asymptomatic, serious disease can occur in the immunocompromised and in fetuses. Despite the organism's significance to human health, there are many gaps in our understanding of *T. gondii's* basic biology. The parasite uses an apical complex comprised of secretory organelles and proteinaceous structures to drive host-cell invasion and is capable of invading virtually any animal cell. However, fundamental details of the apical complex organization and composition are unknown, with observation made difficult by the fact that the entire complex is approximately a single diffraction-limited volume. Numerous proteins have been shown to localize to the apical complex, but many of their functions are yet to be determined and their precise locations within the apical complex are unknown. For example, the motor myosin H has been identified as indispensable for parasite invasion and shown to associate with the conoid, a barrel of uniquely arranged tubulin filaments within the apical complex that protrudes during the process of invasion. However, characterizing the distribution of myosin H and its localization with any further precision is difficult given the small spatial extent of the apical complex. To circumvent this, we use cryogenic electron tomography, single-molecule super-resolution fluorescence microscopy, and a new method of super-resolved cryogenic correlative light and electron microscopy to image parasites with the fluorescent protein PAmKate fused to myosin H. By imaging conoid protruded and conoid retracted parasites, we are determining with high resolution how this vital motor protein changes spatial organization in preparation for host cell invasion, bringing greater detail to a biological process with vast public health implications.

1982-Pos

Single-molecule interaction microscopy revealed pH dependent behavior of monoclonal HA antibody

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Characterizing on-target specificity is essential for the successful employment of antibodies in research, diagnostics, and therapeutics. In standard assays, antibody specificity is typically evaluated at concentrations much higher than the affinity constant, whereas higher antibody concentrations may lead to non-specific binding. Single-molecule fluorescence imaging can examine the binding behaviors of single antibodies, but the irreversible damage of fluorophores due to photobleaching limits its applicability to high-affinity interactions. Here, we report single-molecule interaction microscopy (SMIM), which employed time-lapsed single-molecule imaging to directly visualize the interactions between individual monoclonal hemagglutinin (HA) antibody 12CA5 and the 3xHA epitope tag with extended durations. We discovered that 12CA5 displayed pH-dependent binding