modifications were accompanied by a decrease of membrane/substrate distance and energy of adhesion.

2646-Pos Board B662

Superresolution Microscopy of the T Cell Receptor in the Immunological Synapse

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Single molecule localization-based superresolution microscopy has been used to report the presence of protein nanoclusters at the T cell plasma membrane. In particular, the T cell receptor (TCR) complex has been suggested to be non-randomly organized in resting T cells (1, 2), which has led to a variety of models for specific antigen recognition and T cell activation. We have previously found that characteristic blinking and resulting overcounting of fluorescent proteins and organic dyes may be easily misinterpreted as molecular clusters. As a possible solution to the problem, we developed a method to discriminate between true protein clusters and multiple counts of the same dye molecules (3).

Here, we applied this method to study the organization of the TCR complex in the plasma membrane of resting and activated T cells. While there was clear evidence for the formation of clusters upon activation, no indication of non-homogenous distribution could be obtained under resting conditions. In independent experiments, we confirmed our observations with STED microscopy, yielding similar results. Overall, our data do not support the view of protein nanoclustering being a general ordering principle at the T cell plasma membrane.

(1) Lillemeier et al. Nat Immunol. 2010 Jan;11(1):90-6.
(2) Pageon et al. Proc Natl Acad Sci U S A. 2016 Sep 13;113(37).
(3) Baumgart et al. Nat Meth. 2016;13(8):661-4.

2647-Pos Board B663

Study of Tumor Cellular Damage Induced by Photosensitizing Molecules

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Photodynamic therapy (PDT) is a tumor treatment that uses a combination of otherwise non-toxic chemicals, termed photosensitizers (PSs) and visible light to produce reactive oxygen species capable of destroying tumor cells [Dougherty TJ (1993)][Agostinis P. *et al*. (2011)].

PS molecules are often insoluble in water and form aggregates, which impairs their photophysical properties, generally resulting in a quenching of the productive excited states. To overcome this limit, PSs carriers have been devised [Bechet D. *et al*. (2008)][Konan, Y. N. *et al*. (2002)].

Among them, proteins offer several potential advantages. Hydrophobic PS molecules spontaneously bind to internal hydrophobic cavities of the proper size, preserving their monomeric, photoactive (both photosensitizing and fluorescent) state. [Sharman, W. M. *et al*. (2004)].

Here we identify the uptake of Hypericin and Zinc Phthalocyanine (either delivered alone or using Apomyoglobin as a carrier) in living cells directly from fluorescent emission using various microscopy techniques such as spinning disk, light sheet, and two-photon microscopy. We aim to investigate which cellular components are involved in the damage by the photosensitized ROS, and to understand which are the differences in the accumulation, cellular damage, and general efficacy between these PSs and their respective constructs with Apomyoglobin.

2648-Pos Board B664

Mannan Molecular Sub-structures Control Nanoscale Glucan Exposure in Candida

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N-linked mannans (N-mannans) in the cell wall of *Candida albicans* are thought to mask β-(1,3)-glucan from recognition by Dectin-1, contributing to innate immune evasion. Lateral cell wall exposures of glucan on *C. albicans* are predominantly single receptor-ligand interaction sites and are restricted to nanoscale geometries. *Candida* species exhibit a range of basal glucan exposures and their mannans also vary in size and complexity at the molecular level. We used super resolution fluorescence imaging and a series of protein mannosylation mutants in *C. albicans* and *C. glabrata* to investigate the role of specific N-mannan features in regulating the nanoscale geometry of glucan exposure. Decreasing acid labile mannan abundance and α-(1,6)-mannan backbone length correlated most strongly with increased density and nanoscopic size of glucan exposures in *C. albicans* and *C. glabrata*, respectively. Additionally, a *C. albicans* clinical isolate with high glucan exposure produced similarly perturbed N-mannan structures and exhibited similar changes to nanoscopic glucan exposure geometry. We conclude that acid labile N-mannan controls glucan exposure geometry at the nanoscale. Furthermore, variations in glucan nanoexposure characteristics are clinically relevant and are likely to impact the nature of the pathogenic surface presented to innate immunocytes at dimensions relevant to receptor engagement, aggregation and signaling.

2649-Pos Board B665

STED Nanoscopy of the Centrosome Linker Reveals a CEP68-Organized, Periodic Rootletin Network Anchored to a C-Nap1 Ring at Centrioles

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The centrosome linker proteins C-Nap1, rootletin and CEP68 connect the two centrosomes of a cell during interphase into one microtubule organizing centre. This coupling is important for cell migration, cilia formation and timing of mitotic spindle formation. Very little is known about the structure of the centrosome linker. Here, we used STimulated Emission Depletion (STED)microscopy to show that each C-Nap1 ring at the proximal end of the two centrioles organizes a rootletin ring and in addition multiple rootletin fibres that radiate outwards from the ring into the cytoplasm. Rootletin filaments have a repeat organization of 75 nm and bind CEP68 via its C-terminal spectrin repeat containing region in 75 nm intervals. CEP68 is essential in forming rootletin filaments that branch off centrioles and modulates the thickness of rootletin fibres. Thus, the centrosome linker consists of a vast network of repeating rootletin units with C-Nap1 as ring organizer and CEP68 as filament modulator. The punctual contact model is consistent with the biological properties of the centrosome linker.

2650-Pos Board B666

Quantitative Microscopy Pipeline for Building a Model of the Human Cell

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We aim to replace the typical textbook artistic rendering of a cell with a model created in silico, based on large datasets of 3D images of genome-edited live cells collected by light microscopes.

We have created about 20 human induced pluripotent stem cell lines expressing endogenously EGFP tagged proteins that localize to the major cellular organelles. We plate and feed the cells, using a robot, in 96-well glass bottom plates and image them using spinning disk microscopy. So far, we have collected 3D images from more than 15,000 live cells, comprised of high replicates for each genome-edited cell line. We can also apply drugs externally or store the sample overnight in an incubator. We can prescreen the sample on a slide scanner and then image areas of interest with higher resolution and better image quality on one of the spinning disk microscopes.

As a part of our workflow, we wrote software to identify the same sample area repeatedly and on different microscopes. This will allow us to revisit the same location during long-term time-lapse experiments after imaging other cells. The