STED microscopy case studies

A) Membrane lipid peroxidation in T-cells during activation

In the site of inflammation, immune cells, including T-cells, are exposed to reactive oxygen species (ROS), produced by phagocytic cells and by T-cells themselves. Low level of ROS is indispensable for normal functioning of T-cells, but ROS can also initiate peroxidation of T-cell membranes. By changing physical properties of the membrane (fluidity and viscosity), lipid peroxidation can affect the dynamics of membranous proteins and can interfere with the formation of immune synapse (crucial for Tcell activation). The exact impact of membrane peroxidation on molecular processes underlying T-cell activation is not understood yet. The proposed project aims at performing the real-time imaging experiments on live T-cells and on membranes obtained from T-cells (giant plasma membrane vesicles) to understand the role that lipid peroxidation plays in the activation of T-cells.
Objectives

- To use giant plasma membrane vesicles (GPMVs) isolated from T-cells as a new model system to study the lipid peroxidation quantitatively under biorelevant conditions
- To determine quantitatively the oxidizability of GPMVs derived from T-cells by oxygen-uptake method (studies at the Faculty of Chemistry, University of Warsaw)
- To get knowledge on the impact of lipid peroxidation on the fluidity and viscosity of T-cell membrane (with Laurdan as a membrane stiffness sensor and molecular rotors as viscosity sensors) studies on GPMVs and live T-cells
- To understand the role of membrane peroxidation in T-cell activation. The progress of membrane peroxidation and its impact on the dynamics of macromolecules in live T-cells (upon activation and as a result of exposition to external sources of reactive oxygen species, ROS) will be followed by super-resolution microscopy (STED-FCS).

B) Unravelling functional protein cluster stoichiometry and dynamics during the early stage of T cell activation

Lymphocyte T cells are responsible for cell-mediated adaptive immune responses. Its activation produces membrane microclusters of unknown stoichiometry and mobility. There is poor quantitative information on the early activation process, mainly because there is no proper model for a truly resting state. We aim to deeper understand and quantify the protein homo-and hetero-oligomerisation, and their interaction dynamics. We will use a newly developed a method that keeps Tcells in true resting state, and we will chemically induce the protein interactions leading to early T cell activation. We will control the activation in a dose-response manner by chemical dimerising agents. We will investigate living cells stoichiometry and dynamics under the STED microscope in suspension, using Number and Brightness and Fluorescence Correlation Spectroscopy, respectively.
Objectives

- Engineer and control the early Tcell activation process using a recently established method for observing suspension living cells under the microscope, and implementing a dose-response chemically inducible protein oligomerisation method
- Disentangle the stoichiometry and mobility of TCR and LCK during the early stage of Tcell activation employing live cell quantitative nanoscopy methods (i.e., STED Fluorescence Correlation Spectroscopy and Number and Brightness, as well as the cross-correlation variants) under the previous conditions

C) Construction of super-resolution blueprints of DNA repair factories

Illegitimate genome rearrangements are one of the causes of many human diseases like cancer and genetic disorders. It is therefore crucial for cells to maintain and restore genome integrity after DNA damage. It has been shown that DNA lesions trigger the formation of so-called "DNA damage foci" that accommodate a number of DNA repair proteins. Most of these factors involve in two major DNA repair pathways: non-homologous end joining and homologous recombination, which activities depend on cell cycle stages. It is commonly described that DNA repair factors colocalise at sites of DNA break, but a high- or super-resolution map of their precise positions and organisation within the repair centre is still lacking. By high-precision and high-resolution imaging techniques, we are able to create a preliminary map of the spatial organisation of a DNA repair factory called G1 nuclear body in post-mitotic cells, which is believed to shield DNA lesions inherited from the previous cell cycle. With our expertise in cell biology and advanced immunofluorescence staining, we aim to construct super-resolution blueprints of different DNA repair factories in resting and replicating cells by STED nanoscopy. This study will provide significant impact to understand how cells, in response to DNA damage, organise DNA repair proteins spatially in a DNA repair factory, and will provide important insights on the roles of individual components. Our results will also likely have deep implications extending to the organisation of other subnuclear structures such as replication and transcription factories.

Objectives

- The main objective of this project is to create super-resolution maps of DNA repair factors at sites of DNA damage in non-dividing and dividing cells to understand the hierarchy organisation and functions of DNA repair machineries. Results gained from this study can be extended to study and understand other sub-nuclear structure and replication and transcription factories.

D) Investigating the relationship between EGFR structure and phosphatidyl inositol perturbations in the determination of EGFR endocytic fate.

Epidermal Growth Factor (EGFR) signalling is critically linked to its internalisation and different endocytic routes are known to have different effects on its downstream signalling, downregulating or potentiating it in turn. Additionally, EGFR mutants found in cancer are defective for downregulating endocytosis and this allows their signalling to be amplified in an aberrant fashion. Prior work has suggested a functional linkage between EGFR interactions, oligomerisation and endocytosis, and a critical role for membrane lipids in their regulation. Investigation of this linkage will allow us to gain insight on possible avenues of EGFR signal attenuation.

Objectives

- Here we propose to develop super-resolution endocytosis assays in Structured Illumination Microscopy (SIM) and Stimulated Emission Depletion (STED) to assess the dynamic and structural features of a lipid sequestration-induced endocytic phenotype of EGFR, and to complement the investigation with multicolour Single-Particle Tracking (SPT) to investigate more in detail the role of PI(4,5)P2 in EGFR regulation. This will allow us to build a proof-of-principle case for a future grant application