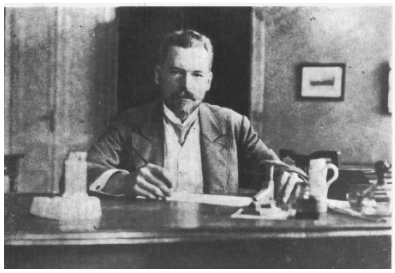

Contribution ID: **13** Type: **Poster**

Single molecule characterization of protein-protein interactions between the heterogeneous plasma membrane and the cytoplasm of living cells

Sunday, 18 September 2022 17:06 (3 minutes)

Based on simultaneous three-color imaging of fluorescently labelled receptors, arrestins and clathrin coated pits, in living cells, we will analyze the dynamics and interaction patterns of membrane bound Adrenergic receptors with cytoplasmic β-arrestin 2 molecules as well as their recruitment to clathrin-coated pits structures (CCP) before internalization.
First, we demonstrate that arrestin molecules naturally undergo surface mediated diffusion alterning between bulk and membrane diffusion. Then, we analyze trajectories of individual receptors and membrane-bound arrestins by identifying over time whether molecules laterally diffuse on the membrane or are confined in some nano-domains by detecting transient trapping events [1]. Next, we study the colocalization of confined trajectories portions with clathrin-coated pits to determine whether confined portions are trapped in CCP or not. Subsequently, we proceed with the analysis of interactions between receptors and arrestins based on colocalization that allows us to quantify the association and dissociation rates. From the combination of information on confinement/trapping in CCP/Colocalization with a different protein we define states of receptor/arrestin over time from which we reconstruct the sequence of events before and after interactions of the molecules as well as the sequences of events leading to CCP recruitment.
Finally, we take advantage of our methodology to compare the effect of a change in biological conditions onto the dynamics and interaction kinetics of receptor/arrestin. This allows us to study the spatio-temporal changes related to receptor activation as well as different receptor affinity to arrestin. Also, we show how at a single-molecule level, one can correlate structural components of arrestin proteins to their role direct effect on receptor/arrestin kinetics and recruitment to CCP based on the analysis of multiple arrestin mutants. Our study [2] sheds new light on spatio-temporal character of receptor/arrestin interaction based on single molecules.

[1] Lanoiselée Y, Grimes J, Koszegi Z, Calebiro D. Detecting Transient Trapping from a Single Trajectory: A Structural Approach. Entropy. 2021; 23(8):1044. https://doi.org/10.3390/e23081044
[2] Grimes J, Koszegi Z, Lanoiselée Y, Miljus T, Mistry R, Stepniewski TM, Medel Lacruz B, Selent J, Hill SJ, Calebiro D. Single-molecule characterization of receptor – β-arrestin interactions. (In revision)

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Session Classification: Poster session party