PhD Thesis Defense GUILLAUME CORDIER 'Unravelling Motor Protein Organization on Lysosomal Membranes with Super-Resolution Microscopy'

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Tuesday April 3, 15:00. ICFO Auditorium

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Single Molecule Biophotonics

ICFO-The Institute of Photonic Sciences

Intracellular transport is of paramount importance to maintain subcellular organization. Failures in intracellular transport can lead to several diseases, especially if the failure

happens in the nervous system.

The key players involved in intracellular transport are the microtubule cytoskeleton and motor proteins. The microtubule cytoskeleton connects the perinuclear region to the cell periphery and acts as tracks for transport. Motor proteins translocate along the microtubule cytoskeleton in order to carry cargoes. There are two kinds of motor protein families that move cargo along polarized microtubules: the kinesin superfamily moves toward the (+) end of the microtubule track whereas dynein moves toward the (-) end of the microtubule track.

Since their first discovery in the 60?s, motor proteins have been extensively studied using in vitro single molecule methods, allowing us to understand the bases of intracellular transport. However, motor proteins, their microtubule tracks and vesicular cargoes have sizes that range from tens to hundreds of nanometers, below the diffraction limit of light microscopy. Therefore, studying intracellular trafficking in the cellular context has been challenging. While the bottom-up in vitro studies are highly valuable for enhancing our understanding of how motor proteins function, they cannot capture the full complexity of the intracellular environment. Therefore, it is highly important to develop new methods to overcome the challenge of studying intracellular transport in intact cells. In the last decade, with the development and advent of super-resolution microscopy methods, a revolution occurred in the field of light microscopy. These methods have made it possible to visualize sub-cellular structures with nanometer spatial resolution, breaking the diffraction limit.

This thesis first describes the development of new methods for high-throughput and multi-color super-resolution microscopy (**Chapters 2 and 3**). Subsequently, I applied these methods to study the organization of motor proteins on the lysosome membrane inside cells with the purpose of determining how intracellular transport can be regulated via motor-protein organization (**Chapter 4**). **Chapter 1** is an Introduction to the state of the art for our knowledge in microtubule-based intracellular transport. Chapter 2 introduces the single molecule localization techniques that improve the spatial resolution of light microscopy. This chapter emphasizes the Stochastic Optical Reconstruction Microscopy (STORM) technique, which I used to study the organization of microtubule based motor proteins around lysosomes as well as the fusion and fission of mitochondria. Chapter 3 describes the development of two new techniques: (i) the use of microfluidic devices to improve the throughput of correlative live-cell and super-resolution microscopy, thus allowing to observe rare events and (ii) sequential multi-color imaging that increases the number of colors that can be imaged with STORM. **Chapter 4** focuses on the biological application of sequential

multicolor imaging to study the 3D organization of dynein and 11 kinesin on lysosomal membranes. Conclusions and Future Perspectives are provided in **Chapter 5**.

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