ICFO



PhD THESIS DEFENSE: Imaging and Analytical Tools to Study the Spatiotemporal Dynamics of Protein Export

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15:00

Auditorium and Online (Teams)

Intracellular trafficking, particularly protein secretion, faces numerous unresolved challenges. This thesis aims to provide and evaluate tools for quantitative investigation of these processes using fluorescent microscopy. Quantitative analysis offers two main benefits: detailed characterization of molecular dynamics for mechanistic understanding and objective measurements for accurate comparisons across experiments.

In Chapter 1, we introduce the secretory pathway, a cellular pathway responsible for the synthesis, processing, sorting and delivery of secretory proteins to the extracellular environment. In Chapter 2, we provide a thorough description of the methodologies used in

this thesis. They include various fluorescence microscopy techniques, automated image analysis, and biological methods tailored to the secretory pathway. The tools were selected to achieve high spatial and temporal resolution, enable quantitative analysis, and allow live-cell characterization.

In Chapter 3, we used fluorescence imaging to objectively evaluate results in four projects addressing protein secretion and intracellular trafficking. These included quantifying colocalization and proximity of structures, measuring fluorescent intensity differences, and characterizing dynamics of particles like ERGIC-derived nanotubules. Consistent sample preparation and image acquisition, coupled with computational analysis, are crucial for accurate, unbiased results.

Chapter 4 focuses on single-particle tracking (SPT) in the secretory pathway, proposing control experiments and parameter descriptors to maximize data quality. We emphasized labeling strategies, imaging, and data analysis considerations for reliable results.

Chapter 5 applied these methodologies to study protein sorting at the TGN, examining the role of ER-Golgi membrane contact sites (MCS) in TGN-derived carrier biogenesis. Using super-resolution fluorescence microscopy, we identified cargo accumulation regions and conducted SPT experiments, revealing confined, slow motion of cargo proteins near MCS. This effect was inhibited by the lipid transfer blocker 25-HC, indicating upstream regulation of cargo localization preferences by MCS.

Friday October 10, 15:00 h. ICFO Auditorium
Thesis Director: Prof. Dr. Maria Garcia-Parajo and Dr. Felix Campelo Aubarell
Hosted by: Prof. Dr. Maria Garcia-Parajo