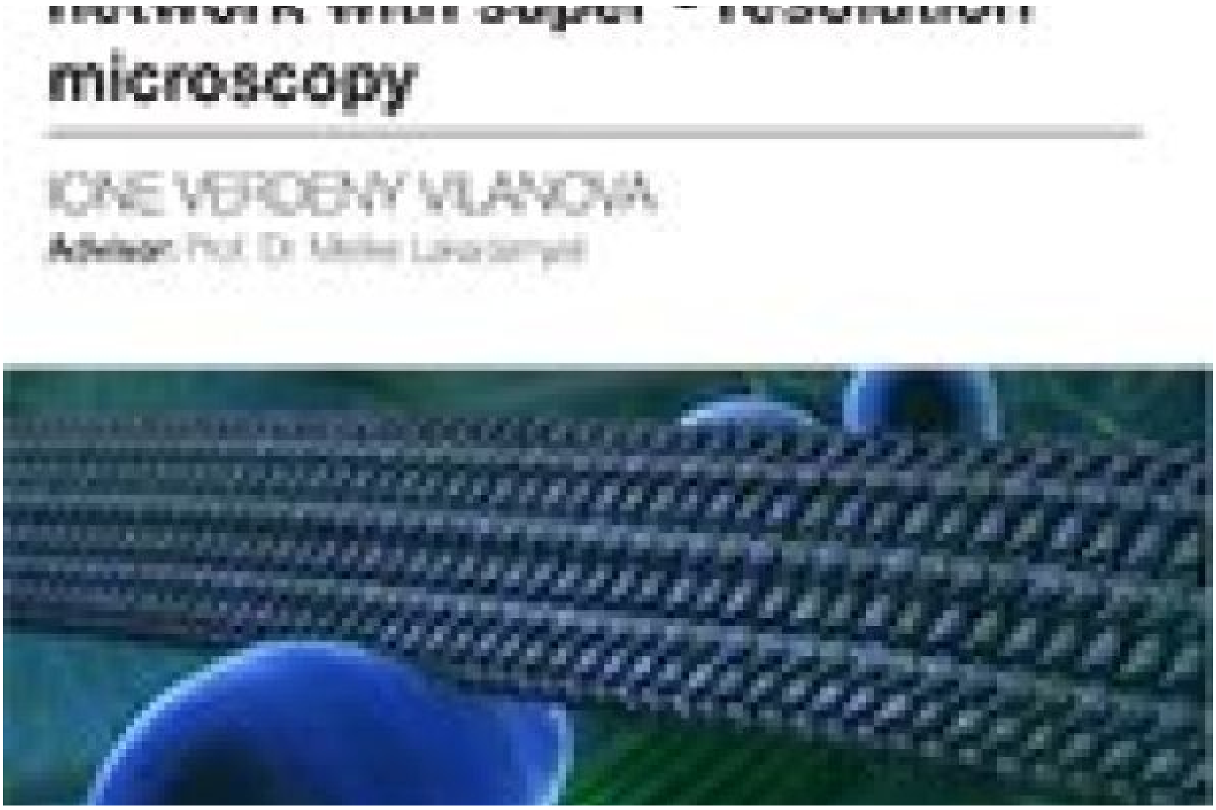

PhD Thesis Defense IONE VERDENY VILANOVA 'Unravelling 3D Cargo Transport Dynamics at the Microtubule Network with Super-Resolution Microscopy'

IONE VERDENY VILANOVA

October 13, 2016

Thursday October 13, 16:00. ICFO Auditorium

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Intracellular transport plays an essential role in a wide variety of fundamental cellular processes required to maintain the internal regulation and organization of cells. Different types of cargos such as organelles, secretory vesicles, mRNAs or protein complexes must be rapidly and efficiently delivered to a specific location at the right time. Molecular motors such as dynein and kinesin actively transport these cargos along the cellular highways that form the microtubule cytoskeleton, by transforming chemical energy into mechanical work. Intracellular transport is tightly regulated at multiple levels, including regulation by scaffolding proteins, motor proteins clustering or the cellular cytoskeleton. Among these regulatory mechanisms, the role of the microtubule cytoskeleton on cargo transport regulation is possibly the least well-understood. The complex three-dimensional geometry of the network along with the crowded environment of the cell cytoplasm complicate vesicular and organelle transport. Indeed, motor proteins have to overcome different types of obstacles and traffic jams in order to reach their final destination. Accumulation of obstacles on microtubules can lead to intracellular transport breakdown, which has been linked to several types of diseases, especially those of the nervous system. Understanding the molecular mechanisms by which transport is regulated as well as how motor proteins overcome obstacles during transport can thus give important clues as to how these mechanisms may break down in disease states.

The main goal of this thesis has been to provide new insights on intracellular cargo transport dynamics in the nanoscale context of the microtubule network and in the physiologically relevant environment of a living cell. The visualization of such biological processes in vivo requires high spatial and temporal resolution and represents an important technical challenge due to the lack of pre-existing, adapted imaging tools with such technical capacities. We thus developed a novel correlative approach combining the cutting-edge technology of single particle tracking and super-resolution microscopy techniques in order to provide nanometer spatial and millisecond temporal resolution. This pioneering approach allowed us to image for the first time in living cells the dynamics of intracellular cargo transport in the nanoscale, three-dimensional context of its underlying network of cytoskeletal tracks.

This thesis explores the role of the microtubule cytoskeleton on cargo transport regulation as well as the cellular mechanisms required to evade different types of obstacles during transport. Microtubule intersections act as distribution centers for cargo transport, providing multiple routes for cargos to be delivered to the proper location of function. The three-dimensional organization of the microtubule cytoskeleton impacts cargo transport dynamics at these intersections such that cargos predominantly pause at tight intersections with an intersection spacing <100 nm, likely because the intersecting microtubule constitutes a roadblock. Moreover, microtubule intersections differentially regulate cargo transport by acting as selective, size-dependent steric filters favoring the smooth trafficking of vesicles <250 nm. Finally, the inherently three-dimensional nature of cargo transport and the capability of cargo-bound motors to perform off-axis motion seems to be a preferential mechanism to evade different types of obstacles such as microtubule intersections or other cargos encountered during transport.

Overall, this thesis provides new insights into the role of the microtubule cytoskeleton on cargo transport regulation, three-dimensional cargo transport motility, and the mechanisms involved in overcoming different types of obstacles during transport. We hope these novel findings will pave the way for a better understanding of several diseases caused by intracellular transport anomalies.

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Thesis Director: Prof Dr Melike Lakadamyali