PhD Thesis Defense JUAN ANDRES TORRENO 'Membrane Protein Nanoclustering as a Functional Unit of Immune Cells - from nanoscopy to function-'

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Friday, October 2, 15:00. ICFO Auditorium

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Single Molecule Biophotonics

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State-of-the-art biophysical techniques featuring high temporal and spatial resolution have allowed for the first time the direct visualization of individual transmembrane proteins on the cell membrane. These techniques have revealed that a large amount of molecular

components of the cell membrane do not organize in a random manner but they rather grouped together forming so-called clusters at the nanoscale. Moreover, the lateral behavior of these clusters shows a great dependence on the compartmentalization of the cell membrane by, e.g., the actin cytoskeleton at multiple temporal and spatial scales. Since these lateral and temporal organizations have been shown to be crucial for the regulation of the biological activity by these transmembrane proteins, the understanding of the spatiotemporal behavior of membrane receptors, and of proteins in general, is a necessary step towards understanding the biology of the cell. Protein nanoclustering and membrane compartmentalization have been shown to play a crucial role on leukocytes, particularly on the surface of antigen presenting cells. Hence, the direct visualization of membrane proteins on the cell membrane of antigen presenting proteins represents a crucial step in understanding how an immune response can be controlled by leukocytes at the molecular level.

In Chapter 1, the immune system, the membrane receptor DC-SIGN and the antigen presenting protein CD1d are briefly introduced. Moreover, recent advances in superresolution microscopy and single particle tracking techniques which allow the study of membrane proteins at the nanoscale are discussed. Finally, an updated review of protein nanoclustering on the cell membrane shows examples of the importance of protein nanoclustering in regulating biological function in the immune system. Chapter 2 presents the quantitative methodology for analyzing STED nanoscopy images and multi-color single particle tracking data used throughout this thesis. Chapter 2 also describes the single-molecule fluorescence sensitive microscopes implemented in this thesis for multi-color single particle tracking experiments and the corresponding data analysis. At the end of Chapter 2, cartography maps combining high temporal with micron-scale spatial information on the basis of single-molecule detection are presented.

The following chapters in this thesis describe the major results obtained on two important receptors of the immune system. In Chapter 3, we address the role of the neck region of DC-SIGN in fine-tuning the nanoclustering degree of DC-SIGN on the cell membrane. Moreover, Chapter 3 also links the nanoclustering capability of DC-SIGN with its virus binding capability. The meso-scale organization of DC-SIGN and its dependence on a glycan-based connectivity is addressed on Chapter 4. This glycosylation network enhances the interaction between DC-SIGN and clathrin beyond stochastic random encountering. In Chapter 5, we showed that DC-SIGN shows subdiffusive behavior and weak ergodicity breaking (wEB) that

cannot be described using the continuous time random walk (CTRW) model. Instead, our data are more consistent with a model in which the plasma membrane is composed of "patches" that change in space in time. In Chapter 6, we demonstrate that the antigen presenting protein CD1d organizes in nanoclusters on the cell membrane of antigen presenting cells whose size and density are tightly controlled by the actin cytoskeleton. Moreover, we also showed that this cytoskeletal control of the CD1d nanoclustering predominantly occurs on the pool of CD1d that has undergone lysosomal recycling, including under inflammatory conditions. Finally, in Chapter 7 we summarize the main results of this thesis and highlight future experiments that will expand the knowledge obtained so far regarding the role of plasma membrane organization and biological regulation.

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