



## **PhD THESIS DEFENSE: Advanced single molecule fluorescent tools to reveal spatiotemporal multi-molecular interactions in living cells**

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April 28, 2023

11:00

Auditorium

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The spatiotemporal organisation and compartmentalisation of molecules in living cells is crucial to regulate cell function. Dysregulation in how molecules dynamically explore their environment and interact with other molecules can lead to disease and death. Therefore, understanding the nature of these dynamic interactions is pivotal in cell biology studies. Fluorescence light microscopy is the preferred approach to perform the necessary biophysical studies and it has led to major findings in the field. Nevertheless, spatial and temporal studies are typically conducted separately due to technical limitations. Moreover,

quantitative imaging and novel analysis toolboxes are required to address new questions in the field. The aim of this thesis is three-fold: (1) develop and implement novel algorithms to analyse super-resolution microscopy data to study the spatial organisation of a variety of proteins at the plasma membrane of cells; (2) develop a novel methodology to study the spatiotemporal organisation of receptors at the plasma membrane of cells based on high-density single particle tracking; and (3) apply our novel methodology in a multi-colour scheme to study the compartmentalisation of DC-SIGN and its multi-component interactions with CD44 and Galectin 9 during viral engagement.

In Chapter 1, we overview how cells are compartmentalised from the intra-cellular organisation to how the cell membrane is compartmentalised by multiple organisers acting most probably in synergy. Also, in Chapter 1, we review the main fluorescence microscopy techniques used in the field of cell biology to study the spatiotemporal organisation of molecules in cells. In Chapter 2, we present the analyses that we have performed to super-resolution microscopy techniques such as stimulated emission depletion (STED) and stochastic optical reconstruction microscopy (STORM). We have used these techniques to elucidate the spatial organisation of proteins at the plasma membrane such as Siglec-1, integrins or PRL-3. We have implemented state-of-the-art algorithms into our analysis workflow to resolve the biological inquiries of our research. In Chapter 3, we change gears and present our novel methodology to analyse high-density single particle tracking, which consists on generating high-density maps (HiDenMaps). In this chapter, we specify the technical requirements to obtain a faithful representation on how molecules explore space. In Chapter 4, we study CD44 which is a transmembrane protein extremely interesting because it can interact with the underlying cortical actin and the extracellular milieu. Moreover, it is thought to act as a key actor in the spatiotemporal compartmentalisation of the plasma membrane for third receptors that do not interact directly with actin. In this Chapter, we have used HiDenMaps to elucidate the hierarchical organisation of CD44 at the plasma membrane of living cells. In Chapter 5, we present a palette of analysis tools to further quantify the patterns revealed by HiDenMaps and resolve the temporal dynamics of these patterns. Moreover, our work reveals a multi-scale organisation of CD44 ranging from fast single molecules dynamics with a mesoscale dynamic compartmentalisation. In Chapter 6, we present a functional study on viral capture by DC-SIGN in immature dendritic cells. We extended our HiDenMap methodology to a multi-colour scheme to study the multi-component interactions of DC-SIGN with CD44 and Galectin 9 during viral engagement. Importantly, we demonstrate the existence of DC-SIGN/CD44/Galectin 9 tripartite pre-docking platform that enhances the successful engagement of HIV-1 and SARS-CoV-2 virus-like particles in immature dendritic cells. Finally, in Chapter 7 we summarise the main results of this thesis and highlight future directions of our research.

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