A SCREENING PLATFORM FOR THERAPEUTIC ANTIBODY/RECEPTOR PAIRS NANOSCALE ORGANIZATION CHARACTERIZATION

M. Cabillic[1], A. Beghin[2], R. Galland[1], F. Levet[1], C. Perrault[3], M. Partiseti[3], J-B Sibarita[1]
[1]Univ. Bordeaux, CNRS, Interdisciplinary Institute for Neuroscience, IINS, UMR 5297, F-33000 Bordeaux, France
[2]Mechanobiology Institute, National University of Singapore, 117411, Singapore.
[3]Sanofi, 13 Quai Jules Guesde, 94400 Vitry-sur-Seine, France
Email: marine.cabillic@u-bordeaux.fr

KEYWORDS: SMLM, high-content screening, high-throughput screening, automation, immunotherapy

Immuno-oncology is a young and growing field at the frontier of cancer therapy. Immuno-oncology therapies aim to stimulate the body's immune system through therapeutic antibodies, by binding and modifying the intracellular signaling of T-cells surface receptors. Understanding how the spatial organization of receptors and signaling proteins is regulated and how it determines lymphocyte behaviors has become a 'holy grail' for cellular immunology. To achieve this goal, a better comprehension of antibodies functions and subcellular trafficking is requested to explain the differential efficacies of therapeutic candidates targeting receptors of interest. Quantitative super-resolution microscopy provides access to the nanoscale organization of membrane receptors playing a physiological role. In combination with high content screening techniques, it offers a new investigation tool for antibody optimization as well as maximizing their functional efficacy.

We characterized receptor/antibodies pairs nanoscale organization by quantitative single-molecule localization microscopy (SMLM) combined with high content screening (HCS). In this context, we have developed a HCS-SMLM platform [1] which we used to characterize several antibodies targeting T-cell membrane receptors. We focused on PD-1 receptor, a control point of the immune system involved in the modulation of immune cells activation. We investigated the impact of known anti-PD-1 therapeutic antibodies used in clinics, on the nanoscale spatial organization of PD-1 receptors in living cells using our HCS-SMLM platform.

Here, we will describe a fully automated workflow for quantitative single-molecule-based super-resolution microscopy operating in 96 well-plates, including the automation of the acquisition and the statistical analysis of the Terabytes of data generated. We will demonstrate that this work provides the proof of concept of the capacity of our HCS-SMLM platform to screen and characterize quantitatively different therapeutic monoclonal antibodies targeting PD-1 on T-cell membrane.

[1] Beghin, A. et al. Localization-based super-resolution imaging meets high-content screening. Nature Methods 14, 1184–1190 (2017).