PhD thesis in experimental biology and theoretical biophysics – Toulouse, France

Biophysical exploration of nanoscale dynamics at the immunological synapse

NanoTCell

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"NanoTCell" is an interdisciplinary project at the interface between quantitative biology and statistical biophysics aiming at elucidating how T cells regulate their cytotoxic activity, a key parameter conditioning the capability of the immune system to eliminate infected or cancer cells. It depends on the assembly of the immunological synapse (see figure), a highly dynamic structure that stabilizes the interaction between the T cell and its target cell, leads to the T cell activation, allows the secretion of lytic granules and potentiates the pore-forming activity of perforin by applying mechanical forces on the target cell plasma membrane.

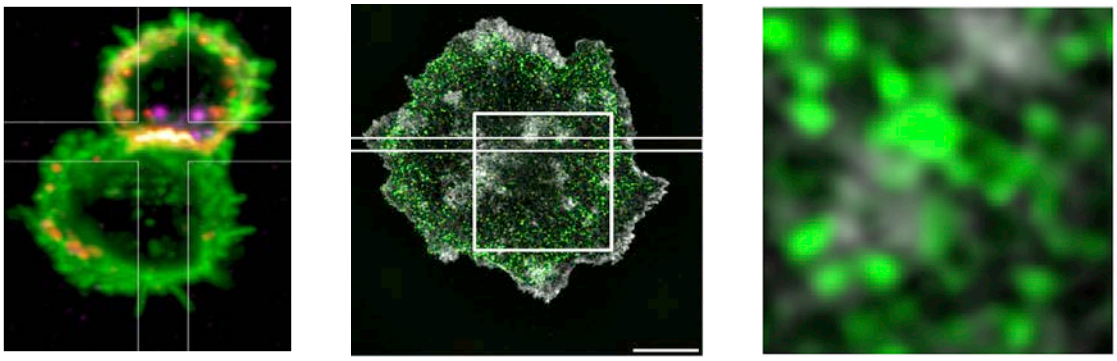

Figure: Left: confocal microscopy image of a human T cell (top) forming an immunological synapse with a target cell (bottom). Green: actin network; purple: perforin; orange: LFA-1 integrin (activated conformation). Center: super-resolution microscopy image (SIM) of the immunological synapse formed by contact on a functionalized glass surface. Grey: actin; green: LFA-1 integrin (activated conformation). Bar: 2 µm. Right: zoom of the same synapse (image size = 1 µm). LFA-1 nanodomains are clearly discernable. Pictures from Raïssa Houmadi Ph.D. thesis [Houmadi2017].

The synaptic cortical actin cytoskeleton is essential in this context as it has been proven to be a key-player of the synapse assembly and its integrity is essential for the cytotoxic activity. Exploring its organization, the molecular control of its remodeling and the ensuing physical forces will be crucial in order to understand how T cells convert molecular signals into a coordinated physical action. The NanoTCell project relies on the study of unique study models consisting in T cells from immunodeficient patients carrying mutations in genes encoding various actin cytoskeleton regulators (WASP, WIP, WRD1, Coronin-1A).

The main objectives of the thesis will be to:

- Characterize by superresolution microscopy (SIM) and dynamical microscopy (TIRF) the synapse spatial organization in T cells from immunodeficient patients and healthy donors, with a focus on the correlation between cortical actin density and the localization of membrane proteins;
- Test the relevance of different physical models used to describe protein organization in nanodomains (fence-and-picket model *vs* models based on protein-protein and/or protein-lipid interactions);
- Measure the physical forces exerted by the T cell at the synapse in the cytotoxic activity context with the help of micro-pillar experiments [Basu2016];
- Elaborate a quantitative numerical multiscale model of immunological synapse describing how actin cytoskeleton remodeling controls lytic granules release and cytotoxic activity.

The **experimental work** (microscopy) will be done under the supervision of **Loïc Dupré**, an immunologist expert in the study of primary immunodeficiencies related to the actin cytoskeleton, at the CPTP (cell imaging facility, directed by **Sophie Allart**, Genotoul network) [Dupré2002, Calvez2011, Dupré2015, Pfajfer2017, Houmadi2018, Pfajfer2018]. The **numerical modeling** will be done under the supervision of **Nicolas Destainville** (LPT), a physicist expert in data analysis and modeling of biophysical **mechanisms** at play at the cell surface [Destainville2016, Gueguen2017, Destainville2018]. The student will share its time equally between both laboratories. The part of the work related to the measurement of physical forces will be done during a stay in **Morgan Huse**'s team at the Memorial Sloan-Kettering Cancer Center, New York, USA.

Expected skills: the candidate must have followed a M.Sc. (or equivalent) in Physical Biology, Biophysics or Biological Physics. He/she must be as familiar as possible with modern fluorescence microscopy techniques, data processing, numerical simulation techniques and physical modeling.

Funding: the 3-year PhD position is funded by the University of Toulouse and is expected to start in September 2019. It aligns with the French national PhD contract and includes health and social insurance coverage.

Application: please send before May 15, 2019 a CV, letter of motivation and 2 references to Loïc Dupré (loic.dupre@inserm.fr) and Nicolas Destainville (destain@irsamc.ups-tlse.fr).

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