Laboratoire : Adhesion et Inflammation, Inserm U1067 / CNRS UMR733
Adresse : Aix Marseille Université, Campus de Luminy, Marseille
Directeur du laboratoire : Olivier Théodoly, DR2 CNRS

Responsables de stage : Pierre-Henri Puech, CR1 Inserm / Laurent Limozin, CR1 CNRS
Adresses électronique : pierre.henri.puech@inserm.fr / laurent.limozin@inserm.fr

Titre du stage : Single cell interrogation of mechanical determinants of T cell activation (under forces).

Résumé
How different TCR- peptide/MHC binding events are processed by the lymphocytes cellular signalling machinery remains a critically important question for both the development and the functioning of the adaptive immune response. Along these lines, the role of the cytoskeletal architecture of the T cell in the activation properties of those cells remains to be clearly established: it allows the T cells to exert forces on the APC, down to single molecule scale, and those forces have been proposed to be a key determinant for the capacity of the T cell to selectively and finely recognize foreign peptide and activate. Our team is investigating how micromechanical properties of T cells (such as adhesion, elasticity, membrane tension) are influenced / influencing the phenomenon of activation, using advanced biophysical techniques based on force application / measurement simultaneous to observation of activation reporters (such as Ca++ sensitive dyes), allowing to measure eg. cell elasticity and adhesion while signalling is occuring. In this context, the way T cell micromechanical microenvironment crucially affects the capacity of T cell to answer to a controlled and defined cue is analyzed, by graduated complexification of this microenvironment (cell / functionnal substrate, cell / APC, APC/ cell / APC) and / or perturbating it (using lipid modification of membranes, drugs against cytoskeleton components, antibodies against receptors, various APC cell types) in original ways.

The master student will be readily included in studies and developments using the avalaible tools and others topics in the team, around the thematics of cell micromechanical properties and transmission / integration of information through the cell's membrane, leading to modification of cell function and behaviour. We will in particular focus on how these tools can provide a very detailled view of the linkage of surface proteins to the cell's cytoskeleton in the context of T cell activation.

This topic built in collaboration with Y. Hamon (Centre d'Immunologie de Marseille Luminy) provides a unique approach to the interface of physics and biology in a young, highly cohesive and multidisciplinary environment (physicists, biologists and physicians) around a theme with strong applications in human health.

Avalaible techniques@LAI
Atomic Force Microscopy (AFM) coupled to fluorescence microscopy, Optical Tweezers coupled with fluorescence microscopy, Micromanipulations / Biomembrane Force Probe (BFP), Fluorescence and TIRF microscopies, interference microscopy (RICM), Image processing, Micropatterning, human and murine cell cultures.

Réferences
- Limozin L*, PH Puech Membrane organization and physical regulation of lymphocyte antigen receptors: a biophysicist's perspective. J MEMBRANE BIOLOGY (2019). 10.1007/s00232-019-00085-2
- Limozin L, Bridge M, Bongrand P, Dushek O, van der Merwe PA, Robert P TCR-pMHC kinetics under force in a cell-free system show no intrinsic catch bond, but a minimal encounter duration before binding. PNAS 116 (34) 16943-16948 (2019).
- Wahl A, Dinet C, Dillard P, Puech P-H, Limozin L*, Sengupta S* Biphasic mechanosensitivity of TCR mediated adhesion of T lymphocytes. PNAS 116:5908-5913 (2019)
- Nano-clustering of ligands on surrogate antigen presenting cells modulates T cell membrane adhesion and organization. Dillard P, Pi F, Lellouch AC, Limozin L, Sengupta K. Integr Biol (Camb). 2016 Mar 14;8(3):287-301.
- Synchronizing atomic force microscopy force mode and fluorescence microscopy in real time for immune cell stimulation and activation studies. Cazaux S, Sadoun A, Biarnes-Pelicot M, Martinez M, Obeid S, Bongrand P, Limozin L, Puech PH. Ultramicroscopy. 2016 Jan;160:168-81.
- Use of TIRF to Monitor T-Lymphocyte Membrane Dynamics with Submicrometer and Subsecond Resolution. Brodovitch A, Limozin L, Bongrand P, Pierres A. Cell Mol Bioeng. 2015;8(1):178-186.
- Ligand-mediated friction determines morphodynamics of spreading T cells. Dillard P, Varma R, Sengupta K, Limozin L. Biophys J. 2014 Dec 2;107(11):2629-38.
- Force measurements of TCR/pMHC recognition at T cell surface. Puech PH, Nevoltris D, Robert P, Limozin L, Boyer C, Bongrand P. PLoS One. 2011;6(7):e22344.
- Atomic Force Microscopy : A Versatile Tool for Studying Cell Morphology, Adhesion and Mechanics Franz CM, Puech PH* Cellular And Molecular Bioengineering 1(4) :1865-5025 2008

Websites
https://labadhesioninflammation.com/ ; http://phpuech.wordpress.fr ; http://laurentlimozin.free.fr/