

Membrane physics as the earliest amplifier of antigen driven T Cell signal initiation: implications for health and disease

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Abstract (10 lines)*

Activation of naïve T cells is the initial and pivotal event in the adaptive immune response, occurring when one to few T Cell Receptors (TCR) engage rare major histocompatibility complex molecules laden with agonist peptides (pMHC) among a sea of self-antigen loaded MHC molecules expressed on antigen presenting cells (APC). This recognition is simultaneously selective, specific and sensitive, and remains incompletely understood. The objective of this PhD project is to identify key molecular and biophysical events as the decision-making steps, that lead to amplification of TCR triggering. Supported by convergent preliminary data, we propose that local TCR-pMHC recognition triggers a propagating change in the electric potential, molecular organization, and biomechanics of the plasma membrane (PM), very finely timed, tuned and coordinated, which serves as an amplification platform to convert discrete molecular-scale events to a cell-scale reaction, all of this taking place very early in the activation processes, prior to canonical T cell biochemical signaling cascades.

Keywords*

T cell activation, plasma membrane, mechanobiology, biophysics, lipids, membrane potential

Scientific question and Objectives (10 lines)*

The project explores the hypothesis that the plasma membrane acts as the earliest platform and amplifier of antigen-driven T cell activation. While classical immunology has focused on biochemical signalling cascades and cytoskeletal forces, recent evidence suggests that biophysical properties of the T cell membrane—its mechanics, electrostatics, and molecular organization—play a decisive role in the very first seconds of immune recognition. Preliminary data show that T cell mechanics and membrane tension, potential, and receptor diffusion rapidly change upon antigen encounter, even when canonical Src kinase-dependent signalling is inhibited. The objectives of the project will be to establish that the plasma membrane itself behaves as a mechano-electro-chemical hub, converting discrete molecular events into a cell-scale response. To this end, multiple experimental approaches at multiple scales will be proposed to the candidate, some already in hands (FCS, FLIM, FRET, AFM...), some to be developed (such as planar patch clamp).

Proposed approach (experimental / theoretical / computational) and research plan (20 lines)*

The project combines biophysics, advanced dynamic imaging, omics on mouse vs human immune cells to identify the molecular players and physical mechanisms governing this membrane-based amplification.

WP1 will investigate how membrane mechanics and opposing substrate stiffness and composition influence T cell activation using atomic force microscopy, optical tweezers, and nanostructured surfaces (LAI/CINAM). This will be complemented with analysis of TCR membrane dynamics (by spot variation fluorescence correlation microscopy, CIML imaging platform) and TCR conformation changes (by Fluorescence lifetime imaging; IBDM imaging platform).

WP2 will integrate bioelectrical measurements via whole cell planar patch-clamp and voltage-sensitive dyes to link activation with membrane potential changes (CINAM, K. Sengupta)).

WP 3 will map the TCR-regulated mechano-interactome, focusing on mechanosensitive ion channels such as Piezo1 which has been shown by us and others to be implicated in T cell activation modulation (R. Roncagalli, CRCM proteomic platform).

WP4 will extend the findings to pathological models, particularly T cells from patients or mice with cholesterol transport

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deficiencies (ABCA1/ABCG1), to assess how altered lipid composition impacts T cell function (collaboration W. Le Goff, IRCAN, Paris).

Interdisciplinarity and Implication of the two labs (15 lines)*

(In this section the collaboration of the two laboratories will be explained in details to explain why the project cannot be conducted by one team alone)

How changes in T cell biophysics and biomechanics are converted into biological signals because of cell-to-cell contacts is of general importance in immunity, both in health and disease. As such, this thematic at the border between biology and biophysics requires multi- and inter-disciplinary skills dispatched at least in the two labs of the present proposal (complemented with long lasting collaborators in other labs, being part of the centuri perimeter, thereby fitting exquisitely in the present call). Biological models are being developed at the CIML (molecular biology, flow cytometry, confocal imaging...), where in addition can be performed analysis of the plasma membrane nano-organisation, T cell signaling.... All the mechanobiology part of the project will be naturally carried out at the LAI, as recognized experts, especially in immune cell biomechanics. In this project, as we will favour natural ligands rather than antibodies on continued vs micropatterned surfaces, those ligands will be either kept immobile or anchored to a lipid bilayer of various composition and fluidity, requiring the expertise of K. Sengupta (CINAM). Newly developed patch clamp device will be developed with CINAM expert as well. Of note, direct electrical measurements on T cells are not existing to our knowledge in the Centuri community, and such tools could be a plus for this community once developed and spread around.

We will highly benefit of the interaction with Centuri engineering team for data processing in order to extract quantitative data from the rich experiments we propose, coupling scalar measurement, resolved in time and space, with imaging techniques, to put in tight correlations and if possible, define clear causality links. They will also help us in designing the T cell whole cell patch clamp assay.

Specify with whom the person recruited will collaborate and on what aspects *

The candidate will collaborate with members of both teams (CIML, HTH lab and LAI), but will benefit from long lasting collaboration with Wilfried Le Goff team (human samples, IRCAN, Paris), Kheya Sengupta (CINAM, planar patch clamp device), Romain Roncagalli (CIML, Proteomics of the mechano-interactome).

PhD student's expected profile*

This topic provides for a cell biologist or an experimental physicist a unique approach at the interface of biology and physics in a young, highly cohesive and multidisciplinary environment (physicists, biologists and physicians) around a theme with strong applications in human health. The candidate should have bases in microscopy and computer programming since the richness of the data may imply to develop data analysis codes, or at least be willing to invest in learning how to use them.

Is this project the continuation of an existing project or an entirely new one?

In the case of an existing project, please explain the links between the two projects (5 lines)*

This project is not a direct continuation of the current PhD project of our co-tutored PhD student (Marie Dessard, 4th year), more focused who deciphered ~~on~~ the role of cholesterol/lipid transporter in T cell activation. This current proposal aims more broadly at identifying the key players and parameters supporting TCR dependent mechanotransduction from the membrane to the cell interior, but will obviously benefit from the results and techniques gathered over the past years.

Two to five references related to the project*

Rossy, J., J.M. Laufer, and D.F. Legler, Role of mechanotransduction and tension in T cell function. *Frontiers in immunology*, 2018. 9: p. 2638.

Ridone, P., et al., Disruption of membrane cholesterol organization impairs the activity of PIEZO1 channel clusters. *Journal*

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of General Physiology, 2020. 152(8): p. e201912515.

Badou, A., et al., Requirement of voltage-gated calcium channel $\beta 4$ subunit for T lymphocyte functions. Science, 2005. 307(5706): p. 117-121

Liu, C.S.C., et al., Piezo1 mechanosensing regulates integrin-dependent chemotactic migration in human T cells. Elife, 2024. 12: p. RP91903

Two main publications from each PI over the last 5 years*

Zak, A., et al., Rapid viscoelastic changes are a hallmark of early leukocyte activation. Biophysical Journal, 2021. 120(9): p. 1692-1704.

Sadoun, A., et al., Controlling T cells spreading, mechanics and activation by micropatterning. Scientific reports, 2021. 11(1): p. 6783.

Project’s illustrating image

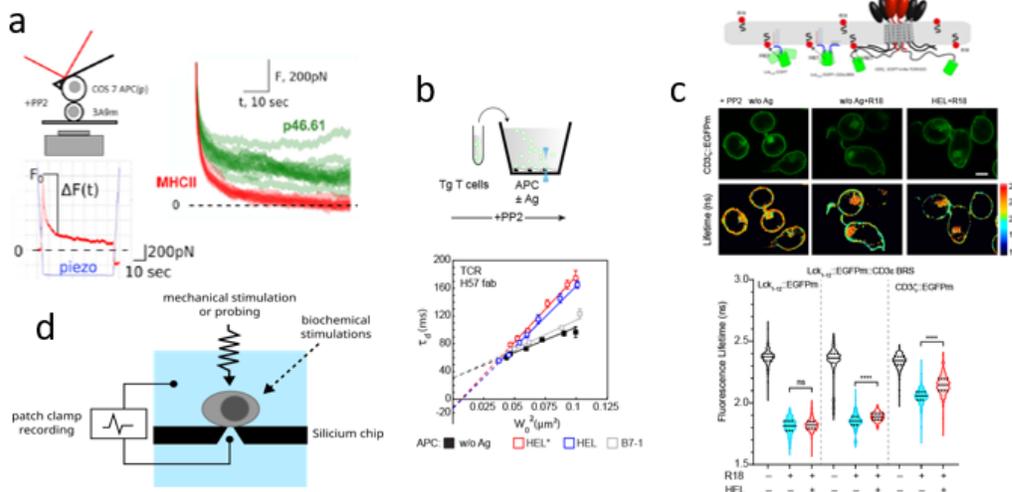


Figure 1 : Src-independent T cell plasma membrane modification upon antigenic challenge . **a**- Atomic force microscopy (AFM) single-cell force spectroscopy (SCFS) of adhered T cells contacted with antigen loaded (HEL 46-61) or not APC stuck on the tip of a cantilever. **b**- svFCS analysis of the TCR diffusion on naive T cells (labelled with monovalent fluorescent antibody) seeded onto APC loaded or not with HEL derived peptides and expressing (or not) the costimulation molecule B7-1. **c**-FRET-FLIM analysis of the lifetime of the EGFP grafted at the c-terminus of the CD3 ζ chain of the TCR complex in proximity of the R18-loaded PM (as acceptor) in T cell in contact with APC loaded or not with HEL derived peptides. Additional GFP-tagged constructs were used as a negative or positive control [30]. **d** : Planar patch-clamp chip, with the tip of the pipette replaced by a micro-aperture in a self-supported film separating two baths (adapted from ref [41]).

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