PhD PROJECT PROPOSAL

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Temporal forces modulation in T cell early recognition and activation

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Abstract (10 lines)*

The interaction between T cells and antigen-presenting cells (APCs) is the bridge between our innate and adaptive immune responses. T cells are activated when the membrane-bound T cell receptors (TCRs) recognize foreign antigenic peptides presented by the major histocompatibility complexes (pMHCs) present on the membrane of the APCs. As the activated T cells multiply, depending on their sub-type, they further differentiate into cytotoxic T cells that directly kill virus-infected and cancer cells, or into helper / regulatory T cells. There is a growing realization that the function of all of these types of T cells is related to their ability to feel, generate, and respond to forces. Traction force microscopy (TFM) has been used to measure cell-scale forces, including proof-of-principle measurements on T cells, but biological exploration was limited since TFM on T cells is particularly challenging when performed in conditions close to physiological ones. Our recent careful TFM shows that T cell subtypes generate forces in distinct temporal patterns, which are related to environmental cues and cell subtype. In this project we shall study (a) relation between stimulation and force generation pattern with the help of novel molecules that bind to TCR with a panel of affinity (b) the mechanism of how the different force patterns are generated, with the help of membrane and cytoskeletal mutations and (c) explore how force patterns are linked with T cell function. This will require the fabrication of novel soft surfaces using tools of nano-technology and Soft-Matter, improvements in force measurement and calculation using advanced image analysis tools by transforming the current analysis using of deep-learning models to quantify the forces from deformation fields, and skills in immunology to dissect the early steps of T cell activation in regard to physiological action.

Keywords*

T cell, immunobiophysics, mechanobiology, spreading, traction force microscopy, forces, cytoskeleton, early activation, adhesion, quantitative biophysics, deep learning

Scientific question and Objectives (10 lines)*

In a collective effort to meet the challenges described in the abstract, T cell forces have been measured using DNA-force probes and new refined TFM modes are being developed by many groups. Nevertheless, these are yet to be used to their full potential to reveal physiologically relevant time evolution of force application, specifically, the feeble forces during early T cell contact and activation, which are in fact the crucial decision making steps, are rarely measured. Moreover, the temporal variations of forces during early T cell contact and activation are rarely dissected in contrast to late time forces, typically after an engagement of circa 30 min.

We recently showed that indeed those early-time feeble forces, ranging from atto to pico Newton, are applied in distinct temporal patterns. The aim in this project is to understand the physical mechanisms a well as biological purpose/implications of these distinct time patterns. The questions we shall ask are: (a) how can we trigger a specific force pattern? Does the strength of the activation signal determine it? (b) what is the mechanism of this temporal variations? Is it related to a specific cytoskeletal or membrane component? (c) is the force generation related to the biological function of the cell sub-type? Throughout, effort will go into improving the sensitivity and throughput of TFM by innovations on improvement of the experiments as well as data analysis, specifically the calculation of the force from images. Finally, we hope to integrate the findings into our mathematical model of cell spreading .

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

This project is mainly experimental, but will involve intensive data analysis with codes and procedure based on already existing and/or published protocols. Obviously, the necessary adaptations of experimental protocols and analysis procedures will be made in coordination with the PIs. If needed, proper introduction or complementary formation will be acquired by the candidate, thanks to Centuri training courses, AMU ones, or dedicated one to one teaching by the PIs of lab members (eg. for using dedicated microscopes or software).

We will base our work on three different work-packages, of gradual complexity and integration of the quantitative aspect of T cell immunobiophysics.

1. Dissection of molecular determinants of the temporal modulations of forces
Here, we will complement the existing data presented in (Cellular forces during early spreading of T lymphocytes on ultra-soft substrates. Farah Mustapha, Martine Biarnes-Pelicot, Remy Torro, Kheya Sengupta, Pierre-Henri Puech. BioRxiv. doi: https://doi.org/10.1101/2022.02.11.480084) by systematically adding to the activating molecules, presented by the substrates, co receptor molecules, adhesion molecules and eventually repulsive molecules. Of note, we will also vary the relative amounts of the different partners at play to obtain a phase diagram of the spreading behaviour (on hard substrates, using interference microscopy (RICM) and/or TIRF) of the T cells and also of the force exertion (on soft substrates, using our TFM set up, see (Protocol for measuring weak cellular traction forces using well-controlled ultra-soft polyacrylamide gels. Mustapha F, Sengupta K, *Puech PH*. STAR Protoc. 2022 Jan 28;3(1):101133. doi: 10.1016/j.xpro.2022.101133)).

2. Impact of the cytoskeleton structures and actions
A direct corollary of point 1 and of our previous work (see for example (Fabio Manca, Gautier Eich, Omar N'Dao, Lucie Normand, Kheya Sengupta, Laurent Limozin, Pierre-Henri Puech. Probing mechanical interaction of immune receptors and cytoskeleton by membrane nanotube extraction. Scientific Reports 13, Article number: 15652 (2023). and Wahl A, Dinet C, Dillard P, Nassereddine A, Puech PH, Limozin L, Sengupta K. Biphasic mechanosensitivity of T cell receptor-mediated spreading of lymphocytes. Proc Natl Acad Sci U S A. 2019 Mar 8. pii: 201811516. doi: 10.1073/pnas.1811516116) will be to interfere with the cytoskeleton components (actin, myosin, but also microtubulis and intermediate filaments) in order to dissect from where the forces are originated and which are the main biochemical control "spots" for the early T cell recognition. For this, we will use drugs, or drugs cocktails, at various concentrations, to tune, again, the effect on spreading and forces. Aside, we have in hand several photoactivable constructs, already tested (see (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. doi: 10.1016/j.ultramic.2015.10.014. Epub 2015 Oct 19.)) that we can also use to tune, at will, in a temporally controlled manner, the cytoskeleton activity. Of note, we will quantify the actin retrograde flow and its structures, using TIRF based microscopies and eventually, super-resolution.

3.Unraveling the impact of subtle physiological differences between physiologically related T cells and processes
Naturally, we will extend these approaches to link them to (1) calcium fluxes at cell scales, which represent a dynamical early hallmark of an efficient activation of the cells (which could be complemented by using phospho-Zap labelling of the cells, after fixation at different time points), (2) the sub-type of cell we consider (we recently observed that the temporal force patterns appear to be deeply modulated by this very crucial parameter, often overlooked in biophysical experiments), and (3) the activation then anergy temporal processes that T cell have to encounter when in physiological immune response, to avoid to over act.

Improving the sensitivity and robustness of the measurements by innovation on the material aspects of TFM as well as use of deep learning in image and data analysis will be intrinsic to the experimental approach. The results will be integrated into the general mathematical models of T cell mechanotrasduction introduced by the host labs (Fabio Manca, Gautier Eich, Omar N'Dao, Lucie Normand, Kheya Sengupta, Laurent Limozin, Pierre-Henri Puech. Probing mechanical interaction of immune receptors and cytoskeleton by membrane nanotube extraction. Scientific Reports 13, Article number: 15652 (2023) ; Wahl A, Dinet C, Dillard P, Nassereddine A, Puech PH, Limozin L, Sengupta K. Biphasic mechanosensitivity of T cell receptor-mediated spreading of lymphocytes. Proc Natl Acad Sci U S A. 2019 Mar 8.

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pii: 201811516. doi: 10.1073/pnas.1811516116)

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)

In this collaboration, CINaM (Physics) will bring expertise on material science and the techniques of micro and nano-engineering of smart substrates, surface microscopies and super-reolution imaging; LAI (Biology) will bring expertise on immunology, and models, especially on T cells, imaging capacities and force based methods such as AFM, TFM and if needed optical tweezers and micromanipulations. Neither partner can hope to achieve the goals alone. LAI lacks relevant expertise in materials and patterning and CINaM lacks competence to access important biological tools. Traditional TFM approaches were developped by Pierre-Henri Puech, coupling imaging techniques and Deep Learning analysis will be helped by Laurent Limozin (LAI), smart substrates were designed by Kheya Sengupta (CINAM) over the last decade, putting this project in the frame of a larger interdisciplinary and inter-lab effort to quantitatively approach forces and their consequences in immunological systems.

In addition, we shall benefit from the technology platform of Centuri for help with fabrication as well as computational support that will be needed for quantifying the distortion and converting detected distortions to a measured force. We hope to use this PhD as a stepping-stone to being the newly developed tools to the Centuri community.

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

The person will collaborate as much as possible equally with the two labs, and will interact with the lab members and collaborators freely. In particular, current PhD students of Pierre-Henri Puech will be involved in helping the person to set the first experiments at the bench and to understand the fundamentals of the experimental lines (Jana El Husseiny, Marie Dessard). In CINAM, in addition to PI Kheya Sengupta, the student will interact with her PhD student Gaurav Varma working on a related topic of immunotherapy, engineers of the cinam microfabrication platform, and will have help from engineer of the department of physics of living systems for imaging.

People involved at the margin of this project, which is included in a long term effort of bothe Pis to dissect the immunobiophysics of early immune cell recongition and activation, are Laurent Limozin (LAI), Yannick Hamon (CIML), Paolo Pierobon (I. Cochin), Julien Husson (LaDHyX), David Gonzalez-Rodriguez (U. Metz).

The project will be instrumental in kick-starting a collaboration with Jay Groves (U. Berkeley, USA) which is about to start in 2025 with both PIs.

PhD student's expected profile*

The candidate should have an academic background in physics/engineering or biophysics. Candidates with previous experience in optical microscopy will be given preference. Reasonable competence in computer programming is expected. We look for highly motivated candidates willing to do experimental and computational work at the interface of physics and biology, in two interdisciplinary laboratories gathering physicists, biologists and medical doctors.

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 a new project, with roots in existing lines of research that have been successfully pursued over the last

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decade independently and sometimes jointly between the two PIs. It is based on results obtained jointly during a co-Pied PhD (Farah Mustapha, defense 2022, doc2AMU funding ; see Mustapha et al. BioRxiv 2024), but takes entirely new directions, especially with the new collaboration with Berkeley.

Two to five references related to the project*

Protocol for measuring weak cellular traction forces using well-controlled ultra-soft polyacrylamide gels. Mustapha F, Sengupta K, *Puech PH*. STAR Protoc. 2022 Jan 28;3(1):101133. doi: 10.1016/j.xpro.2022.101133

Mechanotransduction as a major driver of cell behaviour: mechanisms, and relevance to cell organization and future research Puech Pierre-Henri* and Bongrand Pierre*. Open Biol. 2021 Nov;11(11):210256. doi: 10.1098/rsob.210256

Controlling T cells shape, mechanics and activation by micropatterning. A. Sadoun, M. Biarnes-Pelicot, L. Ghesquiere-Dierickx, A. Wu, O. Théodoly, L. Limozin, Y. Hamon, *P.-H. Puech*, Sci Rep. 2021 Mar 24;11(1):6783. doi: 10.1038/s41598-021-86133-1

Single-cell immuno-mechanics: rapid viscoelastic changes are a hall-mark of early leukocyte activation Alexandra Zak, Sara Violeta Merino Cortés, Anaïs Sadoun, Farah Mustapha, Avin Babataheri, Stéphanie Dogniaux, Sophie Dupré-Crochet, Elodie Hudik, Hai-Tao He, Abdul I Barakat, Yolanda R Carrasco, Yannick Hamon, Pierre-Henri Puech, Claire Hivroz, Oliver Nüsse, Julien Husson, Biophys J. 2021 May 4;120(9):1692-1704. doi: 10.1016/j.bpj.2021.02.042

Wahl A, Dinet C, Dillard P, Nassereddine A, Puech PH, Limozin L, Sengupta K. Biphasic mechanosensitivity of T cell receptor-mediated spreading of lymphocytes. Proc Natl Acad Sci U S A. 2019 Mar 8. pii: 201811516. doi: 10.1073/pnas.1811516116

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

Main publications <u>on the topic,</u> Pierre-Henri Puech

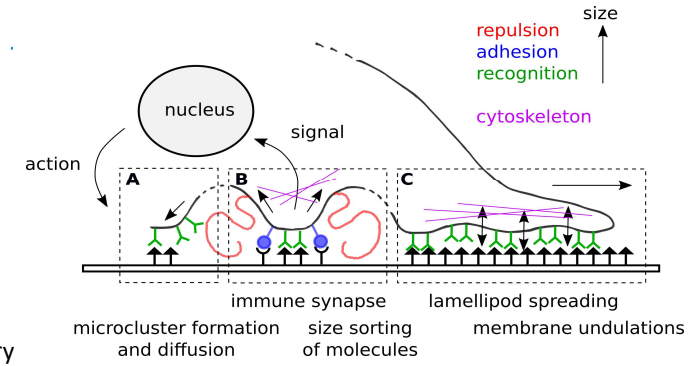
1. Cellular forces during early spreading of T lymphocytes on ultra-soft substrates. Farah Mustapha, Martine Biarnes-Pelicot, Remy Torro, Kheya Sengupta, Pierre-Henri Puech. BioRxiv. doi: https://doi.org/10.1101/2022.02.11.480084

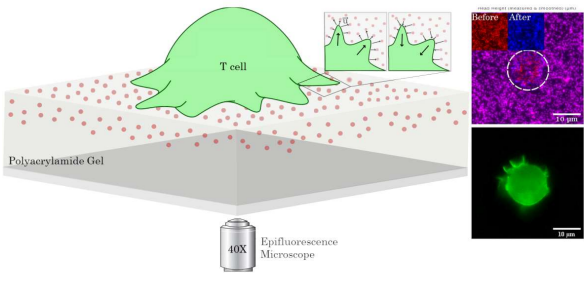
2. Fabio Manca, Gautier Eich, Omar N'Dao, Lucie Normand, Kheya Sengupta, Laurent Limozin, Pierre-Henri Puech. Probing mechanical interaction of immune receptors and cytoskeleton by membrane nanotube extraction. Scientific Reports 13, Article number: 15652 (2023).

Main publications <u>on the topic,</u> Kheya Sengupta

1. Morphodynamics of T-lymphocytes: Scanning to spreading. Sengupta K, et al. Biophys J. 2024. PMID: 38425041
2. Ligand Nanocluster Array Enables Artificial-Intelligence-Based Detection of Hidden Features in T-Cell Architecture. Nassereddine A, et al. Nano Lett. 2021. PMID: 34170136

Project's illustrating image





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(Left) Different molecules types, spatial and temporal scales which modulate early T cell recognition and subsequent activation (from Membrane Organization and Physical Regulation of Lymphocyte Antigen Receptors: A Biophysicist's Perspective. Limozin L, Puech PH .J Membr Biol. 2019 Jul 27. doi: 10.1007/s00232-019-00085-2) ; (Right) Traction force microscopy to unravel the early temporal patterns of forces during T cell recognition, spreading and activation – schematics, before/after superposition of beads images, fluorescent detection of the cell shape (from Cellular forces during early spreading of T lymphocytes on ultra-soft substrates. Farah Mustapha, Martine Biarnes-Pelicot, Remy Torro, Kheya Sengupta, Pierre-Henri Puech. BioRxiv. doi: https://doi.org/10.1101/2022.02.11.480084)

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