PhD PROJECT PROPOSAL

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How reaction-diffusion on a quasi-2D landscape determines the first steps of immune cell response

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Abstract*

T Lymphocytes are able to discriminate self- and foreign- protein fragments (antigen) quickly and with astonishing precision, sensitivity and robustness. How, starting from a single bond between their special receptors (TCR) and an antigen, they can activate the whole cell and eventually the whole organism, remains one of the central mysteries in immunology. It is believed that modulation of its 3D topography coupled to its molecular reorganisation *via* clustering of TCR and/or formation of membrane-proximal condensates plays an important role. We shall combine nanotechnology to manipulate the cluster dimension and placement, Camelidae nanobody know-how to both mimic antigens with variable potency and to precisely locate cellular receptors of interest, and dynamic optical super-resolution techniques to map the membrane topography and to track the receptors. Simultaneously, we shall monitor cell activity in terms of cytoskeletal remodelling, recruitment of signalling molecules, and production of classical activation markers such as cytokines. We shall explore the interplay of molecular diffusion and reaction (bond formation) that ultimately lead to activation.

Keywords*

Biophysics, immunology, super-resolution, nanobodies, nanopatterning, cell membrane.

Scientific question and Objectives*

The aim of this project is to understand **how signal**, in form of bond formation at the molecular scale between the TCR on the surface of an immune cell, and antigen on a target cell, **is amplified**, **leading to cell**, and eventually organism, scale **activation and response**.

We shall ask questions such as:

what determines the dynamics of cluster formation of TCR and its co-receptors? Is there a requirement for TCR cluster size and proximity to favour activation? Does this depend on antigen potency and TCR/antigen bond strength? Can we detect condensation of signalling molecules and if yes, what is its role? How is TCR dynamics and cell activation related to cytoskeletal remodelling?

To attain these objectives, we shall combine reflection interference, capable of imaging the 3D topography of the membrane, with fluorescent tagging via nanobody-oligonecleotide conjugates for total internal reflection fluorescence microscopy and DNA-paint technique to achieve dynamic and super resolution imaging of TCR and other proteins.

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

This is a mainly experimental project with a large component of computational image / data analysis.

Glass surfaces will be nano-patterned with the protein of choice, typically, appropriate camelidae single domain antibodies (nanobodies) that bind to CD3epsilon chain of the TCR complex with various affinities, surrounded by supported lipid bilayer (optionally functionalised with various co-receptors and/or adhesion molecules, **Fig 1 A**). Interaction of primary human T cells with these substrates will be imaged in real time, using reflection interference contrast microscopy (RICM) [1], total internal reflection fluorescent (TIRF) microscopy and super resolution microscopy of the type DNA-paint [2]. The protocols for surface nano-patterning are already available, as is the nanobody technology. DNA-paint microscopy and the associated analysis will be implemented and optimised for this study. Several protein of interest will be marked using nanobodies site specifically conjugated to oligonucleotides. Transient binding of the complementary strand labelled with an appropriate fluorophore will allow tracking of the molecules via localisation-based super-resolution microscopy. This approach will be used to visualise main receptors involved in the immune synapse, including the TCR, CD45, CD2, LFA-1, PD-1 [3].

The trajectories of TCR, as it diffuses and binds to the surface pattern and/or is actively transported via actin, will be traced. Simultaneously, other molecules of interest such as the T cell integrin LFA1 and/or the glycocalyx molecule CD45 that doubles as a phosphatase regulating the TCR signalling process (kinetic-segregation model) [4] can be followed. Such tracking will be combined with mapping of the surface topography using RICM imaging (**Fig. 1 B**) – as is seen in the figure, pattering may strongly impact membrane adhesion and topography. The combination will allow us to co-track 2 molecular species in the quasi-2D landscape of the T cell membrane. The TCR molecules are expected to form clusters that mimic the surface pattern (**Fig. 1 C**). Cell activation markers such as the early marker Zap-70 or later marker CD69 and interleukin-2 secretion will be imaged/measured. By varying the pattern characteristics (from 40 nm to 2 µm size, and appropriately chosen spacing), and the surface nanobody binding strength, **we shall establish the link between the spatial and biochemical characteristics of the input signal and the activation of the cell**. Careful analysis and exploration of membrane/actin dynamics as well as large scale molecular organisation such as condensate formation will be undertaken **to understand the mechanisms of how binding of small number of TCR may lead to activation via cooperative effects**.

Interdisciplinarity and Implication of the two labs*

In this collaboration, CINaM (Physics) will bring expertise in preparation of the innovative antigen presenting surface and in advanced imaging/image analysis, as well as physical conceptualisation of the problem; CRCM (Biology, specialised in immunotherapies of cancer using synthetic biology such as CAR-T and T cell engagers) will bring expertise in immunology, both technological (nanobodie and nanbody-oligo conjugates, cells) and in formulating the biological question. Neither partner can hope to achieve the goals alone. CRCM lacks relevant expertise in materials / patterning and advanced image analysis and CINaM lacks competence to access important biological tools.

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

Kheya Sengupta will supervise all aspects of patterning, imaging, image/cell analysis, and physical interpretation.

Patrick Chames will supervise all aspects of molecules and cells, as well as biological interpretation.

Collaboration with Laurent Limozin for expertise in single molecule image analysis.

PhD student's expected profile*

The candidate should have a Master degree in physics/biophysics/biology. Candidates with previous experience in optical microscopy will be given preference. Basic competence in computer programming is expected. We look for highly motivated candidates willing to do experimental and image analysis 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? No

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

*: Mandatory

Two to five references related to the project*

[1] Abdelrahman A., Smith A.-S., Sengupta K. Observing membrane and cell adhesion via reflection interference contrast microscopy. Methods in Molecular Biology. 2023;2654:123–135. doi:10.1007/978-1-0716-3135-5_8

[2] Schnitzbauer J., Strauss M.T., Schlichthaerle T., Schueder F., Jungmann R. Super-resolution microscopy with DNA-PAINT. Nature Protocols. 2017;12(6):1198–1228. doi:10.1038/nprot.2017.024

[3] Dustin M.L. The immunological synapse. Cancer Immunology Research. 2014;2(11):1023–1033. doi:10.1158/2326-6066.CIR-14-0161

[4] Mariuzza R.A., Agnihotri P., Orban J. The structural basis of T-cell receptor (TCR) activation: an enduring enigma. Journal of Biological Chemistry. 2020;295(4):914–925. doi:10.1016/S0021-9258(17)49904-2

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

Sengupta:

[1] Morphodynamics of T-lymphocytes: Scanning to spreading, BiophysJ.2024 10.1016/j.bpj.2024.02.023

[2] Ligand Nanocluster Array Enables Artificial-Intelligence-Based Detection of Hidden Features in T-Cell Architecture, Nano Letters (2021). DOI: 10.1021/acs.nanolett.1c01073.

Chames:

[1] Combination of T cell-redirecting strategies with a bispecific antibody blocking TGF-β and PD-L1 enhances antitumor responsesOncoimmunology. 2024 Apr 13;13(1):2338558. doi: 10.1080/2162402X.2024.2338558.

[2] Decoupling Individual Host Response and Immune Cell Engager Cytotoxic Potency. ACS Nano. 2025 Jan 21;19(2):2089-2098. doi: 10.1021/acsnano.4c08541.

Project's illustrating image

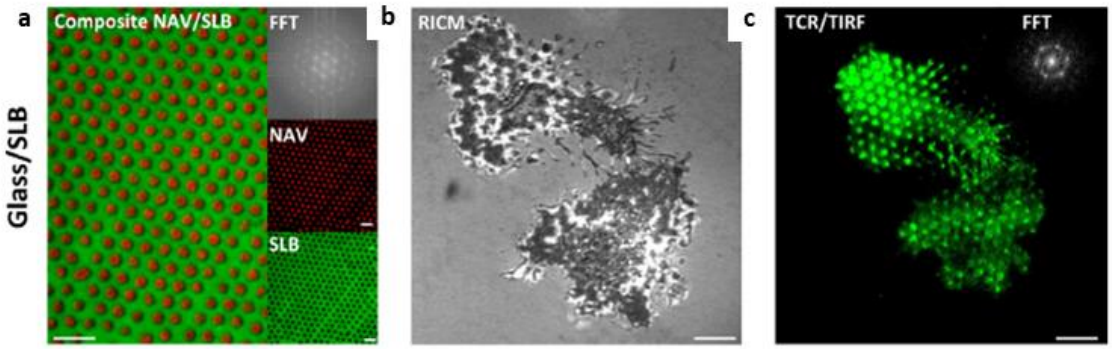

Fig 1. T cells on patterns. a) merged fluorescence image of anti-CD3 coated dots (red, NAV) in a sea of lipids in the form of a supported lipid bilayer (green, SLB). Insets show individual NAV and SLB images and fast Fourier transform (FFT) of the NAV image to illustrate long range order. b) RICM image of T cells on such anti-CD3 pattern. c) TCR of T cells on anti-CD3 pattern imaged in TIRF mode and corresponding FFT. Scale bar = 5 µm .

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