PhD PROJECT PROPOSAL

CENTURI
TURING CENTRE
FOR LIVING SYSTEMS

In silico and in vitro study of curvotaxis: cells surfing of deformation waves

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Abstract (10 lines)*

The project aims to determine whether cell migration could be guided by dynamic changes in curvature and to identify the mechanobiological processes that are involved. We will examine the effects of substrate undulations of different wavelengths and amplitudes on cell migration mechanisms such as Golgi-nucleus axis polarization, actin flux, lamellipodium protrusion, as well as on cell-cell interactions between leaders and followers within epithelia.
The project will propose i) an in silico approach combining stochastic and deterministic migration factors and ii) an experimental setup with planar substrates featuring moving topography in the z direction and undulations in xy plane. Moving topographies could look like travelling crescents to isolate and move single cells or like travelling waves to guide epithelial migration. Moving topography could be a mean for cell engineering to isolate and sort specific cells or to assemble cells from various lines to form preliminary tissue constructs.

Keywords*

Cell migration guidance, dynamic curvotaxis, moving topography

Scientific question and Objectives (10 lines)*

Studies showed that curvature guides cell migration, with cells avoiding convex hills and moving into concave valleys (Pieuchot2018; Vassaux2019; Callens2020). However, static curved substrates such as corrugated or ratchet anisotropic ones only bias rather than direct cell migration (Caballero2015). During embryogenesis, cells migrate over adjacent layers that contract and fold (Schamberger2023). These changes in curvature may guide long-distance cell migration, critical for organ morphogenesis. We hypothesize that migration can be guided over long-distance by deforming the substrate and generating continuously a curvature gradient with concave in front of the cell. We hypothesize that changes in curvature gradient will polarize cells by reorienting nucleus-Golgi axis and actin flux towards concave. The teaming of JL Milan and O Theodoly will also provide a unique opportunity to develop a comparative study of the curvotaxis of mesenchymal cells, which has attracted the most attention in the field, with that of amoeboid cells such as the immune cells studied at LAI (Aoun2020; Seveau de Noray2022), whose curvotaxis properties remain unknown.

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

The project proposes a combined in vitro and in silico approach by developing i) an experimental device capable of culturing cells on a substrate with moving topography in the z direction, with directed undulations in the xy directions, and ii) a physical and computational model of dynamic curvotaxis combining stochastic and deterministic factors of cell migration.

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1) Experimental setup based on pneumatic substrates a) Generating traveling undulations: PDMS substrate film with a rack of pneumatic tubes beneath, based on Theodoly group's device using quake valve technology. Pressuring the tubes sequentially generates undulations (~50-100µm wavelength, 20-50µm magnitude) moving at cell migration speeds.
b) Generating moving topographic landscapes (e.g., crescents). PDMS substrate with crossed pneumatic tube racks that create a pressure grid. Activation of pressure nodes forms convex/concave shapes. Synchronous node activation moves these shapes across the xy plane.

2) Analysis of cell cultured on moving topography a) Track migration and polarization of mesenchymal (e.g., fibroblasts) or amoeboid single cells (e.g., T cells) using optical microscopy. Analyze migration patterns (speed, persistence, polarization). b) Study of cell morphology (nucleus-Golgi axis, actin flux) with optical microscopy modes (DIC, immunofluorescence, TIRF). c) study of cell colonies and epithelium

3) Developing physical and computational models of curvotaxis a) Persistent random walk model of cell migration. Extend Milan's model to simulate migration on curved landscapes, incorporating sensitivity to curvature gradient speed. b) Model of Intracellular processes driving curvotaxis: Develop in silico models to simulate cytoskeletal interactions with the extracellular matrix via focal adhesions. Test whether curvature gradients drive nucleus movement and optimize wave patterns to enhance sensing/migration. c) Epithelium model on moving topography. Scale the model to simulate epithelium as interacting cells, analyzing how curvature-induced polarization interacts with cell-cell mechanical forces.

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)

Milan's group is expert in cell migration modelling. The in silico tools developed by Milan's group is able to put into simulation the physical and biological hypothesis about dynamic curvotaxis and the mechanobiological processes that may be involved. In silico model will help to test hypotheses by implementing them and by observing the emerging response. The in silico is an additional tool to help thinking about cell phenomena.
Theodoly's group is expert in cell migration physics and experimental set ups (Garcia-Seyda2020). Quake valve microfluidic system that is developed by Theodoly's group is fundamental point of the project, because is the source of curvature generation (Bohec2024).
As the experimental set-up can be complex, in silico simulation will be used to explore the effect of mobile curvature on cells, to reduce the design of experiments design and to identify candidates for mobile topographical patterns to guide cell migration.

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

The PhD student will work on i) setting up the experimental device and manipulating the cells using the deformable pneumatic substrate and ii) in silico modelling of dynamic curvotaxis.

The PhD will work with JL Milan on in silico modelling and with O. Theodoly on the experimental aspects

PhD student's expected profile*

Master in (Cell)Physics

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)*

The modelling of dynamic curvotaxis has been a focus of JL Milan's research for several years. JL Milan is currently coordinating an ANR MovingCells project CE45 2022-26. Thanks to the ANR MovingCells project, a preliminary model of cell migration based on a persistent random walk has been developed for static curvature and will be extended to mobile curvature. The ANR MovingCells project serves as the basis for the present CENTURI project. The CENTURI project will

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provide a deeper understanding of the intracellular mechanisms of dynamic curvotaxis and extend the modelling to the scale of the epithelium on mobile curvature. The ANR Moving Cells project is limited to generate travelling waves by forming wrinkles at the surface of the substrate by compressing it, which is a challenge. The present CENTURI propose a promising experimental alternative to generate moving curvature and to generate a freer topographic shape that would not be limited to corrugated shape but would be crescent-shaped or circular to trap and move the cells more efficiently.

Two to five references related to the project*

- [Pieuchot2018] L. Pieuchot, J. Marteau, A. Guignandon, T. Dos Santos, I. Brigaud, P.-F. Chauvy, T. Cloatre, A. Ponche, T. Petithory, P. Rougerie, M. Vassaux, J.-L. Milan, N. Tusamda Wakhloo, A. Spangenberg, M. Bigerelle, K. Anselme, Curvotaxis directs cell migration through cell-scale curvature landscapes, Nat. Commun. 9 (2018) 3995. https://doi.org/10.1038/s41467-018-06494-6.
- [Schamberger2023] Schamberger et al. Curvature in Biological Systems: Its quantification, Emergence and Implications Across the Scales, Advanced Materials 2023. https://doi.org/10.1002/adma.202206110
- [Vassaux2019] M. Vassaux, L. Pieuchot, K. Anselme, M. Bigerelle, J.-L. Milan, A Biophysical Model for Curvature-Guided Cell Migration, Biophys. J. 117 (2019) 1136–1144. https://doi.org/10.1016/j.bpj.2019.07.022.
- [Garcia-Seyda2020] Garcia-Seyda, N.; Aoun, L.; Tishkova, V.; Seveau, V.; Biarnes-Pelicot, M.; Bajénoff, M.; Valignat, M.-P.; Theodoly, O. Microfluidic Device to Study Flow-Free Chemotaxis of Swimming Cells. *Lab. Chip* **2020**, *20* (9), 1639–1647. https://doi.org/10.1039/D0LC00045K.
- [Bohec2024] Pierre Bohec, Florian Dupuy, Victoria Tishkova, Valentine Seveau de Noray, Marie-Pierre Valignat, Olivier Theodoly, Microvalve-based devices for flow-free gradient generators, BioRxiv **2024**, ID#: BIORXIV/2024/626578

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

Jean-Louis Milan:

- [Vassaux2019] M. Vassaux, L. Pieuchot, K. Anselme, M. Bigerelle, J.-L. Milan, A Biophysical Model for Curvature-Guided Cell Migration, Biophys. J. 117 (2019) 1136–1144. https://doi.org/10.1016/j.bpj.2019.07.022.
- [Manifacier2024] Manifacier I, Carlin G, Liu D, Vassaux M, Pieuchot L, Luchnikov V, Anselme K, Milan JL. In silico analysis shows that dynamic changes in curvature guide cell migration over long distances. Biomech Model Mechanobiol. 2024 Feb;23(1):315-333. doi: 10.1007/s10237-023-01777-4. Epub 2023 Oct 24. PMID: 37875692.

Olivier Théodoly:

- [Seveau de Noray2022] Seveau de Noray, V.; Manca, F.; Mainil, I.; Remson, A.; Biarnes-Pelicot, M.; Gabriele, S.; Valignat, M.-P.; Theodoly, O. Keratocytes Migrate against Flow with a Roly-Poly-like Mechanism. *Proc. Natl. Acad. Sci.* **2022**, *119* (48), e2210379119. https://doi.org/10.1073/pnas.2210379119.
- [Aoun2020] Aoun, L.; Farutin, A.; Garcia-Seyda, N.; Nègre, P.; Rizvi, M. S.; Tlili, S.; Song, S.; Luo, X.; Biarnes-Pelicot, M.; Galland, R.; Sibarita, J.-B.; Michelot, A.; Hivroz, C.; Rafai, S.; Valignat, M.-P.; Misbah, C.; Theodoly, O. Amoeboid Swimming Is Propelled by Molecular Paddling in Lymphocytes. *Biophys. J.* **2020**, *119* (6), 1157–1177. https://doi.org/10.1016/j.bpj.2020.07.033.

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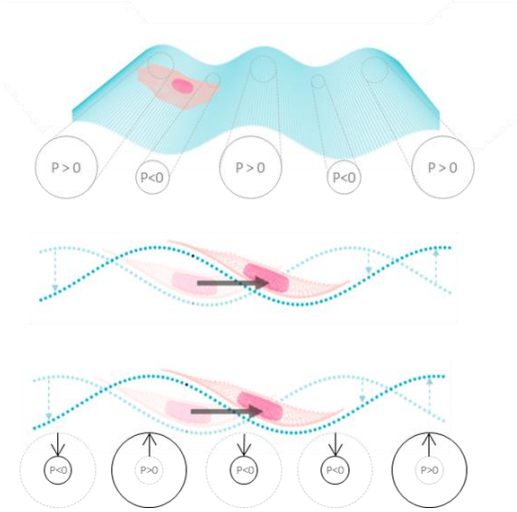
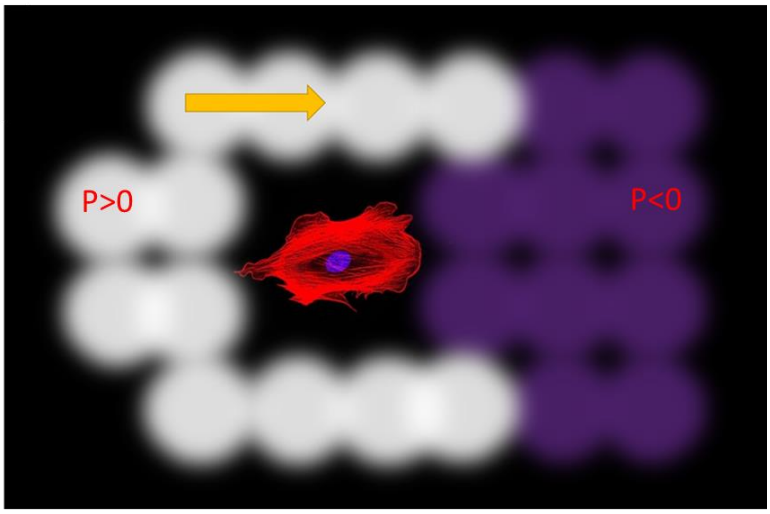
Project's illustrating image

Cell migration guidance by Moving Topography

→ *Pressure Tubes for Moving Waves*



→ *Pressure Nodes for Moving free shapes*



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