PhD PROJECT PROPOSAL

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Memory-driven Active instabilities & Gastruloids modelling

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Abstract (10 lines)*

The local microenvironment, as sensed by cells, is a crucial factor in determining their differentiation state during development and homeostasis. This cellular niche includes interactions with neighboring cells and the dynamically regulated extracellular matrix, which cells continually reorganize. This matrix serves as both a substrate from prior developmental history and a medium facilitating further development and cellular migration. We propose to investigate this mechanism using theoretical and experimental approaches with Gastruloids as our model system. Preliminary data indicate a feedback loop between cell differentiation and migration, where differentiating cells deposit a chemical trail guiding subsequent differentiating cells. At the theory level, we will explore the collective behavior of active agents interacting with their trails, which could be organized into arrested domains. We will test our model predictions in stem cell differentiation within Gastruloids, allowing us to quantitatively explain observed phenomena and guide future experiments.

Keywords* Active matter, Motility-induced phase transition, Gastrulation, Image analysis

Scientific question and Objectives (10 lines)*

Preliminary data Virgile Viasnoff's (VV) team aims to identify the factors determining specific tissue spatial organizations during human gastrulation. The team uses a perturbation approach to locally induce differentiation of human epiblast cells into meso-endoderm, triggering an epithelial-to-mesenchymal transition (EMT). This increases meso-endoderm cell motility and promotes fibronectin (FN) secretion, altering the local microenvironment. Depending on the spatial pattern of differentiation (bead number and positioning within epiblast spheroids), FN deposition forms either large fibers guiding meso-endoderm migration away from the stem cell niche or small fibrils filling the Gastruloid space homogeneously. Large FN fibers lead to spatial separation of epiblasts and meso-endoderm into distinct poles, with directed migration self-reinforced by collective FN deposition. Conversely, small fibrils result in stochastic meso-endoderm

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migration. As FN replaces collagen, epiblasts rapidly differentiate into meso-endoderm, altering the local microenvironment.
The objective of this PhD is to understand how the crosstalk between cell motility, fibronectin deposition and the microenvironment alterations can support an active phase separation in cell types. Beyond this PhD, the objective is to design a theoretical framework that can guide the construction of better cardiac organoids after the gastrulation stage.

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

Theoretical methods We propose to adapt a recently proposed framework [1]. As in [1], we model the fibronectin network as a gel undergoing a transition from an isotropic phase (disorganized filaments displaying a low nematic order $\boldsymbol{Q}_{\mathrm{m}}$) to a nematic phase (filaments stack into a well-defined direction, $\boldsymbol{Q}_{\mathrm{m}}$ is large). In contrast to Ref. [1], the geometry is 3D and confined, and the cells represent a phase separating fluid, with φ a fraction of stem versus differentiated cells. As cells also migrate preferentially along the average orientation of fibronectin filaments; we consider a coupling energy $E(\boldsymbol{n}) = -2\,\mathrm{Tr}[(\boldsymbol{n}\,\boldsymbol{n} - \mathrm{I}/\mathrm{d})(\beta_{\mathrm{cc}}\boldsymbol{Q}_{\mathrm{c}} + \beta_{\mathrm{mc}}\boldsymbol{Q}_{\mathrm{m}})]$, where Q_c is the cell fluid nematic order, β_{cc} and β_{mm} are the strength of the cell-cell and matrix-matrix aligning interactions. We then derive hydrodynamic equations for the orientation fields, expressed in terms of the times needed for cells to differentiate, diffuse, and remodel the fibronectin filaments. We expect a rich phase diagram in terms of 2 competing effects: (1) the stem/differentiated cell phase separation could be maintained by differences in motilities between the two cell types, as self-propelled objects are known to undergo *motility-induced phase separation*, i.e. a tendency to accumulate in areas where their motility is lower, as in high-density regions [3] (2) the matrix deposition induced by the differentiating cells could itself lead to arrested domains, as in Ref. [1], which can either drive or preclude the phase separation process.

Experimental methods VV's team generates microbeads coated with growth factors (Activin A, BMP4, FGF2, Wnt3a) which locally inducing the differentiation of stem cells. VV team will play on the bead number, their spatial positioning, and mesoderm-to-epiblast ratio). The timing and sequence of matrix deposition will be precisely characterized. Live imaging of Gastruloids using RUES cells with Sox17 reporters will enable simultaneous tracking of cell differentiation and migration. Combined with VV's team image analysis pipeline [VV1] and deep-learning-based methods [JFR2; VV2], these experiments will allow for quantitative measures of the fields φ, v, Q_c, Q_{m} defined in the theory.

Research plan: we will establish the phase separation framework in 2D, and then in confined 3D. In parallel, we will extract quantitative maps from experiments. Finally, we will perform the model comparison.

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

Interdisciplinarity: The project is fundamentally interdisciplinary, involving

(1) physics-based, phase-separation theories, as in **Ref. JFR1**.
(2) computational tools, using deep learning-based methods, as in Ref. **VV1, VV2 and JFR2**
(3) a biology-oriented question (see Objective section).

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Specific contributions VV will lead the experimental work, data acquisition, while JFR will lead theoretical predictions. Both teams will collaborate on the quantitative image analysis, as confronting model predictions with experimental data will necessitate advanced quantitative image analysis and tracking, expertise that both teams possess through prior development of deep-learning-based techniques, Ref. **VV1, VV2 and JFR2.**

Our collaboration: Both teams have a strong track record of interdisciplinary research and a history of successful collaborations (e.g., Ref 4 - Fu et al. 24). Achieving our project goals requires close integration between both teams and the PhD student, ensuring a thorough understanding of all project aspects. Even if the candidate focuses on simulation modeling, the candidate will be expected to gain a deep understanding of the experimental conditions and their implications.

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

The PhD student will be closely mentored by J.F. Rupprecht and V. Viasnoff. On experiments, the student will also collaborate closely with M. Marchand, a senior postdoc with four years of experience in Viasnoff's team. The student will also actively participate in the regular Gastruloid meetings initiated by Pierre-François Lenne and Sham Tlili's groups this month. Scientific guidance on theoretical modeling will be enriched through discussions with them, as well as with Simon Gsell and Matthias Merkel, who have common research interests with this project.

PhD student's expected profile* Physics or biophysics student

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. The preliminary experimental observation will be published this year and the follow-up experiments alongside the theoretical results will be conducted in the frame of VV's Chaire d'Excellence.

Two to five references related to the project*

[1] Adar, Joanny *Environment-stored memory in active nematics and extra-cellular matrix remodeling,* PRL (2024).
[2] Adar, Joanny, *Permeation Instabilities in Active Polar Gels*. PRL (2021)
[3] Cates, Tailleur, *Annual Review of Condensed Matter Physics* (2015).
[4] *E-cadherin-dependent phosphorylation of EGFR governs a homeostatic feedback loop controlling intercellular junction viscosity and collective migration modes* C. Fu, F. Dilasser, S.-Z. Lin, M. Karnat, A. Arora, H. Rajendiran, H. T. Ong, N. M. Hoon Brenda, S. W. Phow, T. Hirashima, M. P. Sheetz, J.-F. Rupprecht, S. Tlili, V. Viasnoff, PNAS (2024).

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

Virgile Viasnoff

[VV1] *Automated high-speed 3D imaging of organoid cultures with multi-scale phenotypic quantification.* A. Beghin, … V. Viasnoff, Nat Methods (2022).

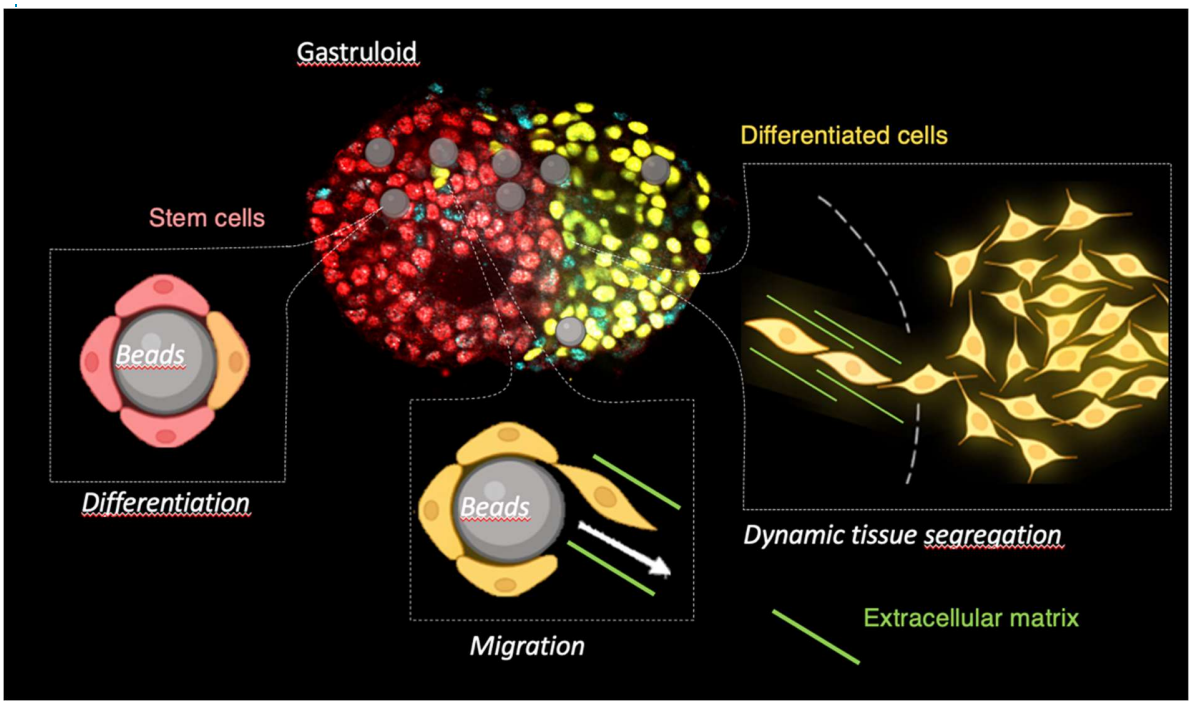
[VV2] Fuentes-Hurtado, Sibarita, Viasnoff *Generalizable Denoising of Microscopy Images using Generative Adversarial Networks and Contrastive Learning.* arxiv *(2023).*

Jean-François Rupprecht (JFR)

[JFR1 *Membrane Tilt Drives Phase Separation of Adhesion Receptors,* Lin, Changede, Farrugia, Bershadsky, Sheetz, Prost, Rupprecht, PRL (2024)

[JFR2] *Inferring the location and orientation of cell divisions on time-lapse image sequences,* Karnat, Saadaoui, Tlili, Karpinski, Rupprecht, bioRxiv 2024.

Project's illustrating image



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