PROPOSAL FOR A M2 internship in Cell Biophysics

Aggregation dynamics in cell populations: experiments and modeling

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Key words: Collective cell motility, Pattern formation, Size regulation, Oxygen sensors
Possibility to pursue a PhD: Yes (This internship is part of the ANR project ADHeC for which a PhD is granted; collaboration Team Eco-évolution mathématique, S. De Monte, IBENS, ENS, CNRS, INSERM, Paris)

Scientific Context

Collective organization and function inside cellular aggregates (from cultured tissues, to multicellular protists, to cancer tumors) is impacted by heterogeneity among the constituent cells. Aggregation, when cells come together in groups, is a key step in determining the structure of multicellular collectives, notably stability, size and composition of groups. Differentiation in different cell types and cell sorting (tissue specific regionalization) are other fundamental developmental processes that can occur simultaneously with aggregation or later.
Both single cell and emergent properties regulate this aggregation dynamics: cell motility, cell-cell adhesion, self generated morphogen gradients (secreted or consumed soluble factors, adhesive tracks on substratum, consumed oxygen…). We aim to develop a simple experimental system associated with a close modeling framework to describe the aggregation dynamics of both homogeneous populations and binary mixtures with controlled levels of diversity, in order to uncover the physical underpinnings of population-level patterning. We will use *Dictyostelium discoideum*, or simply *Dicty*, plating cells in a dish with submerged conditions, a system compatible with long term videomicroscopy and medium/gas control and exchange
Preliminar results have shown that when *Dicty* cells are grown several days on a dish in a nutrient medium (enabling hence cell divisions), they start to aggregate at a critical cell density (Fig. A). Aggregates are very dynamics (as seen by observing the motion of a few marked fluorescent cell inside, Fig. B) but reach an intriguing equilibrium size indicating a probable regulation mechanism by some repulsive field. A purely, yet speculative mechanism will be the existence of a "hypoxic cloud" arising from large oxygen consumption around large aggregates that other neighboring aggregates try to escape. The equilibrium size will hence results from a balance between O_2 diffusion, aggregate size and velocity.

Missions:

1) **Investigations around oxygen.** The M2 student will grow several *Dicty* cell lines such as fluorescent cell lines, and mutants. He/she will first run aggregation experiments using axenic strain AX2 at various controlled O_2 percentages: 40%, 21% (ambient atmosphere), 5%, 2% using gas incubators and timelapse videomicroscopy to observe some eventual changes in the aggregate size distribution. He/she will also use mutants known for having a defect in the O_2 sensing response of *Dicty* [1].A motorized binocular will enable quick screening of various conditions. Finally, he will directly use O_2 sensing films (Fig. C) to

measure some eventual hypoxia under cell aggregates.

2) **Investigations around adhesion.** He/she will also modify cell-cell adhesion by adding EGTA (calcium chelator that lowers cell-cell adhesion), a lectin WGA (that binds to cell adhesion receptor and hence also modifies cell adhesion), and use binary mix of mutants lacking TgrB and TgrC genes involved in cell-cell recognition and adhesion [2] and parent cell lines. A confocal microscope will be used to analyze cell shape and motion within aggregate using a small percentage of fluorescent cells (Fig. B).
3) **Modeling.** The results will be modeled with the cellular Potts model using CompuCell3D free software (http://compucell3d.org/) that enables to simulate both realistic single cell shape changes (using an effective temperature mimicking cell active protrusions), steric (contact) or chemical interactions such as response to the O_2field self generated. We will especially focus on the balance between attractive (adhesion) and repulsive fields for different level of cell protrusive activity.

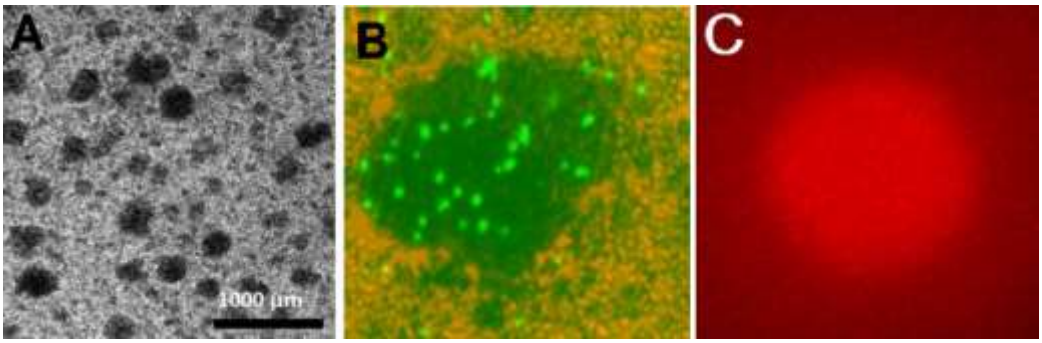

Figure. A) *Dicty* cells at high cell density after several days in culture. Aggregates of cells (black clusters) are surrounded by fastly moving single cells. B) A cluster with 3% of fluorescent cells. C) Fluorescence of O_2 sensing film under a confined dense colony (not a cluster) of *Dicty* cells (intense red indicates hypoxia)

References

[1] Xu et al. Role of the Skp1 prolyl-hydroxylation/glycosylation pathway in oxygen dependent submerged development of Dictyostelium. BMC Developmental Biology 2012, 12:31
[2] Fujimori et al. Tissue self-organization based on collective cell migration by contact activation of locomotion and chemotaxis PNAS | March 5, 2019 | vol. 116 | no. 10 | 4291–4296