**PROPOSAL FOR A M2 internship in Cell Biophysics**

# *Cells in a hypoxic environment: the race for oxygen*

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**Possibility to pursue a PhD**: yes

It has been known for the last three decades that cells are able to detect and adapt to various concentrations of oxygen ($O_2$) as just highlighted by the **Nobel Prize in Medicine 2019** [1]. In a situation of hypoxia (low $O_2$), the HIF (Hipoxia-Inducible Factor) complex associates with DNA to regulate the expression of certain adaptation genes, while in presence of $O_2$ (normoxia) HIF is inactivated by hydroxylation thanks to prolyl 4-hydroxylase (PhyA in protists, PHD2 in animals).

It has also long been known that bacteria, rather than regulating genes for adaptation, **move toward $O_2$, a mechanism called aerotaxis** [2]. Recently, it was demonstrated that epithelial cells also exhibit directed migration toward oxygen using a very simple **spot assay**: after covering an epithelial cell monolayer by a coverglass non permeable to $O_2$, peripheral cells exhibit a strong outward directional migration to escape hypoxia from the center of the colony [3]. Following that assay, we showed at iLM that the social amoeba *Dictyostelium* (***Dicty***) also displays a spectacular phenotype when cells consumed their $O_2$ (Fig. 1A): most cells move quickly outward of the hypoxia area, forming a dense expending ring moving at constant speed. This clear signature of a collective response induced by low $O_2$ can be described by a few readouts on the ring: formation time, speed, shape. Hence, aerotaxis seems a conserved mechanism in various eukaryotic cells. However, the molecular nature of this $O_2$ directed migration remains elusive.

The main objectives of this M2 project are to use *Dictyostelium* mutants and microsystems to quickly progress in understanding $O_2$ driven cell motility at molecular, cellular and multicellular level.

First, the candidate will standardize our spot assay (and related image analysis) using spacers and a motorized binocular in order to test an existing list of mutants available in the lab of C. West (University of Georgia). This collaborator has shown in *Dicty* that PhyA directly regulates protein ubiquitination (thus degradation) in a $O_2$ dependent manner by enabling glycosylation of Skp1, an essential adaptor of ubiquitin ligases [4]. A critical $O_2$ concentration is required for multicellular morphogenesis and cell differentiation [5]. Thus, it is natural to ask whether all these proteins are as well involved in the aerotactic response of *Dicty*.

Second, we are planning to screen an available *Dicty* mutant collection for cells that display higher motility toward oxygen (*Dicty* GWDI mutant library, REMI method https://remi-seq.org). This requires developing a 3D cell sorter to separate fast moving mutants from the other cells (Fig 2). The device will be engineered to be air tight so that cells place at the bottom will quickly consume oxygen, thus generating a vertical gradient. To reach higher oxygen concentration, the cells will have to migrate through a matrix of porous material and collected at the top. Various porous materials will have to be tested for optimal cell migration and $O_2$ gradient formation measured with a selected $O_2$ probe deposited along the edge of the sorter.

An early test shows that a high cell density at the bottom is enough to ensure a quick $O_2$ consumption and to trigger upward migration. Multiple enrichment cycles will be performed as required. Cells that remain at the very bottom and survive hypoxia will also be harvested. The behavior of isolated mutants will be characterized with the spot assay and compared to wild type cells.

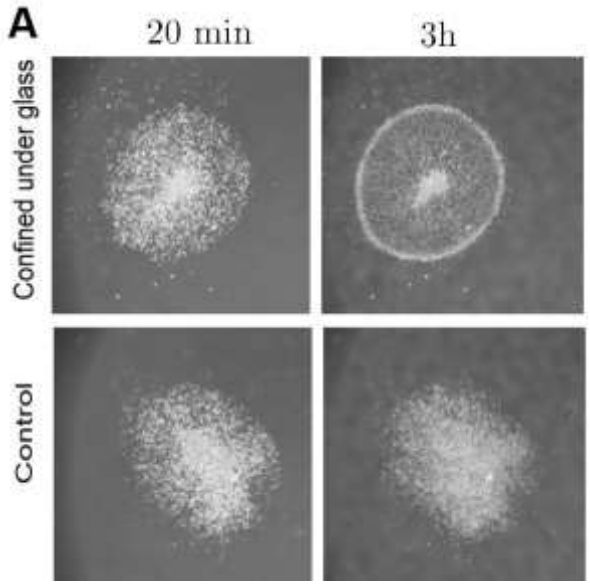


**Figure 1**. A) A spot of initially densely packed *Dictyostelium* cells (20 min column) quickly move outward with the formation of a ring of cells when covered by a coverglass (3h, top row) while they just slowly spread out when there are not covered (3h bottom row). Each spot contains 2000 cells at the beginning of the assay.

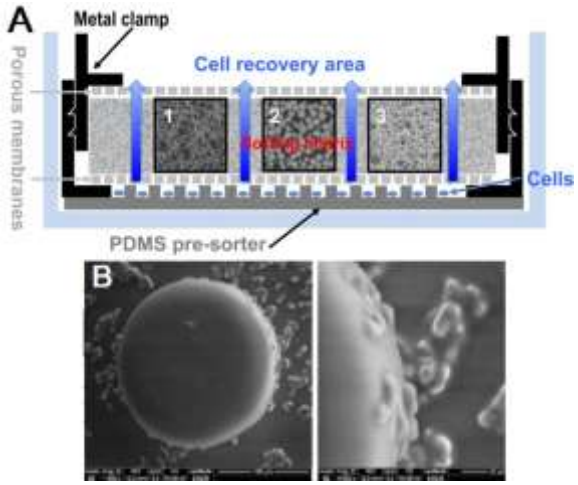


**Figure 2. (A) Side view of the aerotactic cell sorter.** Cells initially plated on a PDMS pre-sorter made of pillars will migrate during a day upward toward O2. Metal clamps will hold the sorting matrix in between 2 porous membranes (one on the pre-sorter pillars, one on the top, removable for cell collection). The 3-mm thick matrix will be either a gel (1), frit glass (2) or a PVA sponge (3). (B) Electron microscopy images of *Dicty* cells climbing PDMS pillars (pre-sorter).


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