PROPOSAL FOR A M2 internship in Cell Biophysics (possible continuation in PhD)

Cells in a hypoxic environment: the race for oxygen

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It has long been known that bacteria move to oxygen a mechanism called aerotaxis [1]. A collaborator team from the CRCL[1] recently demonstrated that epithelial cells also exhibit directed migration toward oxygen using a very simple 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 [2]. Following that assay, we showed at iLM that the social amoeba *Dictyostelium discoideum* also displays a spectacular phenotype when cells consumed their O_2 (Fig. 1A)*:* most cells move quickly outward of the hypoxia area and thus form an expending ring.

These experiments indicate that aerotaxis is probably conserved among all eukaryotes. It may play a role in tumor progression by orienting the migration and invasion of the cells of the primary hypoxic tumor to the blood capillaries, promoting metastatic spread.

However, these are just preliminary experiments and the field is totally open. The CRCL team has already evidence that cells sense oxygen level indirectly through the production of free radicals (peroxide) and its detection by the EGF receptor [2]. However, further work is required to decipher the molecular mechanism behind aerotaxis. There is no easy technic allowing live monitoring of oxygen concentration at cellular level during the aerotaxis experiments. Thus, it is difficult to evaluate quantitatively the oxygen gradient directing the cell motility.

The main objectives of this PhD project are to use *Dictyostelium* mutants and microsystems to quickly progress in the investigation of the molecular mechanisms of aerotaxis. A typical experiment such as the "ring" experiment of Fig. 1 runs for 2 or 3 hours at 22°C while a mammalian experiments runs for 24 to 48h at 37°C. The "ring" phenotype itself is much clearer with *Dictyostelium* than with mammalian cells [2]. Of course, once some interesting findings on the various pathways involved will be obtained with *Dictyostelium*, they will be tested with our colleagues of CRCL on mammalian cells.

On the technological side, we plan to control both spatial and temporal gradients with microfluidics. A simple microfluidic T-junction between an oxygenated and a degased media (green and white channels in the right figure) arriving in a region where cells are plated presents a well-defined and measurable gradient region. Cellular motion will be followed by

[1] CRCL: Cancer Research Center of Lyon, Philippe Gonzalo and Ivan Mikaelian

videomicroscopy allowing the reconstruction of cells trajectories and behavior. Hence, information on the sensibility of cell to spatial quasi-stationary gradients will be determined.
In case of continuation for a PhD, more sophisticated devices to generate travelling pulses of concentration have been used to study *Dictyostelium* chemotaxis in S. Sawai laboratory (Tokyo University) [3]. Dr. K. Funamoto another Japanese collaborator of ILM has developed a unique microfluidic system for controlling oxygen concentration in a culture medium from gas channels [3,4]. We plan to adapt these devices in Lyon with their help. The oxygen sensing mechanism will be analyzed at molecular level using mutants, pharmaceutical and biochemical approaches.

Numerical modeling will be also developed using for instance Active Brownian Particle models as previously done in the host team [5].

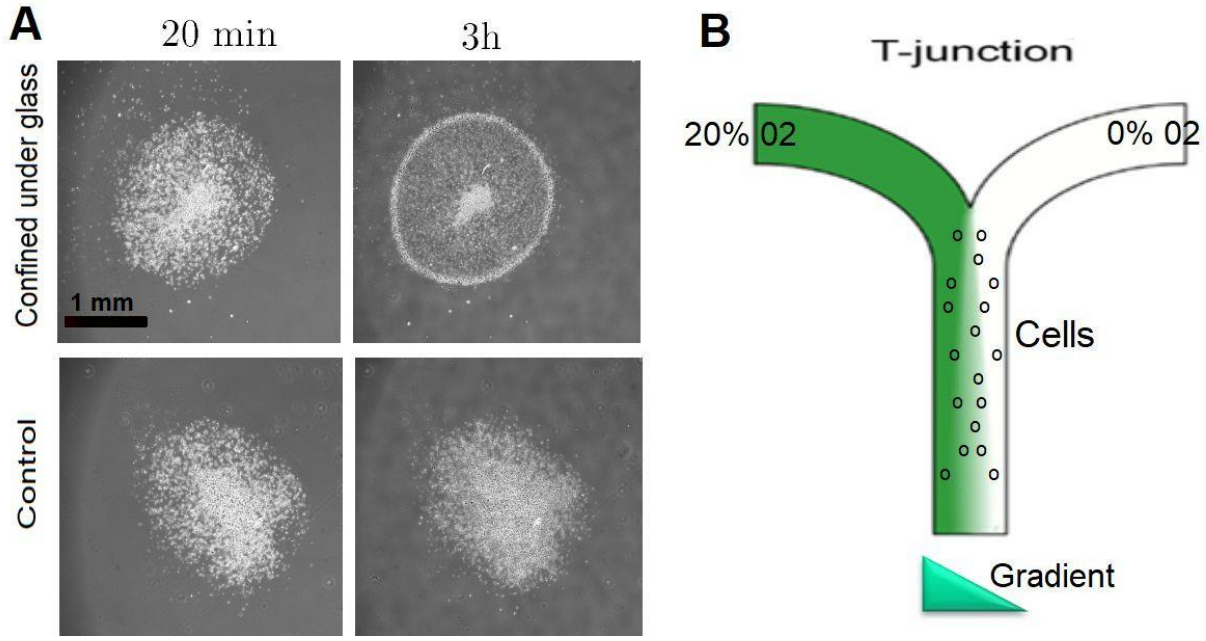

Figure 1. A) A spot of initially densely packed *Dictyostelium* cells (20 min column) quickly move outward with the formation of a ring of cells when covered by a coverglass (3h, top row) while they just slowly spread out when there are not covered (3h bottom row). B) A T-junction microfluidic geometry envisioned to create a gradient region between and oxygenated and a degassed culture media

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Key words: Cell motility analysis (videomicroscopy, tracking, PIV…), micro-fabrication, hypoxia, signaling pathways, active matter modeling**.**