In aerobic organisms, oxygen is essential for efficient energy production and acts as a regulator of gene expression. Excessive oxygen can, however, lead to the production of deleterious reactive oxygen species. Therefore, the directed migration of single cells or cell clumps from hypoxic areas towards a region of optimal oxygen concentration, named aerotaxis, can be considered an adaptive mechanism, playing a major role in several biological and pathological processes. O2 gradients develop in tumours when they grow beyond their vascular supply, leading to heterogeneous areas of O2 depletion and favouring metastatic migration. The social amoeba Dictyostelium (D.d) is a powerful and genetically accessible model organism that has been used to elucidate biological processes, such as chemotaxis. This phenotypic richness in its biology is what makes it such a wonderful model that it is of particular value as biomedical research tools. We present in this poster, data on dynamics of the aerotaxis process in wild type and mutant Dictyostelium cells.

**RESULT 1_ THE DICTYOSTELIUM COLONY REACTS TO THE OXYGEN GRADIENT**

*D.d* cells were seeded as a drop containing 50,000 cells in the center of a 24-well culture plate. After the cells adhered to the substratum, a specific volume of medium, was added and glass coverslips were placed on top to cover and confine the cells cluster. As a control in the not-confined system (NC) cells were seeded without the coverslips. Oxygen concentration was determined over the time by using the VisiSens detector unit DUO1 coupled to the oxygen sensor foil SF-RP'Su4.

A detailed cell tracks analysis revealed after approximately 1 h, a fraction of confinement cells began to sense the oxygen gradient and moved coordinately, assuming an arrangement characterized by a thickened front, named corona, that, once shaped, persistently moved towards the oxygen source. Underneath the corona we observed the cells cluster lining-up in two further regimes with differential cell density. At the corona center, where oxygen concentration was the lowest (.), the cells were rather rounded but adherent and their density was the highest. The central area abutted with a wide region with the lowest cellular density in which the cells displayed an elongated shape. While the size of the central high-density region was approximately constant, the lowest density sector expanded as the corona moved away from the center. (Fig.1 and 2A). The initial of shapes of the cell's clusters either in C and NC were very similar (Figure 2A) displaying a natural circular geometry with a cell's density profile decreasing as approaching the periphery (Fig. 2B).

Surprisingly, the cells that trigger the formation of the corona were not the outermost ones, suggesting that the driving event required to organize such structure is not the relative highest oxygen level.

**RESULT 2_ HYPOXIA SELECTIVELY BLOCKS DICTYOSTELIUM CELL AGGREGATION AND TRIGGERS OXYGEN-DEPENDENT COLLECTIVE MIGRATION**

In *D.d* development is triggered by starvation and results in cells acquiring the ability to gather together into aggregates, by secreting and responding chemokinetically to cyclic AMP, and to stably adhere to each other by tissue- like adhesive bonds. In normoxic conditions (18-21% O2), *D.d* cells aggregate, generate migratory slugs and ultimately culminate to form fruiting bodies. To test the ability of aggregation competent cells to sense oxygen gradient we assessed aerotaxis assays in starved cells. Briefly, approximately 7 hrs after the starvation onset, the aggregation competent cells underwent to confinement. Within the first few minutes the aggregates suffered profound rearrangements getting looser and partially disaggregating. Eventually, within the next 30-40 minutes the cellular aggregates, facing the oxygen source, directionally moved following the oxygen gradient. Surprisingly, we noticed that even in such conditions the single cells still exhibited their elongated shape and arranged them self in star-like structures (Figure 4A). All together these observations suggest that the CAMP sensitivity has been preserved. In non confinement condition the aggregation competent cells complete their development within 24 hrs (Figure 4B). On the whole, these findings indicate that oxygen gradient, coupled to hypoxic environment, led to severe morphological changes of the aggregates.

**RESULT 3_ DICTYOSTELIUM AEROTACTIC MIGRATION RELIES ON INTRACELLULAR HYDROGEN PEROXIDE ACCUMULATION BUT NOT ON CHEMOTAXIS SIGNALLING PATHWAYS**

To identify new players involved in aerotaxis signaling we tested the aerotactic migration of mutants deficient chemotaxis and in catalase activity. In *D.d* the chemosignal pathways is highly conserved including some players as G heterotrimeric protein (Gz), Rictor (HSB1), AKT (pkbr1). The corresponding null strains (Gznull, HSB1, pkbr1null) responded to oxygen gradient by arranging their cell cluster in the three different density regions as we observed in wild type cells. We configured that the pkbr1null strain, in which a moderate decline in the time required for corona formation (T*) and its propagation velocity under confinement. Besides the experimental variability, we found that after a transient period of few hrs the initial velocity vi of the corona propagation decreased until it reached a constant value v£, after at approximately the 10th hr (Figure 3A). The initial velocity (vi) of the corona, measured in a time window of 140 min, was equal to 2.2 mm/min (Figure 3B) but then it sharply, to approximately 30% of the vi (v£=0.67 mm/min), in the range between 10th to 24th hrs (Figure 3C).

In conclusion, the role of hypoxic gradients is relevant in pathological contexts such as cancer metastasis. The use of the Dictyostelium would provide a unique and still underexploited opportunity, to elucidate and identify novel molecular players underlying the aerotaxis process.