Bioelectricity of cell migration

Context: Cell migration is an essential process of life that occurs in many physiological situations, such as food harvesting, development, aggregation, or immunity, as well as in pathological contexts such as metastasis. The ability of cells to migrate relies on an extremely complicated molecular circuitry, where many signaling proteins orchestrate the cell cytoskeleton in time and space, leading to the emergence of a polarity axis and efficient cell locomotion. One of the big challenges is to understand how the activities of all these biomolecules are collectively integrated so that they can work together at the scale of the whole cell. Many scientists have looked for the most "upstream" signaling protein that could perform this global integration, but no universal 'master regulator' could be identified. It has thus been proposed that this integration might occur at a more fundamental and physical level: variations of membrane charges and electric potential. Yet, it remains unclear which aspect of such 'bioelectricity' is involved and what the functional consequences on migration properties are.

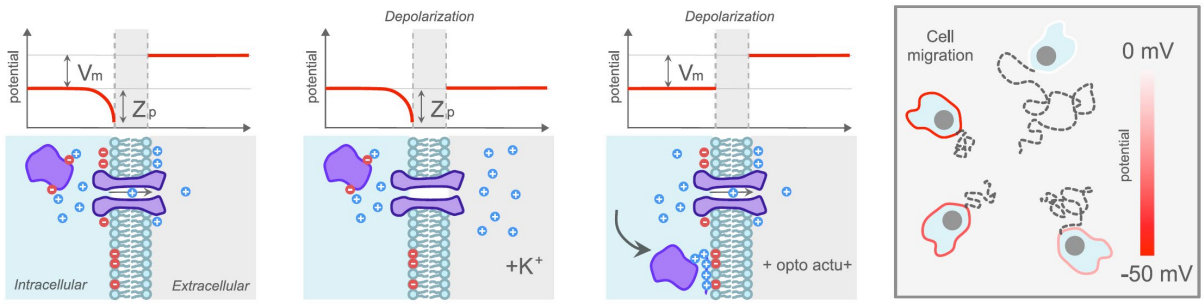
Bioelectricity: bioelectricity as defined here deals with the role of electrical charges at the cell plasma membrane, which separates the intra- and extra-cellular environments. Two main factors[1] contribute to it: the mobile ions that are unevenly distributed across the membrane, leading to an electric field – the transmembrane potential V_m –, and the fixed charges Z_p (for Zeta potential) due to negatively charged lipids and proteins, which are also unevenly distributed and produce a local electric field in the vicinity of the membrane. Regarding V_m, every cell has a so called resting membrane potential that can be measured by whole-cell patch clamp, which is negative and ranges between -90mV and 0mV. This potential stems from the uneven distribution of ions (K+ Cl- and Na+) inside/outside the cell due to active pumps, giving rise to a steady electrochemical gradient characterized by the Goldman equation, and from the large concentration of negatively charged biomolecules (proteins and metabolites) that are impermeable to the membrane and contribute to the uneven distribution of counter-ions, as characterized by the Donnan/Nerst model. V_m can have effects on signaling and cell behavior in many ways, for example it is well known that ions, notably Ca2+, act as signaling molecules. Regarding Z_p, the inner leaflet of the plasma membrane is enriched with about 10% of negatively charged lipids (PS, PIP2 and PIP3). These surface charges generate a very localized field (Ohki model). Despite it being screened over the Debye length (~1nm), it is extremely important for electrostatic interactions of proteins at the periphery of the membrane, which are at the heart of signaling cascades controlling cell behavior[2]. Z_p is hard to measure, given its very local nature, but biosensors have been designed to evaluate its relative strength based on the membrane recruitment of charged protein domains[3]. V_m and Z_p should sum up linearly to establish the total transmembrane potential, but it is currently unknown whether complicated feedbacks between the two exist or not, e.g. through voltage-gated channels. We leave aside here the potential contribution to V_m of extracellular pH (e.g. in hyperkalemia), which may also affect bioelectricity by proton pumps changing the local distribution of charges, and also the extracellular negative static charges coming from matrix proteins (oligosaccharides) that are important for non-specific cell adhesion.

Evidence for a link between bioelectricity and migration: traditionally studied in neurons, V_m was recognized in recent years to play a key role also in non-excitable cells, especially through the work of M. Levine who linked it to cell fate in development. A seminal work[4] has shown a clear correlation between V_m and proliferation. More recently, a causal link between V_m and MAPK signaling, which is central to cell proliferation and migration, has been proposed through protein/lipid clustering at the membrane[5]. Interestingly, cancer cells were found to be more depolarized than their healthy counterparts[6]. Cancer cells are also more migratory, but whether bioelectricity has a causal role in this phenotype is unknown. On the other hand, even though the molecular mechanisms are still debated, it is well established that cells migration is affected by electric fields, in what is known as electrotaxis

or galvanotaxis[7,8]. In a remarkable recent work, it has been shown that surface charges on the membrane can polarize cells and induce their migration[9]. All those evidences point toward a crucial role of V_m and Z_p in shaping the migration properties of cells.

Objective: in this PhD proposal, our goal will be to identify the causal role of cell-autonomous bioelectric properties of cells with regards to their migration using two very different cellular models of eukaryotic migrating cells: mammalian and *Dictyostelium discoideum* cells (called dicty later). By perturbing and measuring V_m/Z_p, while quantifying migration properties of many single cells, we propose to dissect out the relative contributions of V_m and Z_p on two phylogenetically distant biological models to see if general physical principles can apply broadly within the eukaryotic kingdom.

Experimental models and tools: for mammalian cells, we will use an RPE1 cellular model that have extensively studied in the Coppey lab[10]. For dicty cells, we will use the axenic strain AX3 that is routinely used in De Monte lab[11]. For perturbation of V_m, we will use a chemical approach by modifying the extracellular concentration of K+ thanks to potassium gluconate that allow sustained depolarization of cells[12]. For perturbation of Z_p, we will use the recent optogenetic actuator opto-Actu +, which rely on the light-gated recruitment at the plasma membrane of non-specific protein domains bearing positive charges that work both in dicty and mammalian cells[9]. For image-based measurement of relative values of V_m/Z_p, we will use either anionic/cationic dyes[14] or genetically encoded biosensors[3,15]. Finally, to monitor cell migration we will use timelapse microscopy during random/non directed single cell migration, as was previously realized by the two labs[10,11].



Aim 1. causal effect of bioelectricity on migration in mammalian cells: in this task, we will assess whether a sustained depolarization of RPE1 cells, which will mimic cancer cells, has a causal role on their migration properties (mean speed, directionality, persistence). First, we will repeat the optogenetic experiment in[9] to validate the approach on our cellular model. Then, in different sets of experiments together with appropriate controls, we will perturb independently V_m and Z_p in high throughput lens-free microscopy (cytonote[10]) to identify their relative contributions. To know if the putative effects are direct or indirect (through a link between V_m and Z_p), we will use epifluorescence and TIRF microscopy to measure the crosstalks (changing V_m while measuring Z_p and vice-versa).

Aim 2. correlation between bioelectricity and migration in dicty cells: the De Monte lab recently observed a bimodal distribution of migration properties in an isogenic cell line, where a significant fraction of cells (~15%) are much less migratory than others[11]. It was then proposed that the heterogeneity in migration at the population level has a strong influence on the ability of cells to aggregate, and thus may have evolutionary consequences. In this aim, we propose to explore the bioelectric signatures of these cells to see if they correlate with their migrating phenotype. Supporting this hypothesis, a work in dicty as shown an effect of V_m on migration[16]. We will use fluorescent dyes that are known to report V_m/Z_p in bacteria and mammalian cells. We do not aim at having absolute measurements but relative ones, to establish the relationship with the single-cell motility over the whole population. If possible, we will follow cells also during aggregation, where the first multicellular interactions get established.

Aim 3. identify the generic role of bioelectricity in eukaryotic migration: in this task, we will combine our observations to understand the causal effect of V_m/Z_p on the general properties of eukaryotic migration. Depending on the results of task 1, we will also perturb dicty using the same approach to see if the consequences on migration are maintained in highly divergent species. If we do observe similar behaviors, it might be the signature of a general physical principle and a strong hint that a fundamental quantity, such as electrostatics, has been evolutionary conserved to coordinate signaling pathways and biomolecule activities. Being even more speculative, our work may open a new understanding of the evolution of cancer metastasis as a selective trait toward a primitive migration phenotype[17]. Indeed, it has been proposed that cancer could be viewed as an atavistic reversion[18], namely a reversion to an ancestral quasi-unicellular phenotype, akin in behavior to dicty.

Interdisciplinarity and codirection

Mathieu Coppey, at the UMR168 of the Curie Institute, has a long experience in biophysics and an expertise in leading projects at the interface. Over the last year, he extensively developed and applied optogenetic tools to control cell migration. **Silvia De Monte's** research focuses on understanding the eco-evolutionary dynamics at the transition between unicellular and multicellular organization. She used dicty as a model system to bring biological realism into models describing the evolutionary implications of conflicts within multicellular aggregates. **Together**, the supervisors offer an integrated, cross-disciplinary view on the problem of identification of the 'master regulator' processes of cell motility in individual cells.

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