# Funded PhD proposal – 2025

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## Nuclear mechanics and cancer cell invasion

### Context

When cancer cells migrate to form metastases, they must deform and pass through a confined environment formed by other cells and the extracellular matrix (ECM). The nucleus, the largest and stiffest cell organelle [1], may play a critical role in such migration through confined environments and a soft nucleus should thus be an advantage for a cancer cell to invade the surrounding tissues. The stiffness of the nucleus is determined by both chromatin, the compacted structure formed by DNA and histones inside the nucleus, and lamins, intermediate filament proteins present in the nuclear envelope [2]. Within the nucleus, chromatin adopts specific positions relative to the nuclear periphery, with a dense layer of rigid heterochromatin lining underneath the nuclear lamina [3]. These heterochromatin lamina-associated domains (LADs) are characterized by low gene density, high content of repetitive sequences and enrichments of repressive histone modification H3K9me3 (trimethylated histone H3 lysine 9) [4] established by the lysine methyltransferases (KMTs) SETDB1 and SUV39H [5]. This dense layer of rigid heterochromatin together with lamins, strongly influences nuclear stiffness [6]. Moreover, the heterochromatin compartment is an active nuclear mechano-sensor participating not only in protecting genetic material from damage but also in the overall cellular response to mechanical cues [7]. Thus, H3K9me3 establishment and maintenance at the nuclear periphery plays an essential role in chromatin architecture and functionally links gene repression, epigenome, 3D genome and nuclear mechanics [8].

### Objectives and methods

*Cellular systems with modulated levels and organization of H3K9me3 at the nuclear periphery*
Our biologist collaborators (team of Slimane Ait-Si-Ali, Epigenetic and Cell Fate, Université Paris Cité) have shown that the interplay between the lysine methyltransferases SETDB1 and SUV39H1 in A549 cells - an epithelial cancer cell model which is known to overexpress SETDB1 - gives the possibility to modulate H3K9me3 levels specifically at the nuclear periphery. By complementary biological strategies, they have established cell lines in which the H3K9me3 level and spatial organization are modulated, globally or specifically at lamina-associated domains (LADs). More precisely, in the A549 cell line, which is known to overexpress SETDB1, they have shown that SETDB1 loss of function (LOF) triggers a widespread and expected decrease in H3K9me3 inside the nucleus, surprisingly also accompanied by a SUV39H1-mediated increase of H3K9me3 at the nuclear periphery. SUV39H1 LOF, in addition to SETDB1 LOF, abolishes the observed H3K9me3 enrichment at LADs. In agreement with this, SUV39H gain-of-function (GOF) in A549 cells counteracts SETDB1 overexpression, resulting in increased levels of H3K9me3 at the nuclear periphery, which mimics the SETDB1 LOF phenotype at LADs. Taken together, these data give a way to modulate H3K9me3 levels at LADs by shifting the balance between SETDB1 and SUV39H1. In addition to the H3K9 KMTs LOF/GOF models, they also succeeded in modifying LADs heterochromatin by epigenome editing to induce H3K9me3 GOF at LADs. The aim here is to better separate the specific impact of LADs on nuclear stiffness and ultimately

on cellular migration. The H3K9me3 enrichment at the nuclear periphery of the cells with engineered LADs recapitulates that of SETDB1 LOF cells).
The aim of the project is to measure the mechanical properties of the nucleus and to characterize the migration ability of these different cell lines.

*Measurement of nuclear stiffness*
The first objective of the project is to measure the mechanical properties of the nucleus in the different cell lines developed by our collaborators. Our team has developed optical tweezers-based rheometers that allow measuring both cellular and nuclear viscoelastic properties. Internalized micron-sized beads actuated with optical tweezers will be used to create a controlled indentation of the nucleus and infer its stiffness (see Figure) [9]. Preliminary measurements have confirmed that SETDB1 loss of function in A549 cells increases nuclear rigidity. By varying the depth and the speed of the indentation, we will further assess the chromatin *vs* nuclear envelop viscoelastic properties. We will seek to establish a correlation between the rigidity of the nucleus and the level and spatial organization of H3K9me3 in the different cell lines.

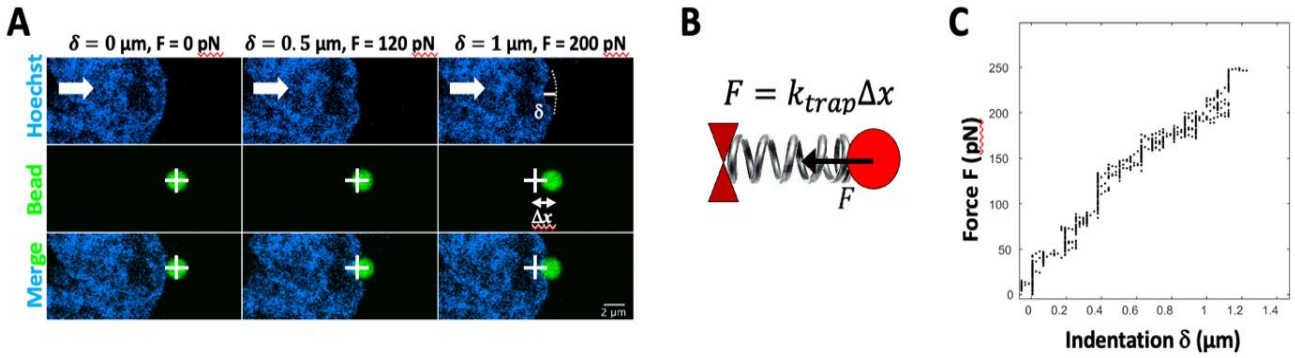


***Figure:*** *Measurements of the nucleus viscoelasticity by indentation in living cells with optical tweezers. (**A**) Images showing a typical nuclear indentation experiment. The white cross represents the centre of the optical tweezers in which the 2 µm-diameter bead is trapped (green). The nucleus (blue, Hoechst) is indented by moving the cell to the right (white arrow) which moves the bead away from the centre of the trap centre by a distance Δx. The indentation $\delta$ of the nucleus is measured by image analysis. (**B**) Scheme of the bead in the optical trap. The force F exerted by the optical trap on the bead is similar to the force exerted by a spring of stiffness $k_{trap}$. (**C**) Force-indentation curve showing force F as a function of indentation $\delta$ in the experiment shown in (A).*

*Motility assays*
The second objective of the project is to quantitatively characterize the *in vitro* migration capacity of the different cell lines, under different confinement conditions We will use the Cytonote lens-free device (Iprasense), which is available in our laboratory. It is based on multi-wavelength LED illumination combined with a holographic reconstruction algorithm that enables fast and efficient reconstruction of the phase images of migrating cells over a large field of view [10]. Thanks to its small size, the system can be placed in any cell culture incubator. We will quantify cell motility (speed, directionality, persistence) at high-throughput, using the TrackMate ImageJ plugin, for cells migrating in different confined environments: i) in 3D or quasi- 2D viscoelastic gels mimicking the extracellular matrix and ii) in PDMS microchannels with different sizes, which allow cells to be confined in a controlled manner. Preliminary measurements have already shown that on free 2D substrate, wild-type A549 cells are slightly faster than SETDB1 LOF cells.

By combining these motility assays in confined environments with direct measurements of nuclear stiffness by optical tweezers, we aim to establish a correlation between nuclear stiffness and the migration ability of the different cell lines.

**References**


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