Proposition de Stage/Thèse de M2 – 2027

Are mitochondria mechanosensitive organelles?

Cells can sense and respond to external forces and **mechanotransduction** events appear to be critical for most cellular functions, including cell migration and invasion. This M2 internship project will study mechanotransduction at the level of **mitochondria**, the intracellular organelle which is central to cell metabolism by producing energy in the form of ATP molecules.

Using a technique combining **optical tweezers** micromanipulation, rapid confocal microscopy and FLIM (Fluorescence Lifetime Imaging Microscopy), we were recently able to measure the effective **bending stiffness** of mitochondria in living cells and show that the **actin and microtubule** cytoskeletons contribute differently to the stiffness of mitochondria, to that of the mitochondrial microenvironment and to the transmission of force from the cytoplasm to the mitochondria[1] (Figure 1). Furthermore, when a force is applied directly to mitochondria, different morphological responses can be observed (fission, pearling) without the **tension** of the mitochondria appearing to be affected.

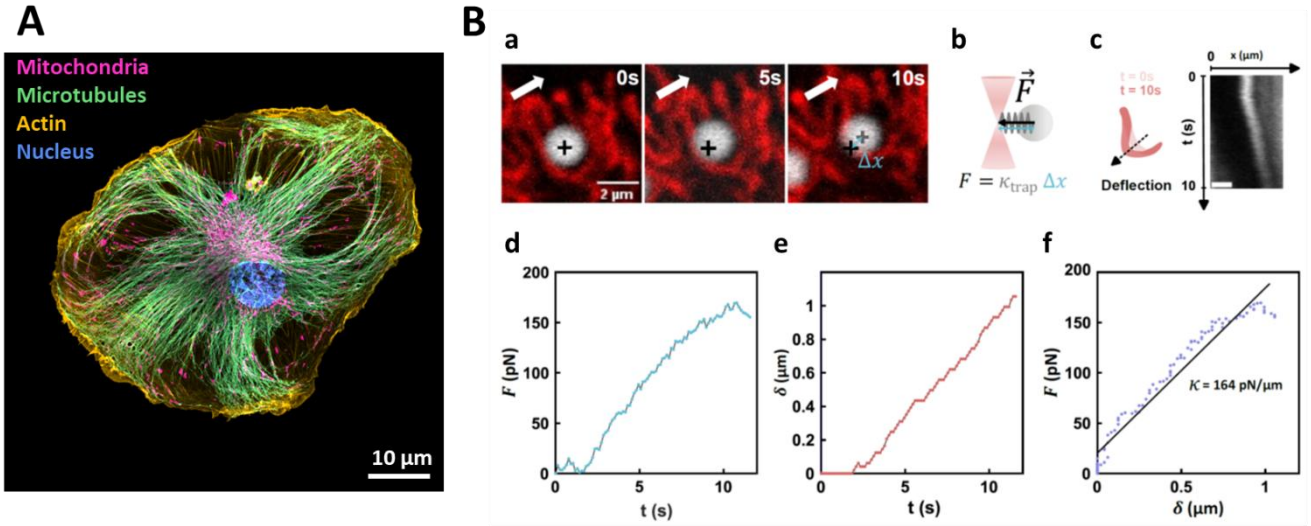


***Figure 1: A**. Typical localization of mitochondria (pink), microtubules (green), actin (yellow) and the nucleus (blue) in human retinal pigment epithelial (RPE-1) cells. **B**. Optical-tweezers based mitochondrial deflection. (a) Confocal images showing a typical deflection experiment of a mitochondrion in RPE-1 cells. A mitochondrion (red) is deflected by moving the cell towards the top-right direction (white arrow) which displaces the bead (gray) away from the trap center (black cross) of a distance Δx. (b) Scheme of the bead in the optical trap. The force magnitude F exerted on the mitochondrion is deduced from the bead displacement Δx and using the calibrated trap stiffness. (c) Scheme of a mitochondrial deflection and kymograph generated along the deflection axis (dotted arrow) for the experiment shown in (A). Scale bar = 1 µm. (d) Plot of the force F (pN) as a function of t (s) obtained by tracking the displacement of the trapped bead. (e) Plot of the mitochondrial deflection δ (µm) as a function of time t (s), obtained from the kymograph in (c). (f) Force-deflection F(δ) curve. The effective stiffness of the mitochondria κ (pN/µm) is obtained by a linear fit of the force-deflection curve at small forces. From reference [1].*

During this internship, we will ask whether an **external force** can be transmitted to the mitochondria and if yes, decipher the role of the cytoskeleton in this mechanotransduction process. We will use a recently developed **cell stretcher** to apply a uniaxial stretch to the cells. Changes in the morphology of mitochondria and of the cytoskeleton will be first measured and correlated using image analysis. We will next ask whether **mitochondrial tension** is modified upon cell stretching using the

Mito-Flipper FLIM probe. The involvement of the cytoskeleton will be further investigated using **pharmacological perturbations** of the actin and microtubule cytoskeleton. If time permits, the role of intermediate filaments will also be quantified using cells depleted of the vimentin intermediate filament protein. Because growing evidence demonstrates strong links between mechanobiology and metabolism in **cancer cells**[2–4], longer term experiments will aim at studying the effects of mechanical forces and of mitochondria mechanoresponses on cancer cell metabolic activity.

References

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