Internship

Role of reactive oxygen species in the mechanical properties of cells

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Excitation of fluorophores in cells induces phototoxicity; photo-excited fluorophores can react with the environment to produce reactive oxygen species (ROS) in the cell. ROS can either break down intracellular structures such as the cytoskeleton or instead cause the formation of intermolecular protein-protein and protein-DNA bonds. The first scenario suggests that the cell will become softer due to phototoxicity, while the second scenario suggests a stiffening of the cell. This link between phototoxicity and cell mechanics is poorly understood and understudied, but deciphering it would provide a better understanding of the detrimental aspects of phototoxicity and highlight ways to use phototoxicity to manipulate cells.

Our preliminary data show that several fluorescent probes lead to a **large stiffening of the cell upon fluorescence excitation**. This stiffening is not dependent on the cell cytoskeletal structure and is also induced in the presence of H_2O_2, which is known to induce the formation of ROS. In this project, we propose to study **this new relationship between intracellular ROS and changes in cell mechanical properties**.

The **specific objectives** of the project are to extend our preliminary results to other cell types. We will also establish if cell stiffening is confined to the area where fluorophore-loaded cells are illuminated. This project might help establish a new role for ROS in living cells, which is that ROS regulate cell mechanical properties. It should also provide a better understanding of the mechanisms of phototoxicity and photodynamic therapy.

During this internship with a major **experimental component**, we will use cell microindentation experiments coupled to epifluorescence (Figure 1; see examples of single-cell micropipette manipulation here: https://cellmechanics.jimdofree.com/videos/). Cell and molecular biology know-how will be provided by biologists through established collaborations.

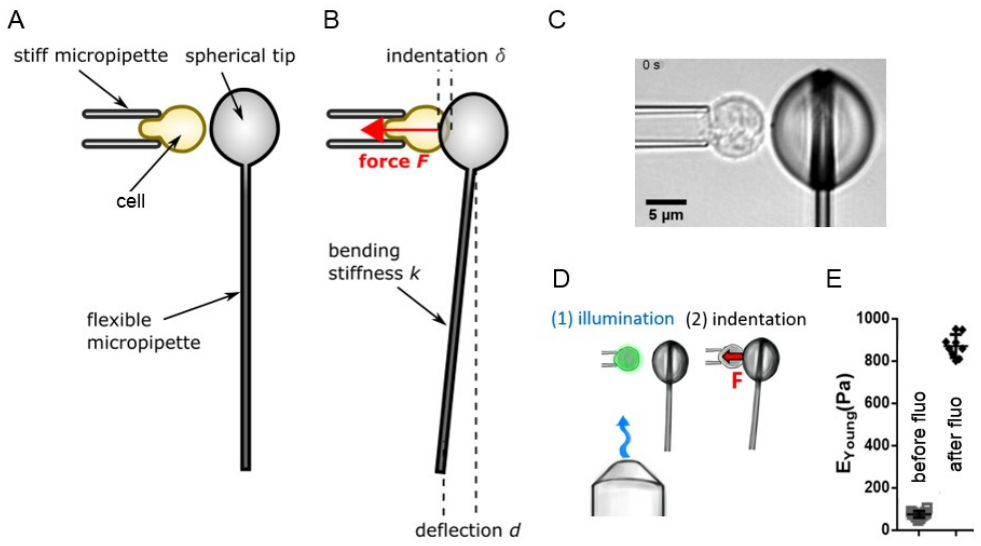

Figure 1. (A) Microindentation setup. Two micropipettes are placed in a Petri dish. A rigid micropipette (left) gently holds a cell. A flexible pipette (right) has a spherical tip. **(B)** The cell is pushed against the flexible micropipette to apply a desired force $F=kd$ to the cell, where d is the deflection of the micropipette and k its bending stiffness. Knowing the applied force F, and the resulting cell deformation δ, one can extract cell viscoelastic properties (Guillou et al., *Sci. Rep.* 2016, 6: 21529; Husson, MIMB 2023, vol. 2600). (C) Image of the setup captured by the microscope camera. (D**)** Coupling the microindentation to epifluorescence microscopy allows combining fluorescence excitation and mechanical measurements. (E) Large increase of the effective cell stiffness (Young's modulus) of cells loaded with a fluorescent probe 2',7'-Dichlorofluorescin diacetate and illuminated with blue light.