Measurement of changes in membrane and actin cortex tension upon induced cell deformation

The plasma membrane is a lipid bilayer that separates the interior of cells from the outside environment. The mechanical tension in the plasma membrane regulates many vital biological processes such as communication with the extracellular environment. The actin cortex, a biopolymer network underneath the plasma membrane, supports the membrane mechanically and regulates cell shape through the generation of active contractile tension via molecular motor proteins.

The goal of the PhD project is the joint measurement of membrane tension and actin cortex tension to understand the response of the two physical quantities upon induced cell shape deformation. We will use the membrane tension reporter Flipper TR (see Figure 1) that allows to infer membrane tension via fluorescence lifetime imaging (FLIM) of a membrane-associated dye (see Figure 1). In conjunction, we will use a parallel plate cell confinement assay based on atomic force microscopy (AFM) that permits to read out cortical tension values via the measured AFM force (see Figure 2).

Furthermore, we will determine how membrane-associated actin cortex molecular regulators such as RhoA respond to variations in membrane tension and how this correlates with Calcium fluxes through membrane-tension-gated ion channels.

Figure 1 - Flipper-TR sensing membrane tension in cell undergoing hyperosmotic shock.

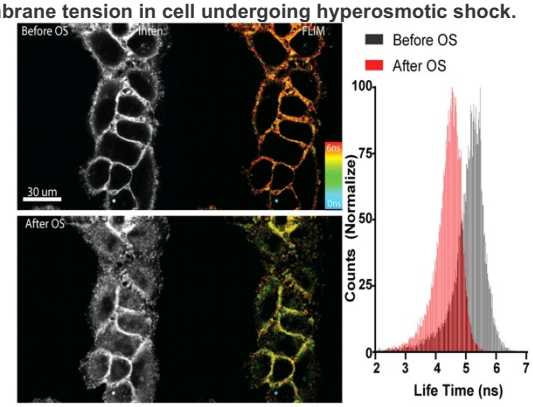

Figure 1: Flipper-TR® staining of cells. Left side: Image of cells stained with Flipper-TR® before (top) and after (bottom) hyperosmotic swelling. Greyscale represents fluorescence intensity, and color codes represent fluorescence lifetime. On the right the histogram shows the lifetime shift after osmotic shock. Images courtesy of Colom et al. 2018.
The fluorescent Flipper-TR® probe works by specifically targeting the plasma membrane of cells and reports membrane tension changes through its fluorescence lifetime changes.

Figure 2 – AFM-based squishing of cells to induce cell deformation and readout cortical tension.

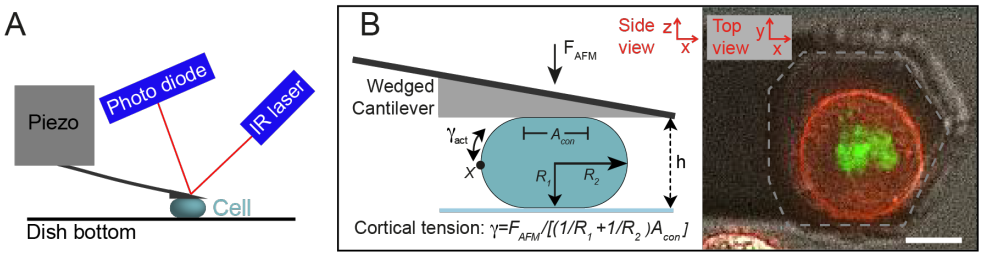

Figure 2: Schematic of the sandwich method for measuring the cellular surface tension of animal cells and the viscoelastic material properties of the actin cortex (1–3). A) Experimental setup as a schematic. Using an atomic force microscope (AFM), an initially round nonadherent cell (blue) is compressed between two parallel plates. The AFM readout allows to measure the force response of the cell upon squishing. B) Left panel: compressed cell in schematic in side view. Right panel: microscopy image of a compressed cell in top view (transmitted light and fluorescence, red fluorescence: cell membrane (mCherry-CAAX), green fluorescence: DNA (EGFP-H2B), dashed gray line: outline of the wedge of the AFM cantilever).

Bibliography

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