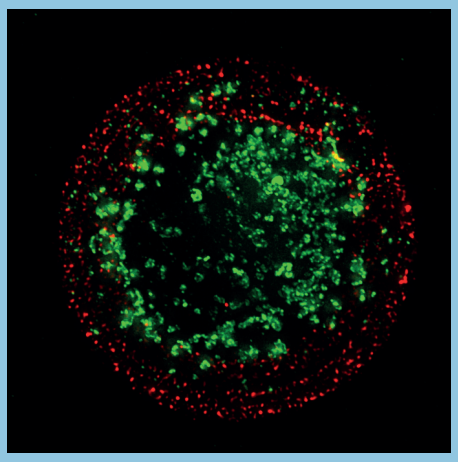
M2 project: Analysis and modeling of lysosomal dynamics in response to biophysical constraints

Lysosomes are essential organelles of the eukaryotic cell responsible of lytic activity. Eukaryotic cells usually have hundreds of lysosomes with a non-random spatial distribution (Ba et al., 2018). Lysosomes are mainly clustered in a fairly immobile perinuclear cloud whereas a minority of mobile lysosomes are moving within the cell periphery (Cabukusta and Neefjes, 2018). It is now clear that this positioning is tightly regulated by diverse biological mechanisms (Pu et al., 2016; Bonifacino and Neefjes, 2017; Cabukusta and Neefjes, 2018). However, at resting state, lysosomal distribution is a non-equilibrium steady state (NESS), which means that the global distribution does not change while lysosomes continue to move individually. The steady-state distribution may vary in response to various stimuli such as cholesterol concentration (Rocha et al., 2009) or pH (Walton et al., 2018).

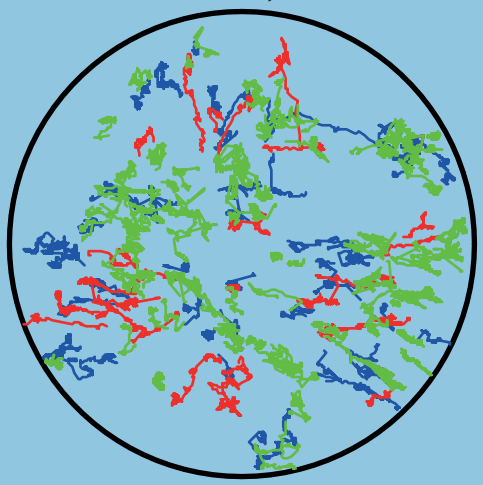


Interestingly, extracellular matrix elasticity also regulates lysosomal distribution (Wang et al., 2018), suggesting that lysosomes form a mechanosensitive compartment. Conversely, it has been shown that lysosomes can influence mechanical properties at immune synapse by regulating contraction of actomyosin network (Kumari et al., 2019). Even if these results suggest an interplay between mechanical stress and intracellular transport, this topic is still poorly investigated.

How lysosomal dynamics is influenced by biophysical constraints?

To study several properties of lysosomal transport in the cell, we have carried out live imaging experiments and used a tracking approach to systematically extract and analyze lysosomal trajectories from dozen of cells. Moreover, we have seeded cells on micropatterns, a microengineering technique that prints fibronectin patterns on coverslips in order to standardize cell shape (Schauer et al., 2010). This morphological normalization and the use of spatial statistics revealed some interesting properties of lysosomal transport and its spatial heterogeneity in the cell. The goal of this project is to extend these results and investigate the influence of mechanical stress on intracellular dynamics.

We can modulate mechanical tension by seeding cells on micropatterns of different sizes and different shapes. The project will focus on the description and comparison of lysosomal dynamics in response to biophysical constraints. The student will use a confocal spinning disk microscope available in the team that is designed for live imaging. The student will also analyze movies and perform data analysis in order to establish a correlation between mechanical stress and lysosomal dynamics. Outstanding questions will be to determine 1) how mechanical stress changes dynamics in terms of velocity and frequency of active transport, 2) whether size and aspect ratio of the micro-pattern influence lysosomal dynamics in the same way and 3) if the whole cell is influenced by mechanical constraints or if there is a spatial heterogeneity at the cell scale in the biological response.



The project may be completed by a modeling approach. Lysosomes switch between active transport and random diffusion. We have start to model this behavior by a hidden Markov model using observed data as model parameters. The student may complete this approach and take biophysical constraints into account in the model.

The student will benefit from a state-of-the-art laboratory with innovative techniques (as micro-pattern) and top-notch microscopes. In addition, the Curie Institute environment, and more specifically our multidisciplinary team, is an ideal setting for a student wishing to learn about cell biology problems with a computational/biophysical perspective. The student will learn how to code in R, to do cell culture and perform high-level microscopy. Nevertheless, preliminary knowledge in these areas is desirable. The essential qualities for this project remain a strong curiosity and a great enthusiasm.

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