QUANTITATIVE MICROSCOPY FOR OPTOGENETICS

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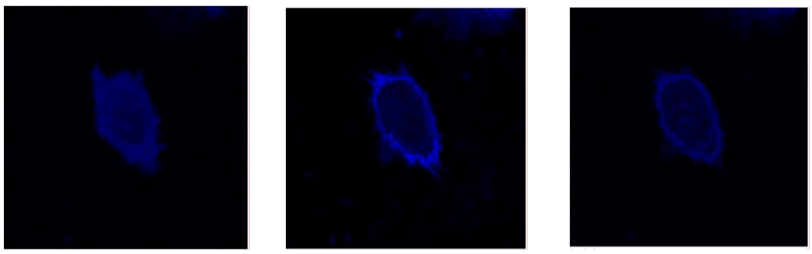
Optogenetics refers to a technique that combines genetic and optical methods to control basic biological processes such as protein dimerization. Light-induced heterodimerization of optogenetics system makes it possible to induce interaction of proteins in a controlled way in space and time. We studied photoactivable cells where RhoGEF is controlled by CRY2-mCherry/CIBN-GFP dimerization. Previous works on this system showed that recruitment of Cry2 to the membrane can be stimulated with blue light and is reversible, i.e. that CRY2-CIBN dissociates spontaneously after photoactivation. However, quantification of the concentration and diffusion constant of the photoactivated molecules is still lacking. In a first time, we have designed experiments to perform quantitative analysis of this optogenetic system using confocal microscopy combined with fluorescence fluctuation techniques. We let the cell adhere on a patterned polyacrylamide hydrogel, in order to control their shape and immobilize them. The confocal setup allows us to get optical sectioning and perform image analysis. We shine the cells with a 488nm laser line to stimulate CRY2 interactions with CIBN and image the fluorescence of mCherry at 561nm, before and after photoactivation (Fig. 1). As a preliminary result, we could characterize the recruitment and dissociation of CRY2 at the plasma membrane, by using the changes of intensity in various segmented regions of the cell. The image analysis showed an increase of the intensity at 561 nm following activation and then a decay when the complex is dissociated with a time constant of 300 seconds (Fig. 2).



(a) (b) (c)

Fig. 1. Illumination and imaging sequences: (a) Control, (b) Activation, (c) Relaxation.

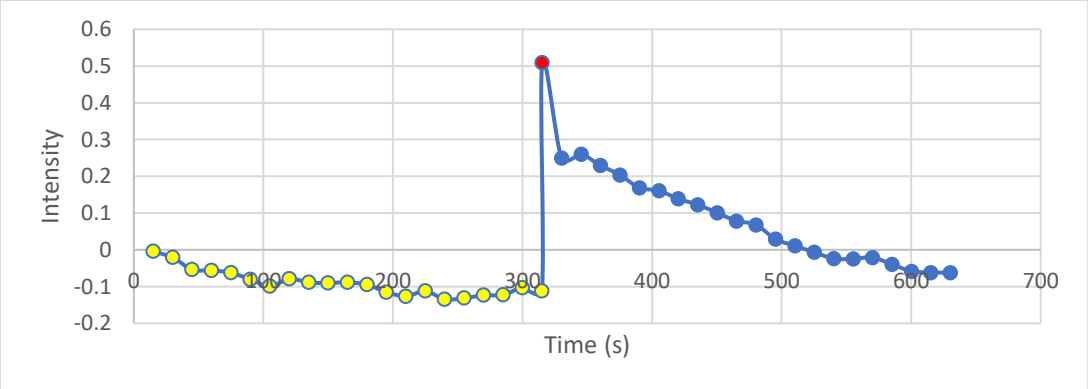


Fig. 2. Segmented fluorescence intensity at 561 nm before acquisition (yellow circles), when activating (red circle) and then during relaxation (blue circles).

In a second time, we will use a TIRF approach to access faster mobility than those accessible in confocal microscopy.

Keywords: Quantification, Optogenetics, Microscopy.