AURKA KINASE ACTIVITY FRET BIOSENSOR: HCS-FLIM FOR DRUG SCREENING AND OTHER REFINEMENTS

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KEY-WORDS: AURKA, FRET biosensor, FLIM, High-Content-Screening

AURKA gene encodes a multifunctional serine/threonine kinase involved in the cell cycle and plays a key role during cell division. The protein is one of the most upstream activator of the mitosis and is involved in the maturation of the centrosomes, the formation of the mitotic spindle and the central spindle. Overexpression of AURKA is a major hallmark of several solid tumors rising from epithelial tissues. So far, no inhibitor of this oncogene has been FDA-approved and therefore it is of great importance to identify new molecules. To be functional, AURKA switch to an activated form through autophosphorylation on the T288 residue leading to a change of conformation.

Our team has developed a FRET (Forster Resonance Energy Transfer) based biosensor for Aurora A consisting of the whole kinase flanking by two fluorophores (Figure 1). We showed that the change of conformation of Aurora A when activated brings closer the fluorophores increasing FRET efficiency and that the biosensor is as functional as the endogenous protein [1]. We have also developed a fast-FLIM prototype (Fluorescence Lifetime Imaging Microscopy) that allows us to image and measure fluorescence lifetime with a higher speed than conventional techniques [2]. As fluorescence lifetime is inversely correlated with FRET efficiency, we are able to track and to image the activation of AURKA within the cells.

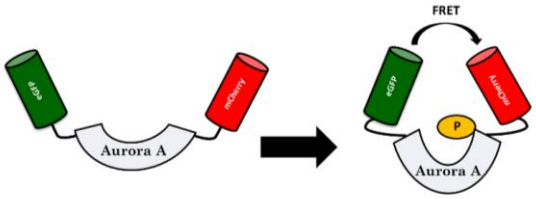

Figure 1. A FRET biosensor of AURKA activation.
The phosphorylation on the T288 residue of the kinase leads to a change of conformation gathering GFP and mCherry and increasing FRET efficiency. By measuring the fluorescence lifetime of GFP, we can have access to FRET efficiency and thus to the activation state of AURKA.

Based on this biosensor, we have already investigated new roles of the AurkA kinase in G1 phase for the establishment of the interphase spindle [1] but also at the mitochondria to control organelle dynamics and energy production [3]. Moreover, our project aims to establish a new method to use AURKA FRET biosensor in a HCS-FLIM manner. We have developed an innovative way to perform a rapid and automated drug screening on a 96-well plate to discover new inhibitors of AURKA. Finally, we have also made effort to improve our biosensor both by changing FRET pairs of fluorescent proteins and by using unconventionally two-color FCS to measure FRET at very low level of expression.

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