ABSTRACT: In vivo BRET microscopy to exploring plasticity during learning process

Signaling pathways underlying inter and intracellular communication underpin responses and adaptation to cellular environment. In order to detect and measure activity in these pathways, powerful optical biosensors have been developed, based on either fluorescence or bioluminescence. Bioluminescence Resonance Energy Transfer (BRET)-based sensors permit such measures with high sensitivity, owing to engineered enzyme substrates such as furimazine (Promega).

This kind of technological development allows to explore the mechanisms at the foundation of brain adaptability to so many internal and environmental stimuli and changes of states, *i.e.* the dynamics of signaling responsible for synaptic plasticity. Even tough it is recognized that cytosolic calcium ion concentration and MAP kinases such as ERK activity are crucial, neither their spatio-temporal patterns or their intermingling have been described to a level of details allowing predictive mathematical modeling.

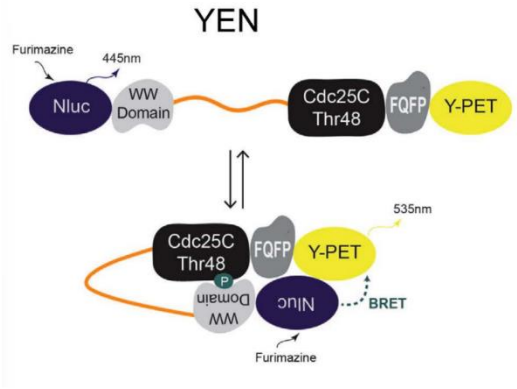

Figure 1: ERK biosensor (doi: 10.3389/fendo.2013.00095)

In order to do so in a neurophysiologically relevant framework, we will undertake an *in vivo* study of calcium and ERK dynamics in mice. To this end, we have developed a fiberscope allowing simultaneous recording of fluorescence signal from a calcium reporter, and BRET signal from an ERK one, with single cell resolution. Proof of principle shows feasibility at the brain surface, and we will now focus on hippocampal place cells, taking advantage of the corpus of evidence supporting Hebbian plasticity in this structure.

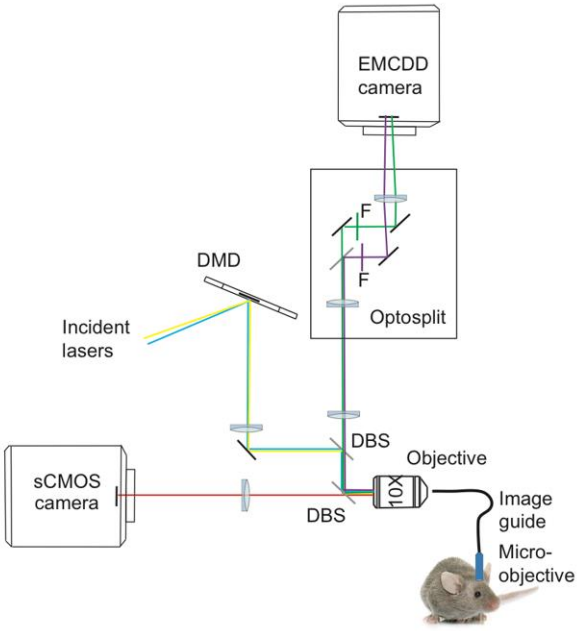

Figure 2 : Fiberscope set up

Current limitations mainly relate to the BRET chemical substrate. First, substrate's fluorescence impairs simultaneous recording of other fluorescent sensors. Second, furimazine intravenous or epidural infusion does not provide a temporally stable luminescence signal, limiting the ability to measure accurately BRET evolution. Third, luciferases substrates are poorly water-soluble, such that solvent toxicity is in fact an issue. We will hence make use of fluoro and/or cephalofurimazine, showing higher water-solubility and blood-brain barrier crossing capability.

To conclude, we will develop and make use of *in vivo* BRET microscopy to explore the intracellular dynamics of signaling pathways inducing synaptic plasticity in hippocampal neurons. Current efforts target luciferases substrates nature and administration route.