

PROBING APP AND BACE1 PROXIMITY BY COMBINING TIME RESOLVED TIRF AND FRET DETECTION IN LIVING NEURONS.

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Förster Resonance Energy Transfer (FRET) imaging is an accurate technique to monitor proteins' interaction within living cells. Measuring the fluorescence lifetime of the donor allows quantifying FRET and relating it to proteins' proximity. To match the high resolution needed in neurobiology, we develop a time resolved Total Internal Reflection Fluorescence Microscope (TIRFLIM) which permits to observe the events occurring on or just below the plasma membrane, with a sub-wavelength axial resolution in wide field[1]. Given our applications, we settled a "through the objective configuration" to perform TIRFM, using a high N.A. of 1.45 (Olympus TIRFM, 60x). To preserve the wide-field approach for time-domain fluorescence lifetime measurements, we use a High Rate Imager (Kentech Ltd) which allows us to smartly sample the fluorescence decay [2]. A 20 MHz supercontinuum laser source (Fianium SC400) permits to excite a large range of fluorophores and measure long fluorescence lifetime or anisotropy decay. The laser's position in the back focal of the objective is finely controlled thanks to a motorized mirror, which leads to an accurate control of the penetration depth. We can also quickly switch between epifluorescence and TIRF configuration, and follow protein's activity from the membrane to the inner part of the cells.

This TIRFLIM set-up is currently dedicated to the study of the Amyloid Precursor Protein (APP), a membrane protein involved in Alzheimer's disease (AD). APP can be cleaved by two enzymes, the β -secretase BACE1 and the γ -secretase complex, to release the A β peptide, which accumulates in the senile plaques, one of the major hallmarks of AD. Recent genetic, epidemiologic and biochemical data point to a link between cholesterol and AD (reviewed in [3]) but data from different groups are still up to now controversial. We hypothesize that a higher concentration of plasma membrane cholesterol could increase APP and BACE1 interaction, which will lead to a higher production of A β peptide. We studied APP and BACE1 proximity under the effect of cholesterol, in HEK-293 cells and primary cultures of embryonic rat hippocampal neurons co-transfected with plasmids encoding BACE1-GFP and APP-mCherry constructs. Thanks to the versatility of the setup, the GFP lifetime was monitored in two complementary types of excitation (TIRF and epifluorescence), with or without addition of cholesterol. The dynamic following-up of the cholesterol effect over 30 minutes will be presented, and new insight on BACE1- APP interaction will be discussed.

In addition, as APP dimers seem to be better substrates for the cleaving enzymes, thus leading to a higher production of A β peptide, we are also studying APP homodimerization. Since homoFRET is the most elegant way to measure it, we implemented a polarization-resolved imager (dualview) on our TIRFLIM set-up, to perform time-resolved fluorescence anisotropy imaging (TR-FAIM). Preliminary results of homoFRET will be presented.

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