

Comparison of FRET in the Chloride Indicator Clomeleon with neuronal development

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A number of techniques have been developed so far to study the dynamic events taking place inside living cells. The drastic fall in FRET efficiency with distance ($k\alpha 1/R^6$) makes it a powerful tool for studying interactions in living cells. FLIM monitors localised changes in the probe fluorescent lifetime, which is concentration independent. In the present work, we have combined FRET and FLIM techniques to study interactions of fluorescent-tagged proteins inside living hippocampal cells by monitoring the fluorescence lifetimes. We have maintained the natural state of the cells throughout the measurement by using very low excitation levels ($<100 \text{ mW/cm}^2$). To get a good S/N ratio, we have taken advantage of the sensitivity of the DL/point (Delay line) and QA/imaging (Quadrant Anode) detectors, based on Time and Space Correlated Single Photon Counting (TSCSPC). Apart from the conventional way of monitoring the donor lifetimes alone, we have observed the donor and acceptor lifetimes simultaneously. Moreover, we have also studied the Decay Associated Spectra (DAS), which give an account of the contribution of the different lifetimes along the different wavelength channels and have shown the changes in the pre-exponential factors as a proof of FRET. Making use of the high sensitivity of YFP on the ionic concentrations, a novel optical indicator called 'Clomeleon' for studying the intracellular Cl^- concentrations was developed by Kuner and Augustine [1]. In Clomeleon, a chloride sensitive variant of YFP called Topaz was linked with a relatively chloride insensitive CFP by using a 24 amino acid linker to form a ratiometric chloride indicator. In the present work, we have studied the fluorescence dynamics of Clomeleon in neurons of hippocampal cell cultures at three different stages of maturation (DIV 7, 11 and 15). In young hippocampal cells (DIV 7), we found a quenching of the YFP moiety of Clomeleon in the majority of neurons, indicating the absence of energy transfer which might be due to the high intracellular Cl^- concentration present. A drastic change in the YFP/CFP ratio as well as kinetics was observed from DIV 7 to 15, with the cells from DIV 15 expressing the highest efficiency of energy transfer. This was in accordance with previous studies [1], where a lowering of intracellular Cl^- concentration with development was observed. FRET was verified by analysing DAS where a change in the pre-exponential values from positive to negative was observed, corresponding to the lifetimes which participate in the energy transfer. A topographical analysis of the measured neurons revealed various lifetimes within different ROIs indicating variability in FRET. This might be explained by the different Cl^- concentrations present in the different intracellular compartments of these cells. Thus, by a combination of FRET and FLIM and using ultra sensitive DL and QA detectors, we were capable of monitoring the differences in energy transfer of Clomeleon at different developmental stages of hippocampal neurons as well as at different intra neuronal compartments.

[1] Thomas Kuner and George J Augustine, "A genetically encoded ratiometric indicator for chloride: Capturing chloride transients in cultured Hippocampal Neurons", *Neuron*, **27**, 447-459 (2000).