

## **LIFETIME DECAY IN LIVING CELLS OF AN OPTIMIZED GENETICALLY ENCODED PROBE FOR FRET EFFICIENCY.**

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Two variants of GFP can be used as fluorescence energy transfer pair in living cells, CFP acting as donor and YFP acting as acceptor. These proteins, genetically fused to the catalytic subunit of PKA (Cat-YFP) and to the regulatory subunit of (RII-CFP), respectively, can be used as an indicator of the concentration of cyclic AMP in cells. In fact, in the presence of cAMP the regulatory and the catalytic subunits dissociates and the fluorescence energy transfer decrease, while in conditions of low cAMP, transfer is maximal. In order to quantify the transfer efficiency of this particular construct, the decrease of the donor fluorescence lifetime has been measured in living CHO cells stably expressing the two chimeras in comparison with CHO cells expressing the regulatory part only. Lifetime decay does not depend upon fluorophore concentration thereby avoiding problem of intensity normalization when comparing different cells. Fluorescence lifetime decays have been collected following two photon excitation at 830 nm by a Ti:Sa femtosecond pulsed laser, through a 485/30 nm bandpass filter in order to collect donor fluorescence only. The photon histograms were built by acquiring more than  $10^5$  photons for each decay. Approximately 100 lifetime decays from at least 10 different cells have been collected for each clone.

When only the donor is present in CHO cells, it has been found that two exponentials well described the CFP decay. Two lifetime distributions results centred at about  $3.60 \pm 0.05$  ns and  $1.0 \pm 0.1$  ns. In the sample where both the chimeras were expressed, the lifetime decays have been found still to be double exponential. In particular the longer component distribution shifts to lower values centred around  $3.07 \pm 0.05$  ns, whereas the shorter component does not show significant changes when compared to the donor lifetime distribution. An analysis of energy transfer efficiency based upon two different CFP populations that might arise from different protonation states of the chromophore is suggested. When the longer lifetime is considered, a transfer efficiency of  $14 \pm 1\%$  is found, showing that this construct has a remarkable sensitivity for cAMP detection in living cells. A careful analysis of the possible artefacts such as spectral bleed-through and acceptor photobleaching are taken into account.