

MEASURING FRET IN LIVING CELLS WITH FLIM

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Interactions between two proteins can be demonstrated by using the FRET/FLIM technique. One protein is labelled with a fluorescent donor molecule, and the other with an acceptor molecule. If both types of labelled proteins are interacting, they are necessarily in close proximity of each other. In that case, fluorescence resonance energy transfer (FRET) occurs between the donor and acceptor molecules. Energy is transferred non-radiatively from the donor to the acceptor, with a rate that is proportional to the inverse sixth power of the separation distance. An energy transfer efficiency of 50% is typically reached at a distance of 5 nm.

The energy transfer from donor to acceptor results not only in a decrease of the fluorescence intensity (which is used in ratio imaging as a FRET-indicator), but also in a decrease of the donor's fluorescence lifetime (Fig. 1). With Fluorescence Lifetime Imaging Microscopy (FLIM) this decrease in fluorescence lifetime can be measured, as described in "Imaging biochemistry inside cells" [1].

For example, to determine if interaction is taking place between antibodies to the EGF receptor on A431 cells, the fluorescence lifetime is measured first using antibodies labelled with a donor molecule only (Fig. 1, top). Then the measurement is repeated using both donor-labelled and acceptor-labelled antibodies (Fig. 1, bottom). The significant decrease in lifetime clearly indicates that FRET, and therefore interaction, is taking place.

Using FLIM instead of ratio imaging to detect FRET as an indicator of protein-protein interactions has several advantages. For example, the fluorescence lifetime is independent of bleaching and bleed-through, so no corrections are needed. Also, the measured lifetimes are independent of local intensity variations of the fluorescence emission.

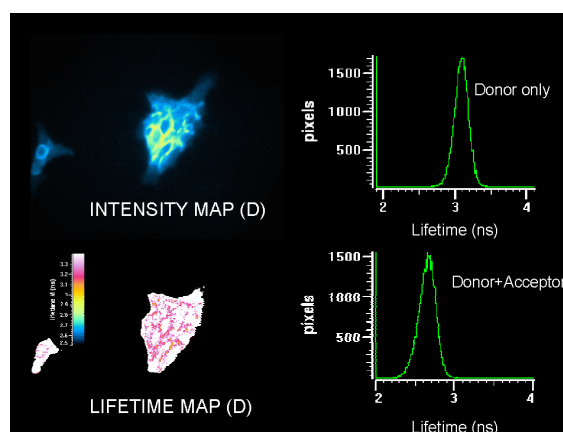


Figure 1. Showing FRET, indicated by a decrease in lifetime, using anti-EGFR (Alexa Fluor 488+/-546) in A431 cells. (A.H.A. Clayton, Ludwig Institute for Cancer Research, Australia)

[1] F.S. Wouters, P.J. Verveer and P.I.H. Bastiaens, "Imaging biochemistry inside cells", *Trends in Cell Biology*, Vol 11 No 5, pp 203--211, May 2001