

REACTIVE OXYGEN SPECIES EVALUATION IN SINGLE LIVING CELL USING PYRENE BUTYRIC ACID LIFETIME VARIATION

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Fluorescent molecules such as pyrene derivatives or ruthenium complexes with long fluorescent decay have oxygen dependent lifetimes. They are also sensitive to free radical quenching. Under nitrogen (i.e. oxygen free atmosphere), free radicals can be quantified using the lifetime decay of pyrene derivatives. Lifetime measurements are not dependent on the absolute intensity of the emitted light and, therefore, independent of the probe concentration. This property is advantageous when the probe is loaded into living cells. We developed a new approach using pyrene butyric acid in single living cells to detect free radical differences between healthy and treated or pathologic cells.

The cells are loaded with Pyrene Butyric Acid and Rhodamine 123. Emission is recorded through adequate filters after excitation by a pulsed laser (337nm, 3ns). In living cells, the energetic state of the cells through NAD(P)H intensity and lifetimes can be obtained. In addition, the total intensity of Rhodamine 123 can be recorded giving information about the energetic state of the mitochondria. In living cells, reactive oxygen species are mainly produced in mitochondria and are important contributors to many forms of cell death. With our method we obtain information about ROS levels and about the cells energetic state.

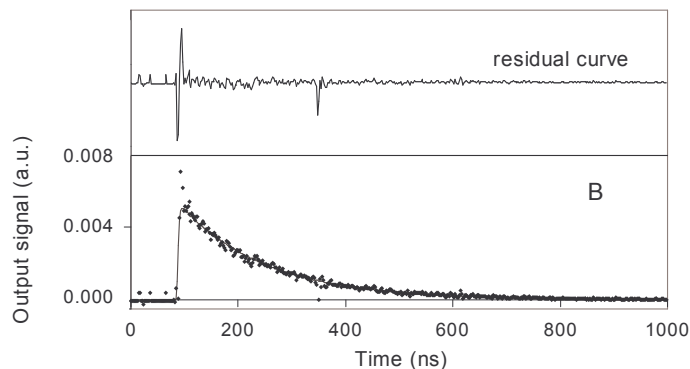


Figure 1: Fluorescence experimental decay of PBA (0.6 μ M) in single living cells together with the simulation.

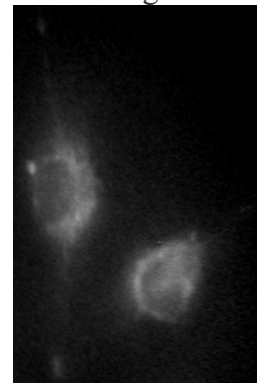


Figure 2 : Fluorescence microscopy image of 3T3 cells labeled with PBA.

PBA lifetime is recorded for living cells under air and nitrogen atmosphere. The decays are compared to the decays obtained for fixed cells as a control for free radical lack. We first confirmed the feasibility of the method on control cells [1]. Then, the cells were treated with several drugs. These drugs were chosen in order to increase or decrease free radical concentration.

[1] A-C. Ribou, J. Vigo and J-M. Salmon. "Lifetime of Fluorescent Pyrene Butyric Acid Probe in Single Living Cells for Measurement of Oxygen Fluctuation", *Photochem. Photobiol.*, 80, 274-280 (2004).