INVESTIGATION OF THE MEMBRANE MICROPOLARITY REGULATION OF THE AUTOPHAGIC FLUX BY SPECTRALLY RESOLVED FLUORESCENCE LIFETIME MICROSCOPY

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Autophagy is a cellular dynamic process aimed to degrade dysfunctional or unnecessary cytoplasmic components. The process, that can be finely monitored by confocal microscopy by using pH sensitive probes [1], consists in a well-regulated sequence of steps. Upon induction, a vesicular sac called the phagophore, elongates, and encloses a portion of cytoplasm, which results in the formation of a double-membrane structure, the autophagosome. Then, the outer membrane of the autophagosome fuses with a lysosome to form an autolysosome, leading to the degradation of the enclosed materials. Recovered amino acids and other small molecules are delivered back to the cytoplasm for recycling or for energy production. Membrane Micropolarity regulation is determinant in controlling the several steps involving the bilayer structure, especially membrane formation and organelle fusion. To unravel these mechanisms, here we present a method based on the spectrally resolved Fluorescence Lifetime Microscopy, which contextually allows for the characterization of the different organelles involved in autophagy and the determination of their membrane polarity. This method is based on the combined use of a genetically encoded pH sensitive protein and Nile Red, a lipophilic probe characterized by an emission shift from red to yellow according to the degree of intracellular micropolarity [2]. The acquisition of spectrally resolved lifetime data, and the implementation of a specific phasor algorithm can provide a new tool to describe not only the autophagic flux but also membrane micropolarity, allowing to investigate the membrane micropolarity regulation of the main steps of the autophagy process.

[1] G. Maulucci, M. Chiarpotto, M. Papi, D. Samengo, G. Pani, and M. De Spirito, “Quantitative analysis of autophagic flux by confocal pH-imaging of autophagic intermediates,” Autophagy, vol. 11, no. 10, pp. 1905–1916, 2015.

[2] G. Maulucci, F. Di Giacinto, C. De Angelis, O. Cohen, B. Daniel, C. Ferreri, M. De Spirito, and S. Sasson, “Real time quantitative analysis of lipid storage and lipolysis pathways by confocal spectral imaging of intracellular micropolarity,” Biochim. Biophys. Acta - Mol. Cell Biol. Lipids, vol. 1863, no. 7, pp. 783–793, Jul. 2018.