Novel probes for simultaneous dual-colour labelling of mitochondria and lysosomes

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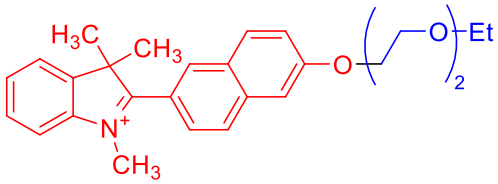
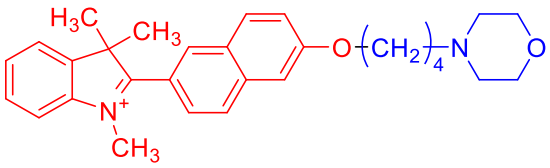
Cellular activity depends on the accurately controlled and synchronised functions of the subcellular organelle ensemble. In this context, lysosomes and mitochondria are central to cellular metabolism as major contributors of chemical energy and building blocks. The coordination of lysosomal and mitochondrial metabolic roles is thus essential for cellular homeostasis, with dysfunction of these organelles associated with many human diseases.

Confocal and two-photon (2P) microscopy are state-of-the-art techniques for live imaging due to their operational simplicity, excellent spatial and temporal resolution and non-invasiveness. Consequently, fluorescent dyes that specifically label subcellular structures have become vital tools for cell biology and biomedical science, and multiple dyes labelling different structures can be combined to follow complex cellular processes. However, using different labels adds cost and complexity to experiments. Thus, for biomedical research applications, a single fluorescent molecule for labelling multiple subcellular structures with organelle-specific photophysical properties has numerous advantages, including lowering cost and experimental complexity while improving the quantification of phenotypes.

Based in our previously described ROS-sensor[1] and cellular pH probe[2], in this communication we describe novel indolium fluorophores for labelling mitochondria and lysosomes with organelle-specific fluorescence properties, which can be readily distinguished by combining confocal and two-photon fluorescence microscopy. Our results show that this unique behaviour relies on aggregation. These probes allow for unambiguous and simultaneous visualisation of mitochondria and lysosomes in separate emission channels by using a single fluorescent marker within living cells.

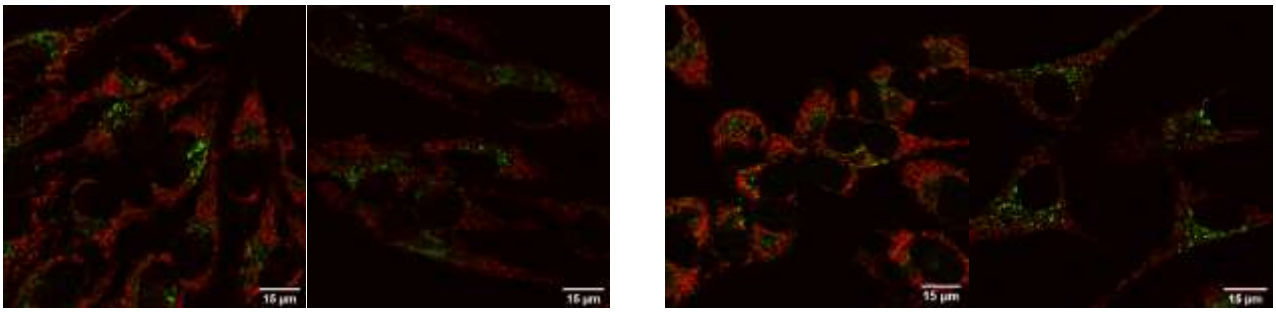


Ind-C



Ind-M

Incubation: 2 hours 24 hours 2 hours 24 hours



HeLa cells. Red channel: Mitochondrias; Green channel: Lysosomes

References:

1. Benitez-Martin, C.; Guadix, J.A.; Pearson, J.R.; Najera, F.; Perez-Pomares, J.M.; Perez-Inestrosa, E.; *Sensors Actuators B Chem.* **2019**, *284*, 744.
2. Benitez-Martin, C.; Guadix, J.A.; Pearson, J.R.; Najera, F.; Perez-Pomares, J.M.; Perez-Inestrosa, E.; *ACS Sensors*, **2020,** *5*, 1068.