**Molecular rotors as tools to study downstream processes in mitochondria upon inhibition of protein-protein interactions**

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Protein-protein interactions (PPIs) play an important role in numerous biological processes and the correct functioning of live cells. Their mis-regulation can often result in the development of disease. Therefore, their inhibition has been the subject of numerous studies and drug discovery campaigns but the quantitative study of this inhibition in relevant biological environments (*in cellulo*) is still limited.

We demonstrate that the inhibition of a PPI between proteins of Bcl-2 family, regulating cell apoptosis, could be monitored by Fluorescence Lifetime Imaging microscopy (FLIM) combined with environmentally sensitive fluorophores termed molecular rotors. Molecular rotors are synthetic fluorescent molecules, in which fluorescence intensity and lifetime strongly depend on the viscosity of their microenvironment.

In this work, we used mitochondria-localised molecular rotors to study the changes in the organisation of the mitochondrial outer membrane (MOM) under different conditions, in different cell lines. Our results have shown that the viscosity within MOM significantly increases with Type-II photodamage and upon starvation. Opposite to these observations, the presence of ABT-737, a well-known inhibitor of above-mentioned PPI, drastically decreases the viscosity, indicating a large perturbation within the MOM structure. These results open up opportunities for direct visualisation of the consequences of PPI inhibition *in live* cells.