Thesis Title
High-content multiplex FRET biosensors to simultaneously monitor mitochondrial functions in cancer cells

3 keywords : **ACRONYM** mitoFRET
cancer / mitochondria / FRET biosensors

Unit/Team of supervising :
IGDR/team « Quantitative Fluorescence Microscopy »

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Socio-economic and scientific context:
This project is proposed by the "Quantitative Fluorescence Microscopy" team. The goal of the team is to develop novel technologies and applied methodologies in fluorescence microscopy to study protein-protien interactions, protein dynamics and catalytic activities in living samples. Giulia Bertolin, recently recruited in the team (CR2 CNRS) is an expert in mitochondria and focus its research in the link between mitochondria and cancer.
The projects benefits from an exceptional environment in terms of equipment – with a fastFLIM prototype microscope developed within the team and co-founded by IBiSA, Rennes Métropole and Région Bretagne, and complementary tools within the team such as multiplex FRET and HCS-FLIM. The project also benefits from the collaboration with the GEO team of Marie-Dominique Galibert, whose expertise in the field of melanoma and the mechanisms of resistance in BRAF mutant melanoma.

Open questions:
Taking into account our current knowledge on mitochondrial physiology, one fundamental question remains unanswered: how are ATP production, mitochondrial turnover and oxidative stress simultaneously regulated, both in physiological and in cancer conditions? To answer this extremely multifaceted issue, multiple FRET biosensors of mitochondrial functions will be used simultaneously. For this project FRET biosensors will be used in conjunction with high-content screening procedures to monitor the global mitochondrial activity, potentially leading to the integration of FRET-based assays to develop innovative strategies of personalised medicine. This innovative approach will be applied to cancer cells of melanoma to underline the contribution of mitochondrial functions to the pathology.

Thesis milestones:
During his/her PhD internship, the selected candidate will develop three main axes:
1. Validation of multiplex FRET approaches applied to mitochondrial biosensors.
We propose to apply our validated multiplex approach to determine how three mitochondrial functions are intertwined: ATP production, ROS production and the NAD+/NADH ratio.
2. Development of new FRET biosensors of mitophagy and ATP production to be used with multiplex approaches.
The aim is to characterize a new FRET biosensor based on the mitophagy marker LC3 relying on the conformational changes of LC3 observed in vitro. A second biosensor we are currently building is based on the ATP5B subunit of complex V of the mitochondrial respiratory chain
3. Adapt the multiplex FRET approaches to high content screening (HCS) methodologies to analyse mitochondrial biosensors in cancer cells.
This screening will be performed in melanoma cancer cells, currently used in the first phases of pharmacological trials. Our screening will be useful to understand the existence of such mitochondrial signatures in a biological context closer to the patient.

Methodological and technical approaches
Development of methods and techniques of quantitative microscopy, multiplex FRET by FLIM in HCS mode.
Development of new FRET biosensors for mitophagy and for ATP production.
Cell biology approaches in cancer cell line paradigm (melanoma) to unveil the existence of mitochondrial signatures in this biological context

Scientific and technical skills required by the candidate
The candidate must have a strong competence in microscopy applied to biology (M2 in biological-health sciences or physics-bio interface), with complementary skills in cell culture and molecular biology. Knowledge of mitochondrial physiology would be a plus.