LUE PhD CAMPAIGN 2019 - Joint PhD with International Partners

Project Title: Developing new optogenetic redox biosensors for sulfur compounds

Acronym: SULSENSE

Workplace: NANCY

Scientific Responsible name: Jérémy Couturier (UMR 1136 IaM, Univ. de Lorraine, France)

Type of Contract: PhD Student contract / Thesis offer

Contract Period: 36 months

Start date of the thesis: 1 October 2019

Proportion of work: Full time

Remuneration: 1 768,55 € gross monthly

Doctoral School: SIReNa

Work context
The thesis will take place with the team "Stress response and redox regulation" of the UMR 1136 Tree Microbe Interactions. This team headed by the Pr. Nicolas Rouhier is composed of around 20 members. During the thesis, the student will have to spend different periods of time in the other laboratories involved in the project (Bruce Morgan (Univ. of Saarland, Germany) and Andreas Meyer (Univ. of Bonn, Germany)).
Team website: http://mycor.nancy.inra.fr/IAM/?page_id=17

Descripition of the thesis topic:
Intracellular concentrations of cysteine are highly controlled to conciliate its potential toxic effect (due to the reactivity of its thiol group) and its role as a major sulfur donor molecule in cells. For instance, cysteine degradation in mitochondria results in the production of 3-mercaptopyruvate (3-MP) and thiosulfate. Developing optogenetic biosensors that allow a better understanding of cysteine metabolism appears necessary. With the experience gained by this consortium on roGFP-based probes and on the biochemical and structural characterization of plant oxidoreductases, we aim at designing new probes, focusing on persulfide-forming proteins, the sulfurtransferases (STRs) notably STR1, STR2, STR16 and STR18 of the model plant *Arabidopsis thaliana*, and a cysteine desulfurase (CD)-STR hybrid protein found in the bacterium *Pseudorhodoferax*. All possess a highly reactive cysteine that is oxidized upon reaction with their substrate, 3-MP, thiosulfate and cysteine, respectively. If the oxidation signal is indeed passed to roGFP2, we could obtain probes specific to these molecules. Basically, we have two main objectives corresponding to two different tasks. The first one is to evaluate *in vitro*, using recombinant proteins, whether the roGFP2 can be selectively oxidized by selected STRs, possessing either one (STR16 and STR18) or two (STR1 and STR2) rhodanese domains and by the selected CD in the presence of their substrate. The same proteins will be fused to the N- or C-terminus of roGFP2. For these fusions, we will keep the backbone used for GSH- and H_2O_2-responsive probes, *i.e.* same linker peptide and optimized

GFP sequences. These constructs will then be used in the second task for examining the changes in the roGFP2 oxidation degree in the presence of their substrates *in vivo*. This will consist in expressing these fusions in yeast cells and in *A. thaliana*. It is important to note that STR1 is located in mitochondria, STR2 and 18 are in the cytosol, and STR16 is in plastids, which can be convenient for their expression in such a physiological context. In this joint project, the three groups located in Nancy, Saarbrücken and Bonn will combine their respective expertise to develop new redox-based fluorescent probes designed to detect changes in 3-mercaptopyruvate, thiosulfate and cysteine, molecules for which there is currently no such sensors.

Application requirements:
Master's degree in biochemistry, plant biology, or microbiology with excellent academic records. You should have as well excellent collaborative skills and strong interests for the studies of redox-dependent modifications of proteins.

Any additional research experience in areas relevant to the research project will be an advantage. This may be practical experience in biochemical, molecular, structural or cellular biology methods... Such additional experience should be demonstrated and included in your application.

Names and contact details of at least two scientists that have offered to act as references for you must also be included, with a clear indication of their address and relationship to you.

Main technical approaches:
- Molecular biology
- Expression and purification of recombinant proteins
- Biochemical characterization
- Functional analyses in yeast and plant
- Possible participation in cell biology studies through collaborations

Keywords: sulfur, biosensors, cysteine metabolism, biochemistry, plant, yeast