**Master 2 internship project**

**Year 2023-2024**

**Laboratory/Institute:** TIMC **Director:** A. MOREAU-GAUDRY

**Team:** TrEE (https://www.timc.fr/TrEE) **Head of the team:** F. PIERREL

**Name and status of the scientist in charge of the project:** Fabien Pierrel, DR CNRS

 **HDR: yes ☒no ☐**

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**Program of the Master’s degree in Biology:**

**☒** Microbiology, Infectious Diseases and Immunology **☐** Structural Biology of Pathogens

**☐** Physiology, Epigenetics, Differentiation, Cancer **☐** Neurosciences and Neurobiology

**Title of the project:**

**Adaptation of *Escherichia coli* to exogenous plastoquinone**

Objectives (up to 3 lines):

Obtain *Escherichia coli* clones with an improved capacity to use plastoquinone, an exogenous quinone of cyanobacterial origin. Reveal the adaptations that underlie the improved phenotypes and characterize the mechanisms involved.

Abstract (up to 10 lines):

Isoprenoid quinones are redox lipids essential to the bioenergetics of most organisms. Indeed, these quinones shuttle electrons between enzymes of respiratory chains which generate the proton gradient used to power ATP synthase. A variety of quinones exist and their biosynthetic pathways are evolutionary related (1). Many bacteria possess several quinones and some of their enzymes are specific for one quinone whereas other enzymes can accommodate several quinones. The reasons for quinone specificity remain obscure and quinone binding sites are often ill defined. We want to approach these issues by analyzing how Escherichia coli may adapt to use plastoquinone (PQ) instead of ubiquinone (UQ), its natural quinone. Through genetic engineering, we have recently succeeded to “replace” UQ with PQ in *E. coli*. The goals of the internship will be to 1) define which quinone-dependent growth phenotypes differentiate best between UQ and PQ, 2) Increase the capacity of *E. coli* cells to utilize PQ, using experimental evolution. 3) Identify the mutations responsible for the evolved phenotypes by whole genome sequencing and reconstruct them in the ancestral strain.

Methods (up to 3 lines):

Molecular biology, microbial cultures under controlled O2 concentrations, western-blot, quinone quantifications by HPLC chromatography coupled to electrochemical and mass spectrometry detections, genome sequencing.

Up to 3 relevant publications of the team:

(1) Abby et al., Advances in bacterial pathways for the biosynthesis of ubiquinone[, BBA Bioenergetics (2020) 1861:148259](https://doi.org/10.1016/j.bbabio.2020.148259)

Requested domains of expertise (up to 5 keywords):

Biochemistry, Genetics, Physiology, Microorganisms, Metabolism