

**Master 2 internship project
Year 2020-2021**

Laboratory/Institute: TIMC-IMAG (CNRS UMR 5525-Université Grenoble Alpes).

Director: Prof. Philippe Cinquin

Team: TRanslational microbial Evolution & Engineering (TREE).

Heads of the team: Prof. Dominique Schneider and Prof. Bertrand Toussaint

Name and status of the scientists in charge of the project: Béatrice Schaack and Corinne Mercier. HDR:

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Program of the Master's degree in Biology:

- Immunology, Microbiology, Infectious Diseases Integrative Structural Biology
 Physiology, Epigenetics, Differentiation, Cancer Neurosciences and Neurobiology
 Planta International

Title of the project: **The Long Term Evolution Experiment (LTEE) in *Escherichia coli*, a tool to investigate the function and the evolution of outer membrane vesicles (OMVs).**

Objectives (up to 3 lines):

1/ to characterize the OMVs produced by the LTEE populations; **2/** to identify the mutations responsible for the changes observed in the evolved populations and **3/** to determine the function(s) of these OMVs produced despite the absence of mammalian cells.

Abstract (up to 10 lines):

Production of OMVs constitutes a privileged process by which Gram negative bacteria deliver key components to distant cells of their mammalian host and control them. Production of OMVs in absence of mammalian cells has been demonstrated. However, whether these OMVs are tools by which bacteria exchange information, independently from the presence of mammalian host cells has never been investigated. The LTEE (R. Lenski, 1988) consists in the daily propagation of 12 independent *Escherichia coli* populations in a minimal medium containing a low glucose concentration, leading to a revivable fossil record of > 70,000 generations. Our preliminary results on one LTEE population have shown that bacterial cells evolved for 50,000 generations (50K) and their ancestor produce OMVs but of different types. The goal is to investigate the potential production of OMVs by the 12 LTEE populations, to characterize these OMVs, to identify the mutations responsible for the changes observed in the evolved populations, and to determine the function(s) of these OMVs.

Methods (up to 3 lines): Bacterial cultures, fluorescence assays, dynamic light scattering, high performance thin layer chromatography, gas chromatography-mass spectrometry, mass spectrometry, microscopy (light, confocal, transmission electron microscopy), genome analyses.

Up to 3 relevant publications of the team:

- Cortes S, Barette C., Beroud R, De Waard M, **Schaack B.** 2018. Functional characterization of cell-free expressed Kv1.3 channel using a voltage-sensitive fluorescent dye. *Protein Expr Purif.* 145: 94-99.
- Renaud S, Cortes S, Bersch B, Henry X, De Waard M, **Schaack B.** 2017. Functional reconstitution of cell-free synthesized purified Kv channels. *Biochim Biophys Acta.* 1859:2373-2380.
- Couce A, Caudwell LV, Feinauer C, Hindré T, Feugeas JP, Weigt M, Lenski RE, **Schneider D**, Tenailon O. 2017. Mutator genomes decay, despite sustained fitness gains, in a long-term experiment with bacteria. *Proc Natl Acad Sci U S A.* 114: E9026-E9035.

Requested domains of expertise (up to 5 keywords):

biochemistry, microbiology, cell biology, molecular biology, statistics