**PhD Project 2019-2022: Biochemical and structural study of [Fe-S]-dependent tRNA modification enzymes involved in genetic translation fidelity**

**PhD thesis director:**

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**Doctoral School: Complexité du Vivant, Sorbonne Universités**

**http://www.ed515.upmc.fr**

Deadline for applying to the doctoral fellowship: June 7th, 2019
Competitive examination: July 3rd-4th, 2019
Delibération: July 5th 2019

**Team :** Molecular and structural enzymology

**Head of Laboratory : FONTECAVE Marc**

**Laboratory:** Chimie des Processus Biologiques. UMR 8229

**Speciality : Structural Biology, Biochemistry**

**Summary of PhD thesis**

Precise decoding of the genetic code is a fundamental process in all living organisms. tRNAs play a critical role in this process by deciphering codon triplets on mRNAs to corresponding amino acids within the ribosome. All tRNA molecules feature post-transcriptional chemical modifications that fine-tune the decoding process at the level of both efficiency and fidelity by stabilizing the tRNA tertiary structure and reduce ribosomal frameshifting (1). In addition, dynamic regulation of tRNA modifications has recently been shown to be critical for cell survival after a variety of stresses (2, 3).
Sulfur, an essential element in life, is present at various positions inside tRNA, in particular at uridine 34 of the anticodon (4, 5). In yeast, the lack of the $s^2$U34 modification results in defects in invasive growth, hypersensitivity to high temperatures, antibiotics or oxidative stress, inability to maintain normal metabolic cycles and protein misfolding and aggregation (6). The synthesis of $s^2$U34-tRNA is catalyzed by specific enzymes called NcsA in archaea, Ctu1 in human and MnmA in bacteria. NcsA belongs to a new enzyme superfamily, the [4Fe-4S]-dependent tRNA thiolation enzymes (7) that uses the [4Fe-4S] cluster as a sulfur carrier, a new function in iron-sulfur enzymology (8).
The PhD project concerns mainly the biochemical and structural characterization of the human Ctu1 enzyme and that of other [Fe-S]-dependent bacterial tRNA thiolases. Biochemical, spectroscopic and biophysical methods, including X-ray crystallography, will be used. *In vivo* studies to test the cellular function of the [Fe-S]–dependent tRNA thiolases and their biosynthetic pathway will be carried out in collaboration with laboratories expert in this field. The candidate should have a good background in protein purification and a strong interest in structural studies. An experience in *in vitro* RNA transcription is advantageous.

**1** Väre et al., *Biomolecules.* 2017, 7, E29. Chemical and Conformational Diversity of Modified Nucleosides Affects tRNA Structure and Function.
**2** Roundtree et al. *Cell*, 2017, 169, 1187-1200. Dynamic RNA Modifications in Gene Expression Regulation.
**3** Gu et al., *FEBS Lett.* 2014, 588, 4287. tRNA modifications regulate translation during cellular stress.
**4** Schaffrath & Leidel. *RNA Biol*. 2017, 14, 1209. Wobble uridine modifications-a reason to live, a reason to die?!
**5** Shigi N. *Front Microbiol*. 2018, 9, 2679. Recent Advances in Our Understanding of the Biosynthesis of Sulfur Modifications in tRNAs.
**6** Cavuzik & Liu Biomolecules 2017, 7, 27. Biosynthesis of Sulfur-Containing tRNA Modifications: A Comparison of Bacterial, Archaeal, and Eukaryotic Pathways.
**7** Bimai et al. submitted. The thiolation of uridine 34 in tRNA, which controls protein translation, depends on a

[4Fe-4S] cluster in the archeum *Methanococcus maripaludis*
**8** Arragain et al., *PNAS*, 2017, 114, 7355-7360. Nonredox thiolation in tRNA occurring via sulfur activation by a [4Fe-4S] cluster.

## Feasibility of PhD project

The laboratory has expertise in the fields of molecular chemistry, inorganic (coordination and organometallic chemistry) and biological chemistry (protein chemistry) (see https://www.college-de-france.fr/site/en-chemistry-of-biological-processes/index.htm). The PhD project belongs to the enzymology and structural biology theme (project 3). The laboratory is equipped with the material needed for the purification (Äkta purifier, Äkta Start and Biorad systems) and crystallization of proteins under aerobic and anaerobic conditions (glove boxes, robots, microscopes; see https://www.college-de-france.fr/site/en-chemistry-of-biological-processes/Protein-Crystallization-Platform.htm), and determination of their three-dimensional structures (Linux stations, graphic stations equipped with 3D vision glasses). We have access to the SOLEIL synchrotron (Saint Aubin) for X-ray diffraction experiments. Potential *in vivo* approaches will be done in collaboration.

**Three recent publications**

"Dynamics of RNA modification by a multi-site-specific tRNA methyltransferase. "
D. Hamdane, A. Guelorget, V. Guérineau, **B. Golinelli-Pimpaneau**.
Nucleic Acids Res. 2014, 42, 11697-706.

"Nonredox thiolation in tRNA occurring via sulfur activation by a [4Fe-4S] cluster. " Arragain S, Bimai O, Legrand P, Caillat S, Ravanat JL, Touati N, Binet L, Atta M, Fontecave M, **Golinelli-Pimpaneau B.** Proc Natl Acad Sci U S A. 2017, 114, 7355-7360

"Dissociation of the dimer of the intrinsically disordered domain of RNase Y upon antibody binding"
Hardouin P., Velours C., Bou Nader C., Assrir N., Laalami S., Putzer H., Durand D, **Golinelli-Pimpaneau B**. Biophysical J. 2018, 115, 2102-2113