Résumé du projet de thèse *(1 page maximum, en anglais)*
Indiquer la participation de chaque co-directeur et structure dans la gestion du projet. Please indicate explicitely the specific contribution of each supervisor to the PhD project.

The proposed PhD project aims to investigate the role of radical S-adenosylmethionine (rSAM) enzymes in anti-phage defense mechanisms and their conservation in eukaryotes. While viperins, a known family of rSAM enzymes, have been demonstrated to synthesize natural anti-phage compounds and anti-viral compounds that could be used as antiviral drugs, this study hypothesizes the existence of additional rSAM enzymes contributing to antiviral defense. The project objectives encompass an extensive search for rSAM enzymes with predicted anti-phage activity in bacterial genomes, followed by experimental characterization of identified candidates. Candidate enzymes will undergo synthesis, cloning, and expression in Escherichia coli for screening of anti-phage activity against a panel of phages. Compound identification will be conducted through liquid chromatography-mass spectrometry (LC-MS) analysis of extracts from engineered E. coli strains. Additionally, purification and in vitro biochemistry studies will be performed for selected proteins. Finally, the conservation of identified rSAM enzyme families in eukaryotes will be assessed through genomic analysis and expression studies in E. coli. Preliminary data indicates the presence of novel rSAM enzyme families with potential anti-phage function, distinct from viperins, suggesting a broader role for rSAM enzymes in antiviral defense mechanisms. This project endeavors to expand our understanding of chemical defense strategies against viral infections and uncover the significance of rSAM enzymes across diverse organisms. If novel anti-phage molecules are identified they could lead to potential novel anti-viral drugs.

Project

Viperins are the first family of rSAM enzymes proven to be involved in the synthesis of natural anti-phage compounds (1)Viperins are conserved across the tree of life and provide anti viral defense not only in bacteria but also in human (2,3). .As such, modified nucleotides produce by viperins across the tree of life also act as anti-viral molecules across a wide range of human viruses (2) We propose that additional rSAM enzymes might be involved in antiviral defense through production of antiphage compounds. The Phd project will identify such enzymes and characterize them in a similar manner as was done for viperins[15]. We will then explore their conservation in diverse organisms across the tree of life.

Project objectives

1. We will perform an extensive search of rSAM enzymes with predicted anti-phage activity in bacterial genomes. Using DefenseFinder (4), we will identify rSAM enzymes present in defense islands, a method used to predict if genes or a group of genes is involved in anti-phage defense. This initial search will uncover interesting families of pVips-like proteins (i.e defensive rSAM). We will follow up with more precise analysis of these families by for example studying their genomic context to lead us to choose candidates to test experimentally. We will characterize the different types of operons ofrSAM in prokaryotic genomes and assess their distribution. (Supervised by Aude Bernheim)
2. For each family, ~5-10 genes will be synthetized and cloned for expression in *E. coli*. We will screen candidates for anti-phage activity by performing viral plaque assays using 12 phages that span major *E. coli* phage families (from our custom and the BASEL collection). Finally, we will identify the compounds produced by enzymes that displayed clear anti- phage activity. Liquid cultures of engineered *E. coli* strains overexpressing defensive enzymes will be used to prepare extracts that contain the compound of interest, and analyzed via LC-MS for product identification. Depending on time, 2-5 of these proteins will be purified as in (1) for in vitro biochemistry studies and follow up mechanistic studies will be performed. (Supervised by Helena Shomar)
3. Using our genomic findings, we will query our custom eukaryotic databases to evaluate the conservation of rSAM enzymes of these novel families across the tree of life. As performed in (3), we will select candidates and express them in E. coli to evaluate if they produce similar molecules as the ones in bacteria (Supervised by Aude Bernheim and Helena Shomar)

Overall, we expect this project to reveal that beyond viperins, rSAM enzymes are a major family of enzymes involved in antiviral defense across the tree of life, and that chemical defense meaning, production of small anti-viral molecules, is a much wider strategy than anticipated. Upon success of identifying novel molecules produces by such enzymes, they could be harnessed to develop novel anti viral drugs.

(1). Bernheim A et al, Prokaryotic viperins produce diverse antiviral molecules. Nature 589, 120-124 (2021)
(2).Gizzi, A. S. *et al.* A naturally occurring antiviral ribonucleotide encoded by the human genome. *Nature* 558, 610–614 (2018).
(3) Shomar H et al Viperin immunity evolved across the tree of life through serial innovations on a conserved scaffold. bioRxiv, 2023.09. 13.557418 (2023)
(4) Tesson F. et al, Systematic and quantitative view of the anti-viral arsenal of prokaryotes Nature Communcations 13:256 (2022)