

# Cell-free synthetic biology platforms for accelerated discovery of modified antimicrobial peptides

## Thesis directors:

### Amir PANDI, HDR

INSERM U1338 (ERL), Laboratory of Computational, Quantitative and Synthetic Biology (CQSB), UMR 7238, CNRS-Sorbonne Université

### Yanyan LI, HDR

Laboratory of Communication Molecules and Adaptation of Microorganisms (MCAM), UMR7245 CNRS/Muséum National d'Histoire Naturelle (MNHN),

## Abstract

The shortage of new antibiotics, combined with rising antimicrobial resistance, has become a critical global health threat of the 21<sup>st</sup> century. Modified peptides, including nonribosomal peptides (NRPs) and ribosomally synthesized and post-translationally modified peptides (RiPPs), represent a vast reservoir of bioactive molecules with antimicrobial potential, yet their discovery and engineering remain limited by slow experimental cycles.

This project aims to develop and optimize lysate-based cell-free transcription-translation systems from both *Escherichia coli* and *Streptomyces venezuelae* as rapid prototyping platforms for the discovery and engineering of novel modified antimicrobial peptides. While *E. coli* cell-free systems are well-established, *Streptomyces* cell-free systems that can provide a native biochemical environment for expressing abundant high GC content biosynthetic gene clusters encoding NRP and RiPP pathways native to actinobacteria, are under-developed.

This PhD project is based on three objectives. First, the doctoral student will develop and optimize an *S. venezuelae* cell-free system using active learning-guided optimization coupled with laboratory automation, similar to previous work that applied this approach to *E. coli* cell-free systems. Second, using both *E. coli* and *Streptomyces* cell-free platforms, the student will reconstitute and characterize NRP and RiPP biosynthetic pathways, focusing on lasso peptides and nonribosomal peptide assembly lines. Third, the student will leverage these platforms for prototyping and identifying novel NRP and RiPP variants with antimicrobial activity.

This interdisciplinary project combines expertise in cell-free synthetic biology (A. PANDI) and in NRP and RiPP chemistry and biosynthesis (Y. LI). The project will uncover rules of combinatorial NRP and RiPP engineering and will deliver optimized cell-free platforms for rapid discovery of novel antimicrobial peptides.