Proposition de projet de doctorat 2024-2027 – ED 406

Analysing interactions between antimicrobial peptides and peptidoglycan by affinity photocrosslinking coupled to MS

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Antimicrobial peptides (AMPs) are a broad class of peptides with promising activity against bacteria or fungi and represent interesting starting points for the development of new therapeutic approaches to treat bacterial infections. Many AMPs act by interacting with bacterial lipid membranes and potentially cell-wall components such as peptidoglycan (PGN), although there are very few direct pieces of evidence of the latter.
We recently isolated DMS-DA6, an AMP showing a specific activity towards Gram-positive bacteria [1]. Gram-negative and Gram-positive cell walls differ greatly in terms of surface glycoconjugates. In the case of Gram-positive bacteria, a very thick layer of PGN and negatively charged lipoteichoic are found in the most outer part of the bacteria. PGN and LTA could act as peptide "sponges", increasing their local concentration, or as peptide "traps", keeping them away from the lipid membrane. We believe DMS-DA6 interacts with PGN but so far, we only have indirect evidence for this interaction.
Our objective is to obtain direct evidence of DMS-DA6/PGN interactions and identify the structural patterns involved in these interactions using affinity photocrosslinking coupled to MS on reconstituted models and live bacteria, and characterise these interactions by calorimetry. The long-term objective is to design new AMPs with optimised sequences.

We will use two complementary affinity photocrosslinking approaches in model systems or live bacteria. First, the peptide will carry a photolabel, that can easily be inserted in the sequence by solid-phase peptide synthesis. With such a design, we aim to identify glycoconjugate partners, based on the expertise we developed for the study of peptide/lipid interactions [2–4]. These peptides will be used in interaction with PGN extracted from bacteria, or directly on live bacteria once the analytical process is well established.
In parallel, we want to develop a new and original approach to study PGN/AMP interactions by metabolically introducing a photoreactive label in the stem peptide of PGN. This approach relies on biochemical engineering of PGN [5], using either modified D-amino acids [6,7] or chemically modified Sortase A substrates [8]. Such approaches have previously been used to introduce molecular handles for post-labeling (alkyne, azide, thiols…) or fluorophores on live bacteria, but to our knowledge, it has never been used to introduce a photoreactive label.

This project should yield valuable information on AMP/PGN interactions. It will give insight on the general mechanism of membrane permeation by AMPs. It should help predict the activity of AMPs on bacterial strains and enable us to rationalise the design of new AMPs with optimised sequences for better bacteria targeting.

This project is at the interface of chemistry and biochemistry and will involve a large set of techniques: peptide synthesis, mass spectrometry, cell culture, model membranes, etc...all available at the host laboratory (LBM).This PhD project will be supervised by Emmanuelle Sachon (HDR) and Astrid Walrant

(HDR in preparation), whose expertise encompass mass spectrometry, affinity photolabelling, membrane active peptides and characterisation of biomolecular interactions.

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