

Jouy-en-Josas, 27 May 2024

Motivation letter of Prix GERLI 2024

Dear Members of Prix GERLI's jury

In this letter, I am honored to present my candidacy for the Prix GERLI 2024 Lipid Science award, based on my research on bacterial lipid adjustments to antibiotics that inhibit the fatty acid synthesis pathway (FASII). I obtained my PhD on 18/12/2023 from the Université Paris-Saclay, conducting research at the Institut Micalis, at INRAE, in Jouy-en-Josas.

My doctoral project addressed a major current concern in medical microbiology, antibiotic resistance. It focused on the renowned pathogen, *Staphylococcus aureus*, which escapes inhibition by a new-generation of antibiotics that target FASII enzymes (called anti-FASII), by incorporating environmental fatty acids (eFAs), which are abundant in animals, to produce its membrane lipids. My results led to the design of drug combinations that work synergistically with anti-FASII to stop *S. aureus* growth. Here are three major discoveries from my doctoral work:

1) We showed that *S. aureus* adapts to anti-FASII by massive protein expression shifts. Importantly, less virulence factors were produced, while stress response functions were increased. Accordingly, anti-FASII treatment led to a delay in infection in an insect model. Importantly, we established that oxidative stress stimulated eFA incorporation, thus establishing a novel connection between oxidation, which is frequent during infection, and eFA assimilation to produce lipid species (published in *iScience*, Wongdontree *et al.*, 2024). This work shows that alteration of bacterial membrane lipids from endogenous to exogenous FAs impacts bacterial virulence and fitness.

2) We discovered that FASII activity is required for *S. aureus* production of lipoteichoic acid (LTA), a key membrane-linked structure involved in cell division, and bacteria-host interactions. Indeed, turning off FASII, such that *S. aureus* relies totally on exogenous FAs, essentially abolishes LTA production, thus changing the membrane lipid balance: FASII turn-off leads to an increase in cardiolipin, and a decrease in diglucosyl-diacylglycerol, the LTA lipid anchor. This discovery shows that LTA is not always required as generally thought, and that FASII activity modulates the balance of lipid species contributing to bacterial adaptation. It also led us to propose and validate a synergistic strategy to inhibit *S. aureus*.

3) We uncovered that the indispensable enzyme in phospholipid synthesis called "PlsX", a reversible enzyme that converts acyl-acyl carrier protein (acyl-ACP) to acyl-PO₄, can be compensated in *S. aureus* by "gain-of-function" mutations in a FASII pathway enzyme (FabF), or in an acyl-CoA thioesterase (FadM). These findings reveal how an "essential" enzyme (PlsX) in lipid synthesis pathway can be compensated by other enzymes belonging to distinct metabolic pathways.

The last two results are in preparation for submission, as shown in Chapter 6 and the Annex of my thesis, respectively. During my thesis, I collaborated on research investigating the role of the FASII regulator FabT in *Streptococcus pyogenes* infection: the manuscript is currently in revision in *Nature Communications*.

It is notable that membrane lipids are relatively little-studied in bacteria for their roles in fitness and virulence. This work opens new perspectives on the roles of bacterial membranes for further studies, for example, in cell division, and in bacterial membrane adaptation during infection. I am now applying for a grant with Dr. Imane El Meouche (Hôpital Bichat, INSERM, Université de Paris Cite) to pursue the role of bacterial membrane proteins and lipids in antibiotic response of *Escherichia coli* in urinary tract infections. This post-doc will give me the opportunity to transmit and apply my studies of lipid biology and techniques towards a new project on medical microbiology. I believe that the fundamental studies carried out during my thesis will be important to apply towards studies of bacterial infection, especially as lipids are highly variable in the host, and may thus have different impacts according to the sites of infection.

Sincerely,

Paprapach WONGDONTREE

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