Fluorescence Lifetime Microscopy as a Tool for Bacterial Cell Envelopes and Antibiotic Susceptibility Testing

Bacterial cells surround themselves with a cell envelope that is fundamentally different to eukaryotic cells and thus is an attractive target for antibiotics. Yet, the rise of antibiotic resistance and is rapidly increasing, while efforts to develop new molecules are slow to pick up. One reason for this is the fact that antibiotic susceptibility testing is based on measuring bacterial growth, which is inherently slow (days to weeks depending on the species) and labor intensive (e.g. plating cells). Access to more rapid testing of antibiotic susceptibility would be beneficial to both screen new drug candidates and administer more efficiently existing drugs in the clinics.

This project aims to overcome this challenge through the use of fluorescence lifetime microscopy (FLIM) in combination with different fluorogenic dyes intercalating to specific compartments of the bacterial cell envelope. This project requires a strong interest in advanced fluorescence microscopy techniques, quantitative data analysis and chemistry.

As a first goal, you will benchmark our approach in *Corynebacterium glutamicum* and *Mycobacterium smegmatis*, both fast-growing, non-pathogenic model organisms for *M. tuberculosis*. You will set up a microfluidic device allowing to expose cells transiently chemical compounds, while imaging them by FLIM. This will allow you to determine the performance of our existing dyes against genetic (e.g. mutants) chemical (e.g. chemical) perturbations. These findings will be used to optimize future reporter dyes (outsourced chemical synthesis), while revealing new aspects on the fundamental characteristics of the mycobacterial cell envelope. Should we be successful, we will expand our approach to different ESKAPE pathogens and validate it in patient derived strains with known antibiotic resistance profiles.

In a second stage of the project, we will aim to screen either chemical compound or genetic mutant libraries to identify new hits disrupting cell envelope biogenesis in a high-thruput manner. This will require a high degree of automation at image acquisition and analysis pipeline.

Further reading:
FLIM: Datta et al. 2020, PicoQuant guide, Park and Gao 2024
For further question please reach out to Andrea Vettiger (andrea.vettiger@unil.ch)