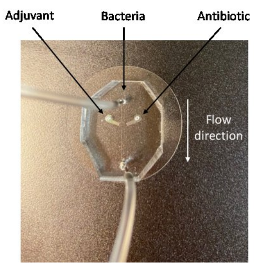
M2 Internship proposal 2024-2025

Laboratory: DISCO Beamline Synchrotron SOLEIL
Duration: 5 months

Subject: Development of a microfluidic system to follow antibiotic accumulation in intact but not growing bacteria under synchrotron DUV light & exploration of the bacterial envelope permeability with second harmonic generation (SHG)

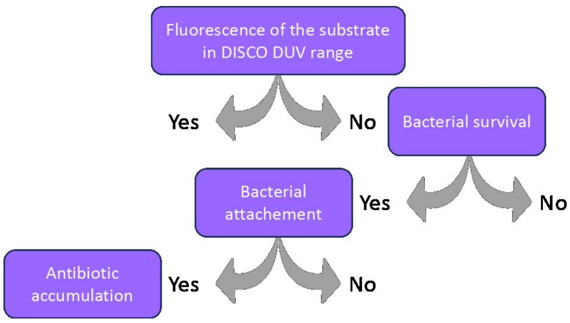
State-of-the-art :
Bacterial drug resistance represents a growing threat to human and animal health worldwide. In Europe, it is responsible for around 25,000 deaths per year; and the medical costs associated with the resulting social costs are estimated at some 1.5 billion euros per year. New forms of resistance are emerging and spreading, leaving clinicians with few options for infection control. At the same time, despite the demand for the development of new antimicrobial compounds, the reality is that only two new classes of antibiotics have been introduced to the market in the last three decades. On the scientific front, there is an urgent need for a better understanding of how antibiotics work, how resistance develops, and the molecular mechanisms that could be exploited to hijack bacterial resistance. The European research program IMI-TRANSLOCATION aimed to better understand antibiotic transport and accumulation, as well as the emergence of multidrug resistance in problematic Gram-negative bacteria of the ESKAPE pathogen group (*Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, *Enterobacter spp.*). Significant results were obtained concerning (i) the translocation of antibiotics through outer membrane porins[1-4], and (ii) the impact of efflux pumps on the accumulation and activity of antibiotics in enterobacteria[1,5-9].

Rational :
For several years, the collaboration between the MCT lab at Aix-Marseille Univ, a recognized expert in the study of bacterial resistance, and the DISCO beamline at SOLEIL, has enabled the development of KMSF (kinetic-micro spectrofluorimetry) to quantify antibiotic accumulation in isolated bacteria[7]. However, this methodology shows two major limitations: (i) the deposition of bacteria between two quartz coverslips does not prevent their mobility in a liquid environment; (ii) the exposure of bacteria to antibiotics at a distance from the microscope does not allow the recording of the fluorescence signal inside bacteria at the very start of the experiment. Consequently, we think that high-resolution DUV imaging with integrated microfluidic devices and cell tracking will provide powerful tools for single-cell bacterial analysis.
A major technical bottleneck with the implementation of single-cell approaches for high-resolution imaging is the immobilization of bacterial cells. Coverslip treatment by adhesive molecules is a potential solution to immobilize bacterial cells and perform microscopy but traditional cationic polymers such as poly-lysine (PLL) deeply affect bacterial physiology.



We have started to prototype an “easy-to-use” microfluidic (MF) device with MF-Lab of SOLEIL based on designs found in the literature (figure)[10]. It is composed of two inlets (for the injection of the antibiotic

or adjuvant), one main channel (for the injection of the bacteria), and one outlet.
Here, the first objective of this internship is to characterize different chemical strategies for their (bio)compatibility when adsorbed to quartz coverslips to immobilize *Escherichia coli* cells.
We identified several commercially available organosilane substrates (i.e., APTES: 3-aminopropyltriethoxysilane, APTMS: 3-aminopropyltrimethoxysilane, GPTS: glycidoxypropyltriethoxysilane, ODMCS: octyldimethylchlorosilane, OTS: octadecyltricholorosilane, and EDS: N-(2-aminoethyl)-3-aminopropyltrimethoxysilane) as well as trademark reagents (i.e. Vectabond®, CellTack™, and Polysin™) that could be used for coating quartz coverslips[11-14].
Typically, PDMS chips will be prepared at the MF-Lab of SOLEIL. Quartz coverslips will be coated with the different substrates and PDMS chips will be sealed on quartz coverslips by oxygen plasma treatment.
First, the putative fluorescence of the substrates will be tested in the UV range used at DISCO. Then, bacterial survival and attachment will be assayed. Bacteria will be imaged immediately after immobilization for 30 min. Cell death will be assayed by using propidium iodide nucleic acid stain to mark dead cells and a conventional fluorescence microscope. Brightfield and fluorescence images will be taken of the same regions to calculate the percentage of dead cells. Tracking methods will be developed on the DISCO beamline to investigate the transition to irreversible attachment (different from flipping, rotating, or detaching bacteria) for bacteria deposited during liquid flow (i.e., rapid rinsing flow and slow working flow) onto the different substrates. We suspect that the degree of flagellar-driven movement of bacteria on the surface plays an important role on the rate of deposition. With this, we hope to identify a substrate that will improve the bacterial deposition rate, thereby increasing the likelihood that an initially moving bacterium will become immobilized.
Accumulation assays will be performed with commonly used *E. coli* strains AG100 (wild-type), AG100A (drug efflux-defective), and ciprofloxacin as a well-characterized antibiotic of the fluoroquinolone family (see flowchart below).



Antibacterial drugs are small molecules. To understand the reasons behind their successes and failures, it is necessary to understand their initial interaction with the bacterial envelope responsible for regulating small-molecule uptake. The envelope of each bacterial species, however, is composed of a unique combination of proteins and phospholipids that can significantly alter its interaction with small molecules. In particular, Gram-negative bacteria are characterized by a highly impermeable outer membrane composed of lipopolysaccharide and outer membrane proteins (i.e., porins), which arrange channels for the diffusion of small hydrophilic compounds. Here, the second objective of this internship is to explore second harmonic generation (SHG) simultaneously with two-photon fluorescence (TPF) to examine differences between Gram-positive and Gram-negative bacteria[15-18]. With the combination of these methods, we will have access to the kinetics of the initial molecule-membrane interaction and subsequent transport processes, thus providing an avenue to describe the underlying mechanisms of action. We will use the multiphoton confocal Nikon A1 MP+ microscope available at SOLEIL. As a first step to further developing this approach, we will use malachite green as an SHG-active reference molecule, strains of Gram-positive (i.e., *Streptococcus pneumoniae* and *Staphylococcus aureus*) and Gram-negative (i.e., *E. coli* and *Pseudomonas aeruginosa*). We expect that initial adsorption to the outer leaflet of the bacterial envelope will cause an increase in the SHG signal, whereas molecules that have flipped into the inner leaflet will generate SHG with the opposite phase, leading to destructive interference of the detected signal over time. As such, one and two sequential rise and decay of the signal with Gram-positive and Gram-negative bacteria, respectively. Competition assays with permeating antibiotics could also be experienced.

References:

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Mission:

- Microfluidics under DUV light: characterize different chemical strategies for their (bio)compatibility when adsorbed to quartz coverslips to immobilize bacterial cells; perform antibiotic accumulation assays and compare data with the previous setup ("quartz coverslip sandwich").
- SHG/TPF: examine membrane permeability of Gram-positive and Gram-negative bacteria using malachite green as an SHG active molecule; perform competition assays with antibiotics.

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Deadline for application: 01/12/2024

PhD possibility: Yes (Contrat de formation doctorale SOLEIL ; Concours de l'ED569 Innovation Thérapeutique : du fondamental à l'appliqué)