Antibiotics conjugated small tag for bacterial labeling and monitoring of drug uptake/efflux

K. Jantarug[1], V. Tripathi[2], B. Morin[3], A. Iizuka[3], D. Bumann[2], N. Khanna[3], P. R. Fuentes[1]*

[1]Department of Chemistry, University of Zurich, Winterthurerstrasse 190, 8057 Zurich, Switzerland, [2]Biozentrum, University of Basel, Spitalstrasse 41, 4056 Basel, Switzerland, [3] Department of Biomedicine, University of Basel, Hebelstrasse 20, 4031 Basel, Switzerland

The NCCR AntiResist is developing novel *in vitro* conditions mimicking patient samples for drug screening and development. However, to create efficient patient-like conditions, the physiological properties of single-cell bacteria need to be studied. To detect bacteria in patient samples and study the drug uptake and efflux in bacteria, we functionalized antibiotics of interest to have a small tag, for example, a relatively small fluorophore or a Raman tag. These small tags would allow us to visualize bacterial infections in patient samples and monitor the uptake and efflux of antibiotics of interest with decreased interference in cell permeability, chemical and biological properties. As a first example, we modified the antibiotic vancomycin with a silicon-rhodamine-based fluorophore, Janelia Fluor 669 (JF669), to label *Staphylococcus aureus (S. aureus)* in patient samples. Using this probe (VanJF699), we could detect *S. aureus* in human cells with low background, minimal non-specific binding, and using a suitable wavelength for human tissue imaging. Additionally, VanJF669 could be used for super-resolution bacterial imaging. In a second example, we explored even smaller tags to minimize the interference of the tag with antibiotic trafficking. Raman tags were utilized instead of small fluorophores. Alkyne and azide moieties could exhibit Raman signals around 2100 cm^{-1}, which are in a silent region of human cells. We modified trimethoprim to have an alkyne or azide that would allow us to monitor the efflux and uptake by confocal Raman microscopy. Regarding these small tag-conjugating antibiotics, *E. coli* treated with trimethoprim-alkyne can be imaged by Stimulated Raman scattering (SRS) microscopy showing Raman signal around 2143 cm^{-1}. In the future, we will use our probes to understand the physiological properties of bacteria at the single-cell level and accelerate the development of patient-like conditions.

Samir Gautam et al, *Trends* in *Biotechnology,* **2013**, 31, 258-67.
Jonathan B. Grimm et al, *Nature Methods,* **2020**, 17, 815–821.
Lu Wei et al, *Nature Methods,* **2014**, 11, 410–412.
Wanida Phetsang et al, *ACS Infectious Diseases,* **2016**, 2, 688-701.