Imaging and biosensor strategies in drug discovery for Vector-Borne Diseases

Friday, 26th September 2025 14:00-17:00 (CET)

The event will be on Teams platform

WG2/WG3 Workshop of the COST Action CA21111
One Health drugs against parasitic vector-borne diseases in Europe and beyond
OneHealthdrugs

Program and abstracts

Workshop

„ Imaging and biosensor strategies in drug discovery for Vector-Borne Diseases ”

26th of September 2025
(online event)

Chairs: Theodora Calogeropoulou (WG2 leader) and Guy Caljon (WG3 leader)

Time (CET)	Topic/Presenter
14:00-14:10	Welcome Theodora Calogeropoulou (National Hellenic Research Foundation, Greece) Guy Caljon (University of Antwerp, Belgium)
14:10-14:20	Update on OneHealth*drugs* COST Action Maria Paola Costi (University of Modena and Reggio Emilia, Italy)
14:20-15:00	“Investigating *Leishmania* proton pumps as possible drug targets” Eva Gluenz (University of Bern, Switzerland)
15:00-15:40	“From bioinspiration to functional fluorescent molecular tools for parasite cell imaging” Mourad Elhabiri (CNRS-Unistra-UHA, Strasbourg, France)
15:40-16:00	“Design and Synthesis of Fluorescent Probes for the direct InhA inhibitor NITD-529” Konstanina Stavropoulou (National and Kapodistrian University of Athens, Greece)
16:00-16:20	“Subcellular nano-chemical characterization in photothermal spectroscopic imaging of antimicrobial interaction in model system *Bacillus subtilis* & vancomycin” Maryam Ali (University Jena, Jena, Germany)
16:20-16:30	Closing remarks

Investigating *Leishmania* proton pumps as possible drug targets

Alagöz, C [a], Alburquerque-Wendt A. [a,b,c], Gluenz, E [a]

[a] University of Bern, Switzerland; [b] Swiss Tropical and Public Health Institute, Switzerland, [c] Charles University, Czech Republic
eva.gluenz@unibe.ch

The environments in which *Leishmania* reside in the course of their life cycle vary significantly. The sand fly alimentary tract is a changeable environment with fluctuating temperature and nutrient availability; constant higher temperature and acidic conditions dominate in the mammalian phagolysosome. This offers the parasite reliable cues to modulate proliferation and metabolism; this also necessitates mechanisms to maintain organelle physiology including regulation of ion fluxes across membranes. We took an unbiased, gene-centered approach to identify the most vital ones for normal fitness, by screening CRISPR knockout cell lines in different life cycle stages. These screens identified a P-type H^+ ATPase important for survival in the mammalian host and the vacuolar-type H+ ATPase (V-ATPase; a conserved multi-subunit proton pump), as a key factor in mediating the tolerance to different stress conditions, including tolerance of low external pH [1]. Further characterization of the V-ATPase gene deletion mutants found them capable of near-normal proliferation in rich cell culture media but highly sensitive to low external pH, elevated temperature and exposure to hypoosmotic media, and moderately sensitive to alkaline pH and starvation. Using a combination of gene deletion and tagging methods and live-cell imaging, we found that exposure to these conditions leads to the formation of distended vacuolar structures, which contain molecular markers of autolysosomes: cysteine peptidase A and Atg8. We are currently using genetically encoded biosensors to measure organellar pH in wild-type *Leishmania* and deletion mutants to understand how loss of the V-ATPase and P-Type H^+ ATPase impact on the physiology of different parasite organelles. Treatment of *Leishmania* with the V-ATPase inhibitor Bafilomycin A phenocopies the V-ATPase deletion, indicating that pharmacological inhibition of the V-ATPase could offer a route to eliminating *Leishmania* from host cells. The challenge will be to identify compounds specific to the parasite V-ATPase.

References

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Acknowledgment

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From bioinspiration to functional fluorescent molecular tools for parasite cell imaging

Davioud-Charvet E. and **Elhabiri M.**

[a] Laboratoire d'Innovation Moléculaire et Applications, UMR 7042, CNRS-Unistra-UHA, ECPM, 25 Rue Becquerel, 67200 Strasbourg (France)
elhabiri@unistra.fr

Our team's research efforts are focused on developing anti-malarial drug candidates (i.e., benzyl-menadione) that act as prodrugs with efficient redox-cycling activity upon bioactivation.[1] The next milestone in their development is to elucidate their mechanism of action and identify the main biological targets. Although (pro-)ABPP approaches have been successfully developed in these recent years,[2] we have also chosen to develop lab-made fluorescent molecular tools for 'click & fish' approaches, transport and cellular localization tracking, and measuring pH or redox state alterations within parasitized red blood cells. This presentation will therefore focus on the development of these fluorescent molecular tools, from engineering to investigating their physico-chemical and photophysical properties and reactivity (e.g., ratiometric pH probes,[3] redox-sensitive pro-fluorophores or azide derivatives for bioorthogonal chemistry)[4], and their applications in cellular imaging (e.g., parasitized red blood cells and BY-2 tobacco cells used as a model). We will also discuss the Trojan horse approach used to vectorize chloroquine analogues functionalized with a fluorescent probe. These serve as a relevant model for discriminating between chloroquine-sensitive and chloroquine-resistant strains,[5] as well as for evaluating transport across the parasitophore vacuolar membrane.[6] Finally, we will focus on valuable derivatives of our antimalarial compounds. When subtly modified, they demonstrate valuable emission properties and protein alkylation properties,[7] paving the way for a new strategy for identifying biological targets.

References

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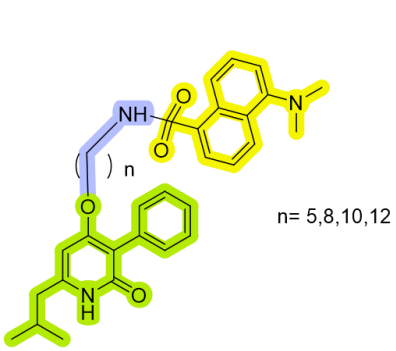
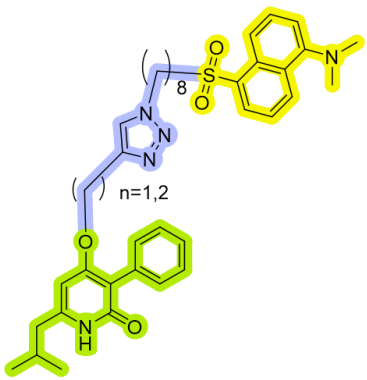
Design and Synthesis of Fluorescent Probes for the direct InhA inhibitor NITD-529

Stavropoulou K., Chorianopoulou A., Kolocouris A., Papanastasiou I.[a]
[a] National and Kapodistrian University of Athens, Greece
konstavro@pharm.uoa.gr

Tuberculosis (TB), caused by Mycobacterium tuberculosis (Mtb), remains a persistent global health challenge, exacerbated by the rise of multidrug-resistant (MDR) and extensively drug-resistant (XDR) strains. To address this challenge, a new class of drugs have been developed—**direct InhA inhibitors, such as 4-hydroxy-2-pyridone analogues**. Unlike isoniazid, these compounds do not require enzymatic activation, making them effective even against KatG-mutant strains, which are responsible for the mycobacterial resistance.[1]

Herein, we present the rational design and synthesis of novel fluorescent NITD-529 derivatives to investigate drug–target interactions. We synthesized a series of NITD-529 analogues with varying chain lengths, conjugated with dansyl chloride as a fluorophore, due to its high quantum yield and selective reactivity with primary amines[2]. Molecular docking studies revealed that longer linkers enhance the proper orientation of the probe within InhA's binding pocket and facilitate cavity exit of the fluorescent probe. The resulting fluorescent product will be applied in TB drug research and live-cell imaging of drug–enzyme dynamics.





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Subcellular nano-chemical characterization in photothermal spectroscopic imaging of antimicrobial interaction in model system *Bacillus subtilis* & vancomycin

Authors: Maryam Ali,[a,b] Robin Schneider,[b,c] Sebastian Unger, [a,b] Astrid Tannert,[b] Rainer Heintzmann, [a,b] Anne-Dorothea Müller,[e] Christoph Krafft, [a,b] Ute Neugebauer, [a,b,d] Daniela Täuber, [a,b]

[a] *Friedrich Schiller University Jena, Jena, Germany.* [b] *Leibniz Institute of Photonic Technology, Jena, Germany.* [c] *Aalen University of Applied Sciences, Aalen, Germany.* [d] *Jena University Hospital, Jena, Germany.* [e] *Anfatec Instruments AG, Oelsnitz, Germany.*
Email: maryam.ali@uni-jena.de

Spectroscopic imaging using mid-infrared photo-induced force microscopy (PiF-IR) provides nanoscale access to the chemical characterization of the surface of microbes[1,2] and tissue sections[3] with spatial resolution of ≈ 5nm. We applied PiF-IR to *Bacillus subtilis* (*B. subtilis*), a well-known model system for antimicrobial interaction. We studied *B. subtilis* with and without treatment with vancomycin. High-resolution images of the cell wall surface obtained as RGB merges of succeeding PiF-IR scans at illuminations in glycan (pink) and amide (green) bands show local variations of the chemical composition; see Figure 1. A chemometric analysis of PiF-IR spectra from treated and untreated *B. subtilis* allowed to identify spectral shifts resulting from the formation of hydrogen bonds between vancomycin and D-alanyl-D-alanine and to localize these bonds in hyperspectral PiF images. This work demonstrates the potential of PiF-IR to localize targeted drug delivery in VBD and to visualize chemical interaction of drugs on a scale of few nm.

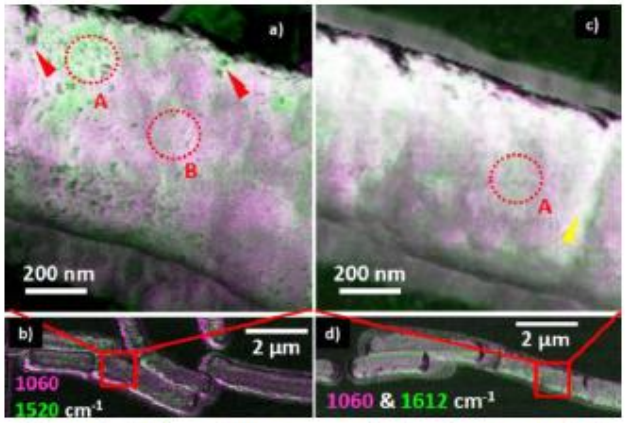

***Figure 1**: High-resolution images (a,c) and overviews (b,d) showing chemical contrasts in glycan (pink) and peptide (green) of treated (a,b) and untreated (c,d) B. subtilis cells. A) loosely and B) densely organized glycan strands. Red and yellow arrows: possibly developing septa or depressions and piecrust at a forming septum, respectively [reproduced from Ali et al. arXiv:2505.10249 under CC BY 4.0 license[2]].*

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