VECTORIZATION AND INNOVATION AGAINST PATHOGENS RESISTANCE

SUMMER SCHOOL OF ITI Innovec

Innovating vectorization of biomolecules | InnoVec

The **Interdisciplinary thematic** institutes of the **University** of Strasbourg & cnrs & **Inserm**

funded under the **Excellence Initiative** program

5-6 September 2022
Forum Faculté de Médecine
4, rue Kirchleger
STRASBOURG

PROGRAM

	5th September 2022	6th September 2022
9.00-9.10	Introduction	Introduction
	CHAIR : Dr. Olivier Cunrath	**CHAIR** : Prof Michaël Ryckelynck
9.10-10.00	**The global challenge of antibioresistance** Pr. Patrick Plésiat (CNR Besançon, F)	**Nucleic acid conjugates for biomedical applications** Pr. Philippe Barthelemy (U. Bordeaux, F)
10.00-10.50	**Enhancing the delivery of antimicrobial agents into biofilms through lasers and nanoparticles** Pr. Kevin Braeckmans (U. Gand, B)	**Antisense therapy against bacteria** Pr. Nuno Azevedo (U. Porto, P)
10.50-11.20	Coffee Break	Coffee Break
11.20-11.40	**Vectorization of Iridium(III) Complexes using Siderophore Surrogates: a Trojan Horse Strategy against Gram-Negative Pathogenic Bacteria** Aline Faucon (CNRS, U. Strasbourg, F)	**Selection of enzyme inhibiting aptamers by ultrahigh-throughput microfluidic screening** Claire Husser (CNRS, U. Strasbourg, F)
11.40-12.00	**Design of new pyoverdine analogues using synthetic biology** Hélène Puja (CNRS, U. Strasbourg, F)	**A novel inhibitor of GcpE, a target for the development of antimicrobials** Cléa Witjaksono (CNRS, U. Strasbourg, F)
12.00-12.20	**Iron acquisition *in Pseudomonas aeruginosa*: mathematical modelisation of phenotypic switches** Thibaut Hubert (CNRS, U. Strasbourg, F)	**Rationally modifying Neomycin, an antibiotic from the aminoglycosides family** Julia Revillo Imbernon (CNRS, U. Strasbourg, F)
12.20-13.30	Lunch and Poster Session	Lunch and Poster session
	CHAIR : Dr. Gaëtan Mislin	**CHAIR** : Dr. Gilles Prévost
13.30-14.20	**Ultrasound-activated microbubbles to treat bacterial infections** Pr. Klazina Kooiman (Erasmus MC, NL)	**Antivirulence drugs and new generation antibiotics, two examples of strategies against microbial resistance**

		Dr. Nicolas Levy (Mutabilis, F)
14.20- 15.10	**Inspired by nature´s design: Biomimetic enterobactin analogues for antimicrobial drug conjugates** Pr. Philipp Klahn (U. Göteborg, SW)	**Novel antimicrobials by innovative mining of natural products and development of antibiotic hybrids** Dr Lamya El Mortaji (Deinove, F)
15.10- 16.00	Coffee Break/poster Session	Coffee Break/poster Session
16.00- 17.30	Round Table : **Intellectual property** Presentation of CEIPI, Pr. Jean-Marc Deltorn, TTO Conectus Invited plenary speakers : guided visit (in English) of cathedral and surrounding historical buildings	Round Table : **Innovation** FabLab/Start up/ TTO Conectus
17.30	Closing remarks	Closing remarks and prizes giving

PLENARY LECTURES

THE GLOBAL CHALLENGE OF ANTIBIORESISTANCE

Patrick Plésiat

Faculté de Médecine-Pharmacie, 19 rue Ambroise Paré, 25030 Besançon

The discovery of antibiotics has been a tremendous milestone in the history of modern medicine. Since the mid 1940s, these “miraculous drugs” have saved million people suffering from severe bacterial infections. During the golden age of antibiotics (1940s-1980s), thousands of natural, hemisynthetic, and synthetic molecules have been found to have antimicrobial properties *in vitro*. However, only a few of these that showed a low toxicity level for Man and acceptable pharmacokinetic-pharmacodynamic parameters proved to be useful for the clinical practice. They are grouped in families according to their structure and mode of action.

Antibiotics differ from antiseptics by their ability to bind to and then inhibit specific bacterial targets, that are not present in eucaryotic cells. When important physiological functions are impaired, bacteria stop growing, and finally die if strongly hit. Because these microorganisms are able to divide much more rapidly than human cells, and are haploids, they stochastically accumulate mutations during their growth. Some of these mutations may provide individual cells (mutants) with the capacity to survive harsh conditions (exposure to biocides), and colonize new environments (hospital, new hosts). Bacteria can also acquire new functions and phenotypes thanks to interspecies sexual and asexual genetic transfers involving mobile genetic elements such as plasmids, transposons, and ICE. Multiple strategies have been developed by bacterial strains to counter the action of industrial antibiotics, preventing these molecules from interacting with their cellular targets. Some mechanisms of resistance are more common and more efficacious than others, e.g. the enzymatic alteration of drug molecules, the enzymatic or mutational modification of antibiotic targets, (iii) the impairment of drug penetration, and (iv) increase of drug extrusion via active efflux systems. When individual strains accumulate several resistance-related mutations and/or transferable resistance genes, they become refractory to most of the drugs available commercially to treat patients. The emergence and diffusion of such multidrug resistant (mdr) bugs in the community or in the hospital is a global health concern, as these latter cause therapeutic failures (relapses or deaths), with a concomitant increase in the length of hospital stays, and treatment costs. A short list of nosocomial bacteria that form the ESKAPE group, has concentrated most of the problems linked to antibioresistance worldwide, namely *Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Enterobacter cloacae*. Within these species, so-called “high-risk clones” have emerged these past decades. These epidemic strains are able to diffuse rapidly between humans, or between animals and humans, are adapted to the hospital environment, and can collect multiple resistance genes from various animal or environmental reservoirs. Some have become untreatable because of their panresistance to old and new antibiotics.

Because the bacterial structures that can be used to develop new therapeutic agents are few, and are already targeted by natural antibiotics exploited by Man, innovation in the field is difficult. The antibiotic crisis started in the 1990s when the pipeline of new molecules became dry while antibioresistance was still growing. Of all the new strategies attempted by pharmaceutical companies (e.g., combinatorial chemistry, multiomics approaches, drug design), none has resulted in innovative drugs to combat mdr superbugs. The last agents reaching the market are optimizations of older antibiotic molecules. To control antibioresistance at the global scale (a no-frontiers challenge), the overuse and misuse of antibiotics must be tackled first in both the community and the hospital sectors. Academic research is crucial to find out novel strategies against human and veterinary pathogens, that not necessarily involve antimicrobials, but may rely on the administration of drugs able to inhibit bacterial virulence or the formation of persistent biofilms on indwelling materials.

References

[1] ARC group. The Lancet, **2022**, https://doi.org/10.1016/S0140-6736(21)02724-0
[2] Cassini A *et al.* The Lancet, **2018**, http://dx.doi.org/10.1016/S1473-3099(18)30605-4
[3] GLASS report **2021**. https://apps.who.int/iris/bitstream/handle/10665/341666/9789240027336-eng.pdf
[4] ECDC report **2020**. https://www.ecdc.europa.eu/en/publications-data/
[5] Karakonstantis S. *et al.* J Antimicrob Chemother, **2020**, 271-282
[6] Tacconelli E. *et al.* The Lancet, **2018**, 18, 318-317
[7] Prasad N. *et al.* Antimicrob Agents Chemother, **2022**, 66, 1-18
[8] Silver L. *et al*. Clin Microbiol Rev, **2011**, 24, 71-109
[9] Barczak A. Curr Op Microbiol, **2009**, 12, 490-496
[10] Yang L. *et al*. FEMS Immunol Med Microbiol, **2012**, 65, 146-157.

ENHANCING THE DELIVERY OF ANTIMICROBIAL AGENTS INTO BIOFILMS THROUGH LASERS AND NANOPARTICLES

Kevin Braeckmans

Biophotonics Research Group
Lab. General Biochemistry & Physical Pharmacy
Ghent University
Ottergemsesteenweg 460, 9000 Gent, Belgium
Kevin.Braeckmans@UGent.be

One of the reasons for bacterial tolerance to antimicrobial agents is hindered penetration through biofilms. We tried to improve the delivery of antimicrobial agents into biofilms by a combination of laser irradiation and photothermal nanoparticles. When laser light is delivered as a brief but intense pulse, the photothermal nanoparticles become extremely hot, leading to the generation of water vapour nanobubbles when they are present in hydrated tissues, like biofilms. The bubbles will expand up to several hundred nm until the thermal energy from the particles is consumed, after which the bubbles violently collapse. The bubble implosion generates high-pressure shock waves which can cause mechanical damage to the surrounding structures.[1] We evaluated if laser-induced vapour nanobubbles can interfere with biofilm integrity and reduce the diffusion barrier to antimicrobial agents. The concept was explored on gram negative (Burkholderia multivorans LMG18825; Pseudomonas aeruginosa LESB58) and gram positive (Staphilococcus areus Mu50) biofilms. In line with previous studies of ours on nanoparticle diffusion in biofilms[2,3] we found that 70 nm gold nanoparticles can penetrate deep into cell clusters. Subsequent treatment with 7 ns pulsed laser light caused vapour nanobubbles to be formed inside biofilms, as confirmed with dark field microscopy. The laser induced vapour nanobubbles induced structural changes into the biofilm. Thanks to a better penetration of tobramycin deep into the cell clusters, its potency could be increased by up to 3 orders of magnitude.[4] Following these encouraging findings, we investigated whether vapour nanobubbles can enhance the efficacy of a broad range of commercially available antimicrobials used for treating wound infections. Only in a limited number of cases we found a potentiating effect, namely for benzalkonium chloride (~ 21x) in P. aeruginosa biofilms, and cetrimonium bromide (~ 24x) and mupirocin (~ 53x) in S. aureus biofilms.[5] We could show that the lack of improvement for the other tested antimicrobial – biofilm combinations can be attributed to the absence of a diffusion barrier in those cases.
Taken together we find a substantial synergistic effect between VNB formation inside biofilms and tobramycin treatment of gram positive and gram negative biofilms. Future work should focus on evaluating this novel concept in more relevant biofilm model systems and eventually in vivo.
Text[1ref]

References

[1] R. Xiong, S.K. Samal, …, K. Braeckmans. *Adv. Phys.,* **2016**, 1, 596-620.
[2] K. Forier, …, K. Braeckmans. *Nanomedicine*, 8, **2013**, 8, 935-949.
[3] K. Forier, …, K. Braeckmans. *J. Control. Release*, **2014**, 195, 21-28.
[4] E. Teirlinck, …, K. Braeckmans. *Nat. Comm.*, **2018**, 9, Art. Nr. 4518.
[5] E. Teirlinck, …, K. Braeckmans. *Biofilm*, **2019**, 1, Art. Nr. 100004.

ULTRASOUND-ACTIVATED MICROBUBBLES TO TREAT BACTERIAL INFECTIONS

Klazina Kooiman[1], Inés Beekers[1], Robert Beurskens[1], Tammy Gonzalez[2], Andrew B. Herr[2], Christy K. Holland[3], Nico de Jong[1,4], Alexander L. Klibanov[5], Joop J.P. Kouijzer[1], Simone A.G. Langeveld[1], Kirby R. Lattwein[1], Mariël Leon-Grooters[1], Frits Mastik[1], Gaëtan L.A. Mislin[6], Estefania Oliva[7], Antonius F.W. van der Steen[1], Tom van Rooij[1], Willem J.B. van Wamel[8], Himanshu Shekhar[3], Jean-Marc Strub[9], Martin D. Verweij[1,4]

[1]Biomedical Engineering, Dept. Cardiology, Erasmus MC University Medical Center Rotterdam, Rotterdam, the Netherlands

[2]Cincinnati Children's Hospital Medical Center, Division of Immunobiology, Center for Systems Immunology, and Division of Infectious Diseases, Cincinnati, Ohio, USA

[3]Department of Internal Medicine, Division of Cardiovascular Health and Disease, University of Cincinnati, Cincinnati, Ohio, USA

[4] Laboratory of Medical Imaging, Department of Imaging Physics, Delft University of Technology, Delft, the Netherlands

[5]Cardiovascular Division, Department of Medicine, University of Virginia, Charlottesville, USA

[6]CNRS/University of Strasbourg UMR7242, Illkirch-Graffenstaden, France

[7]Faculté de Pharmacie de Strasbourg, Plateforme d'Analyse Chimique de Strasbourg-Illkirch (PACSI), Illkirch-Graffenstaden, France

[8]Department of Medical Microbiology and Infectious Diseases, Erasmus MC, Rotterdam, the Netherlands

[9]CNRS/University of Strasbourg UMR 7178, Institut Pluridisciplinaire Hubert Curien, Laboratoire de Spectrométrie de Masse Bio-Organique, Strasbourg-Cedex 2, France

In the clinic, ultrasound is being used to diagnose bacterial infections such as infective endocarditis, a biofilm infection on the heart valves and/or cardiac devices, for example a pacemaker. Treating bacteria and bacterial biofilms using ultrasound and microbubbles (1-10 μm in size), which we have termed *sonobactericide*, has shown preclinical potential to eradicate bacteria and biofilms[1]. The ultrasound activates the microbubbles so they will vibrate with the frequency of the ultrasound[2]. For example, microbubbles vibrate two million times per second in a 2 MHz ultrasound field, an ultrasound frequency used clinically for ultrasound imaging of the heart. In this talk, I will cover the background of the ultrasound-activated microbubbles as well as four different types of microbubbles being used for sonobactericide: 1) non-targeted microbubbles either administered with or without a drug; 2) drug-loaded microbubbles; 3) targeted microbubbles; and 4) droplets which upon an ultrasound stimulus become microbubbles. The focus is on the research of the past three years.

References

[1] K.R. Lattwein, H. Shekhar, J.J.P. Kouijzer, W.J.B. van Wamel, C.K. Holland, K. Kooiman, Sonobactericide: An Emerging Treatment Strategy for Bacterial Infections, *Ultrasound in Medicine and Biology*, **2020**, 46, 193-215.

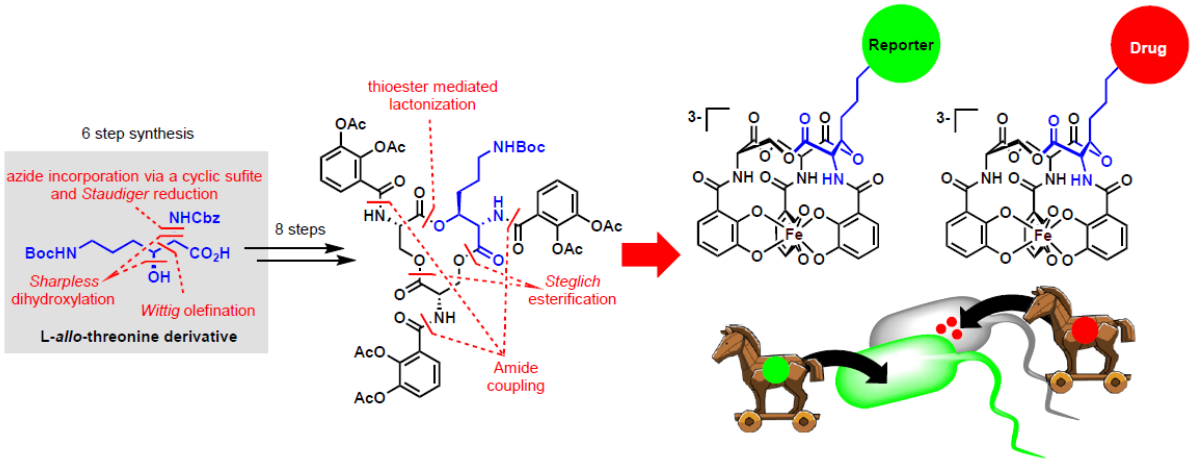
[2] K. Kooiman, S. Roovers, S.A.G. Langeveld, R.T. Kleven, H. Dewitte, M.A. O'Reilly, J.M. Escoffre, A. Bouakaz, M.D. Verweij, K. Hynynen, I. Lentacker, E. Stride, C.K. Holland, Ultrasound-Responsive Cavitation Nuclei for Therapy and Drug Delivery, *Ultrasound in Medicine and Biology*, **2020**, 46, 1296-1325.

INSPIRED BY NATURE'S DESIGN: BIOMIMETIC ENTEROBACTIN ANALOGUES FOR ANTIMICROBIAL DRUG CONJUGATES

Philipp Klahn

Department of Chemistry and Molecular Biology, Division of Organic and Medicinal Chemistry, Kemigården 4, Room 8020, 41 296 Göteborg, Sweden, philipp.klahn@chem.gu.se

In the context of growing resistance of bacteria against established antibiotics, the development of novel antimicrobial drugs and concepts against bacterial pathogens is of vast importance.[1-3] In particular, Gram-negative pathogens provide big challenges in drug development due to the effective permeation barrier of the Gram-negative cell envelope. A smart concept for the development of novel antimicrobials is based on the conjugation of antimicrobial drugs with siderophores, small molecule iron(III)chealtors as carrier molecules showing an active uptake mechanism for translocation over the cell envelope barrier.[1] In this context, analogues of enterobactin, a *tris*-catecholate siderophore, playing an important role during host infection are of high interest.[3]



In our approach,[4] we utilize a γ-modified L-allo threonine derivative as building block for the synthesis of biomimetic enterobactin analogues, showing an attachment point for drug or reporter molecule conjugation in the backbone of the siderophore and simultaneously retain the hydrolyzability of the *tris*-lactone structure, important to enter the cytosol. Based on this approach we synthesized novel siderophore-fluororophore conjugates and evaluated their siderophore-receptor mediated uptake into bacteria via different methods. In addition, first drug conjugates are reported and evaluated for their antibacterial activity.[5,6]

References

[1] P. Klahn, M. Brönstrup, *Nat. Prod. Rep.,* **2017**, *34*, 832–885.
[2] P. Klahn, M. Brönstrup, *Curr. Top. Microbiol. Immunol.,* **2016**, *389*, 365–417.
[3] P. Klahn, R. Zscherp, C. C. Jimidar, *Synthesis,* **2022**, 10.1055/a-1783-0751.
[4] R. Zscherp, J. Coetzee, J. Vornweg, J. Grunenberg, J. Herrmann, R. Müller, P. Klahn, *Chem. Sci.,* **2021**, *12*, 10179–10190.
[5] C. Rohrbacher, R. Zscherp, S. C. Weck, C. Ducho, P. Klahn, *unpublished results* **2022**.
[6] R. Zscherp, J. Coetzee, J. Herrmann, R. Müller, P. Klahn, *unpublished results* **2022.**

Nucleic acid conjugates for biomedical applications

Philippe Barthélémy

ARNA Laboratory, INSERM U1212 / UMR CNRS 5320 https://arna.cnrs.fr
ChemBioPharm http://chembiopharm.fr
University of Bordeaux

Forty years ago, Zamecnik and Stephenson[1,2] reported that short synthetic DNA fragments (called oligonucleotides) possessing sequence binding complementary to RNA molecules, belonging to the Rous sarcoma virus, inhibited viral replication. This seminal discovery documented replication prevention of a viral RNA strain using a specific oligonucleotide - today known as antisense treatment. Several studies have also elucidated many of molecular mechanisms underlying different human and animal pathologies. Thus, altering the expression of pathological genes, whether genetic or modified by mutation, is of keen interest and technologies are being developed/pursued for such purposes. Among the different approaches, antisense DNA (ASO) and RNA interference (RNAi) are two very promising technology for inhibiting specific genes.

In this presentation we will present our recent results on amphiphilic nucleoside,[3] nucleotide[4] and oligonucleotides featuring antisenses[5] and/or G-quadruplex-prone sequences.[6]

References

[1]Zamecnik, P. C.; Stephenson, M. L. *Proc. Natl. Acad. Sci. U. S. A.* **1978**, 75 (1), 280–284.
[2]Stephenson, M. L.; Zamecnik, P. C. *Proc. Natl. Acad. Sci. U. S. A.* **1978**, 75 (1), 285–288.
[3](a) Latxague, L. *et al. Journal of Colloid and Interface Science,* **2021**, 857 63. https://doi.org/10.1016/j.jcis.2021.03.026. (b) Bansode, N.D. *et al. Biomater. Sci.*, **2020**, DOI: 10.1039/D0BM00508H. (c) Baillet, J., *et al. Soft Matter,* **2020**, doi:10.1039/D0SM01055C.
[4]Dessane, B. *et al. Sci Rep* **2020**, 10 (1), 2850. https://doi.org/10.1038/s41598-020-59632-w
[5] (a) Karakia, S. *et al. Journal of Controlled Release* **2017**, 258, 1–9. (b) Benizri, S. *et al. Biomaterials Science*, **2021**. https://doi.org/10.1039/D1BM00273B.
[6]Vialet, B. *et al. Chem. Commun*. **2017** DOI: 10.1039/C7CC05693A.

VECTORIZATION AND INNOVATION OF NUCLEIC ACID MIMICS

Azevedo NF 1,2

1LEPABE - Laboratory for Process Engineering, Environment, Biotechnology and Energy, Faculty of Engineering, University of Porto, Rua Dr. Roberto Frias, 4200-465 Porto, Portugal
2ALiCE - Associate Laboratory in Chemical Engineering, Faculty of Engineering, University of Porto, Rua Dr. Roberto Frias, 4200-465 Porto, Portugal

Vectorization of nucleic acid mimics (NAMs) or nucleic acid analogues for therapeutic applications is now entering a golden age, and several oligonucleotides have recently been approved for use in humans by regulatory authorities[1]. Up until now these applications have been mainly targeting human cells, but strategies aimed at microorganisms, and more specifically bacteria, are starting to emerge[2]. Like for human cells, NAMs can be engineered to hybridize with specific sequences in bacteria with high affinity and hence be used to inhibit the translation of essential genes. However, as the bacterial envelope barrier hinders NAMs internalization, suitable and bacteria-specific delivery vectors are in dire need (Figure 1).

In here, I will start by introducing the most relevant types of NAMs that are being studied to tackle antimicrobial resistance in bacteria. Then, I will discuss limitations and advantages of coupling NAMs to different vectors such as liposomes[3], vitamin B12[4], dendrimers and cell-penetrating peptides. Important aspects to discuss includes the stability of the conjugate, the ability of the conjugate to cross the bacterial envelope or deliver the NAMs inside the cell and also possible applications in biofilms.

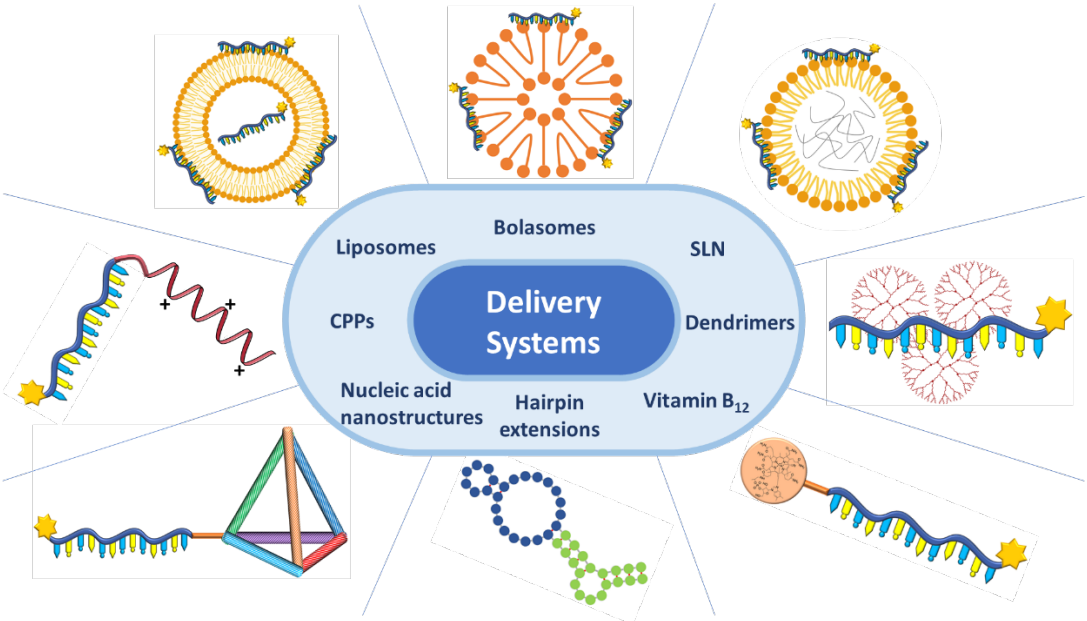


Figure 1. Schematic representation of existing oligonucleotide delivery systems in bacteria.

References
[1] T.C. Roberts et al *Nature Reviews Drug Discovery*, **2020**, 19, 673–694.
[2] R.S. Santos et al *Advanced Drug Delivery Reviews*, **2018**, 136-137, 28-48.
[3] L. Moreira et al *ACS Infectious Diseases,* **2022**, 8, 1218–1230.
[4] S. Pereira et al *Antibiotics,* **2021**, 10, 379.

Antivirulence drugs and new generation antibiotics, two examples of how structural biology can help in the development of strategies against microbial resistance

Nicolas Levy [1 2], Jean-Michel Bruneau [1], Erwann Le Rouzic [1], Damien Bonnard [1], Frédéric Le Strat [1], Audrey Caravano [1], Francis Chevreuil [1], Julien Barbion [1], Sophie Chasset [1], Benoît Ledoussal [1], François Moreau [1], Marc Ruff [2]

1) Mutabilis , 102 Avenue Gaston Roussel , 93230 Romainville , France.
2) IGBMC , 1 Rue Laurent Fries , 67404 Illkirch , France.

Worldwide spread of antibiotic-resistant germs pushes research towards new chemotherapeutic strategies besides the development of new generation antibiotics. Antivirulence drugs differ from later in their ability to disarm pathogens against natural defences or hinder their potency to invade and spread in human body without placing them under immediate selective pressure or harming commensal species. Lipoteichoic acid (LTA) is a major cell wall component in Gram + bacteria. This polymer of polyphosphoglycerol is substituted with D-alanyl ester or a glycosyl residue and anchored in bacterial membrane. D-alanylation of LTA involves activity of four proteins (dltA to dltD) and dltA has been shown to be a target of choice in this pathway. Here we describe the crystal structure of dltA from *Staphylococcus aureus* (SAU), *Enterococcus faecalis* (EFA), and *Streptococcus pyogenes* (SPY) alone or in complex with inhibitors. We describe the general binding mode of molecules in the substrate pocket and differences between species. Analysis of apo forms brings light to the difference in druggability of EFA and SPY dltA despite same active site residues composition. Finally, we provide insights into the unique binding mode of an allosteric inhibitor specific of Streptococci. Altogether these results pave the way to the design of high affinity inhibitors of the alanylation of LTA in gram + bacteria.

Penicillin-binding proteins (PBPs) are the targets of the β-lactams, the most successful class of antibiotics ever developed against bacterial infections. Unfortunately, the worldwide and rapid spread of large spectrum β-lactam resistance genes such as carbapenemases is detrimental to the use of antibiotics in this class. New potent PBP inhibitors are needed, especially compounds that resist β-lactamase hydrolysis. Here we describe the structure of the E. coli PBP2 in its Apo form and upon its reaction with 2 diazabicyclo (DAB) derivatives, avibactam and CPD4, a new potent PBP2 inhibitor. Examination of these structures shows that unlike avibactam, CPD4 can perform a hydrophobic stacking on Trp370 in the active site of E. coli PBP2. This result, together with sequence analysis, homology modeling, and SAR, allows us to propose the DAB CPD4 as potential starting scaffold to develop molecules active against a broad range of bacterial species at the top of the WHO priority list.

NOVEL ANTIMICROBIALS BY INNOVATIVE MINING OF NATURAL PRODUCTS AND DEVELOPPEMENT OF ANTIBIOTIC HYBRIDS

El Mortaji Lamya

DEINOVE CAP SIGMA, ZAC Euromédecine II
1682 rue de la Valsière, 34790 GRABELS

Antimicrobial resistance (AMR) has emerged as one of the major public health issues of these last decades[1]. The increasing occurrence of antibiotic resistance and superbug outbreaks has led to the urgent need of new antibiotics[2].

Although, synthetic chemistry has undoubtedly been crucial for developing successive evolutions of known antimicrobials, the vast majority of these molecules were originally discovered as natural compounds produced by living microorganisms.

DEINOVE's platform is dedicated to exploit the microbial world to discover tomorrow's compounds arising from an unprecedent metabolomic diversity. Thanks to its unique expertise in microbial ecology coupled to its ultra-high-throughput screening capabilities, DEINOVE aims at discover, tame and preserve this unlimited source of new metabolites and accelerates the discovery of new molecules. By integrating omics technologies, synthetic biology and data science into a high-throughput fermentation, bioprocess engineering and know how in advanced activity assays our platform is able to decrypt the unlimited metabolic potential of microorganisms and thus participate to tackle the AMR challenge.

In parallel, DEINOVE pursues its drug development programme with its proprietary antibiotic molecule, DNV3837, a hybrid antibiotic converted, *in vivo*, into DNV3681, a powerful dual-action gram-positive antibiotic. Having demonstrated its safety in humans, DNV3837 is currently evaluated in a phase II clinical trial for the treatment of severe Clostridioides difficile infections, a pathogen responsible for certain healthcare-associated infections, representing a major threat to human health and recognized as a priority by the World Health Organization.

References

[1] (a) J O'Neill, *Review on Antimicrobial Resistance, 2016*
[2] (b) Antimicrobial Resistance Collaborators, *The Lancet*, 2022, 399, 629-655

JUNIOR LECTURES

VECTORIZATION OF IRIDIUM(III) COMPLEXES USING SIDEROPHORE SURROGATES: A TROJAN HORSE STRATEGY AGAINST GRAM-NEGATIVE PATHOGENIC BACTERIA

Aline L. Faucon[1], Julien Renault[2], Julie Couchot[3], Françoise Hoegy[1], Jean-Luc Renaud[2], Patrick Plésiat[3], Sylvain Gaillard[2], Gaëtan L. A. Mislin[1]

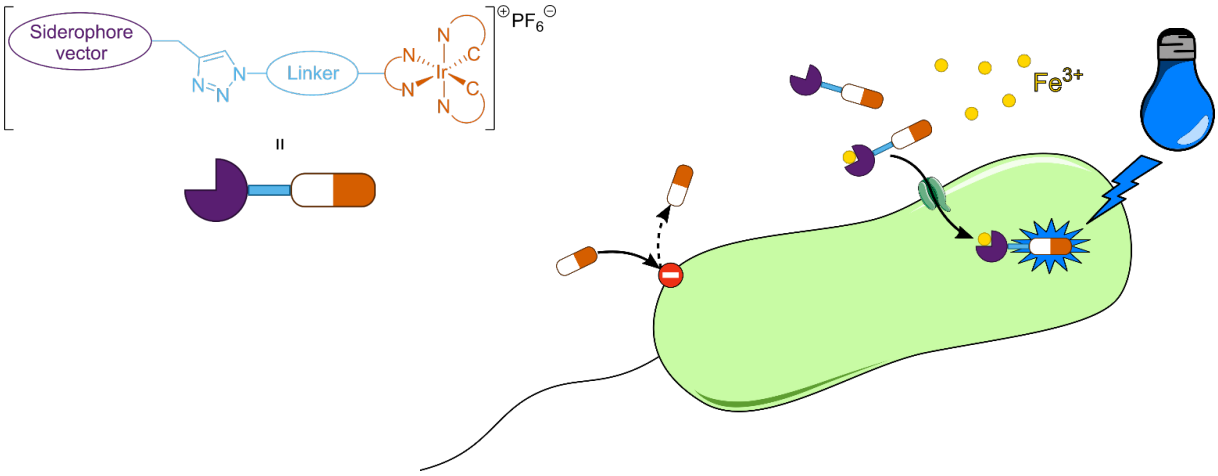
1 : UMR7242 Biotechnologie et Signalisation Cellulaire (BSC), CNRS, Université de Strasbourg, 300 Boulevard Sébastien Brant, 67400 Illkirch-Graffenstaden, France.

2 : UMR6507 Laboratoire de Chimie Moléculaire et Thio-organique (LCMT), ENSICAEN, 6 Boulevard Maréchal Juin, 14050 Caen, France.

3 : Centre National de Référence de la Résistance aux Antibiotiques, Service de Bactériologie, CHU de Besançon, 3 Boulevard Fleming, 25030 Besançon, France.

Bacterial resistance to antibiotics is a major threat towards humanity, and requires innovative solutions to fight infections caused by Gram-negative bacteria, such as *Pseudomonas aeruginosa*. Antibacterial photodynamic therapy can be used as treatment against infections of skin or tissues. Photosensitizers (PS) are excited with visible light, leading to the production of reactive oxygen species, toxic to cells. However, the penetration of these PS is a challenge, especially in Gram-negative bacteria due to their low membrane permeability. It is nonetheless possible to hijack the iron uptake systems in order to promote PS uptake, using a Trojan horse strategy.[1] Bacteria acquire iron, a nutrient essential for their growth, using siderophores, which are small chelating molecules that can form a complex with iron(III).[2] The conjugation of a PS to a siderophore analogue could therefore increase the penetration of a drug in the bacterial inner space. The PS used in this project are iridium(III) complexes, having intrinsic and photo-induced antibacterial activities.[3]

This project is a collaboration with Pr. Jean-Luc RENAUD and Dr. Sylvain GAILLARD's team (LCMT, EnsiCaen), as well as *Centre National de Référence de la Résistance aux Antibiotiques* directed by Pr. Patrick PLÉSIAT (CHU Jean-Minjoz, Besançon).



References

(1) G. L. A. Mislin & I. J. Schalk *Metallomics* **2014**, *6* (3), 408–420.

(2) R. C. Hider & X. Kong *Nat Prod Rep* **2010**, *27* (5), 637–657.

(3) E. Sauvageot *et al. Metallomics* **2017**, *9* (12), 1820–1827.

DESIGN OF NEW PYOVERDINE ANALOGUES USING SYNTHETIC BIOLOGY

Hélène Puja, Anne Forster, Noelle Potier-Holler, Laurent Bianchetti, Annick Dejaegere, Coraline Rigouin

Biotechnologie et Signalisation Cellulaire UMR 7242, CNRS
Equipe « Métaux et Microorganismes : Biologie, Chimie et Applications »
ESBS, 300 Boulevard Sebastien Brant 67412 ILLKIRCH-GRAFFENSTADEN Cedex

Pseudomonas aeruginosa is an opportunistic pathogen frequently isolated in intensive care units, widely acknowledged for its capacity to develop high antibiotic resistance and to cause therapeutic failure. It is therefore considered a research priority to develop new therapeutic strategies. In this context, our team chose to target the iron uptake pathway, a critical component for bacterial growth during infections. To efficiently scavenge iron, *P. aeruginosa* produces and exports a siderophore, the pyoverdine, capable to hexacoordinate ferric iron and to import it through an uptake pathway specifically recognizing ferri-pyoverdine[1]. Our aim is to produce a pyoverdine-antibiotic conjugate recognized by the iron-specific uptake pathway, and through a “Trojan Horse” strategy, deliver the conjugate into the periplasm[2]. To achieve the production of the conjugate, we chose a hemi-synthetic method, consisting in the preliminar production of pyoverdine by bacterial biosynthesis and subsequent conjugation of the antibiotic by click-chemistry. To perform a stereo-specific and effective click-chemistry, our objective is to incorporate a non-natural amino acid (nnAA) containing an azido group, at the C-terminal side of the siderophore by modifying the biosynthesis pathway of pyoverdine through synthetic biology methods[3]. The presence of the azido group in the newly-synthetized pyoverdine will allow easy copper-catalyzed click-chemistry with an alkyne-modified antibiotic. Pyoverdine is assembled by four non-ribosomal peptide synthetases (NRPS), multi-modular enzymes in which each module is responsible for the selection, activation, and condensation of a single amino acid on the growing peptide chain. The substrate selectivity is controlled by the Adenylation (A) Domain, that will bind the selected substrate and activate it. To incorporate the nnAA into the biosynthesis, we tried to change the substrate specificity of the A-domain of PvdD, the last NRPS of the pyoverdine pathway responsible for the addition of the last threonine in C-ter. We performed a 3D modelization of the substrate binding pocket of PvdD(A) and determined the residues involved in substrate recognition. In a modified strain derived from PAO1, we constructed a set of derived mutants by performing site-directed mutagenesis on these key residues, to modify the structure of the pocket and accommodate azido-nnAA. We cultivated the mutants to produce the pyoverdines, and analyzed them by MALDI-ToF Mass spectrometry. A few mutants on the same position, a phenylalanine directly interacting with the substrate and closing the pocket, produced pyoverdines with a lower m/z, and performing a ESI-MS/MS on these pyoverdines showed that PvdD had incorporated preferentially L-serine instead of L-threonine in the molecule.

We cultivated these mutants in the presence of several nnAA (3-azido-L-alanine, 4-azido-L-homoalanine, L-Homopropargylglycine) and analyzed the produced pyoverdines, but were unable to detect azido-pyoverdine by MALDI-ToF analysis. However, these preliminary results indicate that it is possible to change the substrate specificity by performing targeted rational mutagenesis on the binding site.

References

[1] Schalk IJ. Metal trafficking via siderophores in Gram-negative bacteria: specificities and characteristics of the pyoverdine pathway. *J Inorg Biochem*, **2008**. 102, 5-6 (1159-1169). [2] Mislin GLA, Schalk IJ. Siderophore-dependent iron uptake systems as gates for antibiotic Trojan horse strategies against Pseudomonas aeruginosa. *Metallomics*, **2013**. 6, 408-420.[3] Kries H, Wachtel R, Pabst A, Wanner B, Niquille D, Hilvert D. Reprogramming Nonribosomal Peptide Synthetases for “Clickable” Amino Acids. *Angew Chem Int Ed*. **2014.** 53, 10105-10108.

IRON ACQUISITION IN PSEUDOMONAS AERUGINOSA: MATHEMATICAL MODELISATION OF PHENOTYPIC SWITCHES

Thibaut HUBERT[1,2], Morgan MADEC[2] , Isabelle SCHALK[1]

[1] Biotechnologie et signalisation cellulaire, UMR7242, ESBS, Pôle API, 300 Bd Sébastien Brant, 67400 Illkirch-Graffenstaden

[2] Laboratoire des sciences de l'ingénieur, de l'informatique et de l'imagerie, UMR7357, TPS, Pôle API, 300 Bd Sébastien Brant, 67400 Illkirch-Graffenstaden

Bacteria secrete high-affinity iron-chelating compounds called siderophores in their environment to access iron, a key nutrient with low bioavailability and source of strong competition between microorganisms in most biotopes. Many bacteria also use siderophores produced by other microorganisms (xenosiderophores) in a hacking strategy. *Pseudomonas aeruginosa*, an opportunistic human pathogen, produces two siderophores and is also able to use many xenosiderophores thanks to about twenty different iron uptake pathways present in its genome[1]. Depending on the environmental stimuli, *P. aeruginosa* will specifically express the pathway(s) best suited to access iron[1,2]. Few data are available concerning the phenotypic plasticity related to the expression of these different iron uptake pathways. The objective of the project is to model this phenotypic plasticity in *P. aeruginosa* in response to the external environment.

Two mutants of *P. aeruginosa* were constructed, each carrying fluorescent reporters allowing to follow the expression of an iron uptake pathway using a specific xenosiderophore. These mutants were first cultured in the presence of increasing concentrations of the xenosiderophores of interest. The fluorescence data obtained allowed the construction of a mathematical model of the transcriptional activity of genes involved in the two iron uptake pathways studied according to the concentration of the xenosiderophore. The results show that each pathway studied has an expression profile that is function of the xenosiderophore concentration and that is specific to it. Other culture conditions will be further tested, such as with mixtures of xenosiderophores, and the experimental data obtained will be used to develop a more complex mathematical model to decipher the regulatory network involved.

References

[1] Perraud Q, Cantero P, Roche B, Gasser V, Normant VP, Kuhn L, Hammann P, Mislin GLA, Ehret-Sabatier L, Schalk IJ. Phenotypic Adaption of *Pseudomonas aeruginosa* by Hacking Siderophores Produced by Other Microorganisms. Mol Cell Proteomics. **2020** Apr;19(4):589-607. doi: 10.1074/mcp.RA119.001829. Epub 2020 Feb 5. PMID: 32024770; PMCID: PMC7124469.

[2] Perraud Q, Cantero P, Munier M, Hoegy F, Zill N, Gasser V, Mislin GLA, Ehret-Sabatier L, Schalk IJ. Phenotypic Adaptation of *Pseudomonas aeruginosa* in the Presence of Siderophore-Antibiotic Conjugates during Epithelial Cell Infection. Microorganisms. **2020** Nov 18;8(11):1820. doi: 10.3390/microorganisms8111820. PMID: 33218210; PMCID: PMC7699141.

SELECTION OF ENZYME INHIBITING APTAMERS BY ULTRAHIGH-THROUGHPUT MICROFLUIDIC SCREENING

Claire Husser (1)**, Janis Hötzel** (2)**, Beatrix Suess** (2)**, Michael Ryckelynck** (1)

(1) Université de Strasbourg, Architecture et Réactivité de l'ARN (UPR9002 du CNRS), Team "Digital Biology of RNA", Institut de Biologie Moléculaire et Cellulaire du CNRS

(2) Technische Universitat Darmstadt; Department of Biology, Team "Synthetic RNA Biology"

Because of their ability to catalyze a wide range of chemical reactions, enzymes are at the basis of the vast majority of the mechanisms supporting cell life. Yet, they can also be involved in various pathologies. Among them, secreted enzymes play central roles during bacterial infections (e.g., beta-lactamase) or tumors development (e.g., matrix metalloproteinases), making them particularly relevant druggable targets.

Drugs targeting enzyme are usually small chemical compounds, whom the development is long and costly. On the contrary, aptamers (i.e., short oligonucleotides able to adopt a three-dimensional structure allowing them to specifically bind a target molecule) can be rapidly and efficiently selected through in vitro evolutionary processes. Aptamers represent therefore a new promising source of drugs to fight against diseases and infections. Yet, to be used in vivo, these aptamers should remain stable and functional in complex biological environments (e.g., blood). To this end, a solution consists in selecting aptamers made of 2'-modified (e.g., 2'-fluoro or 2'-oMe) non-natural nucleotides turning them more resistant to hydrolysis[(1)].

Our group is specialized in the development of synthetic RNA modules (especially light-up RNA aptamers) and of Fluorogenic RNA-based Biosensor using µIVC-seq pipeline[(2)] (a technology coupling the use of In Vitro Compartmentalization assisted by microfluidics with Next Generation Sequencing and bioinformatics). In the work presented here, we repurposed this technology to search for nuclease-resistant aptamers able to bind a target enzyme with good affinity while also displaying significant inhibition properties. To do so, we combined: (i) the use of a library pre-enrichment step by Systematic Evolution of Ligands by EXponential enrichment (SELEX)[(3)] to isolate aptamers displaying affinity to the target, with (ii), a functional ultrahigh-throughput microfluidic screening (µIVC-seq) to select them for their capacity to inhibit enzyme activity.

In this presentation I will present you how we used this innovative selection process to identify new nuclease-resistant inhibitory aptamers targeting metallo-beta-lactamase, a class of secreted enzymes responsible for resistance to beta-lactams, the most widely prescribed antibiotics,[(4)] and involved in medically relevant resistant bacterial infections.

References

[1] Thirunavukarasu, D., Chen, T., Liu, Z., Hongdilokkul, N. & Romesberg, F. E. *J. Am. Chem. Soc.*, **2017**, 139, 2892–2895

[2] Trachman, R. J. et al., *Nat Chem Biol*, **2019,** 15, 472–479

[3] Ellington, A. D. & Szostak, J. W., *Nature*, **1990**, 346, 818–822

C. Tuerk, L. Gold, *Science,* **1990**, 249(4968) 505-10.

[4] L.C. Ju, Z. Cheng, W. Fast, R.A. Bonomo, M.W. Crowder, *Trends in pharmacological sciences*, **2018**, 39(7) 635-647. (2018)

A NOVEL INHIBITOR OF GCPE, A TARGET FOR THE DEVELOPMENT OF ANTIMICROBIALS

Clea Witjaksono[a], Vivien Herrscher[b], Jean-Bernard Behr[b], Myriam Seemann[a]

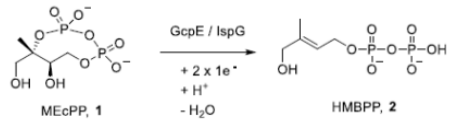
[a] Institut de Chimie UMR 7177, Université de Strasbourg, CNRS, Equipe Chimie Biologique et ApplicationsThérapeutiques, 4 rue Blaise Pascal, 67070 Strasbourg

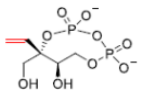
[b] Univ. Reims Champagne-Ardenne, ICMR, CNRS UMR 7312, 51687 Reims, France

cwitjaksono@unistra.fr

GcpE, also called IspG, is the penultimate enzyme of the methylerythritol phosphate (MEP) pathway[1] involved in the biosynthesis of isopentenyl diphosphate (IPP) and its isomer the dimethylallyl diphosphate (DMAPP), the universal precursors of isoprenoids. These latter are present in all living organisms and fulfil a variety of crucial biological functions such as electron transport, membrane stabilisation and signalling.[2]

The MEP pathway is present in many pathogenic bacteria involved in nosocomial infections such as *Escherichia coli*, *Pseudomonas aeruginosa*, *Acinetobacter baumannii* and *Salmonella* spp. but not in humans that utilise an alternative route, making the enzymes of the MEP pathway, including GcpE, interesting targets for the development of new antibacterial agents to overcome antibacterial resistance. GcpE harbours an oxygen-sensitive $[4Fe\text{-}4S]^{2+}$ cluster and catalyses the conversion of 2-*C*-methyl-D-erythritol 2,4-cyclodiphosphate (MEcPP) **1** into (*E*)-4-hydroxy-3-methylbut-2-enyl diphosphate **2** (Scheme 1).[3] The reaction involves the transfer of two electrons to the substrate **1** and a water elimination.





Scheme 1: The reaction catalysed by GcpE and the 2-vinyl analogue of the substrate

With the aim to identify new potent inhibitors of GcpE, a 2-vinyl analogue of MEcPP was designed (Scheme 1), whose double bond could possibly be delocalised during enzymatic catalysis, potentially leading to new reactive centres capable of forming covalent bonds with surrounding amino acid residues.

The inhibition potential of the substrate analogue was evaluated against GcpE from *Escherichia coli* and was found to be a promising irreversible inhibitor of GcpE.[4]

* This work was supported by the "Fondation pour la Recherche Médicale"

References

[1] N. Campos, M. Rodríguez-Concepción, M. Seemann, M. Rohmer, A. Boronat *FEBS Lett.*, **2001**, 488, 170–173.
[2] S. Heuston, M. Begley, C. G. M. Gahan, C. Hill *Microbiology*, **2012**, 158, 1389–1401.
[3] M. Seemann, B. T. S. Bui, M. Wolff, D. Tritsch, N. Campos, A. Boronat, A. Marquet, M. Rohmer *Angew. Chem. Int. Ed.*, **2002**, 41, 4337–4339.
[4] V. Herrscher, C. Witjaksono, M. Buchotte, C. Ferret, F. Massicot, J.-L. Vasse, F. Borel, J.-B. Behr, M. Seemann *Chem. Eur. J.*, **2022**, 28, e202200241.

Rationally modifying Neomycin, an antibiotic from the aminoglycosides family

Julia Revillo Imbernon*[1] and Esther Kellenberger

[1]Laboratoire dÍnnovation Thérapeutique – Centre National de la Recherche Scientifique : UMR7200, université de Strasbourg, Institut de Chimie du CNRS – France

Abstract

Aminoglycoside antibiotics (AGs) have occupied a time-honoured position in the hierarchy of antibacterial agents for almost 80 years. The therapeutic class contains several molecules such as gentamicin, amikacin, tobramycin, neomycin, streptomycin... All of them targeting cellular ribosomal ribonucleic acid (rRNA) to inhibit bacterial replication.
In this project, we focus on neomycin, which exerts excellent antibacterial activities against Gram-positive and Gram-negative bacteria. Due to nephrotoxicity and ototoxicity, neomycin is mainly used in topical ointments or is administered by unique injections in emergency. Resistances to neomycin involve Aminoglycoside-Modifying Enzymes deactivations (AME) accounting for about 60-70% of acute resistances, drug efflux and 16S rRNA mutations.

Four laboratories are collaborating to develop original derivatives of neomycin in order to fight infection by aminoglycoside-resistant bacteria. The main goal is to maximize the bactericidal effect by minimizing antimicrobial resistance and toxicity.

For this purpose, we have modelized by homology all the AMEs of the strains of *E. coli* and *S. aureus* (5 models in total). The starting 3D structure of the derivatives-AME complexes was achieved in all models by constrained docking. Representative complexes, including those containing neomycin (taken as a control), were simulated by molecular dynamics. For each complex, three trajectories of 40ns were calculated. The stability of the complexes was studied to determine a possible defect in the metabolization of the aminoglycosides. In addition, the binding modes were considered as indicators of the possible catalysation of the derivatives. For this, the RMSD of the active site, the distance of the derivatives to the catalytic center as well as the presence of the essential interactions were evaluated.

The collected data aims to determine whether the modifications applied to neomycin are favorable or unfavorable to metabolism.
In certain models, we observe that the steric hinderance caused by the introduced substitutions leads to a different binding mode from the one of the control neomycin. These results suggest that the optimization of those substitution chains should be explored to avoid the action of these AMEs. In other cases, the MD productions present various binding modes, not allowing any conclusions to be drawn, but giving information on possible system instabilities.

*Speaker