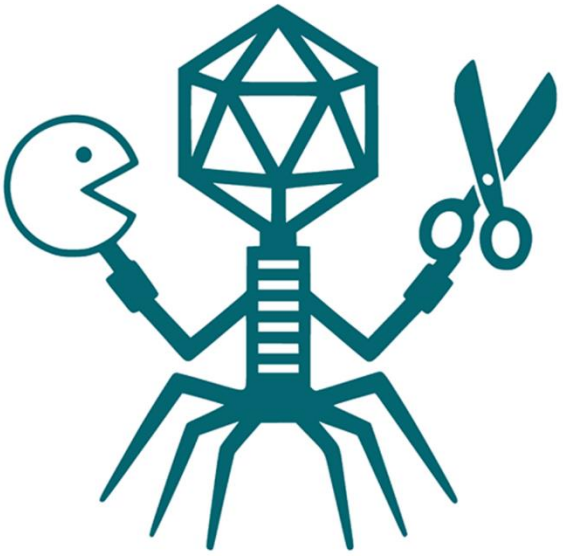

THE PHAGE PROTEIN MEETING

10-11 SEPTEMBER 2025

GHENT

ABSTRACT BOOK

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EXOLYTICS

PROGRAMME

Wednesday September 10

8:00-9:00 Registration, set-up posters and coffee

Welcome session (Chairs: Prof. Yves Briers and Prof. Carlos São-José)

9:00-9:10 Welcome by **Prof. Yves Briers** (Ghent University, Belgium) on behalf of Organizing and Scientific Committees

9:10-9:40 O01 **Keynote lecture: Prof. Lone Brøndsted** (University of Copenhagen, Denmark): The advancement of phage proteins: unlocking new frontiers in antimicrobial solutions

9:40-10:10 O02 **Invited speaker: Prof. Daniel Nelson** (University of Maryland, USA): Targeting Group B *Streptococcus* with ClyX-2: A chimeric endolysin for vaginal biofilm clearance and reduced inflammatory response

10:10-10:40 O03 **Invited speaker: Prof. Zuzanna Drulis-Kawa** (University of Wroclaw, Poland): A catalog of capsule depolymerases encoded by *Klebsiella* phages

Coffee break (10:40-11:10)

Session 1: Computational research in phage proteins (Chairs: Prof. Rafał Mostowy and Dr. Roberto Vázquez)

11:10-11:40 O04 **Invited speaker: Prof. Hang Yang** (Wuhan Institute of Virology, China): Recognition, mining and engineering of active lysins against Gram-negative pathogens

11:40-12:00 O05 **Selected speaker: Dr. Moritz Ertelt** (Fraunhofer Institute for Translational Medicine and Pharmacology, Germany): *In silico* prediction and in-depth analysis of phage receptor binding protein structures, enzymatic activities and host receptor interactions

12:00-12:20 O06 **Selected speaker: Victor Klein-Sousa** (University of Copenhagen, Denmark): The tail fiber atlas & structural insights into phage infection

Poster pitch session 1 (4 x 3 min) (Chair: Dr. Kevin De Muynck)

12:20-12:23 P02 **Selected Pitch: Robby Concha-Eloko** (Institute for Integrative Systems Biology, Spain): Unlocking data in *Klebsiella* lysogens to predict capsular type-specificity of phage depolymerases

12:24-12:27 P05 **Selected Pitch: Ilaria De Angelis** (Ghent University, Belgium): Structural and functional analysis of K1- and K2-specific depolymerases for targeted therapy against multidrug-resistant *Klebsiella pneumoniae*

12:28-12:31 P26 **Selected Pitch: Dr. Jéssica Duarte da Silva** (Institute of Biology, University of Campinas, Brazil): Diversity of endolysins and depolymerases encoded by rare bacteriophages isolated by the B3-CEPID Project, a Brazilian Center for Research on Bacteria and Phages

12:32-12:35 P15 **Selected Pitch: Aleksandra Otwinowska** (University of Wrocław, Poland): Prophage-derived depolymerases as a promising source of capsule-targeting tools against *Klebsiella pneumoniae*

Lunch & poster session (12:35-14:00)

Session 2: Phage proteins for biocontrol: from basic to translational research (Chairs: Dr. Mark van Raaij and Dr. Mathias Schmelcher)

14:00-14:30 O07 **Invited speaker: Prof. Yves Briers** (Ghent University, Belgium): Synthetic biology of modular phage proteins

14:30-15:00 O08 **Invited speaker: Dr. Lucía Fernández** (Instituto de Productos Lácteos de Asturias, Spain): Impact of temperature on endolysin LysRODI inactivation and killing dynamics of *Staphylococcus aureus* in milk

15:00-15:20 O09 **Selected speaker: Dr. Danielle Peters** (National Research Council, Canada): Harnessing depolymerases: Enhancing phage therapeutics against resistant pathogens

15:20-15:40 O10 **Selected speaker: Prof. Magdalena Plotka** (University of Gdansk, Poland): Practical applications of extremophilic endolysins and their derivatives

15:40-16:00 O11 **Selected speaker: Dr. Sharon Shui Yee Leung** (The Chinese University of Hong Kong): Combating *Acinetobacter baumannii* induced lung infections with phage-encoded antibacterial enzymes: Promise and challenges.

Group picture (in front of the conference venue) and coffee break (16:00-16:30)

Session 3: The ESCMID Study Group for Non-Traditional Antibacterial Therapy (ESGNTA) session (Chair: Prof. Zuzanna Drulis-Kawa)

16:30-16:50 O12 **Selected speaker: Dr. Peter Braun** (Fraunhofer Institute for Translational Medicine and Pharmacology, Germany): Receptor binding proteins as novel diagnostic tools for bacterial infections

16:50-17:10 O13 **Invited speaker: Prof. Tristan Ferry** (University of Lyon, France): Clinical trial with anti-staphylococcal lysin CF-301 for joint infections

17:10-17:30 O14 **Invited speaker: Dr. John H. Rex** (AMR.Solutions): Is it rational to work on phage proteins (and phage) as therapeutics? Yes! No! Carefully!

17:30-18:15 **Panel Debate**: Dr. Lorenzo Corsini (BioNTech R&D, Austria), Prof. Daniel Nelson (University of Maryland, USA), Dr. Kristof Van Emelen (Obulytix,

Belgium): The hurdles for phage proteins towards clinical and market applications

Conference dinner (19:15-22:30)

- Apéro from 19:15 – **the boat leaves at 19:30**
- Upon registration only
- Find dinner ticket and directions to the meeting point in your name tag

Thursday September 11

Session 4: Fundamental research on phage proteins (Chairs: Prof. Rob Lavigne and Dr. Rayén León Quezada)

9:00-9:30 O15 **Invited speaker: Prof. Carlos São-José** (University of Lisbon, Portugal): Endolysins: insights into their mode of action and regulation for enhanced applications

9:30-9:50 O16 **Selected speaker: Samiya Siddiqui** (University of Leuven, Belgium): PIT3: A phage-derived protein with anti-virulence activity against the Type III Secretion System in *Pseudomonas aeruginosa*

9:50-10:10 O17 **Selected speaker: Prof. Urmi Bajpai** (University of Delhi, India): Bacteriophage-encoded novel endolysins targeting *Mycobacterium tuberculosis*

10:10-10:30 O18 **Selected speaker: Hannelore Longin** (University of Leuven, Belgium): Fold first, ask later: structure-informed protein function prediction in *Pseudomonas* phages

10:30-10:50 O19 **Selected speaker: Marta Gomes** (University of Minho, Portugal): Exploring phage early genes to combat drug-resistant *Acinetobacter baumannii*

Coffee break (10:50-11:20)

Session 5: Tailocins (Chairs: Dr. Agnieszka Latka and Prof. Hang Yang)

11:20-11:40 O20 **Selected speaker: Prof. Hernán Burbano** (University College London, UK): Tracing the evolution of tail fibers and their receptors in historical metagenomes

11:40-12:00 O21 **Selected speaker: Dr. David Baltrus** (University of Arizona, USA): Exploring the genetic basis of interspecies killing by tailocins

12:00-12:20 O22 **Selected speaker: Prof. Talia Karasov** (University of Utah, USA): Constraints on the evolution of tailocin tail fibers in host-associated bacteria

Poster pitch session 2 (4 x 3 min): (Chair: Dr. Kevin De Muynck)

12:20-12:23 P42 **Selected Pitch: Prof Haidai Hu** (Novo Nordisk Foundation Center for Protein Research, Copenhagen University, Denmark): Zorya Anti-phage defense at the membrane boundary

12:24-12:27 P31 **Selected Pitch: Magdalena Pełka** (University of Warsaw, Poland): Phage-borne proteins with lytic activity against *Neisseria gonorrhoeae*

12:28-12:31 P34 **Selected Pitch: Samuel Staubli** (ETH Zürich, Switzerland): Phage-derived endolysins as precision tools against vancomycin-resistant enterococci

12:32-12:35 P36 **Selected Pitch: Gilles Vandermarliere** (Ghent University, Belgium): You get what you screen for: benchmark analysis of three expression hosts for the selection of new antibacterial enzybiotics

Lunch & poster session (12:35-14:00)

Session 6: Phage lysins for therapy (Chairs: Prof. Daniel Nelson and Dr. Lucía Fernández)

14:00-14:30 O23 **Invited speaker: Dr. Mathias Schmelcher** (Zurich University of Applied Sciences, Switzerland): Engineered phage lysins – tackling hurdles for clinical application

14:30-14:50 O24 **Selected speaker: Dr. Lamya El Mortaji** (Aurobac Therapeutics): Antibacterial mechanism of action of Gram-negative targeting endolysin ATX401 (formerly CF-370) is primarily through N-terminal AMP rather than murein hydrolase activity

14:50-15:10 O25 **Selected speaker: Dr. Guy Hermans** (Obulytix): Systematic engineering of lysins yields highly potent, serum resistant activity towards drug-resistant *Acinetobacter baumannii*

15:10-15:30 O26 **Selected speaker: Dr. Tim Thysens** (Eurofins): Endolysins as therapeutics: bridging preclinical development and clinical manufacturing

15:30-15:50 O27 **Selected speaker: Prof. Hak-Kim Chan** (University of Sydney, Australia): Formulations of inhalable endolysin Cpl-1 and their efficacy in a murine lung infection model

15:50-16:10 O28 **Selected speaker: Dr. Niels Vander Elst** (Karolinska Institute, Sweden): Bacteriophage-derived endolysins restore antibiotic susceptibility in β-lactam- and macrolide-resistant *Streptococcus pneumoniae* infections

Coffee break (16:10-16:40)

Session 7: Phage receptor-binding proteins: basic research (Chairs: Prof. Malabika Biswas and Dr. Peter Braun)

16:40-17:00 O29 **Selected speaker: Prof. Rafal Mostowy** (Jagiellonian University, Poland): Unravelling the host range of *Klebsiella* phages: depolymerases and beyond

17:00-17:20 O30 **Selected speaker: Dr. Veronika Theresa Lutz** (University of Copenhagen, Denmark): Gp38 adhesins of Straboviridae phages target specific extracellular loops of outer membrane receptors

17:20-17:40 O31 **Selected speaker: Celia Ferriol-González** (University of Valencia, Spain): Receptor-binding protein evolvability is a major determinant of phage host range in capsule-diverse hosts

17:40-18:00 O32 **Selected speaker: Dr. James Hodgkinson-Bean** (University of Copenhagen, Denmark): Exploring flagellotropic phage adsorption by cryoEM

18:00-18:20 O33 **Selected speaker: Dr. Mark van Raaij** (Centro Nacional de Biotecnología, Spain): Crystallographic structures of bacteriophage receptor-binding proteins and endolysins

18:20-18:30 **Poster and pitch awards by Dr. Kristof Van Emelen** (Obulytix)

Closing

Friday September 12

4th Annual Symposium of the Belgian Society for Viruses of Microbes (Ghent, **Campus Schoonmeersen**) www.bsvom.be/en/symposium

WELCOME SESSION

Keynote Lecture O01 *Lone Brøndsted*

The Advancement of phage proteins: unlocking new frontiers in antimicrobial solutions

Brøndsted L1

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This presentation will highlight the exciting advancements in phage proteins that possess antimicrobial properties. It will explore their history and how these remarkable molecules can enhance our understanding of phage biology. Finally, the application of phage proteins in medicine and agriculture will be discussed, examining the pros and cons of how these innovations can contribute to a healthier future.

Invited Speaker O02 *Daniel Nelson*

Targeting Group B *Streptococcus* with ClyX-2: a chimeric endolysin for vaginal biofilm clearance and reduced inflammatory response

Dave UM1, Kirian RD1, El-Baz N2, Zhu JC1, Riley SP1, Zierden HC1, Doster RS2, Nelson DC1

(1). University of Maryland (USA)
(2). University of Louisville (USA)

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Group B *Streptococcus* (GBS) is a major contributor to severe maternal and neonatal outcomes, including stillbirth, meningitis, and long-term developmental complications. Despite its high prevalence, with one in four women carrying GBS vaginally, there are no licensed vaccines, and diagnostic efforts remain limited to high-income settings. Rising antibiotic resistance, patient allergies, and concerns regarding antibiotic use during pregnancy further highlight the need for alternative therapies. Bacteriophage-derived endolysins, which rapidly degrade bacterial cell walls, offer a promising non-antibiotic approach. In this study, we report on the anti-streptococcal efficacy of ClyX-2, a novel chimeric endolysin synthesized by our group. ClyX-2 demonstrated potent activity against multiple GBS strains at concentrations as low as 2 μM, significantly reducing both planktonic burden and mature biofilms. Within 30 minutes, ClyX-2 eradicated biofilm biomass, and confocal microscopy confirmed disruption of the biofilm matrix. In an epithelial cell culture model, ClyX-2 sterilized co-inoculated GBS within 4 hours and achieved > 3-log reduction in 24-hour preformed biofilms. Importantly, treatment with ClyX-2 also lowered pro-inflammatory cytokine secretion associated with GBS infection. In cervicovaginal mucus, ClyX-2 significantly inhibited GBS metabolism without affecting the commensal bacterium *Lactobacillus crispatus*. These findings suggest that ClyX-2 offers a targeted, low-toxicity approach to prevent and clear GBS colonization, with potential to reduce maternal carriage and interrupt neonatal transmission.

Invited Speaker O03 *Zuzanna Drulis-Kawa*

A catalog of capsule depolymerases encoded by *Klebsiella* phages

Drulis-Kawa Z1, Otwinowska A1, Olejniczak S1, Latka A1, 2, Pozniak M1, Majkowska-Skrobek G1, Maciejewska B1, Koszucki J3, Panicker V3, Jablońska S1, Olszak T1, Hulsens M2, Brouns S4, Tournebize R5, 6, Bugert JJ7, Briers Y2, Mostowy R3

(1). Department of Pathogen Biology and Immunology, University of Wrocław, Wrocław (Poland)
(2). Department of Biotechnology, Ghent University, Ghent (Belgium)
(3). Malopolska Centre of Biotechnology, Jagiellonian University, Kraków (Poland)
(4). Department of Bionanoscience, Delft University of Technology, Netherlands
(5). Centre of Immunology and Microbial Infections, Sorbonne University, Paris, France
(6). UTechS Photonic BioImaging Direction de la Technologie, Centre de Recherche et de Ressources Technologiques, Institut Pasteur, Paris, France
(7). Bundeswehr Institute of Microbiology, Munich, Germany

E-mail: zuzanna.drulis-kawa@uwr.edu.pl

The diversity of *Klebsiella* phage capsule depolymerases is probably greater than 160 cps locus types for capsule biosynthesis in *Klebsiella* spp. discovered so far. The knowledge of depolymerases structure and function, the genomic annotation and detection tools to predict their enzymatic specificity are still very limited.
We present the dataset of experimentally verified *Klebsiella* phage depolymerases, comprising 106 enzymes targeting 59 distinct capsule types with 46 prepared and tested by our team. Their activity cover both classical K-types and genomically defined KL-types reported in clinical *K. pneumoniae* strains.
Notably, 19 of the characterized enzymes originated from prophages—an underexplored but rich source of depolymerase diversity. Some capsule types (e.g., K23, K28, KL111) were independently recognized and degraded by enzymes produced by both lytic and temperate phages suggesting convergent evolution. While most proteins exhibited narrow serotype specificity recognizing only one type of CPS, 15 depolymerases showed activity on bacterial strains belonging to 2 or 3 cps locus types.
Six major architectural classes were identified based on the arrangement of the central β-helical catalytic domain and the C-terminal substrate recognition modules. Structural modeling revealed extensive domain arrangements including insertions within the β-helix, tail fiber-like extensions, and chaperone-associated peptidase folds. Depolymerases targeting the same capsule type typically share structural traits, although notable exceptions suggest multiple evolutionary strategies for CPS degradation. Altogether, this dataset provides a foundation for comparative and functional studies of phage-encoded capsule-degrading enzymes.

SESSION 1: COMPUTATIONAL RESEARCH IN PHAGE PROTEINS

Invited Speaker O04 *Hang Yang*

Recognition, mining and engineering of active lysins against Gram-negative pathogens

Zhang L1, Hu F2, Zhao Z1, He J1, Yao F3, Wei H3, He J1, Yang H3

(1). Huazhong Agricultural University
(2). Fujian Medical University
(3). Wuhan Institute of Virology, Chinese Academy of Sciences

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With the increased emergence of multidrug-resistant bacteria, new antimicrobials with mechanisms of action different from conventional antibiotics are urgently needed. Bacteriophage-encoded lysins are bacteriolytic proteins produced at the end of the phage lytic cycle that destroy elements of bacterial cell wall and enable the release of phage progeny from host cells. More frequently, lysins are considered promising alternatives to conventional antibiotics due to their direct peptidoglycan degradation activity and low risk of resistance development. However, the discovery of these enzymes is often hampered by the limited availability of phage genomes. Herein, we will report a new strategy for active lysin discovery against Gram-negative pathogens, *i.e.*, the recognition-mining-engineering approach. In the recognition module, we aim to identify the main factors that determine the membrane-penetrating and bactericidal activity of Gram-negative lysins; in the mining module, we developed several new approaches that help to mine new lysins from bacterial proteomes; and in the engineering module, we combined the knowledge from the recognition and mining modules and designed several engineered lysins with enhanced bactericidal activity. Taken together, these studies investigate the challenges of Gram-negative lysin development in deep and set up new workflow clues for accelerating the medical translation of lysins as alternative antimicrobials against Gram-negative pathogens.

Selected Speaker O05 *Moritz Ertelt*

In silico prediction and in-depth analysis of phage receptor binding protein structures, enzymatic activities and host receptor interactions

Ertelt M1, 2, Dunne M1, 2, 3, Reetz L1, Braun P1,2

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(2). Institute of Infectious Diseases and Tropical Medicine, University Hospital Ludwig-Maximilian University Munich, Munich (Germany)
(3). Micreos GmbH, Wädenswil, (Switzerland) (present address; not connected to this research)

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Machine learning (ML) approaches are reshaping the functional annotation of bacteriophage proteins, particularly receptor-binding proteins (RBPs). Traditional sequence-based bioinformatic methods frequently fall short in reliably characterizing RBPs and are not able to predict their potential enzymatic activity or specific interactions with bacterial host receptors. Here, we present a computational pipeline integrating ML-based functional annotation and structural modelling to characterize RBPs from phage genomes. The pipeline combines protein structure prediction and in-depth comparative analyses against datasets of known phage protein structures and enzymes. Specifically, active sites within RBPs are computationally identified by leveraging comparisons of geometrical spatial arrangements of protein residues, providing insights into reaction mechanisms. Additionally, the pipeline incorporates methods for predicting potential interactions between identified RBPs and bacterial surface receptors, offering valuable hypotheses for experimental validation. This integrative approach of existing novel tools streamlines the prediction of phage-host interactions, enabling high-throughput analyses with implications for phage therapy, bioengineering and microbial ecology. Our results suggest that AI-enhanced structural analyses substantially improve the functional annotation and biophysical understanding of phage receptor-binding mechanisms compared to conventional methodologies, accelerating their characterization.

Selected Speaker O06 *Victor Klein-Sousa*

The tail fiber atlas & structural insights into phage infection

Klein-Sousa V1, Roa-Eguiara A1, Kielkopf CS1, Sofos N1, 2, Taylor NMI1

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(2). Core Facility of Integrated Microscopy at University of Copenhagen (CFIM), Copenhagen, (Denmark)

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Phage infection begins with host recognition and proceeds through genome translocation across the cell membrane. Here, we investigate both processes using a combination of computational and experimental structural biology. Receptor-binding proteins (RBPs) are essential for phage adhesion, yet their sequence and structural diversity remain poorly characterized. Tail fibers, a major class of RBPs, are elongated, flexible trimers, making their full-length structures difficult to resolve experimentally.
To address this, we developed RBPseg, a computational pipeline that integrates monomeric predictions with a structure-based domain identification strategy. This approach segments tail fiber sequences into tractable units for high-confidence modeling using AlphaFold-Multimer (AFM), outperforming AFM alone. Using RBPseg, we generated complete tail fiber models for mostly *E. coli* phages. We validated our predictions through single-particle cryo-electron microscopy on three BASEL phages.
A structural classification of 67 tail fibers revealed 16 distinct classes and 89 domains, uncovering patterns of modularity, convergence, divergence, and domain swapping. These classes account for an estimated 24% of the known tail fiber sequence space. We are currently expanding this atlas with RBPseg models for over 4,000 RBPs from diverse phages.
Part of the work has been recently published at Science Advances: https://www.science.org/doi/full/10.1126/sciadv.adv0870

SESSION 2: PHAGE PROTEINS FOR BIOCONTROL: FROM BASIC TO TRANSLATIONAL RESEARCH

Invited Speaker O07 *Yves Briers*

Synthetic biology of modular phage proteins

Briers Y1

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Bacteriophages drive an unparalleled evolutionary arms race. Their short replication cycles and rapid turnover accelerate protein evolution in phages far beyond any other known system. Our research group has a keen interest in two highly modular phage proteins, lysins and receptor-binding proteins (RBPs), which are characterized by frequent recombination. For phage lysins, we have developed a comprehensive pipeline, inspired by hit-to-lead strategies in small molecule drug discovery. This pipeline integrates design-build-test-learn cycles, spanning from discovery (via the PhaLP database 2.0, http://phalp.ugent.be), and (meta)genomic prediction and analysis tools like SUBLYME and SPAED) to high-througput lysin engineering. This approach has yielded engineered lysins with enhanced functionality under harsh conditions that have been evaluated for diverse applications. In parallel, we aim to advance the field of RBPs with a similarly comprehensive pipeline, starting with community tools, including the novel PhaRBP database (http://pharbp.ugent.be), which integrates in-house developed tools with others for RBP/depolymerase detection, and PhageHostLearn for specificity prediction. We have dissected the modular architecture of complex RBPs in *Klebsiella* phages at both phage and protein level. These insights directed a standardized strategy for engineering *Klebsiella* phages and tailocins with branched RBPs, enabling cross-genus targeting capabilities. Our integrated approach to phage protein discovery and engineering not only deepens our understanding of phage biology but also paves the way for next-generation biocontrol agents.

Invited Speaker O08 *Lucia Fernandez*

Impact of temperature on endolysin LysRODI inactivation and killing dynamics of *Staphylococcus aureus* in milk

Fernandez L1, Agun S1, Lopez C1, Alvarez J1, Garcia P1

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Artisanal cheese production requires the implementation of strict control measures to inhibit growth of pathogenic and spoilage bacteria, while preserving the viability of microorganisms involved in fermentation and ripening. In this context, phage lytic proteins can be a very interesting antimicrobial choice due to their specificity, while exhibiting a broader spectrum of action than phages themselves. Several studies have demonstrated the potential of lytic proteins against *Staphylococcus aureus* in milk. However, the impact of the existing environmental conditions during treatment must be considered to maximize its efficacy. Temperature is a particularly crucial parameter in dairy production, and it is also known to influence the enzymatic activity and stability of lysins. Here, we estimated different parameters related to the killing activity and stability of protein LysRODI at different temperatures (12 ºC, 25 ºC, 32 ºC and 37 ºC). The results obtained at 12 ºC revealed good protein stability but lack of enzymatic activity and very slow bacterial growth. At the other temperatures, bacterial growth and death due to endolysin killing were estimated based on previous data. Interestingly, the killing rates estimated at the three temperatures were similar, indicating a similar enzymatic activity within the analyzed range. However, the protein displayed much greater stability at 25 ºC compared to 32 ºC and especially to 37 ºC. The estimated parameter values will be subsequently used to develop a mathematical model to further study the endolysin killing dynamics under different production scenarios, considering temperature variation during milk storage and fermentation.

Selected Speaker O09 *Danielle Peters*

Harnessing depolymerases: enhancing phage therapeutics against resistant pathogens

Lam S1, Beaudoin G1, Martynov Y1, Arbour M1, Chen W1, 2, Peters DL1

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(2). Department of Biology, Brock University, St. Catharines, Ontario (Canada)

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The Gram-negative opportunistic pathogen, *Acinetobacter baumannii*, is categorized as a priority one pathogen by the World Health Organization due to intrinsic antibiotic resistance and virulence factors. The bacterium is protected by a thick capsule polysaccharide (CPS) layer of diverse sugars that shields it from immune defenses, antibiotics, and bacteriophages. Specialized phages have evolved mechanisms to circumvent this barrier by encoding depolymerases in their tail appendages to degrade CPS and access bacterial receptors. In this novel study, we leveraged a panel of seven lytic phages from Ottawa, Canada, alongside six recombinant depolymerases, to broaden the panel host range without genetic engineering. A synergy assay demonstrated that combining varying concentrations (0.8 – 50 ng/mL) of depolymerases with 102 – 108 PFU/mL phages significantly inhibited the growth of multiple *A. baumannii* strains, outperforming high doses of phage or depolymerase alone. Area under the curve analysis showed up to 85% growth inhibition compared to controls. Remarkably, phage-depolymerase combinations achieved up to six-log increases in endpoint titre, offering a cost-effective solution to phage propagation challenges. Efficiency of plating studies further confirmed expanded host ranges in the presence of depolymerases, facilitating broader therapeutic applications without genetic engineering. Preparations for murine models are underway to study combined phage-depolymerase treatment *in vivo*. These findings highlight the transformative potential of phage-derived depolymerases across research, therapeutic, and industrial applications, paving the way to enhanced phage therapeutics with improved host ranges.

Selected Speaker O10 *Magdalena Plotka*

Practical applications of extremophilic endolysins and their derivatives

Plotka M1

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Studies on endolysins derived from thermophilic bacteriophages, although important for ***e.g.*** the protein's stability point of view, are still limited. This may be due to difficulties in accessing sites where thermophages occur, e.g. hot springs or hydrothermal vents, or difficulties in culturing extremophiles in laboratory conditions. We successfully analyzed three extremophilic type 2 amidases, one type 3 amidase, and one endopeptidase focusing on structure-function relationship. We also analyzed a number of mesophilic proteins that are structurally similar to the thermophilic endolysins. Although the temperature optimum for activity of extremophilic endolysins is around 60 °C, the proteins are also active against mesophiles at lower temperatures. MIC values have been determined for clinical strains of bacteria including *Pseudomonas aeruginosa* and carbapenem-resistant *Acinetobacter baumannii*. The activity of lysins against forming/mature biofilms and persister cells was also assessed. Although *in vitro* studies are very promising and have shown, for example, the elimination of 107 bacteria exposed to endolysins, *in vivo* studies are much more challenging. We observe inactivation of endolysins in the presence of human serum as well as their weak activity in *in vivo* models such as *Galleria mellonella*. We would like to discuss these aspects of endolysin activity during The Phage Protein Meeting.

Selected Speaker O11 *Sharon Shui Yee Leung*

Combating *Acinetobacter baumannii*-induced lung infections with phage-encoded antibacterial enzymes: promise and challenges

Wang H1, Zhang P1, Leung SSY1

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Many of the WHO identified priority bacteria, including *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae* and *Escherichia coli*, are major causative pathogens of pneumonia. It is sought-after to develop novel antibacterials and treatment strategies to combat pneumonia induced by these Gram-negative superbugs. Recently, antibacterial proteins encoded by phage, particularly endolysins and depolymerases, have emerged as novel antibacterial agents. Our team has focused on strategies to weaponize these two classes of phage-derived enzymes against lung infections induced by *A. baumannii*.
To endow endolysins with the capability of trespassing the outer membrane (OM) barrier is believed to be the key in turning the naturally existing endolysins to antibacterial weapons against Gram-negative bacteria, including the resistant strains. We employed two different strategies, (i) protein engineering and (ii) combination treatment with colistin, to boost the OM permeability of endolysins. Enhanced antibacterial efficiency was demonstrated with an engineered lysin (LysAB2-KWK) and colistin-LysAB2 combination *in vitro* and *in vivo* using an immunocompromised mice pneumonia model.
As for depolymerases, they have been widely investigated in treating bloodstream infections due to their dependency on serum-mediated bacterial killing. To extend their application to low-serum local infections, like lung infections, we reported the use of depolymerases in combination with antibiotics to achieve synergistic bacterial killing. An *A. baumannii* phage encoded depolymerase, Dpo71, was confirmed to be capable of potentiating seven commonly used antibiotics and reversing their bacterial resistance in the presence of 5% serum. The *in vivo* efficacy of a selected combination, Dpo71-ceftazidime in controlling *A. baumannii*-induced pneumonia was also validated in a mice model.
Collectively, endolysins and depolymerases have demonstrated great potential in controlling bacterial pneumonia, but significant challenges remain in translating these novel treatments.

SESSION 3: THE ESCMID STUDY GROUP FOR NON-TRADITIONAL ANTIBACTERIAL THERAPY (ESGNTA) SESSION

Selected Speaker O12 *Peter Braun*

Antibody alternatives from bacterial viruses: phage receptor binding proteins as novel diagnostic tools for bacterial infections

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Phage receptor binding proteins (RBPs) are emerging as an innovative alternative to antibodies for the detection of pathogenic bacteria. While antibody-based assays are widely used for identifying specific bacterial antigens, they often suffer from limitations in specificity, particularly due to the close genetic relationship of target pathogens to their non-pathogenic relatives. RBPs, on the other hand, have evolved over millennia to bind to specific bacterial surface receptors, providing a more precise and, a priori, real-life-tested approach for pathogen detection. We focus on the identification and engineering of RBPs that may serve as highly specific detection tools in clinical and biodefense-related settings for a wide range of pathogenic bacteria including notorious biothreat agents. To achieve this objective, we employ in silico RBP prediction tools and structural analyses to uncover diverse RBPs with most likely optimal binding capabilities. For initial testing of new RBPs, their respective genes are funnelled as synthetic open reading frames into our standardized heterologous protein production pipeline and coupled with reporter moieties, either fluorophores or chromogenic enzymes. If enrichment of target bacteria from clinical or environmental matrices is required, RBPs can be easily coupled to magnetic beads to enhance the capture and isolation of target bacteria from such complex samples. From there, options arise to improve sensitivities in diagnostic tests or to obtain pure live cultures for further study. In summary, by leveraging the unique properties of RBPs, we seek to advance the field of pathogen detection and to overcome key limitations associated with traditional (antibody-based) diagnostic methods.

Invited Speaker O13 *Tristan Ferry*

Compassionate use and clinical trial with anti-staphylococcal lysin CF-301 for prosthetic joint infections

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Prosthetic joint infection (PJI) is the most dramatic complication after joint arthroplasty. *S. aureus* and coagulase-negative staphylococci are frequently involved in patients with PJI. These bacteria could be involved in recurrence as they can produce biofilm and persist at the implant surface. Phage therapy has been developed for patients infected with *S. aureus*, but it seems to be more difficult to develop phage therapy to target coagulase-negative staphylococci. In patients with biofilm-associated coagulase-negative staphylococci PJI, the standard of care is to explant/exchange the infected prosthesis, a complex surgical procedure at risk of complication and that could be associated with bone loss, loss of function and potentially with amputation in case of iterative failures. Lysin CF-301 (also called exebacase) is an anti-staphylococcal lysin with potent bactericidal activity against coagulase-negative staphylococci and shown to disrupt mature biofilms formed by these bacteria. In 2019, we set-up at the referral centers for the management of complex Bone and Joint Infections called “CRIOAc Lyon”, a conservative approach for patient experiencing coagulase-negative staphylococci PJI called LysinDAIR, that is the intraarticular administration of CF-301 during arthroscopy, a mini-invasive surgical technic. We treated the first case as compassionate use in 2019, and a clinical trial was started but then stopped, due to the bankruptcy of the company that developed CF-301 in December 2023. In the work presented here, we report this unique experience of first consecutive applications of a phage-derivated lysin in humans, with feedback about its safety and efficacy during a long-term follow-up.

Invited Speaker O14 *John H. Rex*

Is it rational to work on phage proteins (and phage) as therapeutics? Yes! No! Carefully!

Rex, JH

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Working from the theme of “Do we want / could we make use of therapeutics focused on phage/phage proteins?”, this talk is focused on 3 ideas that I think would be most pertinent to this community. The core exam question of “Is it rational to work in this space?” is approached from the perspectives of “Yes, as we desperately need new (and novel!) therapeutic choices,”, “No, as development and economics are both very hard,”, and finally “Carefully, as the science makes sense and much is now known about the pitfalls of this area.” I hope some of you will conclude that “Carefully” is your answer – and if so, that this talk helps you get started on the path.

SESSION 4: FUNDAMENTAL RESEARCH ON PHAGE PROTEINS

Invited Speaker O15 *Carlos São-José*

Endolysins: insights into their mode of action and regulation for enhanced applications

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Gram-positive (G+) bacteria are normally considered more susceptible to the exolytic action of endolysins due to the absence of an outer membrane. However, they can still exhibit some tolerance to the lytic enzymes, especially under isotonic conditions that support growth. Previous studies have shown that this tolerance requires both an intact proton motive force (PMF) and the presence of wall teichoic acids (WTA).
We investigated how the interconnected PMF and WTA factors modulate the activity of the anti-*S. aureus* endolysin Lys11. Our findings showed that the chemical (ΔpH) and electrical (Δψ) gradients of the PMF affect Lys11's functional domains differently. Dissipation of ΔpH primarily enhanced the lytic action via the enzyme's peptidase domain, while Δψ dissipation unexpectedly boosted binding through the amidase domain. WTA acted as major tolerance determinants by inhibiting peptidoglycan (PG) cleavage and by severely impairing binding mediated by the canonical cell binding domain.
The enhanced lytic activity under PMF-collapsed conditions mirrors the natural mechanism of phage infection, where endolysins act only after holins dissipate the PMF. Supporting this, we showed that antimicrobial peptides mimicking holins eliminate PMF/WTA-mediated tolerance, greatly increasing *S. aureus* susceptibility to endolysins. Moreover, endolysins targeting G+ bacteria are often termed single-use agents due to strong binding to CW debris post-lysis. We found that removing the amidase domain can reduce binding, while improving bacteriolysis kinetics. Finally, PG-cleaving domains from virion-associated lysins (VALs), which naturally evolved to target energized cells externally, may offer alternatives to overcome tolerance.

Selected Speaker O16 *Samiya Siddiqui*

PIT3: A phage-derived protein with anti-virulence activity against the Type III Secretion System in *Pseudomonas aeruginosa*

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Pseudomonas aeruginosa is a Gram-negative, nosocomial, opportunistic pathogen capable of causing a variety of infections, particularly in immunocompromised individuals. Over the past decades, *P. aeruginosa* has rapidly developed multidrug resistance (MDR) against a wide range of antibiotics, posing a significant challenge to modern medicine. As a result, alternative therapeutic strategies targeting bacterial virulence rather than viability can be a promising approach to combat infections. One such strategy is the use of anti-virulence agents, including phage-derived proteins, to attenuate bacterial pathogenicity by disarming the pathogen without exerting selective pressure for resistance.

This project investigates the anti-virulence potential of phage-derived proteins, focusing on YuAgp37, designated as PIT3 (Phage-encoded Inhibitor of Type III Secretion System)—identified in a screen for inhibitors of the Type III Secretion System (T3SS). The study was conducted in two phases. First, candidate phage proteins were screened for their ability to reduce T3SS activity, with PIT3 demonstrating a consistent reduction in ExoS activity. Second, PIT3 was heterologously expressed in *E. coli* and purified for downstream analyses. The purified protein was then used to assess molecular interactions at both the DNA and protein levels. Electrophoretic mobility shift assays indicated concentration-dependent DNA binding, while pull-down assays were conducted to identify potential protein-protein interactions. These findings support a potential regulatory role for PIT3 and lay the groundwork for further exploration of phage proteins as anti-virulence agents in the fight against multidrug-resistant *P. aeruginosa*.

Selected Speaker O17 *Urmi Bajpai*

Bacteriophage-encoded novel endolysins targeting *Mycobacterium tuberculosis*

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Tuberculosis (TB) is a curable infectious disease. However, the lengthy treatment regimen and drug toxicity, and the multidrug-resistant infections, pose significant challenges to TB patients, causing extensive suffering and disability compounded by economic hardships. The incidence of drug-resistant non-tuberculous mycobacterial (NTM) infections is also increasing globally. Hence, there is an urgent need to discover alternative antimycobacterial solutions.
Endolysins, with their rapid and precise action against planktonic cells and biofilms, can be promising adjuncts to TB drugs and potentially reduce drug dosage and associated toxicity. We have a repertoire of mycobacteriophage-encoded endolysins (LysinA & LysinB), demonstrating varied domain architecture.
In my talk, I will share data on the endolysins we have purified as recombinant proteins and functionally characterized using biochemical and antibacterial assays such as plate lysis, log-kill and biofilm-inhibition assays. Some endolysins possess intrinsic antibacterial properties and induced cell lysis upon external application, an essential trait for natural lysins to act as antibiotics.
Also, will present biochemical and antibacterial profiling of a LysinB that shows significantly high esterase activity (5.1 U/mg), indicating catalytic efficiency, inhibits *M. smegmatis* biofilm formation by >75% and exhibits lytic activity against H37Rv and MDR strains of *M. tuberculosis* and *M. fortuitum*, making it a promising antimycobacterial candidate.

Selected Speaker O18 *Hannelore Longin*

Fold first, ask later: structure-informed protein function prediction in *Pseudomonas* phages

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Phages proteins are an inexhaustible source of inspiration for biotechnological and clinical applications. However, many more could be hiding in plain sight. Indeed, up to 70% of predicted phage proteins are annotated as proteins of unknown function. Despite significant interest in unravelling these proteins' functions, phage proteins are absent from recent large-scale structure-based efforts (such as AlphaFold database).
Here, we investigate the efficacy of structure-based protein annotation for *Pseudomonas*-infecting phages. Briefly, we collected every protein annotated as 'hypothetical/phage protein' in NCBI and of at least 100 amino acids in length, of 887 *Pseudomonas*-infecting phages. These 38,025 proteins (31% of all proteins) were then clustered into 10,453 groups of homologs. Protein structures were predicted with ColabFold and structural similarity to the PDB and AlphaFold database was assessed with FoldSeek. Of all proteins, 59% displayed significant similarity to at least one structure in these databases. We benchmarked various post-processing strategies for extracting function from these FoldSeek hits (different information resources, hit selection methods, and structure-based clustering of hits). The resulting annotations were then compared with state-of-the-art phage annotation tools Pharokka and Phold.
On average, up to 42% of the phage proteins of unknown function could be annotated using structure-based methods, depending on the post-processing strategies applied. While caution is warranted when transferring protein annotations based on similarity, these methods can significantly speed up research into new antimicrobials and biotechnological applications inspired by nature's finest bioengineers: phages.

Selected Speaker O19 *Marta Gomes*

Exploring phage early genes to combat drug-resistant *Acinetobacter baumannii*

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Acinetobacter baumannii is a critically important multidrug-resistant pathogen associated with severe hospital-acquired infections. Bacteriophages have emerged as a promising alternative; however, the mechanisms underlying phage-host interactions remain poorly understood. Early phage genes, expressed shortly after infection, play a crucial role by hijacking the host's cellular machinery to prioritise phage replication. Notably, some of these early genes can independently inhibit bacterial growth, highlighting their potential as novel therapeutic agents. In this study, we explored the interaction between two phages – a podovirus-like and a siphovirus-like phage – and their drug-resistant *A. baumannii* hosts, using transcriptomic approaches, such as RNA-seq. We have identified both phages' early genes, selected those with unknown function and individually evaluated their ability to inhibit bacterial growth. Among them, one gene of each phage exhibited a strong inhibitory effect, reducing CFU/mL counts by up to 4 logs. Additionally, although these phages can only infect their host's specific capsular type, we confirmed that these genes are effective across different *A. baumannii* strains, while remaining non-toxic to commensal species, such as *E. coli*. Further ongoing studies are focused on understanding if these genes can act synergistically.
Furthermore, uncovering the mechanisms by which phages infect their hosts is essential, not only for the development of phage-derived therapeutics but also to find essential bacterial pathways that may serve as targets. Thus, elucidating the mechanism of inhibition is equally important. Ongoing studies are focused on identifying the bacterial interaction partner of the inhibitory genes.

SESSION 5: TAILOCINS

Selected Speaker O20 *Hernán Burbano*

Tracing the evolution of tail fibers and their receptors in historical metagenomes

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Tail fibers of bacteriophages and phage tail-like elements such as R-type tailocins evolve rapidly, giving rise to extensive genetic diversity within phage and bacterial populations. This diversity is reflected in the co-existence of highly divergent tail fiber variants segregating at different frequencies. In wild populations of *Pseudomonas viridiflava* associated with *Arabidopsis thaliana*, we previously identified such divergent tailocin variants, highlighting the dynamic nature of these molecular weapons. However, the evolutionary timescale over which tail fiber turnover occurs—and by extension, the durability of bacterial sensitivity or resistance to specific tailocin variants—remains poorly understood. To investigate this, we combined the analysis of present-day *P. viridiflava* genomes with metagenomic data derived from historical *A. thaliana* herbarium specimens spanning the last 200 years. By applying ancient DNA extraction and analysis techniques, we reconstructed tailocin gene clusters and identified the tail fiber haplotypes that have segregated across centuries. Additionally, we examined the presence and absence of genes involved in the biosynthesis of tailocin receptors, focusing on components of the bacterial outer membrane, particularly the O-antigen. Our findings reveal long-term maintenance of tail fiber diversity and suggest a co-evolutionary dynamic between specific tail fiber variants and genes involved in receptor biosynthesis. This study demonstrates how historical metagenomes offer a powerful lens through which to explore microbial genetic diversity across time, uncovering the evolutionary pressures that shape tail fiber interactions in natural populations.

Selected Speaker O21 *David Baltrus*

Exploring the genetic basis of interspecies killing by tailocins

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Tailocins are generally considered to be highly specific in their targeting and killing activity, and are further thought to be used for intraspecies competition. We have previously shown that, despite previous dogma and assumptions, that tailocins produced by various *Pseudomonas* species can possess highly specific interspecies killing activity. Notably, we have shown that tailocins from *P. syringae* can target some *Salmonella* strains and that tailocins from one strain of *P. fluorescens* can target *E. coli* O157:H7. Our follow up experiments suggest that tailocins produced by *P. syringae* and fluorescens both attach to the LPS, and therefore that this broad activity persists despite highly different LPS structures in the targets. This presentation will highlight our efforts to genetically demonstrate that differences in target specificity are due to allelic variation in tailocin tail fibers and further that differences in tailocin targeting can be localized to a handful of amino acid changes within the tail fibers.

Selected Speaker O22 *Talia Karasov*

Constraints on the evolution of tailocin tail fibers in host-associated bacteria

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Pseudomonas plant pathogens encode a conserved mechanism of competition — a phage-derived element termed a tailocin, which they use to kill competitors. With a mechanism of action similar to phage, the tailocin tail fibers encode specificity and target subsets of co-occurring pathogenic strains. Surprisingly, the most lethal tailocin variants are at low frequencies in the pathogen populations and have not outcompeted their neighbors. This finding suggests the presence of a trade-off between broad-spectrum killing and an unknown pathogen fitness trait. Here we investigate constraints on the evolution of the tail fibers and killing spectrum. We find that the most lethal tailocins are associated with LPS variants that compromise *Pseudomonas* survival in the host. The pathogens have evolved a limited repertoire of tail fibers and LPS variants in part due to this trade-off, suggesting strong constraints on the evolution of new tail fiber and LPS variation

SESSION 6: PHAGE LYSINS FOR THERAPY

Invited Speaker O23 *Mathias Schmelcher*

Engineered phage lysins – tackling hurdles for clinical application

Röhrig C1, Keller AP1, Sobieraj A1, Huemer M2, Chang CC2, Mairpady Shambat S2, Keller N2, Phothaworn P3, Blanco Massani M4, Zinsli L1, Meile S1, Eichenseher F1, Shen Y1, Bernkop-Schnürch A4, Korbsrisate S3, Zinkernagel AS2, Loessner MJ1, Schmelcher M1, 5

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In the light of the current antimicrobial resistance crisis, bacteriophage endolysins have gained increasing attention as a promising class of antibacterial agents, due to their strong bactericidal activity, high specificity for their target bacteria, activity against biofilms, persister cells, and drug-resistant strains, and their low probability of resistance development. Owing to their modular architecture, endolysins and other peptidoglycan hydrolases (PGHs) can readily be engineered to create protein chimeras with novel properties. Our lab has compiled a large collection of engineered staphylococcal PGHs, which can be rapidly screened for candidates featuring desired characteristics with respect to potential therapeutic applications, such as high activity against staphylococci in human serum or intracellular environments. Despite the aforementioned advantages, systemic administration of PGHs is currently hampered by several factors such as a lack of cell-penetrating properties, insufficient accumulation at infection sites, short circulation half-lives, and immunogenicity. In order to tackle such hurdles, we further modify our pre-selected chimeric PGHs, e.g., by fusion to various functional peptides. PGHs equipped with cell-penetrating peptides (CPPs) effectively reduced intracellular staphylococci, both in vitro and in vivo; cell-penetrating homing peptides (CPHPs) were able to specifically direct PGHs to bone cells and significantly reduce *S. aureus* in a murine bone infection model; and fusion of PGHs to an albumin binding domain increased their circulation half-life and, consequently, their therapeutic efficacy. Taken together, such improvements could bring PGHs one step closer to the clinic.

Selected Speaker O24 *Lamya El Mortaji*

Antibacterial mechanism of action of Gram-negative targeting endolysin ATX401 (formerly CF-370) is primarily through N-terminal AMP rather than murein hydrolase activity

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Rapidly lytic phage endolysin is an attractive novel modality for treating serious bacterial infections. To enhance penetration into Gram-negative (GN) bacteria, these peptidoglycan (PG) cell wall hydrolase enzymes are classically fused to an antimicrobial/cell penetrating peptide (AMP/CPP). A prototype of these engineered endolysins is ATX401 (formerly CF-370), described for its broad *in vitro* activity and potentiation of meropenem in animal models [Sauve et al. JID 2024]. We investigated the *in vitro* activity and in vivo efficacy of ATX401 and deciphered the key components driving the antibacterial activity but also limiting its potency in the presence of serum.

ATX401 showed promising *in vitro* activity against carbapenem-resistant GN isolates in modified culture medium (DCAAT). However, activity was abolished by serum and efficacy in a rat lung infection model was very limited. Investigations of serum inhibition identified albumin binding rather than enzymatic degradation as the primary cause. Surprisingly, PG hydrolysis activity was maintained in the presence of serum, plus disruption of the lysin active site, while abolishing enzymatic activity, still facilitated antibacterial activity. The N-terminal CPP was subsequently shown to be as potent as ATX401, indicating limited contribution of the lysin domain to its antibacterial activity.

These findings highlight challenges that must be overcome when developing GN lysins. (1) It is crucial to confirm the antibacterial MOA and that activity of the fusion product is greater than each component alone. (2) The product should retain antibacterial activity in the presence of serum. (3) Ideally, it should show activity in standard AST protocols.

Selected Speaker O25 *Guy Hermans*

Systematic engineering of lysins yields highly potent, serum-resistant activity towards drug-resistant *Acinetobacter baumannii*

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Phage lysins offer a promising alternative to traditional antibiotics, yet their efficacy against Gram-negative pathogens in human serum remains a major limitation. Leveraging the VersaTile DNA shuffling platform, a proprietary lysin domain library, and high-throghput functional screening, we explored an expansive design space to engineer lysins with enhanced properties. This approach yielded novel constructs exhibiting potent *in vitro* activity against *Acinetobacter baumannii* and exceptional serum resistance. Broad-spectrum efficacy was confirmed across clinical isolates, and safety profiling demonstrated no cytotoxicity in blood cells, cell lines, or animal models. Lead candidates underwent further optimization for potency, stability, and manufacturability, with top performers advancing towards *in vivo* efficacy studies and preclinical development. These engineered lysins represent a significant step towards the development of systemically active, next-generation antimicrobials for use in multidrug-resistant infections.

Selected Speaker O26 *Tim Thysens*

Endolysins as therapeutics: bridging preclinical development and clinical manufacturing

Thysens, T1

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Selected Speaker O27 *Hak-Kim Chan*

Formulations of inhalable endolysin Cpl-1 and their efficacy in a murine lung infection model

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Endolysin Cpl-1 is known to have antimicrobial activity against *Streptococcus pneumoniae*, which causes lung infection. Administration of Cpl-1 as an inhalation aerosol to the lungs will target the infection directly, potentially minimizing the dose required and adverse effects, while optimizing the efficacy. We have previously shown that Cpl-1 remained stable when aerosolized by vibrating mesh nebulization, which confirmed the feasibility to nebulize endolysins for inhalation delivery. We have recently further investigated the efficacy of inhaled Cp1-1 powder and liquid formulations to treat *S. pneumoniae* in a murine lung infection model. Single treatment of Cpl-1 liquid or powder (40 µg per animal, n=4) decreased the pulmonary bacterial load by approximately 1-log. In contrast, a combination liquid formulation containing Cpl-1 and another endolysin Pal (20 µg each per animal) induced a 2-log reduction of pulmonary bacteria counts in mice. The results provide proof-of-concept that inhalable endolysin formulations can treat bacterial lung infections, and that combining endolysins may enhance antimicrobial activity compared to monotherapy.

Selected Speaker O28 *Niels Vander Elst*

Bacteriophage-derived endolysins restore antibiotic susceptibility in β-lactam- and macrolide-resistant *Streptococcus pneumoniae* infections

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Streptococcus pneumoniae causes serious infections worldwide, including bacteremia, pneumonia, and meningitis, with the latter often leading to long-term neurological damage. Rising resistance to key antibiotics such as β-lactams and macrolides underscores the urgent need for novel therapies. This study investigates the bacteriophage-derived endolysin Cpl-1 as a potential adjunct to antibiotic treatment. Cpl-1 was tested in human blood and cerebrospinal fluid (CSF) against multidrug-resistant clinical strains, showing potent bactericidal activity. When combined with penicillin or erythromycin, Cpl-1 displayed synergistic or additive effects, restoring antibiotic efficacy. In differentiated SH-SY5Y human neuronal cells infected with pneumococci, these combinations significantly reduced bacterial load and protected neurons from cytotoxic damage. Using a transwell system with human endothelial cells, Cpl-1 demonstrated the ability to cross the blood-brain barrier, a finding corroborated in a mouse model of bacteremia-derived meningitis. Systemic Cpl-1 treatment, particularly in combination with penicillin, cleared bacteria from the brain, improved survival, and prevented disease symptoms. These results highlight Cpl-1 as a promising adjunctive therapy that enhances antibiotic performance, combats resistant pneumococcal strains, and effectively treats meningitis. For press release, see: https://news.ki.se/researchers-restore-antibiotic-effect-in-the-event-of-resistance.

SESSION 7: PHAGE RECEPTOR-BINDING PROTEINS: BASIC RESEARCH

Selected Speaker O29 *Rafal Mostowy*

Unravelling the host range of *Klebsiella* phages: depolymerases and beyond

Koszucki J1, Panicker V1, Otwinowska A2, Olejniczak S2, Leconte J1, Holt KE3, Enright M4, Feil EJ5, Rocha EPC6, Smug B1, Maciejewska B2, Drulis-Kawa Z2, Mostowy R1

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Understanding the molecular basis of host range in *Klebsiella pneumoniae* phages is key to elucidating phage–bacteria co-evolution and guiding both ecological and therapeutic applications. Here, I present two complementary approaches focused on receptor-binding proteins (RBPs), each rooted in a distinct phage lifestyle. First, leveraging the lysogenic cycle, we conducted a genome-wide association study (GWAS) across an ecologically representative dataset of 2,527 *Klebsiella* genomes to identify prophage-encoded RBPs associated with capsule type. While classical beta-helix depolymerases were confirmed for a number of K-types, many GWAS hits belonged to enzymatically distinct families, notably SGNH hydrolases, suggesting capsule modification—not only degradation—may underlie infection. Second, we analyzed 192 lytic phage genomes sequenced during host range experiments. Here, via structural modelling, we found that depolymerases are mosaics in sequence and structure, enabling diverse enzymes to target the same capsule, and single enzymes to recognise multiple capsules. Furthermore, while most viruses carried expected depolymerases, 19% of them (n=36) lacked classical depolymerase folds, instead encoding SGNH hydrolyses or previously uncharacterized RBP structures. Altogether, these results point to a broader, underappreciated complexity in RBP–capsule interactions, underscoring the need for integrative approaches to predict and engineer phage host range.

Selected Speaker O30 *Veronika Theresa Lutz*

Gp38 adhesins of *Straboviridae* phages target specific extracellular loops of outer membrane receptors

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Phages recognize their hosts by binding bacterial surface receptors with receptor-binding proteins (RBPs). The RBP Gp38 of *Straboviridae* phages is particularly noteworthy due to its modular structure featuring conserved glycine-rich motifs (GRMs) interspersed with hypervariable segments (HVS), which enable the phage to interact with outer membrane protein receptors via their extracellular loops. Through a targeted isolation approach, we established a collection of *Straboviridae* phages from three different genera: *Tequatrovirus*, *Mosigvirus*, and *Krischvirus*, all expressing the Gp38 adhesin protein. We identified the outer membrane proteins OmpA, Tsx, OmpF, and TolC as phage receptors and found that the similarity of Gp38 adhesin proteins correlated with receptor recognition. Focusing on OmpA, we combined *in silico* adhesin-receptor binding predictions, phage plaque assays on *E. coli* expressing OmpA variants with diverse outer loop sequences, and a host range analysis across the diverse *E. coli* reference collection (ECOR). Our findings show that Gp38 variants encoded by phages FL08 and AV119 bind to many OmpA outer loop variants. In contrast, the Gp38 of phages FL12, FL18, and FL20 bind only to a limited number of outer loop variants, resulting in a narrower host range. Finally, phylogenetic analysis of Gp38 revealed distinct groups corresponding to their bacterial receptors, facilitating future receptor predictions for phages.
In summary, we identified specific molecular interactions involved in host binding of phages in our *Straboviridae* phage collection. The phylogeny of Gp38 allows prediction of receptors of *Straboviridae* phages, which is crucial for the efficiency of phage therapy applications.

Selected Speaker O31 *Celia Ferriol-González*

Receptor-binding protein evolvability is a major determinant of phage host range in capsule-diverse hosts

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Capsulated bacterial hosts usually have a broad capsule diversity, representing the major determinant for phage infectivity. Phage receptor-binding proteins (RBPs) recognize surface receptors, as the bacterial capsule. This interaction is essential to initiate the infection process. Here, we designed an experimental evolution approach to explore host range adaptation in a diverse 12-phage community interacting with a *Klebsiella* spp. population containing 39 distinct capsular types. Phages included were diverse in genomic and host range terms. Our findings revealed RBP-evolution as a key driver for host range modification. Generalist phages had highly evolvable RBPs that accumulated non-synonymous mutations modulating their host range. On the contrary, specialist phages acquired fewer mutations, maintaining their narrow host range. Additionally, recombination between co-infecting closely related phages promoted rapid host range modification through RBP swapping. This study advances our understanding of phage host range evolution and provides new insights for optimizing phage-based applications.

Selected Speaker O32 *James Hodgkinson-Bean*

Exploring flagellotropic phage adsorption by cryoEM

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Bacteriophages of the class *Caudoviricetes* infect bacterial hosts through deployment of specialized tail machinery capable of adsorption and penetration of host bacterial cell walls. The mechanism of adsorption and penetration has been studied extensively using model *Enterobacteriaceae* phages such as T4, T7, P22, P1 and λ. These model phages primarily adsorb initially to the bacterial cell wall, binding to LPS or a protein-based surface receptor. While highly informative, these studies largely neglect flagellotropic bacteriophages. Flagellotropic bacteriophages adsorb initially to bacterial flagella, followed by translocation and re-adsorption to the host cell wall. To date, much remains unknown regarding these flagellotropic processes. We decided to investigate this process using a known flagellotropic bacteriophage.

We used cryogenic electron microscopy (cryo-EM) single particle analysis to investigate the structure of the flagellotropic virion, with a focus on flagella binding fibers in the context of the pre-contraction baseplate. Due to the unique conformation of the fiber, we were able to reconstruct most fiber regions to near atomic resolution (2.9-3.5 Å). Using these reconstructions, we were able to identify flagella binding proteins and analyze their structure. Considered with negative stain data and previous literature, these structures allow us to put forth a hypothetical model regarding flagella adsorption and translocation.

Selected Speaker O33 *Mark J. van Raaij*

Crystallographic structures of bacteriophage receptor-binding proteins and endolysins

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Most bacteriophages recognize their host cells via specialized spike or fibre proteins. The overall goal of our group is to determine their structures in complex with their natural receptors. We also study the high-resolution structures of endolysin proteins, involved in bacterial wall degradation.
Bacteriophage epsilon15 infects *Salmonella enterica* subspecies *enterica,* serovar Anatum A1. This bacterium has a lipo-polysaccharide O-antigen consisting of units of D-O-acetyl-galactose-alpha1-6-D-mannose-beta1-4-L-rhamnose. Lysogenic epsilon15 blocks acetylation of the O-antigen and favours production of beta-linked O-antigen. The receptor-binding fibre protein is gp20. Gp20 consists of an N-terminal triple coiled-coiled virus binding domain (not resolved in our structures), a central beta-helix domain with O-antigen hydrolysis activity, a lectin domain and a C-terminal esterase domain. The O-antigen hydrolysis and esterase activities were proven by mutational analysis and NMR spectroscopy. The O-antigen hydrolysis proceeds through an inversion mechanism. The importance of the esterase activity for infection is unknown.
The RBP of bacteriophage S24-1 has been identified as orf16, a 642 amino acid protein. We have solved the structure and shown it is very similar to the RBP of Staphylococcus phages phi11 and P68. Teichoic acid may be a receptor for the protein, and we were able to co-crystallize the protein with a teichoic acid analogue.
Pseudomonas phage JG004 endolysin Pae87 is a monomodular lysozyme. We solved its structure without and with a peptidoglycan fragment NAG-MurNAc-LAla-DGlu, suggesting that a region of the muramidase domain functions as a de facto cell wall binding domain.

POSTER PRESENTATIONS

Poster Presentation P01 *Li Cheng*

Exploring molecular determinants of *Klebsiella* bacteriophage host range

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Klebsiella pneumoniae is classified as a critical multidrug-resistant (MDR) pathogen. To tackle the rising antibiotic resistance, bacteriophages – natural predators of bacteria are being developed as adjunct therapies. However, the therapeutic capacity is often limited by narrow host ranges. This limitation is primarily due to the specificity of phage recognition, which depends on the interaction between the phage receptor binding proteins - RBPs (e.g. tail fiber or tail spike) and bacterial receptors (e.g. capsular polysaccharides (CPS) and lipopolysaccharides (LPS)). Here, this research aims to explore two common types of paired phage-host interactions (RBP-CPS and RBP-LPS) by expressing, characterizing and engineering *Klebsiella* phage RBPs, and by comparing their genetic and structural features related to specificity. It is anticipated that this research will bring new insights into phage-host interaction providing the groundwork for their therapeutic use.

Poster Pitch P02 *Robby Concha-Eloko*

Unlocking data in *Klebsiella* lysogens to predict capsular type-specificity of phage depolymerases

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Capsular polysaccharides act as a primary barrier against phage infection in many clinically relevant bacteria, including *Klebsiella pneumoniae*. To overcome this defense, most phages encode depolymerases that specifically degrade the capsule. However, the extensive diversity and frequent horizontal gene transfer of both capsules and depolymerases hinder efforts to predict phage infectivity from sequence data alone.
To address this limitation, and the scarcity of lytic phage data, we leveraged genomic information from *Klebsiella* lysogens, retrieving prophage-encoded depolymerase domains alongside host K-locus (KL) types. KL tropism was framed as a multi-label classification task using a two-tiered machine learning framework. KL-specific binary classifiers were first trained to isolate depolymerase features linked to individual KL types. Their outputs were then integrated by a recommender system to rank the most likely KL targets. Two complementary strategies were applied: a directed acyclic graph-based model (TropiGAT) and a sequence clustering-based model (TropiSEQ).
Both approaches showed strong predictive performance and transferred well to lytic phages, particularly for KL types prevalent in clinical settings. The combined models enabled the construction of a comprehensive database linking depolymerase domains to capsular types.
These results show that prophage-encoded information can be mined to model phage-host specificity at the subspecies level, supporting phage design and capsule-targeted applications in therapeutics and biotechnology.

Poster Presentation P03 *Maria João Costa*

Phage driven diagnostics: a novel RBP for the Targeted Detection of Pseudomonas aeruginosa

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Pseudomonas aeruginosa is a highly adaptable Gram-negative pathogen and a leading cause of healthcare-associated infections, including pneumonia, wound infections, and bacteraemia. Its intrinsic resistance to antibiotics, coupled with its ability to persist in diverse and hostile environments, presents a major clinical challenge. Current diagnostic methods are often slow and lack specificity, underscoring the need for rapid, reliable detection tools.
Phage-derived receptor binding proteins (RBPs) offer a powerful alternative for pathogen detection, combining high specificity with modularity and ease of engineering. In this study, we characterised a novel RBP from the *P. aeruginosa*-infecting phage PAO1_E5.1 as a tool for rapid bacterial identification. Bioinformatic analyses (BLASTp, HHpred, Pfam, InterProScan, Motif Search) identified potential genes in the phage E5.1 genome responsible for *P. aeruginosa* binding. Those genes were fused to the fluorescent protein AceGFP and heterologously expressed in *Escherichia coli* BL21 for functional analysis. Using epifluorescence microscopy, one of those genes confirmed to encode a protein with strong, specific binding to multiple *P. aeruginosa* strains, with no detectable cross-reactivity against *Salmonella enterica*, *Klebsiella pneumoniae*, *Listeria monocytogenes*, or *Staphylococcus aureus*.
The high specificity of the phage E5.1 RBP paves the way for its integration into next-generation biosensors and clinical diagnostics, representing a valuable tool for timely infection control of *P. aeruginosa* and improved antimicrobial stewardship.

Poster Presentation P04 *Dorien Dams*

RBP grafting and rebooting of engineered *Klebsiella* phages with an altered host range

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The limited host range of many *Klebsiella* phages, driven by high capsular diversity, has led to the evolution of branched receptor-binding protein (RBP) systems that expand host specificity. These systems have a highly modular architecture, allowing rapid host adaptation through horizontal domain swapping. We investigated this modularity in Klebsiella phages K11 and KP32, which possess a dual RBP system with a branching-docking structure. RBP1 includes an N-terminal anchor-branching domain linking the variable C-terminal receptor-binding domain (RBD1) to the phage tail. RBP2 carries a conserved N-terminal docking peptide (CP) that attaches a second RBD2 with different specificity to the RBP1 branching domain. Using the VersaTile technique, we swapped full-length RBPs as well as RBDs at both positions between the closely related phage scaffolds K11 and KP32. Most engineered phages showed the expected host specificity switch. The KP32 CP domain demonstrated autonomous function, proper folding, and interchangeability, confirming its structural role as a docking domain in branched RBP systems. Our findings highlight the modularity of dual RBP systems in *Klebsiella* phages and demonstrate how rational RBP swapping can mimic natural host adaptation, offering a Lego-like approach to engineer phages with altered host ranges.

Poster Pitch P05 *Ilaria De Angelis*

Structural and functional analysis of K1- and K2-specific depolymerases for targeted therapy against multidrug-resistant *Klebsiella pneumoniae*

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Phage infection begins with receptor-binding proteins (RBPs) attaching to specific receptors on the bacterial surface. In *Klebsiella pneumoniae*, the capsular polysaccharide is the primary receptor for attachment. *Klebsiella* phage RBPs include serotype-specific enzymatic domains, called depolymerases, that degrade the capsule, enabling phage adsorption and genome entry. The *K. pneumoniae* capsule is a key virulence factor that inhibits phagocytosis. its removal by depolymerases attenuates virulence and promotes immune clearance. Due to their specificity, rapid action, and efficacy at low doses, depolymerases are promising antivirulence agents. *K. pneumoniae* displays high capsular diversity, with K1 and K2 among the predominant hypervirulent K-loci. 26 depolymerases (12 K1- and 14 K2-specific) were selected from: 1) the genomes of monospecific phages, 2) from the literature with confirmed depolymerase activity, 3) prophage genomes. After clustering by sequence identity, 10 representatives (5 K1 and 5 K2) were chosen for evaluation of enzymatic activity, production yield, and physicochemical stability to identify the best-performing depolymerase with therapeutic potential. To further understand specificity determinants, sequence–structure differences between K1- and K2-specific depolymerases were explored, revealing that the selected K1 depolymerases possess an insertion domain absent in the selected K2 variants. This structural distinction is also reflected in the sequence based phylogenetic analysis, where K1 and K2 enzymes form separate evolutionary branches. This study aims to identify the molecular determinants of depolymerase serotype specificity, which is essential to develop phages and depolymerase-based therapies.

Poster Presentation P06 *Ana Hernanz-Grimalt*

Functional and structural characterization of a novel receptor-binding protein from a *Klebsiella pneumoniae* phage

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Klebsiella pneumoniae is an opportunistic pathogen with significant clinical relevance, against whom phages have been proposed as a promising therapeutic strategy. Due to *K. pneumoniae*'s highly diverse capsular polysaccharide, phage receptor-binding proteins (RBPs) with depolymerase (Dpo) activity are key determinants of host range. Most *Klebsiella* spp. phages have a narrow host range, conferred by specific RBPs with a right-handed Dpo domain. However, some phages capable of infecting several capsular types have also been found. These phages carry structurally different RBPs that seem to contribute to a broader host range. In this work, we sought to gain further insights into how phages with broad spectrum target the capsule of its host by expressing the RBP from Klebsiella phage vB_Kpn_K50PH164C1 capable of infecting several capsular serotypes (K43, K50, K53, K61, K69). The full-length protein and a N-terminally truncated version were produced in recombinant form and purified via size exclusion chromatography (SEC), and structural studies were conducted by X-ray crystallography and cryo-electron microscopy. Even though this protein was predicted to have depolymerase activity, experimental approaches proved otherwise. However, the functional and structural characterization of this novel RBP will improve the feasibility of detecting depolymerase activity using phage genome sequences by expanding the database with experimentally validated data. In addition, it will provide insights into phage–host interactions, which is crucial to design personalized treatments against *Klebsiella* spp.

Poster Presentation P07 *Zuzanna Kazmierczak*

Structural and functional characterization of phage protein orf096: A novel anti-MRSA agent

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The rising threat of antimicrobial resistance, particularly in methicillin-resistant *Staphylococcus aureus* (MRSA), urgently requires new therapeutic approaches. This study characterizes orf096, a bacteriophage protein with promising antibacterial properties against drug-resistant *S. aureus* strains.
Recombinant His-tagged orf096 was produced and purified, with phage baseplate localization confirmed via immunogold electron microscopy. Orf096-specific antibodies significantly inhibited phage-mediated bacterial killing, confirming its essential lytic role. Time-kill assays demonstrated potent antibacterial effects, substantially reducing viable *S. aureus* cell counts within 24 hours across all tested strains, including MRSA. Preliminary SEM studies confirmed *S. aureus* biofilm formation on sapphire discs using cryo-EM technique, with orf096's impact on biofilm formation subsequently evaluated. AlphaFold modeling revealed fiber-like architecture, while bioinformatics identified N-acetylglucosamine and rhamnose substituents of wall teichoic acids within the bacterial cell wall structure as likely binding targets. No cytotoxic effects against human endothelial cells were observed.
These results establish orf096 as a functionally critical phage protein with potent, selective antibacterial activity against multidrug-resistant S. aureus. The protein's apparent specificity for bacterial cell wall components, combined with its lack of mammalian cytotoxicity, positions it as a promising candidate for developing novel antimicrobial therapeutics.
This work was supported by the National Science Centre in Poland grant 2023/51/D/NZ7/02145.

Poster Presentation P08 *Agnieszka Latka*

From puzzle to picture: Dissecting phage depolymerase–bacterial capsule interactions via modular and amino acid engineering of *Klebsiella* phage RBP KP32gp38

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Although phage biology and its clinical potential have advanced considerably, phage specificity remains poorly understood and largely reliant on empirical testing. This study explores the receptor-binding protein (RBP) KP32gp38 of Klebsiella phage KP32, which features a rare C-terminal tandem composed of a carbohydrate-binding module (CBM) and a lectin-like (LEC) domain. Through the creation and analysis of an extensive set of RBP truncations, mutants, fusions, domain swaps, and engineered phages, we systematically dissected the roles of these modules in trimerization, substrate binding, and specificity. Our results demonstrate that the LEC domain is essential for trimerization, while the CBM domain is crucial for enzymatic activity and capsule binding. The CBM–LEC tandem cannot be easily replaced without compromising the binding capacity and enzymatic activity, whereas the trimerization process appears more tolerant to structural changes. Engineered phages lacking these domains confirmed the necessity of both CBM and LEC for full functionality. Notably, two conserved residues within the LEC domain in the region facing the CBM—W492 and F494—were identified as critical for capsule binding and trimerization, respectively. Interestingly, a W492I mutation had no drastic impact on phage infectivity, suggesting that strong binding affinity may not be essential for successful phage infection. This study provides valuable insights into the molecular determinants of phage–host interactions and offer a foundation for advancing phage-based antibacterial strategies.

Poster Presentation P09 *Jintao Liu*

Essential phage component induces resistance of bacterial community

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Despite extensive knowledge on phage resistance at bacterium level, the resistance of bacterial communities is still not well-understood. Given its ubiquity, it is essential to understand resistance at the community level. We performed quantitative investigations on the dynamics of phage infection in *Klebsiella pneumoniae* biofilms. We found that the biofilms quickly developed resistance and resumed growth. Instead of mutations, the resistance was caused by unassembled phage tail fibers released by the phage-lysed bacteria. The tail fibers degraded the bacterial capsule essential for infection and induced spreading of capsule loss in the biofilm, and tuning tail fiber and capsule levels altered the resistance. Latent infections sustained in the biofilm despite resistance, allowing stable phage-bacteria coexistence. Last, we showed that the resistance exposed vulnerabilities in the biofilm. Our findings indicate that phage lysate plays important roles in shaping phage-biofilm interactions and open more dimensions for the rational design of strategies to counter bacteria with phage.

Poster Presentation P10 *Ivana Mašlaňová*

Structural engineering of staphylococcal *Kayvirus* using CRISPR-Cas10 for imaging and biosensing

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Recent advances in CRISPR-Cas genome editing have expanded the scope of bacteriophage engineering, enabling innovations in medicine, nanotechnology, and synthetic biology. While staphylococcal phage genomes have been modified previously, targeted structural protein engineering has remained largely unexplored. Here, we used a two-strain strategy combining homologous recombination with CRISPR-Cas10-assisted counter-selection to insert a poly-histidine tag into a surface-exposed, flexible loop of the tail sheath protein of Staphylococcus phage 812h1 (*Kayvirus*). Guided by structure–function relationships, the site was chosen to preserve assembly and infectivity. The His-tag enabled antibody-mediated detection and precise functionalization of the phage surface. Functional validation by bio-layer interferometry, ELISA, flow cytometry, and fluorescence microscopy confirmed that the modification maintained biological activity. The labeled phages were effectively immobilized on biosensor platforms, visualized microscopically, and applied to flow cytometric bacterial identification. These findings underscore the importance of high-resolution structural data for reporter tag design and establish CRISPR-Cas10-mediated structural engineering of *Kayvirus* phages as a versatile platform for generating application-specific viral particles for diagnostics, biosensing, and potentially therapeutics.
Acknowledgment: Supported by the National Institute of Virology and Bacteriology (Programme EXCELES, ID Project No. LX22NPO5103), funded by the European Union–Next Generation EU and MZ CR (NU22-05-00042).

Poster Presentation P11 *Melinda Mayer*

Variant tail fibers in prophages of a human gut pathobiont

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The sulfite-reducing anaerobe *Bilophila wadsworthia* is a common member of the human gut microbiome. It produces hydrogen sulfide and has been associated with inflammatory conditions, including ulcerative colitis, colorectal cancer and liver steatosis. However, it is also frequently found in healthy subjects, suggesting that bacterial lifestyle factors such as abundance, metabolic activity, or interaction with the diet may be associated with its harmful effects. Understanding and controlling *B. wadsworthia* populations is crucial to clarify its role in disease and to develop potential therapeutic strategies.

To explore phage-mediated control methods, we induced prophages using mitomycin C and observed long-tailed phages in most *B. wadsworthia* strains tested via electron microscopy; some strains also released tailocins. Genome analysis indicates that *B. wadsworthia* strains carry multiple prophages, which show interesting variation in their predicted tail fibres. Some prophages carry single annotated tail fibre genes, while others have two to three, with similar amino acid sequences interspersed by variable regions. Some tail fibres are conserved between strains, while others appear unique. The diversity in tail fibre proteins identified presents opportunities for future engineering of tailocins to target virulent strains. These could be used to design therapeutic interventions which could function in the competitive environment of the gut microbiome, enabling precise modulation of *B. wadsworthia* populations.

Poster Presentation P12 *Valeria Napolitano & Flavia Squeglia*

Structural insight into the CPS degradation machinery of bacteriophages against *Klebsiella pneumoniae*

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The ESKAPE pathogens (*Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Enterobacter* species) are the leading cause of nosocomial infections worldwide. Among the others, *K. pneumoniae* is particularly concerning due to its ability to develop multidrug resistance and hypervirulent genotypes. The bacterial capsule, composed of capsular polysaccharides (CPS), protects the microorganism from phagocytosis and directly disrupts the vulnerable host response system and thus is a major virulence factor. Phage-encoded depolymerases represent a promising therapeutic approach being able to hydrolyze the bacterial CPS and expose the bacteria to the host immune system.
Over the years, we have characterised several phage-originated depolymerases acting against *K. pneumoniae*. Aiming to a thorough understanding of serotype specificity of these enzymes, we combine x-ray crystallography, CPS degradation assays, mass spectrometry and computational analyses to identify the mode of action of depolymerases acting against different serotypes. We also show that the CPS degradation product can induce the maturation and differentiation of Monocyte-Derived Dendritic Cells which, in turn, induce lymphocyte proliferation and Th polarisation. Altogether, our findings provide structural insights into the CPS degradation machineries against *K. pneumoniae* and a strong basis for considering depolymerases as potent anti-virulence agents, due to their dual role in depleting bacteria of their CPS shield while also releasing CPS fragments that stimulate the host immune response.

Poster Presentation P13 *Victor Németh*

PhaRBP: A comprehensive and searchable receptor-binding protein database for phage research

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Bacteriophage therapy, the application of viruses that target specific bacteria, has known a renaissance in the last decade to treat antibiotic resistant infections. However, the scale-up process of this medical application proves difficult. The specificity of phages, often species-level, necessitates for a time-intensive screening round for each patient. Recent advancements in modular receptor-binding protein (RBP) design and synthetic biology have the potential to alleviate this problem by engineering the main proteins responsible for phage-specificity. However, RBP annotations in current genome and protein databases are often missing or wrong. This project builds the foundation for further RBP-research by providing the first phage RBP database (PhaRBP). This open-source web-searchable database offers a standardized and complete collection of phage RBPs. The collection process is automated and starts from UniProt and Genbank, where all phage related proteins and genomes are extracted, these are scanned for annotations and simultaneously put through current state-of-the-art RBP detection tools. The platform enables filtering by detection method, host, taxonomy, domain architecture, and class. Alphafold3 or ESMfold structure predictions are made and added for the proteins in the database, streamlining RBP-research. The first application of this database has been to build a new state-of-the-art RBP-detection tool, RBPdetect2, that is able to differentiate between the two types of RBP's, tail spike and tail fiber proteins, a task in which most current tools fail. It has been able to identify new RBPs that were not detected by the other tools. RBPdetect2 is now a feature of PhaRBP.

Poster Presentation P14 *Victor Németh*

ProteinTinder: An interactive platform for intuitive protein structure annotation

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Protein structure predictions tools such as AlphaFold3 and ESMFold have advanced protein research significantly. However, analyzing and manual annotating protein structures is a time-intensive process. To reduce the friction in this process, 'ProteinTinder' an open-source mobile-friendly web-based platform for visual and intuitive annotation of protein structures, has been designed. The platform enables easy domain annotation, domain correction and protein filtering by swiping. The backend is powered by a MySQL database managed by Python package Django, allowing for robust data-storage and management. User-authentication allows for easy sharing of annotations between users and standardized export options allow for easy processing of the results. What started off as a small side-project has become a polished tool that significantly speeds up the process of protein annotation.

Poster Pitch P15 *Aleksandra Otwinowska*

Prophage-derived depolymerases as a promising source of capsule-targeting tools against *Klebsiella pneumoniae*

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Klebsiella pneumoniae remains a major clinical threat due to its extensive capsular diversity and resistance to treatment. One promising antivirulence strategy involves phage-derived depolymerases, enzymes that specifically degrade capsular polysaccharides (CPS). While lytic phages have been the primary focus of depolymerase discovery, prophages embedded within bacterial genomes represent a largely unexplored but potentially rich source of such enzymes.
We mined prophage regions from 99 *K. pneumoniae* strains (KASPAH collection), identifying 469 candidate proteins that met strict criteria of amino acids length, tail fiber/spike annotation, and lyase or hydrolase domain found. Based on AlphaFold2 modelling, we clustered 124 protein groups, with only 32 clusters displaying parallel β-helix fold typical of depolymerases. 50 high-confidence candidates for experimental validation were chosen from these 32 clusters. We identified 14 enzymatically active depolymerases targeting 11 distinct KL-types, including KL23, KL28, KL32, KL38, KL46, KL52, KL60, KL62, KL64, KL127, and KL143. Remarkably, 9 of these capsule types had not previously been associated with any known depolymerase. Several enzymes showed activity against non-host serotypes, potentially reflecting historical serotype swaps in the prophage host strains.
The remaining proteins could not be expressed or lacked activity, likely due to accumulation of mutations in prophage genomes.
Our results highlight both the potential and the limitations of prophage-derived depolymerases and emphasize the importance of combining computational mining with experimental validation.
Project supported by National Science Center grant Sonata-BIS10 2020/38/E/NZ8/00432.

Poster Presentation P16 *Leonie Reetz*

Rapid and specific detection of pathogenic bacteria using recombinant receptor binding proteins of bacteriophages

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For infections caused by highly pathogenic bacteria, such as *Bacillus anthracis* or *Yersinia pestis*, timely antibiotic therapy for infected patients is paramount. To ensure the correct treatment regimen, rapid and unambiguous pathogen detection is essential. While PCR is the gold standard for diagnostics of most infectious diseases, antibody-based assays that detect specific antigens of the pathogen are commonly used for rapid POC testing or as confirmatory methods in diagnostic laboratories. Nevertheless, antibodies often feature insufficient specificity due to the high degree of relatedness of these pathogens to their non- or less pathogenic relatives. Receptor binding proteins (RBPs) of bacteriophages, which mediate recognition and binding to host bacteria, represent a promising alternative to antibodies. Here, we identified RBPs derived from various phages targeting *Bacillus anthracis* and *Yersinia pestis*. These RBPs were engineered into bio-probes and recombinantly expressed, incorporating fluorescent proteins or enzymes to enable specific detection of the target pathogens. Additionally, the RBPs were coupled to magnetic beads to serve as highly specific capture molecules for the enrichment and isolation of bacterial pathogens from different matrices.
We are currently expanding our library of RBP bio-probes to specifically target other pathogens of interest, including *Burkholderia* spp., *Klebsiella pneumoniae*, and *Mycobacterium tuberculosis*.

Poster Presentation P17 *Maria Stroyakovski*

Phage specific to K16 capsular-type *Klebsiella pneumoniae* encodes capsule depolymerases with equivalent specificities

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Klebsiella pneumoniae is a common opportunistic pathogen associated with hospital and community acquired infections, as well as the leading cause of neonatal-sepsis in low- and middle-income countries (LMICs). One of the main virulence factors of *K. pneumoniae* are capsular polysaccharides (CPS), which can be degraded by polysaccharide-degrading enzymes encoded by their natural predators – bacteriophages (phages). Here we have isolated novel phages against K16 capsular-type strains of *K. pneumoniae*, derived from neonatal blood stream infections (BSI). We show that all of the phages isolated require the K16 capsule for infection, and a subset can infect all *Klebsiella* K16 strains tested, despite their varied source location (Malawi and UK), pointing towards their suitability for phage-typing. Putative depolymerases were identified in the K16- specific phages, of which two novel capsule depolymerases, K16-Dep46 and K16-Dep47 from bacteriophage MSKp6, were further characterised. To the best of our knowledge, we describe here the first K16 specific depolymerase, as well as the first instance of a *K. pneumoniae* phage carrying two depolymerases with the same capsule specificities. Pre-treatment with the K16-specific depolymerases enabled phage infection of previously ineffective phages, highlighting their potential to be used synergistically, which was further confirmed in liquid culture assays.

Poster Presentation P18 *Seila Agun*

Understanding the role of amidase domains in bicatalytic staphylococcal endolysins

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Endolysins targeting *Staphylococcus* often exhibit modular architectures combining multiple enzymatically active domains (EADs), such as CHAP and amidase domains. While amidases have been described as enhancing muralytic activity (particularly through increased substrate binding), conflicting reports show that their deletion can enhance lysis efficiency in some cases. In this work, we explored the structural and functional diversity of amidase domains in staphylococcal endolysins. We analyzed a curated set of sequences from the PhaLP database and used phylogenetic clustering and AlphaFold-based structure predictions to compare amidases from monocatalytic and bicatalytic lysins. To test their functional relevance, we constructed different lysin libraries using VersaTile assembly and screened them against several staphylococcal strains using a high-throughput halo assay. Additionally, turbidity reduction assays were performed to quantitatively evaluate lytic activity in suspension.
Differences in activity and architecture patterns revealed that amidase function depends on domain context and structural arrangement. These results support a context-dependent role for amidases and raise the hypothesis that, in some cases, they may act as a regulatory element, modulating enzymatic activity in response to environmental cues during phage-induced lysis. Overall, this work provides new insights into the diverse functions of amidase domains in endolysins and lays the groundwork for the rational engineering of more effective lysins for antimicrobial therapy.

Poster Presentation P19 *Marta Barbosa*

A Deep Dive into Phage Lytic Machinery: Structural and Bioinformatic Profiling of Lytic Proteins from Newly Isolated Pseudomonas aeruginosa Bacteriophages

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Antimicrobial resistance (AMR) is a major global health threat, with *Pseudomonas aeruginosa* as a leading cause of healthcare-associated infections and high mortality in immunocompromised patients. Bacteriophages and their lytic proteins are emerging and promising alternatives to conventional antimicrobials.
This study focused on the characterization of 10 newly isolated *P. aeruginosa* phages and performed an in-depth comprehensive bioinformatic analysis to investigate the genomic and structural features of their lytic cassettes.
Morphological characterization via transmission electron microscopy, revealed five siphoviruses, four myoviruses, and one podovirus. Four phages followed a temperate life cycle and the remaining six showed varying lytic spectra profiles, with the broadest-range phages infecting >50% of tested clinical strains. Whole-genome sequencing uncovered genomes ranging from 39.5 to 93.4 kb, with no detectable virulence genes.
An extensive bioinformatic analysis of their lytic cassettes using structural homology-based tools and searching (among others) for conserved domains, predicted structures, transmembrane regions and membrane topologies, revealed considerable diversity in endolysin enzymatic domains, holins from distinct families with differing architectures, and multiple spanin organizations.
These findings underscore the structural and functional heterogeneity of phage lytic systems and provide a valuable reservoir of lytic proteins with high potential for phage-derived antimicrobials targeting multidrug-resistant *P. aeruginosa*.

Poster Presentation P20 *Meagan Beatty*

A deep dive into the marine halophilic viral glycoside hydrolase endolysin guided by sequence-based viromics

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Extremophilic marine viruses are equipped with enzymes adapted to operate in challenging environments. However, only a small fraction of these viruses can be cultivated *in vitro*, hence viral metagenomics (or viromics) is a pivotal tool to identify enzymes that could be applied to today's global challenges. Unfortunately, there is a lack of fully characterized viral carbohydrate-active enzymes (CAZymes) reported in literature which hinders their success as antimicrobial agents in both health and agricultural fields. In our collaborative research, we dive into virology datasets acquired from Spanish hypersaline marine-sourced solar salterns in search for novel viral halophilic CAZymes. Within the halovirome, glycoside hydrolase 24 (GH24) was the most abundant GH, acting as phage lysins on extremely halophilic bacteria. We identified one full-length sequence (LysBDP) found in crystallizers containing >35% NaCl with high originality among >30,000 reported GH24 sequences. LysBDP was successfully heterologously expressed, purified and confirmed to have β-1,4-N-acetylmuramidase activity by several assays. At high concentrations of salt, we found an increase in stability but a decrease in activity – a surprise for a protein of halovirus origin. We speculate that LysBDP is an endolysin that contains an N-terminal catalytic domain and an additional helix responsible for cell wall binding. The biological importance of the observed salt-intolerance and modularity hints to possible hosts. This detailed study sheds light on the types of carbohydrate-active endolysins used by halophilic viruses and is the first culture-independent study on CAZymes from halophilic-marine environments.

Poster Presentation P21 *Joni Bert*

Isolation and treatment of a pandrug-resistant *Staphylococcus pseudintermedius* infection from canine pyoderma with a bacteriophage-derived endolysin

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The rise of antimicrobial resistance (AMR) in veterinary pathogens poses a growing challenge, particularly in companion animals where treatment options are becoming more limited. *Staphylococcus pseudintermedius*, a common cause of canine pyoderma, is increasingly exhibiting multidrug and even pandrug resistance, complicating clinical management. In this study, we report a clinically relevant case involving a pandrug-resistant *S. pseudintermedius* isolate from a dog with chronic, recurrent pyoderma unresponsive to all approved antibiotic classes.
Given the failure of conventional therapies, a bacteriophage-derived endolysin was evaluated as a targeted alternative. The enzyme was tested in vitro against the isolate, showing rapid and potent bacteriolytic activity, with significant CFU reductions and a low MIC. Importantly, no cytotoxicity was observed in canine keratinocyte cultures. The endolysin was formulated into a hydrogel and applied topically to the lesions. Treatment was well tolerated and led to marked clinical improvement within days. Bacterial counts declined rapidly, and the pathogen was undetectable after one week.
This case highlights the therapeutic potential of endolysins in treating infections caused by highly resistant veterinary pathogens. It underscores the need to integrate such biologics into clinical practice, especially where traditional antibiotics fail, and supports their role in One Health strategies to combat AMR.

Poster Presentation P22 *Malabika Biswas*

Dissecting the phage lytic machinery: harnessing holin-endolysin systems from bacteriophage phi11 as a novel strategy against multidrug-resistant *Staphylococcus aureus*

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Emergence of multidrug resistant staphylococcal strains has necessitated an urgent development of alternative therapies, among which bacteriophage therapy can prove to be a promising one, to counter antibiotic-resistant bacteria. Bacteriophage Phi11 which infects *Staphylococcus aureus*, produces two essential proteins, gp52 (holin) and gp53 (endolysin) both of which exhibit anti-staphylococcal activity.

We investigated the impact of Gp52 on the viability and the morphology of the cells and generated a series of Gp52 mutants to identify the functional domains. Each construct was tagged with green fluorescent protein to study its intracellular localization. The first 12 amino acid residues in the N- terminal domain seemed critical for correct topological insertion and functionality of Gp52. The deletion of the transmembrane domains significantly hampered the antibacterial property of Gp52.

Gp53 and its mutant have been cloned and purified and the recombinant wildtype and the mutant variants were tested for their in vitro antibacterial activity against various staphylococcal strains. Gp53 exhibited a strong lytic activity confirming its bactericidal potential while its single amino acid deletion variant demonstrated complete loss of function. MTT and LDH release assays were performed to test the biosafety profile of Gp53 using mammalian cell lines and no significant cytotoxicity was observed.

Our work also addressed bacterial biofilm-associated infections in medical implants such as prosthetics, catheters, and orthopedic implants by immobilizing Gp53 onto glass or metal surfaces. Using crystal violet and CFU assays, we are investigating the ability of the protein to function as a biofilm-preventive coating.

Poster Presentation P23 *Karolina Ciemińska*

Prophage diversity and potential endolysins in clinical *P. aeruginosa* strains

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We established a collection of approximately 250 bacterial strains isolated from urinary tract infections, catheter-associated infections, and bloodstream infections. Among them, three *Pseudomonas aeruginosa* strains drew particular attention due to spontaneous induction of bacteriophages: *P. aeruginosa* 431, 6297, and 8553. After isolating the phages, we tested their infectivity against 48 other clinical *P. aeruginosa* strains; however, in most cases, only lysis from without was observed. Whole-genome sequencing revealed that each strain harboured between six and eight prophages. We focused on strain 431, from which we successfully isolated phages producing plaques of three distinct morphologies. Bioinformatic analyses indicated that two of these phages encode endolysins. The study of prophages from clinical isolates may enhance our understanding of their functional roles and, although these phages are not suitable for phage therapy, they may serve as a valuable source of endolysins with promising antibacterial potential.

Poster Presentation P24 *Riet Dehaene*

What else can we use lysins for? Engineering a dual-action kill switch for *Pseudomonas*

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Pseudomonas species are used in a wide range of applications across biotechnology, environmental science, agriculture, and medicine due to their metabolic diversity, robustness, and adaptability. However, controlled cell disruption and safe disposal remain key challenges, particularly in applications involving intracellular product recovery or environmental release. A major bottleneck in these processes is the high viscosity of lysates caused by genomic DNA release during cell lysis. We present a dual-action kill switch, that integrates a highly efficient bacteriophage-derived lysis cassette from phage phiKMV—comprising a pinholin, SAR endolysin, and spanin complex—with a periplasmically localized Benzonase nuclease. The phage lysis proteins enable rapid and complete disintegration of the bacterial envelope, mimicking the precise and sequential lysis strategy evolved by phages. Simultaneously, the nuclease degrades genomic DNA released upon lysis, reducing lysate viscosity and minimizing the risk of horizontal gene transfer. The system is tightly regulated to prevent leaky expression and ensure host viability until induction. While developed for *Pseudomonas putida*, the modular design supports adaptation to other *Pseudomonas* species. This technology not only enhances downstream processing in industrial settings but also offers valuable tools for biocontainment, environmental biosafety, and programmable cell clearance in synthetic biology applications.

Poster Presentation P25 *Nikita Deshwal*

River Ganga: a reservoir of virulent phages for mitigation of antimicrobial resistance

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Antimicrobial resistance (AMR) is emerging as a major global threat. Therefore, alternative strategies to fight AMR are essential. Numerous studies have confirmed that one effective method to mitigate AMR is phage therapy. The River Ganga acts as a hub to isolate such phages. Various reports claimed that its water possesses self-healing properties, with a vital role governed by bacteriophages. Our research work focuses on isolating lytic phages from this river that specifically target ARB. For our study, the Ghar site, an area with significant sewage discharges, was chosen as a sampling site, & key pollutants were mapped. Moreover, the FFR method was used to estimate the total viral load, & this concentrate was further used to purify specific phages. The isolated phages belonged to the families Myoviridae, Siphoviridae, Podoviridae, & Inoviridae, as determined by TEM. Also, a spot test was done on the lawns of *E. coli, Staphylococcus, & Pseudomonas* from this FFR concentrate. Clear plaques appeared against *E. coli & Pseudomonas*, while turbid plaques were observed against *Staphylococcus*. A halo ring around the *E. coli* plaque suggested the depolymerase activity of the phage lysin, & lytic action was observed on erythromycin & tetracycline-resistant hosts. We have isolated phages via enrichment against opportunistic pathogens - *Enterobacter* & *Staphylococcus warneri*. *Enterobacter* phages were able to lyse both erythromycin & ESBL-resistant hosts, whereas the *Staphylococcus* phages were able to lyse erythromycin-resistant hosts specifically. These findings suggest that the Ganga River contains diverse lytic bacteriophages with potential therapeutic usage against multidrug-resistant bacteria, & the isolated phages can be further explored for phage therapy.

Poster Pitch P26 *Jéssica Duarte da Silva*

Diversity of endolysins and depolymerases encoded by rare bacteriophages isolated by the B3-CEPID Project, a Brazilian Center for Research on Bacteria and Phages

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The antimicrobial resistance (AMR) crisis remains a major global threat, further exacerbated by the Covid-19 pandemic. Phages—viruses that infect bacteria—were once seen as promising tools for treating bacterial infections. The recent rise of multidrug-resistant (MDR) strains has renewed interest in phage therapy, particularly in phage cocktails and phage-derived enzymes, such as endolysins and depolymerases. The B3-CEPID (Center for Research in Bacterial and Phage Biology) is a multidisciplinary research center funded by FAPESP, dedicated to advancing molecular knowledge of bacteria and their viruses. It brings together over 160 students, researchers, and technicians, across 10 thematic modules coordinated by 20 principal investigators from five academic institutions in São Paulo, Brazil. This study aimed to identify and characterize endolysins and depolymerases encoded by bacteriophages recently isolated within the CEPID project. These phages belong to rare or understudied genera. CEPID-B3 Kp10 and Kp07 infect *Klebsiella spp*. and belong to *Sichuanvirus* and *Jiaodavirus*, respectively. CEPID-B3 Enc01 infects *Enterobacter spp*. and is classified as *Henuseptimavirus*, while Csak03 infects *Cronobacter* spp. and belongs to *Loughboroughvirus*. Kp07 encodes a lysozyme-type endolysin; Kp10 encodes two: a lysozyme-type and a putative murein transglycosylase with a signal peptide and transmembrane domain. Csak03 and Enc01 also encode lysozyme-type endolysins with signal peptides. Kp07 encodes nine depolymerases, Kp10 eight, Csak03 three, and Enc01 one. Phylogenetic analyses revealed that enzyme clustering does not strictly follow phage morphotype or taxonomy, which gives us some interesting insights to be further explored.

Poster Presentation P27 *Soma Mondal Ghorai*

Expression and characterisation of novel chimeric phage endolysin CHAPk-SH3bk against biofilm forming Methicillin Resistant *Staphylococcus aureus*

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Methicillin resistant *Staphylococcus aureus* is one of the major pathogens in hospitals, community and livestock associated infections. The biofilm-forming ability of MRSA is increasing the risk of infections leading to mortality in human and animal health sectors. Phage endolysins with peptidoglycan hydrolases activity against peptidoglycan layer of bacterial cell wall present themselves as a promising antibacterial agent when applied externally against Gram-positive bacteria. The study involved *in silico* analysis and designing of novel recombinant endolysin CHAPk-SH3bk. The chimeric endolysin was expressed, characterized and investigated for bacteriolytic and antibiofilm activity against MRSA in *in vitro* studies. The bioinformatics analysis identified critical amino acids ASP47, ASP56, ARG71, and Gly74 of CHAPk domain responsible for peptidoglycan lytic activity. The CHAPk-SH3bk effectively bound peptidoglycan fragment using these critical amino acids forming a maximum of 14 hydrogen bonds. The *in vitro* bactericidal assays displayed a higher activity of single domain construct CHAPk compared to chimeric CHAPk-SH3bk against planktonic MRSA. Whereas the novel chimeric endolysin CHAPk-SH3bk displayed an effective biofilm reduction activity against 24-hour biofilms of hospital-associated and bovine origin MRSA. The CHAPk was ineffective against hospital-associated MRSA biofilms. The results displayed that the presence of cell wall binding domain SH3bk enhanced the catalytic activity CHAPk against sessile MRSA providing a reference for using chimeric endolysin as an alternative to antibiotics to combat MRSA-associated biofilms persistent in hospital devices and to investigate them further as a therapeutic agent.

Poster Presentation P28 *Ana Lisac*

Bacteriophage T4 propagation in *E. coli* exposed to substrate limitation

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Understanding bacteriophage propagation on bacteria in different physiological conditions is imperative for predicting phage therapy efficacy on various bacterial infections, especially chronic ones. We investigated phage T4 propagation on bacteria *E.coli* grown in a chemostat at very low dilution rates extending down to 0.027 h-1 and bacteria exposed to nutrient deprivation. An increase in adsorption constant and latent period with dilution rate decrease and burst size being proportional to dilution rate was confirmed, consistent with previously published results, extending validity of previous findings. Additional bacterial exposure to starvation, either through nutrient cessation or transferring bacteria into SM buffer, sustained phage propagation during first hours of starvation and diminished to formation of a single phage per infected cell after 24 hours. Nutrient deprivation effects were investigated on fast growing bacteria and bacteria in a death phase. While no phage generation was observed within bacteria in death phase, fast growing bacteria transferred into SM buffer generated a single phage within 48 hours without lysis, indicating that bacterial exposure to nutrient depleted conditions triggers a so called “scavenger response” whose intensity depends on starvation exposure time.

Poster Presentation P29 *Julia Marin-Navarro*

Enzybiotic activity of phage endolysins against *Listeria* is enhanced by glucose oxidase

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Listeria monocytogenes represents a major concern for food safety worldwide. Glucose oxidase (GOX) has long been used as an antibacterial agent based on the release of hydrogen peroxide in its catalytic reaction. However, Gram-positive bacteria, such as *Listeria*, generally exhibit greater resistance to GOX, likely due to their thicker cell-walls. In contrast, endolysins can hydrolyze the peptidoglycan layer of bacteria with high target specificity, leading to cell lysis and death. Here, we show that the combination of the two types of enzyme-based antibiotics (enzybiotics) results in a powerful synergistic antibacterial effect against different Listeria species. When assayed individually, high doses of GOX were required to exert an effective antibacterial action on *Listeria innocua*, while the anti-*Listeria* amidase alone exhibited only limited antibacterial activity, even at high doses. Strikingly, the combined treatment resulted in a 4-log reduction in viable cell counts (log N_0/N), compared to reductions of just 1.2 and 0.2 logs achieved with the amidase and GOX alone, respectively. Flow cytometry and microscopy analyses revealed that the amidase treatment alone induced aggregation of dead *L. innocua* cells. Although *L. monocytogenes* showed higher resistance to single treatments with either GOX or amidase than *L. innocua*, the synergistic combination was also effective against this more resilient species. Overall, these findings represent a novel, efficient, and eco-friendly antimicrobial strategy against one of the most lethal foodborne pathogens.

Poster Presentation P30 *Paulina Miernikiewicz*

PolaR, a novel antimicrobial lysin for *Rothia mucilaginosa*

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PolaR is the first lysin specifically targeting *Rothia spp*. and the first antibacterial enzyme identified from the human gastric mucosal virome. *Rothia spp*. are Gram-positive, non-motile, encapsulated bacteria that colonize the oral cavity and respiratory tract, and can act as opportunistic pathogens, causing infections such as pneumonia, endocarditis, and sepsis. *Rothia mucilaginosa* (RM) coexists with *Pseudomonas aeruginosa* in cystic fibrosis lungs, where cooperative mechanisms may contribute to chronic colonization and treatment resistance. Here, we present PolaR's anti-RM activity, initial safety assesment, and selected physicochemical characterization results.
PolaR was expressed in *Escherichia coli* and purified using chromatography techniques. Its lytic activity was confirmed by Sytox Green fluorometric assay, showing dose-dependent bacteriolytic effects. Cytotoxicity tests on eukaryotic cells indicated that PolaR is non-toxic. Physicochemical analysis demonstrated that PolaR maintains structural integrity under physiological-like conditions.
These findings highlight PolaR as a promising targeted antimicrobial agent against RM, combining specificity, safty and stability, and supporting its potential for development as a novel therapeutic.
This research was funded by National Science Centre, Poland, Grant number 2023/51/D/NZ6/02589

Poster Pitch P31 *Magdalena Pełka*

Phage-borne proteins with lytic activity against *Neisseria gonorrhoeae*

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As antibiotic resistance among pathogenic bacteria becomes more threatening, new alternatives for treatment must be researched. The emergence of new multidrug resistant gonococcal strains indicates that research on novel therapy is highly relevant. We're investigating phage-borne proteins as antimicrobials against *N. gonorrhoeae*, the etiological agent of gonorrhoea.
The workflow started with screening of N. gonorrhoeae FA1090 dsDNA prophage sequences for genes encoding proteins with potential lytic activity. Based on bioinformatic analysis, we selected genes which potentially encode lysins. Gene cloning and protein expression optimization was performed using the pET28a(+) system in E. coli. Proteins were purified by nickel affinity chromatography. Activity tests of studied proteins included zymogram, TRA (Turbidity Reduction Assay), effect on gonococcal biofilm and cytotoxicity evaluation against human cells.
Bioinformatic analysis revealed that two identified proteins carry the NlpC/p60 domain with endopeptidase activity. We optimized synthesis and purification conditions for both endolysins. They showed muralytic activity against M. lysodeikticus peptidoglycan in zymogram assays. Lytic activity was confirmed with TRA assay, adapted to gonococci. Formed N. gonorrhoeae biofilm was disrupted by endolysins enhanced with outer membrane permeabilization reagents. We also proved that both endolysins are not cytotoxic.
Further research will focus on proteins engineering and investigating their influence on infection-related gene expression. We suggest that phage proteins may help develop future treatments for gonorrhea.
Presented research is a part of project 2021/43/O/NZ6/00379 supported by National Science Centre, Poland

Poster Presentation P32 *Iris Pottie*

A functional metagenomic pipeline for phage lysin discovery from environmental samples

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The growing threat of antibiotic-resistant bacteria has intensified the need for alternative antimicrobial strategies. Phage lysins, enzymes derived from bacteriophages that break down bacterial cell walls, represent a promising new class of antibacterial agents. This study explores the discovery of novel lysins using functional metagenomics, a cultivation-independent approach that enables the identification of bioactive genes through heterologous expression and activity-based screening.
We developed a comprehensive pipeline for mining viral genomes and metagenomes, consisting of: (i) partial DNA digestion with a restriction enzyme, (ii) adaptor ligation, (iii) size selection, (iv) PCR amplification, (v) a second size selection, (vi) cloning into a bi-directional expression vector, and (vii) muralytic activity screening. This workflow enabled the construction of genomic libraries and was validated by the identification of four lysins from *Enterococcus* phage AQEF5 and *Escherichia* phage λ. When applied to metagenomic libraries, sampled from sewage and pig faeces, the pipeline yielded four more lysins, active against *E. coli, E. faecalis*, or both.
Our findings highlight the effectiveness of this pipeline in uncovering novel lysins from environmental samples, offering a valuable tool to fill the discovery pipeline in the ongoing battle against antibiotic resistance.

Poster Presentation P33 *Timofey Skvortsov*

Two novel *Proteus* phage endolysins with different metal cofactor requirements

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Proteus mirabilis is a Gram-negative uropathogenic bacterial species responsible for many catheter-associated urinary tract infections (CAUTIs). Due to the growing rates of antimicrobial resistance among CAUTI pathogens, novel antimicrobial solutions are urgently needed. Bacteriophage endolysins are an emerging class of antimicrobial agents, but no endolysins from *P. mirabilis* phages have been reported to date. Here we describe two new *Proteus* phage endolysins, LysPM1 and LysPM2, containing Peptidase_M15_3 and CHAP domains, respectively. We experimentally confirmed that both enzymes have antibacterial activity against several *Proteus spp*. members when used in combination with outer membrane permeabilizers, including EDTA, citric acid, chloroform, as well as against frozen bacteria. Both enzymes have pH optimum around 7-8 and are thermally stable up to 60°C. Both endolysins completely lost the lytic activity upon treatment with EDTA, suggesting that divalent cations participate in the catalytic mechanism; the addition of Ca^{2+} and Zn^{2+} to LysPM1, and Ca^{2+} and Mn^{2+} to LysPM2 partially restored the activity. No detectable effect of NaCl concentration was observed in the range of concentrations tested.
Our study confirms LysPM1 and LysPM2 as first experimentally characterised *Proteus* phage endolysins. These findings could guide further development of phage-derived lytic enzymes for treatment of *Proteus* infections.

Poster Pitch P34 *Samuel Staubli*

Phage-derived endolysins as precision tools against vancomycin-resistant enterococci

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Antibiotic-resistant infections are increasing globally, presenting a major public health challenge. Gram-positive pathogens such as *Enterococcus faecalis* and *Enterococcus faecium* are of particular concern, especially in clinical settings. As conventional antibiotics lose efficacy and the development of new drugs stagnates, alternative antimicrobial strategies are urgently needed.
Endolysins - bacteriophage-derived peptidoglycan hydrolases - offer a targeted and efficient approach to bacterial killing. These enzymes cleave conserved bonds within the bacterial cell wall, causing rapid lysis. Their ability to act exogenously makes them especially suitable against Gram-positive bacteria, including enterococci.
We conducted a systematic analysis of native and engineered endolysins targeting *Enterococcus* spp. The panel included enzymatically active domains (EADs) - glycosidases, amidases, and endopeptidases - each cutting distinct peptidoglycan bonds. These were paired with diverse cell wall binding domains (CBDs), featuring α-helices, β-sheets, or α–β sandwich architectures. Chimeric endolysins were created through rational domain swapping, using flexible linkers to enhance function and stability.
Our findings show that these enzymes possess potent bactericidal activity against *Enterococcus*, including vancomycin-resistant strains. Their modularity allows for precision engineering, while targeting highly conserved structures limits resistance development.
Together, these results highlight endolysins as promising candidates for next-generation antimicrobials. Their structural versatility, robust efficacy, and low resistance potential position them as viable tools in combating multidrug-resistant Gram-positive infections.

Poster Presentation P35 *Wannes Van Wassenbergh*

The quest for hidden lysins: exploring viral dark matter using sequence-based metagenomics

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Traditionally, protein discovery relied on cultivating microbes under defined culture conditions. However, estimations indicate that 98% of bacteria cannot be grown under laboratory conditions, a phenomenon known as the great plate count anomaly. Consequently, also accessing phage proteins remains a challenge, underscoring the value of culture-independent approaches. Among these, sequence-based metagenomics enables direct access to the vast genetic diversity of the environment, facilitating rapid discovery and characterization of potential lysin candidates through next-generation sequencing. In this work, an in-depth analysis of six putative endolysins from an existing metagenomic dataset was conducted. This included structural and sequence distance-based analyses, functional characterization, and host strain evaluation via turbidity reduction assays. Our efforts resulted in the identification of previously unannotated domains and endolysins active against both Gram-negative and Gram-positive strains. In addition, a new standard is introduced to assess muralytic activity, incorporating three complementary metrics that capture both the rate and extent of peptidoglycan degradation over time. Together, they offer a nuanced understanding of enzymatic performance, enabling consistent and comprehensive comparisons across experiments. Integrating and refining the pipeline developed in this study can significantly enhance the speed and efficiency of discovering and characterizing potential lysin candidates for both therapeutic and industrial use.

Poster Pitch P36 *Gilles Vandermarliere*

You get what you screen for: benchmark analysis of three expression hosts for the selection of new antibacterial enzybiotics

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Microbial contamination, particularly by lactic acid bacteria, remains a frequent challenge in commercial bioethanol fermentations, reducing yields by up to 5%. The emergence of antibiotic resistance further complicates their control, highlighting the need for new antimicrobial strategies. Phage lysins offer a targeted and effective alternative to conventional antibiotics. Their modular architecture can be exploited for high-throughput engineering using the VersaTile technique. However, downstream selection of lead molecules remains a critical bottleneck. In this study, we compared three expression hosts: *Saccharomyces cerevisiae*, *Pichia pastoris*, and *Escherichia coli*. We analysed their screening performance in identifying lysins targeting *Limosilactobacillus fermentum*, a common industrial contaminant. Additionally, we evaluated *S. cerevisiae* both in a secretion and surface display mode. Our results revealed significant differences in both the number and type of hits identified across hosts: *S. cerevisiae* resulted in higher diversity, while screening in *E. coli* appeared most stringent. Several new lysins were identified, across the three expression systems, with strong activity against L. fermentum, even in ethanol-rich conditions mimicking bioethanol fermentation tanks. Moreover, one novel lysin achieved up to a 2-log bacterial reduction. Our findings highlight the influence of the expression host on screening outcomes, demonstrating the principle: “You get what you screen for”, and establish a versatile platform for development of next-generation antimicrobials against industrial contaminants.

Poster Presentation P37 *Roberto Vázquez*

‘Exquisite specificity’, if the context allows: endolysin evolution is a contingent outcome of phage-host molecular landscapes

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(Endo)lysins are very often described as ‘specific’ molecules, and it is held that their specificity is determined by cell wall binding domains (CBDs) in Gram-positive hosts. These notions were backed by success in deriving exquisitely specific CBDs against *Streptococcus pneumoniae* or *Listeria*. Sparked by this framework, we devised a systematic characterization of CBDs from lysins mined from phages infecting *Staphylococcus, Enterococcus,* and *Streptococcus*. After probing 66 eGFP-CBD fusions against 71 bacterial strains, we observed that (1) ‘exquisite specificity’ is rare, and CBDs seldom preferentially bind their original host, from which we deemed that (2) CBD specificity is not a trait under evolutionary pressure, but rather a possible consequence of evolutionary optimization of spatial-temporally constrained host lysis. The few cases of distinct binding that we observed could be explained by equally distinct cell wall features and/or particular anchoring domains available in the phage-host system. Thus, (3) the evolution of strict specificity depends on the case-dependent availability of suitable molecular resources at the phage-host interface. Finally, we explored how CBD specificity influences full-lysin activity range and found that the catalytic domain is often more determinant. We finally concluded then that (4) lysin specificity ultimately arises from the complex interaction between catalytic and binding domains.
Therefore, producing specific lysin-based antimicrobials remains an empirical problem, although further understanding on the structure-function relationships of lysins, within an evolutionary context, may ultimately yield useful insights for these engineering efforts.

Poster Presentation P38 *Roberto Vázquez*

PhaLP 2.0: a community-oriented database and portal for phage lysin research with new tailored computational tools and metagenomic data

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Biology is becoming increasingly data-dependent, and computation is in the course of becoming in-dispensable for many research avenues. The field of phage lysins is not an exception to this new paradigm. But, to make the most of it, meaningful, easily accessible data is a major requirement. To this end, PhaLP was created four years ago as a centralized resource of phage lysin sequences and associated metadata intended to empower lysin researchers around the world. Now we present PhaLP 2.0 as the necessary next step. In its original architecture, PhaLP mined and classified lysin sequences from UniProt, and made them available for the community. In PhaLP 2.0, we have integrated two specifically tailored computational tools to make the most of the newly available resources.
Using SUBLYME, a protein-embedding-based machine learning tool designed to identify and classify phage lysins, 755,000 new sequences were mined from the metaviromic dataset EnVhog and added to PhaLP 2.0. This represents a significant increase in diversity in comparison to the original training dataset, bringing the total number of clusters from only 1,000 clusters in PhaLP 1.0, to 41,000 clusters in PhaLP 2.0.
With SPAED, we took advantage of the predicted aligned error (PAE) matrix obtained from AlphaFold predictions to identify domain-linker boundaries and detect disordered regions. In PhaLP 2.0, we leverage SPAED to improve domain-level functional annotations and facilitate subsequent choices for modular engineering.
Both SUBLYME and SPAED are available as independent tools in GitHub, the latter also in a dedicated website (https://spaed.ca), and PhaLP 2.0 will be made accessible through a new server (https://phalp.ugent.be).

Poster Presentation P39 *Maarten Boon*

pPUT: a novel phage-based orthogonal expression system optimized for *Pseudomonas*

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Bacteriophages are master manipulators of bacterial physiology, encoding compact yet powerful genetic toolkits to hijack host machinery. Among these, T7-like phages are particularly notable for their orthogonal transcriptional systems, which have inspired numerous synthetic biology (SynBio) tools and enabled high level protein expressions in *E.coli* with the T7 RNA polymerase (pET system).
The SynBio field is increasingly shifting to other organisms for targeted applications, and *Pseudomonas* species have gained notable attention as highly versatile and robust production hosts. However, many of the parts and tools used for their engineering are designed and optimised for E. coli, leading to aberrant performance in other hosts. To fully unlock the potential of *Pseudomonas* species for SynBio, a well-characterized and tailored set of genetic parts is thus indispensable.
We mined the genomes of several T7-like Pseudomonas phages for novel genetic parts adapted for *Pseudomonas*, as these phages encode their own orthogonal transcriptional machinery. We have developed an RNA sequencing method termed ONT-cappable-seq that guided the large-scale identification of new (orthogonal) promoters and terminators. These were subsequently validated *in vivo* resulting in a broad set of characterised parts applicable in synthetic DNA circuits. In addition, we introduced and optimized four T7-like RNAP polymerases, their corresponding phage promoters and lysozymes, leading to a tailored version of the multifaceted T7 expression system for *Pseudomonas* species. These different phage elements were introduced in a vector set (pPUT) as well as dedicated expression strains, enabling flexible and high level protein expression in pseudomonads.

Poster Presentation P40 *Melissa-Jane Chu Yuan Kee*

Prophage activation by *Staphylococcus aureus* pathogenicity Islands

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Staphylococcus aureus pathogenicity islands (SaPIs) are mobile genetic elements that encode superantigens and toxins. They are parasites that exploit bacteriophages for their reproduction, packaging and dissemination. Normally, SaPIs reside stably in the host chromosome until they are induced to excise by anti-repressor proteins from helper phages they parasitize. Recently, we discovered that a SaPI-encoded small protein (inducer) can activate prophages to enter the lytic cycle in an SOS-independent manner. Using genetic and biochemical analyses, we showed that the binding partner for the SaPI inducer is the phage-encoded master repressor. Pull-down assays and Western blot analyses demonstrated a direct interaction between the wild-type SaPI inducer and the wild-type prophage CI repressor proteins. A SaPI inducer mutant, identified previously through a genetic screen, did not bind to wild-type prophage repressor; however, the interaction was restored between the SaPI inducer mutant and a prophage CI suppressor mutant. We also demonstrated that the SaPI inducer causes the dissociation of the CI repressor from the cI-cro intergenic DNA, thereby activating the lytic life cycle of the prophage. Further work elucidating the structural basis of prophage activation by SaPI-encoded inducers will shed light on these interactions and could pave the way for a novel approach in our ongoing fight against antibiotic-resistant *S. aureus* infections.

Poster Presentation P41 *Klara-Isabell Garbe*

Tailored phage selection for the treatment of chronic airway infections due to MDR *Pseudomonas aeruginosa*

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Chronic *Pseudomonas aeruginosa* infections are a major cause of morbidity and mortality in cystic fibrosis (CF) patients. Treatment remains challenging due to antibiotic resistance, biofilm-associated persister cells, and the rapid genetic adaptation of hypermutator strains to the CF lung environment. While phage therapy offers a promising alternative, its efficacy may be limited if phages are not selected under conditions that mimic chronic infection.
We isolated and characterized bacteriophages capable of infecting *P. aeruginosa* strains lacking key phage receptors—type IV pili, flagella, or lipopolysaccharides (LPS)—which are frequently lost during adaptation in chronic infections. Additionally, we identified phages that target strains overexpressing alginate, another common adaptive phenotype. To assess the impact of hypermutator strains, we used the PAO1ΔmutS strain, representative of clinical isolates carrying mutations in the mismatch repair gene mutS. In this hypermutator background, phage resistance emerged more rapidly than in the wild-type PAO1. We also investigated phage evolution during serial co-culture in PAO1 and PAO1ΔmutS strains. Finally, using polarized Calu-3 airway epithelial cells, we demonstrated that phages from our library could delay or prevent disruption of epithelial cell layer integrity.
Our findings highlight the potential of tailored phage selection to target antibiotic-resistant *P. aeruginosa* in chronic infections by accounting for its phenotypic adaptations and resistance mechanisms. The experiments suggest that directed phage evolution could enhance efficacy and receptor specificity for personalized therapeutic applications.

Poster Pitch P42 *Haidai Hu*

Zorya anti-phage defense at the membrane boundary

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Zorya is a recently identified and widely distributed bacterial immune system that protects bacteria from viral (phage) infections. Three Zorya subtypes have been discovered, each containing predicted membrane-embedded ZorAB complexes paired with soluble subunits that differ among Zorya subtypes, notably ZorC and ZorD in type I Zorya systems. Here, we investigate the molecular basis of Zorya defense using cryo-electron microscopy, mutagenesis, fluorescence microscopy, proteomics, and functional studies. We present cryo-EM structures of ZorAB and show that it shares stoichiometry and features of other 5:2 inner membrane ion-driven rotary motors. The ZorA5B2 complex contains a dimeric ZorB peptidoglycan binding domain and a pentameric α-helical coiled-coil tail made of ZorA that projects approximately 70 nm into the cytoplasm. We also characterize the structure and function of the soluble Zorya components, ZorC and ZorD, finding that they harbour DNA binding and nuclease activity, respectively. Comprehensive functional and mutational analyses demonstrate that all Zorya components work in concert to protect bacterial cells against invading phages. We provide evidence that ZorAB operates as a proton-driven motor that becomes activated upon sensing of phage invasion. Subsequently, ZorAB transfers the phage invasion signal through the ZorA cytoplasmic tail to recruit and activate the soluble ZorC and ZorD effectors, which facilitate degradation of the phage DNA. In summary, our study elucidates the foundational mechanisms of Zorya function as an anti-phage defense system.

Poster Presentation P43 *Anastasiia Nadieina*

Activation of a bacterial immunity protein by a viral-host factor complex

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Bacteria have evolved diverse immune systems to defend against phage infections, often triggered by direct detection of phage proteins. However, whether bacterial immunity can sense phage-induced perturbations of host cellular functions remains unclear. In our work, we investigate the fused toxin-antitoxin system CapRelEch from *Enterobacter chengduensis*, which is activated not by a phage protein alone but by a complex formed between the T7 phage effector Gp0.4 and the bacterial cytoskeletal protein FtsZ. It was previously discovered that Gp0.4 binds FtsZ to inhibit its polymerization, arresting bacterial cell division. Our data reveal that CapRelEch detects this specific Gp0.4-FtsZ complex, which relieves autoinhibition of CapRel's toxic domain and triggers an immune response. This mechanism is similar to that of effector-triggered immunity (ETI) in plants and animals, where immune systems respond to pathogen-induced disruptions in host, rather than the pathogens themselves. Using structural biology approaches, including hydrogen-deuterium exchange mass spectrometry (HDX-MS), we aim to elucidate the allosteric activation mechanism of CapRelEch by this effector-host protein complex. Our findings uncover a novel bacterial immune sensing strategy that detects pathogen-induced host perturbations and suggest that recognition of cytoskeletal disruptions may be a conserved feature of innate immunity across life domains.

Poster Presentation P44 *Sean Jia Le Pang*

Genomic landscape of complete *Acinetobacter* phages: clustering, core-shell genes, and synteny insights

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Acinetobacter baumannii is a major clinical threat due to its multidrug resistance and frequent involvement in nosocomial outbreaks. Bacteriophage therapy offers a targeted alternative, but its success depends on a deep understanding of phage genomics and proteomics. This study aims to characterise publicly available genomes of *Acinetobacter* phages, identify therapeutically promising clusters and protein families valuable for phage engineering. A total of 250 complete *Acinetobacter* phage genomes were downloaded from NCBI database and clustered using PhamClust. A phylogenetic tree using the terminase large subunit and portal protein was constructed. Gene synteny analysis was performed using Clinker. Protein families identified by PhaMMseqs were analyzed for conserved and cluster-specific phams. *Acinetobacter* phages were classified into 12 clusters. A novel group, Cluster 10, contained three phages with exceptionally broad host ranges, highlighting its strong potential for therapeutic development. While the terminase large subunit and portal protein were useful markers for cluster-level grouping, they lack sufficient resolution for fine-scale phylogenetic discrimination. Three conserved enzymes (endolysins, DNA helicase and HNH homing endonuclease) were found across multiple clusters, making them candidate targets for synthetic design. Meanwhile, cluster-specific phams offer biological insights and could be employed as cluster-specific markers for molecular diagnostics. Collectively, these findings expand the genomic landscape of Acinetobacter phage diversity and provide potential protein targets for advancing phage-based therapies and diagnostics.

Poster Presentation P45 *Yang Shen*

Potential and challenges of applying phage proteins in aquaculture

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The sustainable growth of aquaculture is increasingly threatened by bacterial diseases that undermine fish health and farm productivity. While vaccines and antibiotics have been the main solutions of disease control, limitations such as strain variation, delayed onset of immunity, and antimicrobial resistance demand alternative yet sustainable solutions. Bacteriophages offer one promising route, but their complexity in regulatory approval, host range restrictions, and environmental variability can limit consistent application. In this context, phage-derived proteins represent an attractive alternative as defined, modular, and effective agents for pathogen control.

This poster highlights both the potential and challenges of applying phage proteins in aquaculture. As a proof-of-concept, we reference the successful deployment of a *Yersinia*-targeting strategy in a well-boat setting, which effectively prevented *Yersinia ruckeri* infections during salmon transport. Building on this, we explore the functional diversity of phage proteins, including endolysins, cell wall-/receptor-binding proteins, depolymerases, and tailocins for therapeutic or diagnostic applications.

Despite their promise, several barriers must be addressed for successful deployment in aquaculture. These include ensuring protein stability in aquatic environments, developing scalable production and purification methods, navigating regulatory frameworks, and mitigating the risk of resistance development.

By integrating field studies with a survey of emerging protein classes, this poster aims to spark discussion on how phage-derived proteins can complement existing disease management strategies and contribute to a more sustainable aquaculture industry.

Poster Presentation P46 *Dennis Grimon*

From design to function: an automation-enabled pipeline for the development of Precision Lysin Therapeutics

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Phage lysins hold significant promise as next-generation antimicrobials due to their unique mode of action, low propensity for resistance development, and amenability to engineer both specificity and bactericidal activity for application-specific needs. However, systemic applications against Gram-negative bacteria remain limited and phage lysins' activity is often abolished under physiological conditions.

At Obulytix, we are leveraging these unique traits to develop Precision Lysin Therapeutics (PLTs) that overcome the current limitations of phage lysins and are fit for purpose in real world clinical settings. Using our proprietary VersaTile technology, we are able to generate up to millions of PLTs, unlocking a massive reservoir of diversity. Using pipeline automatization and the integration of high-throughput expression, purification, and functional screening under physiological conditions, we are able to exploit this diversity, enabling rapid and scalable identification of viable drug candidates.

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