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Book of Abstracts

Contents

Mycobacteriophages and their host interactions	2
Discovery of capsid-packaged phage proteins injected into bacteria	3
Decoding NAD-RNAs in Bacteriophage Infection	4
Function of Phage Transfer-Messenger RNA in a crAss-like phage phi14:2	5
FibriLysins: playing the Reverse Uno card against Staphylococcus aureus	6
ASO-based functional genomics reveals new phenotypes of essential genes in nucleus-forming jumbo phages	7
Functional dissection of the Bacillus subtilis SP β prophage lysogeny-lysis system	8
The bully phage: A Shiga toxin-encoding prophage interferes with the induction of co-hosted prophages	9
The Phage Nucleus shapes the Virulence Spectrum in Chimalliviridae	10
Poster #2: ASO-Mediated mRNA Silencing Reveals a Link Between Tail Assembly and Genome Packaging in Phage Φ KZ	11
Poster #5: The impact of chronic viral infection on archaeal cell morphology	12
Poster #9: A prophage-encoded anti-phage defence system that prevents phage DNA packaging by targeting the terminase complex	13
Poster #11: Protein fusion analysis reveals new phage sponges	14
Poster #12: Phage Fitness Landscapes and Butterfly Effects in Evolving Phage Populations	15
Poster #13: Interbacterial competition triggers stress-induced CRISPR immunity against bacteriophages	16
Poster #14: Molecular mechanisms of the synergistic interactions of Druantia III and AR-MADA II in the antiphage defense of enterohaemorrhagic Escherichia coli strain EDL933	17
Poster #15: Guide amplification bootstraps immune specificity in a bacterial Argonaute .	18
Poster #18: Decoding the phage unknown with protein language models	19
Poster #19: Hidden Functional Diversity among Type V-A CRISPR Effectors	20

Poster #20: RNAylation as a Potential Modulator of Translation in bacteriophage T4 Infection of <i>Escherichia coli</i>	21
Poster #21: Insights into plasmid elimination by a pAgo guided anti plasmid immune system	22
Poster #24: Temperate versus virulent phage lifestyle impacts microdiversity in soil environments	23
Poster #26: The Z-Project: Expanding the Bacteriophage Toolbox	24
Poster #28: Domain swapping reveals deep conservation of death-domain immune signaling	25
Poster #29: Living together: molecular mechanisms of phage-bacterial coexistence in the gut	26
Poster #30: Phage-encoded single-guide RNAs subvert CRISPR-Cas9 immunity	27
Poster #31: tRNA epitranscriptome plasticity optimizes phage infection in <i>E. coli</i>	28
Poster #32: Mechanism of activation of the defense-associated reverse transcriptase DRT4 by a phage single-stranded DNA-binding protein	29
Poster #33: Gasdermin-mediated phage defense as a model for intercellular immune signaling in <i>Streptomyces</i>	30
Poster #35: Identification of host factors involved in cystovirus replication	31
Poster #37: Unveiling the regulatory landscape of the double-stranded RNA phage $\phi 6$ using ribosome profiling	32
Poster #38: Establishment of phage-based biocontrol strategies against phytopathogens for sustainable plant disease management	33
Poster #39: Novel insight into the infection and egress dynamics of an Inovirus in <i>Shewanella oneidensis</i> MR-1	34
Poster #40: The temporal regulation of crAss-like phage phi14:2 vRNAP across its transcription cycle	35
Poster #41: pDolos: A plasmid-based phage satellite of the <i>Shewanella</i> inovirus Dolos	36
Poster #42: Spatial regulation of bacterial antiphage defense systems within biofilms	37
Poster #45: Regulation of Transcription by Jumbo Phage AR9 Encoding Two Multisubunit RNA Polymerases	38
Poster #46: Pyocin-Mediated Growth Suppression and Bacterial Regrowth Dynamics	39
Poster #47: Phage-encoded ribosome-targeting factors in bacterial takeover	40
Poster #48: Quantitative and Biophysical Analysis of Phage-Induced Outer Membrane Vesicles in <i>Salmonella</i>	41
Poster #49: Drivers and Constraints of Prophage Persistence in Natural Communities	42

Poster #50: The permanent replication cycle potential in Chimalliviridae	43
Poster #51: The role of the phage encoded auxiliary metabolic gene <i>pebS</i> during the infection of marine cyanobacteria	44
Poster #52: Systematic studies of bacteriophage confinement in glycan-based biofilms of <i>Erwinia amylovora</i>	45
Poster #53: DNA loaded outer membrane vesicles after bacteriophage treatment	46
Poster #55: Dissecting antiviral defense systems in Actinobacteria – from single cells to the community level	47
Poster #56: Stepwise R-Loop Unwinding and Cas3-Mediated Target Degradation in a Multiprotein CRISPR– Cas System	48
Poster #59: Sulfate-Reducing Bacteria and Their Viral Fingerprints in the Gut Microbiome: Uncovering Adaptation and Disease Markers in Inflammatory Bowel Disease	49
Poster #60: Antibiotics and diet modulate phage-bacteria interactions in the gut microbiome	50
Poster #61: Towards the human gut microbiome: expanding phage-plasmid detection with tyPPing	51
Poster #62: Phage P2-encoded Orf30 protein as a novel regulator of virion formation	52
Poster #63: Differences in regulatory genes drive divergent evolutionary outcomes under phage challenge	53
Poster #65: Fun/Z is an abortive immunity system related to archaeal and eukaryotic replication component	54
Poster #66: Tin: A Minimalist Abortive PD-(D/E)XK Nuclease Fusing Sensor and Effector Functions for Anti-Phage Defense	55
Poster #67: Need for speed: early lysogen formation following rapid lysis drives temperate phage persistence	56
Poster #69: Characterization of a new NAD ⁺ -processing domain involved in antiphage defense and shared across the tree of life	57
Poster #70: Exploring potential mechanisms of programmed cell death in <i>Legionella</i> phage defense: Interplay of NAD(H) depletion and the effector phospholipase PlaB	58
Poster #71: New insights into the Mrr-domain-associated prokaryotic Argonaute systems SPARMA and SPARMTA	59
Poster #72: Investigating the mechanism of mRNA export from the early phage infection compartment of jumbo phage PhiKZ	60
Poster #73: Deoxydinucleotides are potential mediators of bacteria–phage interactions	61
Poster #75: Who starts, who follows: reading assembly grammar in bacterial and metazoan death domains	62

Poster #76: Regulation of the lysis-lysogeny switch in temperate actinobacteriophages of <i>Corynebacterium glutamicum</i> ATCC 13032	63
Poster #78: Adaptive trajectories of bacteriophages: Evolutionary dynamics across contrasting environments	64
Poster #80: 3D spatial analysis of fluorescence foci from z-stack microscopy using cell reconstruction	65
Poster #82: Too Early or Too Late? Trade-Offs in the Timing and Specificity of Bacterial Defence Systems Against Mobile Genetic Elements	66
Poster #83: Multicellular defense in <i>Streptomyces</i> : a collective shield against phage predation	67
Poster #84: Systematic characterization of prophage integration sites in <i>Staphylococci</i>	68
Poster #86: A widespread protein family inhibits both restriction-modification and CRISPR-Cas defenses	69
Poster #87: Transcriptional Reprogramming by Cyanophage Syn9: Investigating Host-Phage Interactions in <i>Synechococcus</i> WH8109	70
Poster #88: Characterization of the <i>Methanosarcina</i> Spherical Virus	71
Poster #93: The role of phage Thanatos ADP-ribosyl-transferases Alt1 and Alt2 in disarming and preparing the host	72
Poster #94: The role of phages in microbial communities	73
Poster #95: Universal host contaminant protein identification and quantification for any production host by LC-MS/MS	74
Poster #96: Structural and functional characterisation of a membrane associated anti-phage defence system	75
Poster #97: Cryo-EM structure of the <i>Escherichia coli</i> siphophage Bas24	76
Poster #98: Exploring flagellotropic phage adsorption by cryoEM	77
Poster #99: A diverged ubiquitination pathway mediates antiviral defense in bacteria	78
Poster #100: Balancing of immune activation and suppression by N4-like <i>Pseudomonas aeruginosa</i> phages.	79
Poster #102: Exploring auxiliary metabolic genes (AMGs) from cyanophage Syn9	80
Poster #107: An Lsr2-like Xenogeneic Silencer Confers Immunity Against AT-Rich Bacteriophage Infection	81
Poster #108: Identification of a non-WTA bacteriophage receptor in <i>Staphylococcus aureus</i>	82
Poster #122: Zorya-mediated defence results in distinct cellular outcomes following phage infection	83

Poster #123: ATP-dependent oligomerization triggers tRNA degradation by Schlafen-fused prokaryotic Argonaute proteins	84
Temperate bacteriophage populations in the human gut: culturomics and experimental insights	85
No Escape – The ARTs of host takeover	86
The Enemy of My Enemy Carries a Colicin: Engineering phages to redirect evolutionary trajectories of uropathogenic <i>E. coli</i>	87
Targeted Antimicrobial Peptide Discovery and Phage Cocktail Development Against ES-KAPE Pathogens	88
PhaDRiM - Phage induced Strain Replacement in Microbiomes	89
Genetic perturbation of gut bacteria with engineered phage vectors and CRISPR-Cas systems	90
Exploring virus dynamics in a <i>Nanobdellati</i> Co-culture system	91
Viral Take-over of Host Photosynthetic Electron Transport: Investigating the role of Cyanophage-encoded Plastocyanin	93
Metabolic reconstitution in the NAD ⁺ battlefield: phages and bacteria in a molecular arms race	94
Understanding the role of bacterial anti-phage defences to inform phage therapy	95
Phage-induced membrane remodelling is a vulnerability targeted by bacterial immunity	96
ApeA cleaves genomic RNA to defend against RNA phage infection	97
Deoxydinucleotides activate the bacterial anti-phage defense system ApeA	98
Bacterial multicellular behaviour in antiviral defense	99
Using LC-MS/MS for identification and quantification of host contaminant protein during phage production	100
Horizontal Gene Transfer Shapes Bacterial Defense in <i>Vibrio cholerae</i>	101
Sulfonamide antibiotic rescues bacteria from phage-induced collapse	102
Zorya-mediated defence results in distinct cellular outcomes following phage infection .	103
Hidden Functional Diversity among Type V-A CRISPR Effectors	104
From genetic conflicts to molecular innovation	105
Diverse responses of an archaeal CBASS	106
The ancient evolution of gasdermin-mediated immunity	107
Dozens of phage proteins block retron defense by targeting its toxicity on the ribosome .	108
Bacteria-inspired exploration of immune functions in eukaryotes	109

A novel signaling system originating from a core metabolic enzyme	110
Hidden Amun defensive proteins within defense islands broaden protectivity against phage families	111
A compact Druantia defense clears phage infections via single-stranded DNA recognition and directional duplex unwinding	112
Processing of forked DNA activates a helicase-nuclease immune system	113

Abstracts

Organisation and regulation of the viral infection cycle / 112**Mycobacteriophages and their host interactions****Author:** Graham F. Hatfull¹¹ *University of Pittsburgh*

Mycobacteriophages are natural predators of *Mycobacterium* strains. We have gained insights into mycobacteriophage biology through science education programs such as PHIRE and SEA-PHAGES, in which many students have collected over 30,000 phages, half of which infect *Mycobacterium*. This set of phages isolated on a single bacterial strain (*M. smegmatis* mc2155) reveals the amazing genetic diversity of the phage population. These phages has provided insights into the mycobacteria and facilitated development of tools that are broadly useful for the *Mycobacterium* research community. Elucidating phage diversity and development of engineering tools enable first therapeutic phage intervention for treating an *M. abscessus* infection. We now have received 650 requests for phage evaluation of NTM strains, which we have screened with a phage panel, although the phage repertoire is limited. Most strains are *M. abscessus*, of which about 50% have smooth colony morphotypes and are rarely killed efficiently by any phages. The other 50% have rough colony morphotypes, and we can identify one or more phages that infect and kill about 50% of these efficiently. To date we have provided phage preparations for clinical treatment of over 50 patients, providing insights into the opportunities and challenges for *Mycobacterium* phage therapies. However, a serious challenge to broadening therapeutic use is the narrow host preference of the phages and unpredictability of phage infection profiles. This likely arises from a diverse set of integrated prophages prevalent in *M. abscessus* strains, a rich and varied plasmid repertoire, and an abundance of known phage defense systems in genomic islands.

Organisation and regulation of the viral infection cycle / 10**Discovery of capsid-packaged phage proteins injected into bacteria**

Authors: Avigail Stokar-Avihail¹; Tara Katarina Bartolec¹; Lena Sarah Müller¹; Mikhail Savitski¹; Athanasios Typas²

¹ *European Molecular Biology Laboratory, Heidelberg, Germany*

² *European Molecular Biology Laboratory (EMBL)*

The typical phage infection cycle begins with adsorption of a phage particle to a bacterial cell, followed by injection of the phage genome and production of new progeny phages. However, it is often overlooked that, in addition to their genomes, some phages also inject proteins into the host cell. Although a handful of such proteins have been described, their prevalence and diversity across phages remain largely unexplored.

Here, we developed a mass spectrometry-based proteomics approach to discover phage-injected proteins. Inspired by the classic Hershey–Chase “Waring blender” experiment (1952), we label all phage particle proteins with heavy isotope amino acids and infect unlabeled bacteria. This enables us to distinguish proteins originating from the infecting phage particle (heavy-labeled) from proteins synthesized *de novo* during infection (unlabeled). Following phage adsorption and DNA injection, empty phage particles are removed from the bacterial surface by the shearing force of a blender. As most phage structural proteins remain outside the cell, they are removed during this step, whereas proteins injected into the host cell are protected from blending and retained within infected cells.

Initial validation demonstrates that the method effectively distinguishes injected proteins from non-injected phage particle proteins. We are applying this approach to a diverse collection of phages to identify previously unknown phage-injected proteins and investigate their roles during infection.

Organisation and regulation of the viral infection cycle / 25**Decoding NAD-RNAs in Bacteriophage Infection****Author:** Katharina Höfer¹¹ *Leibniz Institute for Immunotherapy, Division "Nucleic Acid technologies", Regensburg*

Bacteriophage infection is shaped by small metabolites and dinucleotides, which can act as crucial regulatory molecules in virus–host interactions. One prominent example is nicotinamide adenine dinucleotide (NAD⁺), a central redox cofactor that also serves as an RNA cap. In this project, we investigate how NAD-capped RNAs influence bacteriophage infection using the T4–*Escherichia coli* system as a model. We identify the first NAD-capped RNAs in this infection context and show that RNA modifications can modulate phage–host processes, including RNAlation, a recently discovered reaction in which the T4 ADP-ribosyltransferase ModB transfers NAD-capped RNAs to proteins. Together, our results indicate that NAD-RNAs represent an additional regulatory layer in bacteriophage infection and suggest that similar RNA modification-dependent mechanisms may operate in other bacteria–phage systems.

Reference #1:

Wolfram-Schauerte M, Keuthen H, Moskalchuk A, Ueffing T, Pozhydaieva N, Ramírez Rojas AA, Schindler D, Kaiser S, Paczia N, Höfer K. T4 phage RNA is NAD-capped and alters the NAD-cap epitranscriptome of *Escherichia coli* during infection through a phage-encoded decapping enzyme. *bioRxiv*. 2024. doi: 10.1101/2024.04.04.588121.

Reference #2:

Wolfram-Schauerte M, Pozhydaieva N, Grawenhoff J, Welp LM, Silber I, Wulf A, Billau FA, Glatter T, Urlaub H, Jäschke A, Höfer K. A viral ADP-ribosyltransferase attaches RNA chains to host proteins. *Nature*. 2023;620:1054–1062. doi: 10.1038/s41586-023-06429-2.

Organisation and regulation of the viral infection cycle / 36**Function of Phage Transfer-Messenger RNA in a crAss-like phage phi14:2****Author:** Nandika Sanjog Sahani¹**Co-authors:** Elizaveta Buianova²; Baturay Altay¹; Huanzhang Tang¹; Barbara Steigenberger¹; Mikhail Fofanov³; Cedric Lood³; Milan Gerovac⁴; Bas Dutilh³; Jörg Vogel²; Maria Sokolova¹¹ Max Planck Institute of Biochemistry² Helmholtz Institute for RNA-based Infection Research (HIRI), Helmholtz Centre for Infection Research (HZI), Würzburg, Germany³ Friedrich Schiller University, Jena, Germany⁴ Helmholtz Institute for RNA-based Infection Research (HIRI), Helmholtz Centre for Infection Research (HZI), Würzburg, Germany, Helmholtz Centre for Infection Research, Braunschweig, Germany

CrAss-like phages are a group of viruses that include some of the most abundant phages in the human gut. These phages infect bacteria of the Bacteroidetes phylum. Despite their abundance, their molecular biology remains poorly understood.

In this study, we use phi14:2, a crAss-like phage isolated from the marine microbiome that infects *Cellulophaga baltica*, as a model system to investigate the role of phage-encoded transfer-messenger RNA (tmRNA). In bacteria, tmRNA rescues stalled ribosomes from defective mRNA transcripts lacking a stop codon and directs incomplete proteins for degradation through a process known as trans-translation. The role of phage tmRNA remains unknown.

We show that the phage tmRNA is one of the most abundant transcripts produced during infection. The entire pool of phage tmRNA co-fractionates with host ribosomes and interacts with host SmpB, a protein required for tmRNA activity. We demonstrated that the phage tmRNA-encoded tag peptide is a degradation tag, promoting more efficient degradation than the host one. To reveal the function of the phage tmRNA, we established a reporter system in *C. baltica* to monitor trans-translation.

In parallel, we established in-cell crosslinking mass spectrometry for phage-infected bacteria, enabling detection of phage-host protein interactions. We identified phage protein gp40 interacting with host RNase R, an enzyme responsible for degrading non-stop transcripts in coordination with trans-translation. This suggests that the phage may contribute to the disposal of non-stop transcripts.

Reference #1:

Drobysheva, A.V., Panafidina, S.A., Kolesnik, M.V. et al. Structure and function of virion RNA polymerase of a crAss-like phage. *Nature* 589, 306–309 (2021).

Reference #2:

Janssen, B.D., Hayes, C.S. The tmRNA ribosome-rescue system. *Advances in Protein Chemistry and Structural Biology* 86, 151–191 (2012).

Reference #3:

O'Reilly, F.J., Graziadei, A., Forbrig, C. et al. Protein complexes in cells by AI-assisted structural proteomics. *Mol. Syst. Biol.* 19, e11544 (2023).

Organisation and regulation of the viral infection cycle / 113**FibriLysins: playing the Reverse Uno card against *Staphylococcus aureus*****Author:** Rob Lavigne¹¹ *KU Leuven*

In fracture-related infections (FRI), antibiotic-resistant *Staphylococcus aureus* frequently persists within fibrin-encapsulated biofilms and abscess communities that restrict immune clearance and antibiotic penetration. This trait is mediated by prophage-encoded accessory genes, which serve as major drivers of *Staphylococcus aureus* virulence.

We here present FibriLysins, a first-in-class antibacterial strategy that effectively plays a “Reverse Uno card” on the pathogen by repurposing staphylokinase, a prophage-encoded virulence factor used by *S. aureus* for dissemination, as a targeted fibrin-degrading module fused to a potent bacteriophage-derived endolysin. This dual-action strategy dismantles the protective fibrin barrier surrounding bacterial communities and subsequently eradicates the exposed bacteria.

From a library of screened FibriLysins, the lead candidate, FL-028, showed broad activity against diverse clinical *S. aureus* isolates. In vitro, FL-028 disrupted fibrin-encapsulated abscess communities and biofilms, reducing bacterial burdens by 3.4 and 2.3 log₁₀, respectively, outperforming vancomycin. In a murine FRI model, FL-028 reduced bacterial burden approximately one log₁₀ more effectively than vancomycin and eliminated abscess communities from infected tissues. Importantly, local administration showed no detectable adverse effects on bone healing.

These findings establish FibriLysins as a therapeutic platform that turns a key bacterial virulence mechanism against its host, illustrating how phage-derived antibacterials can exploit pathogen biology to overcome persistent, antibiotic-resistant staphylococcal FRI.

Reference #1:

De Maesschalck, V. et al. FibriLysins disrupt *Staphylococcus aureus* biofilms and abscess communities. *Nature Microbiology* (2026) in press.

Reference #2:

De Maesschalck, V., Gutiérrez, D., Paeshuyse, J., Lavigne, R. & Briers, Y. Advanced engineering of third-generation lysins and formulation strategies for clinical applications. *Critical Reviews in Microbiology* 46, 548-564 (2020).

Organisation and regulation of the viral infection cycle / 114**ASO-based functional genomics reveals new phenotypes of essential genes in nucleus-forming jumbo phages****Author:** Jörg Vogel¹¹ 1 Helmholtz Institute for RNA-based Infection Research (HIRI), 2 University of Würzburg

Jumbo phages with large genomes (200-600 kb) are fascinating models to study RNA translocation across membranes and intracellular structures. Many such jumbo phages including the *Pseudomonas* phage phiKZ build and replicate with an intracellular structure in the infected bacterium, the so-called phage nucleus. This protects their genome from cytosolic phage defense proteins, but it also necessitates phage mRNA export into the host cytosol for translation by host ribosomes. The mRNA export machinery is currently unknown, and functional screens have been severely hampered by the general genetic inaccessibility of nucleus-forming jumbo phages. To overcome this limitation, we have introduced non-genetic mRNA knockdown using exogenous delivery of programmable antisense oligomers (ASOs) to silence genes of both DNA and RNA phages (Gerovac M et al. 2025). A systematic screen of core and accessory genes of phage ΦKZ, coupled to RNA-sequencing and microscopy analyses, has discovered previously unrecognised proteins that are essential for phage propagation and that, upon silencing, elicit distinct phenotypes at the level of the phage and host response. I will give an update on where we stand with microbial ASO technology in general and with nucleus-forming phages in particular.

Reference #1:

Gerovac M, Buhlmann L, Zhu Y, Đurica-Mitić S, Rech V, Carien S, Gräfenhan T, Popella L, Vogel J (2025) Programmable antisense oligomers for phage functional genomics
Nature 646(8087):1195-1203

Organisation and regulation of the viral infection cycle / 92**Functional dissection of the *Bacillus subtilis* SP β prophage lysogeny-lysis system****Author:** Katharina Kohm^{None}**Co-authors:** Robert Warneke¹; Najiba Azemi¹; Juri Rappsilber¹; Fabian M. Commichau²¹ Institute for Biotechnology, FG Bioanalytic, TU Berlin, Germany² Institute for Biology, FG Molecular Microbiology, University of Hohenheim, Stuttgart, Germany

SP β -like phages possess a unique lysogeny control system [1]. Our recent work identified the master repressor of SP β and an essential activator of the lytic cycle [2]; however, the regulatory network governing the lysis-lysogeny decision requires further elucidation [1]. Furthermore, fundamental mechanisms such as virion assembly or the adsorption of SP β to its bacterial hosts remain poorly understood [3].

We are using time-resolved quantitative proteomics to gain a better understanding of the regulatory dynamics of SP β . This involves studying (1) MrpR-dependent induction and (2) infection. Analyses of a non-inducible lysogen and an inducible lysogen revealed different dynamics of phage protein accumulation following induction. Time-resolved proteomics suggests that the absence of *yosL* affects the temporal organization and/or progression of the lytic phage program. Moreover, the study is investigating which temporal changes in the bacterial proteome are associated with successful lytic induction. During phage infection a rapid and sequential accumulation of phage proteins occurs. Most phage proteins were first detected during the early phase of infection, whilst other proteins accumulated at a later stage. This suggests a staggered expression program during infection.

In addition, we investigated the mechanisms underlying phage–host recognition. Isolation and whole-genome sequencing of *B. subtilis* strains resistant to SP β infection confirmed the involvement of the minor teichoic acid biosynthesis genes *ggaA* and *ggaB* in phage resistance [4]. To identify a potential secondary receptor involved in SP β adsorption or entry, transposon mutagenesis is currently being employed.

Reference #1:

[1] Kohm K, v. Clanner A, Hertel R, Commichau FM (2025) Closely related and yet special - how SP β phages control lysis-lysogeny decisions. *Trends Microbiol.* 33: 387-396.

Reference #2:

[2] Kohm K, Jalomo-Kharyova E, Krüger A, Basu S, Steinchen W, Bange G, Frunzke J, Hertel R, Commichau FM, Czech L (2023b) Structural and functional characterization of MrpR, the master repressor of the *Bacillus subtilis* prophage SP β . *Nucleic Acids Res.* 51: 9452-9474.

Reference #3:

[3] Kohm K Hertel R (2021) The life cycle of SP β and related phages. *Arch Virol* 166: 2119-2130.

[4] Estrela AI, De Lencastre H, Archer LJ (1986) Resistance of a *Bacillus subtilis* mutant to a group of temperate bacteriophages. *Microbiology* 132:411–415.

Organisation and regulation of the viral infection cycle / 44**The bully phage: A Shiga toxin-encoding prophage interferes with the induction of co-hosted prophages**

Authors: Yael Litvak¹; Rachel Hashuel¹; Tamara Wellins¹; Rachimi¹; Gutman¹; Melki¹; Taktuk¹; Yechezkel¹; Alshech¹; Wasserzug Pash¹; Avissar¹; Roitman¹; Tzarum¹

¹ *The Hebrew University*

Shiga toxin (Stx) prophages lysogenize various bacterial species, converting them into dangerous pathogens and driving the ongoing emergence of outbreaks. These pathogens are often polylysogens, harboring multiple prophages within their genomes. Yet, the impact of Stx prophage acquisition on resident prophages remains largely unknown. We show that upon infection the Stx prophage lysogenizes commensal *Escherichia coli* within the intestine. Markedly, this lysogenization altered the induction of resident prophages encoded within each new host strain. Moreover, Stx prophage lysogenization abolished the induction of ϕ NP, the most active prophage of *Citrobacter rodentium*. We further show that ϕ NP inhibition is mediated by the Stx Cro repressor, which exhibits dual-functionality, promoting its own lytic cycle while repressing the expression of ϕ NP lytic genes. Our findings reveal a novel competition strategy among prophages, and suggest that interfering with the induction of co-hosted prophages plays a role in the emergence and evolution of new Stx-producing pathogens.

This work has been deposited on bioRxiv and is currently under peer review at Nature Communications.

Organisation and regulation of the viral infection cycle / 79**The Phage Nucleus shapes the Virulence Spectrum in Chimalliviridae****Author:** Milan Gerovac¹¹ *Helmholtz Centre for Infection Research*

Chimalliviridae reorganise infected bacterial cells by assembling a phage nucleus for replication of their genomes. However, the role of the phage nucleus in host takeover and unique infection dynamics is poorly understood (1,2,3). Here, we investigate how these phages reshape the organisation of protein complexes in infected cells and how this helps sustain phage replication under stress. Using gradient fractionation coupled with proteomic analysis, we identified multiple phage protein complexes during infection. Additionally, we observed that host protein complexes associated with stress response, translation, and central metabolism were remodelled, suggesting the suppression of stress for prolonged phage replication. After nucleus-forming phages appeared enriched in bacterial isolates (1), we examined infection dynamics using electron microscopy and fluorescence imaging. Strikingly, we observed phage replication in dividing cells. These observations reveal an infection state in which nucleus-forming phages can replicate permanently rather than following a strictly virulent replication style that suggests an extended virulence spectrum. Together, our findings link the phage nucleus to extensive remodelling of the host cell and identify permanent replication as a distinct infection mode of nucleus-forming phages.

Reference #1:

Dougherty, P. E., Bernard, C., Carstens, A. B., Bumunang, E., Gerovac, M., Müsken, M., Stanford, K., McAllister, T. A., Rocha, E. P. C., & Hansen, L. H. (2026). Persistent virulent phages exist across bacterial isolates. *Nature Microbiology*, 11(1), 31–41, doi: 10.1038/s41564-025-02207-0

Reference #2:

Gerovac, M., Buhlmann, L., Zhu, Y., Đurica-Mitić, S., Rech, V., Carien, S., Gräfenhan, T., Popella, L., & Vogel, J. (2025). Programmable antisense oligomers for phage functional genomics. *Nature*, 646(8087), 1195–1203, doi: 10.1038/s41586-025-09499-6

Reference #3:

Gerovac, M., Chihara, K., Wicke, L., Böttcher, B., Lavigne, R., & Vogel, J. (2024). Phage proteins target and co-opt host ribosomes immediately upon infection. *Nature Microbiology*, 9(3), 787–800, doi: 10.1038/s41564-024-01616-x

Poster-Session / 2**Poster #2: ASO-Mediated mRNA Silencing Reveals a Link Between Tail Assembly and Genome Packaging in Phage Φ KZ****Author:** Leandro Buhlmann¹**Co-authors:** Milan Gerovac²; Jörg Vogel²¹ *Institute of Molecular Infection Biology (IMIB), University of Würzburg, Würzburg, Germany*² *Helmholtz Institute for RNA-based Infection Research (HIRI), Helmholtz Centre for Infection Research (HZI), Würzburg, Germany*

The *P. aeruginosa* infecting jumbo bacteriophage Φ KZ encodes 371 predicted genes, most of which are of unknown function. This limited characterization is partly attributable to its classification within the Chimalliviridae, a family of phages that assemble a nucleus-like compartment within the bacterial host. The phage nucleus encloses the viral genome to protect it from host defense systems such as restriction nucleases, thereby rendering the phage highly recalcitrant to traditional genetic manipulation.

To overcome this limitation, we established a platform for programmable antisense oligomer (ASO)-mediated gene silencing, enabling the targeted knockdown of essential genes in genetically intractable phages. This approach allows phenotypic characterization of phage mRNA knockdowns during infection. For example, knockdown of transcripts encoding the essential tail sheath and tail tube proteins resulted in a distinct phenotype. Fluorescence microscopy revealed atypical DNA signals in the cytoplasm, consistent with defects in virion assembly. To date, the mechanism by which the phage genome is exported from the phage nucleus and packaged during virion assembly remains unknown.

Future studies will investigate the coupling between tail assembly and genome packaging and determine whether defects in structural protein expression result in impaired DNA encapsidation or accumulation of unassembled genomic material.

Reference #1:

Gerovac, M., Buhlmann L. et al. Programmable antisense oligomers for phage functional genomics. *Nature* 646, 1195–1203; 10.1038/s41586-025-09499-6 (2025)

Poster-Session / 5

Poster #5: The impact of chronic viral infection on archaeal cell morphology**Authors:** Hanna Martin¹; Sabine Schwarzer¹; Tessa Quax¹¹ University of Groningen

Archaeal viruses represent a distinct and diverse group of infectious agents that challenge current models of virus–host interactions. Unlike viruses infecting other domains of life, archaeal viruses exhibit remarkable morphological and genetic diversity. To date, the number of archaeal viruses that have been isolated and characterized is relatively limited, which hinders the study of their infection cycles. As the cell envelope of archaea differs fundamentally from that of bacteria, viral mechanisms of entry and egress are likely to be different. In contrast to the predominantly lytic lifestyle observed in many bacterial and eukaryotic viruses, several halophilic archaeal viruses, including His1 and His2, establish chronic infections and continuously release progeny without apparent host cell lysis. His1, a spindle-shaped virus, is classified among the *Halspiviridae* [1]. In contrast, His2 belongs to the recently proposed *Pleolipoviridae* family, comprising pleomorphic viruses with minimalistic virion architecture [2]. It has been demonstrated that both viruses infect the halophilic euryarchaeon *Haloarcula hispanica*, a species characterized by its growth phase-dependent morphological changes [3].

The impact of chronic viruses on host cell function and morphology remain poorly understood. Consequently, we employed time-resolved infection assays with His1 or His2 and observed that this led to reduced motility of the cells. Closer inspection with light and electron microscopy revealed that infected cultures exhibited a substantial accumulation of cell clusters, surrounded by a thickened cell wall. Our findings offer insight into the impact of chronic infection on archaeal cell morphology, thus aiding the exploration of this enigmatic viruses.

Reference #1:

Bath, C. and M.L. Dyll-Smith, His1, an archaeal virus of the Fuselloviridae family that infects *Haloarcula hispanica*. *Journal of virology*, 1998. 72(11): p. 9392-9395.

Reference #2:

Demina, T.A. and H.M. Oksanen, Pleomorphic archaeal viruses: the family Pleolipoviridae is expanding by seven new species. *Archives of virology*, 2020. 165(11): p. 2723-2731.

Reference #3:

Schwarzer, S., et al., Growth phase dependent cell shape of *Haloarcula*. *Microorganisms*, 2021. 9(2): p. 231.

Poster-Session / 9

Poster #9: A prophage-encoded anti-phage defence system that prevents phage DNA packaging by targeting the terminase complex**Author:** Nadejda Sigal¹**Co-authors:** Anat Herskovits¹; Avijit Das¹; Dan Koenigsfeld¹; Einat Biderman¹; Gil Azulay¹; Ilya Borovok¹; Keren Tzohar Rabinovich¹; Lev Rabinovich¹; Olga Stadnyuk¹; Polina Lisitzin¹; Raz Zarivach²; Yonatan Cohen¹¹ *Tel Aviv University*² *Ben Gurion University of Negev*

A unique feature of temperate phages is the ability to protect their host bacteria from a second phage infection. Such protection is granted at the lysogenic state, where the phages persist as prophages integrated within the bacterial chromosome, expressing genes that defend the host and themselves from predation. Here, we report a prophage-encoded anti-phage defence system that inhibits DNA packaging of invading phages in *Listeria monocytogenes*. This system includes a defence protein, TerI, and two self-immunity proteins, anti-TerI1 and anti-TerI2. TerI targets the terminase complex of invading phages to prevent DNA translocation into procapsids without halting the lytic cycle, thereby leading to the release of unpacked non-infectious procapsids upon bacterial lysis. In contrast, the self-immunity proteins, anti-TerI1 and anti-TerI2, counteract TerI during prophage induction to allow virion production. We found this unique prophage-encoded anti-phage defence system, TERi, to be prevalent in *Listeria* phages, providing population-level host protection without compromising the prophage lytic lifecycle.

Poster-Session / 11**Poster #11: Protein fusion analysis reveals new phage sponges****Author:** Romi Hadary¹¹ *Weizmann institute of science*

Immune systems in animals, plants, and bacteria often rely on intracellular nucleotide signaling to activate immune responses in response to viral infection. Bacteriophages (phages) can evade these defenses by producing sponge proteins that bind and sequester immune signaling molecules. However, the full arsenal of phage sponges is currently unknown. We found that phage sponge proteins tend to be fused to one another in phage genomes. Exploiting this property, we discovered Sequestin, a previously uncharacterized family of phage proteins that manipulates bacterial immune signaling. Experimental validation revealed that Sequestin inhibits the Thoeis defense system by acting as a nucleotide “sponge” that binds the Thoeis signaling molecule 1’–3’ glycosylated adenosine diphosphate–ribose (3’cADPR). Structural and mutational analyses explain the molecular basis of signal recognition. Thousands of Sequestin homologs occur in phage genomes, highlighting the abundance and diversity of viral strategies that evade nucleotide-based immunity.

Reference #1:

Tal, Hadary et al. ,Structural modeling reveals phage proteins that manipulate bacterial immune signaling.Science391,eaea1761(2026).DOI:10.1126/science.eaea1761

Poster-Session / 12

Poster #12: Phage Fitness Landscapes and Butterfly Effects in Evolving Phage Populations**Authors:** Sebastian Leptihn¹; Zhanybek Selpiev²; Belinda Loh²¹ *HMU Erfurt*² *Fraunhofer Institute IZI*

Our study investigated evolutionary dynamics in *Klebsiella* bacteriophage populations, focusing on a spontaneously emerged mutant exhibiting a bulls-eye plaque morphology. Whole-genome sequencing identified point mutations in tail proteins as the genetic basis for this variant. *In vitro* competition experiments revealed that independent runs did not always produce the same winner, with phage variants reaching near-fixation in different experiments, reminiscent of butterfly-like effects where small differences cause distinct outcomes. This indicates that small changes in genetic background and variant characteristics can bias phage populations to distinct evolutionary outcomes, even in nominally identical environments.

To understand the impact of mutations on fitness landscapes, we determined attachment rates, burst sizes, and latent periods *in vitro*. While the bulls-eye plaque variant “Porky” and its parental phage “Shorty” did not differ significantly in attachment rate, both latent period and burst size were affected. Despite these differences, competitive advantage was not fixed: in some runs one phage outcompeted the other, and in others the outcome was reversed. In contrast, a third mutant (“Sporty”) showed stronger attachment and consistently outcompeted both Shorty and Porky. To determine contribution of each fitness parameter, we performed simulations of the evolutionary dynamics using discrete mathematical models constrained by infection and replication parameters and found the adsorption rate to be a key fitness parameter. Our integrated approach shows how genetic mutations in phage populations can change phage fitness and illustrates the importance of sensitive variant detection methods, like deep sequencing, for quality control in phage preparations.

Reference #1:

Zhanybek Selpiev, Sebastian Leptihn, Mathias Müsken, and Belinda Loh. Deciphering Shared Receptor Usage in Genomically Unrelated Bacteriophages Infecting Hypervirulent *Klebsiella pneumoniae* K1 ST23 FEMS Microbes (2025)

Poster-Session / 13

Poster #13: Interbacterial competition triggers stress-induced CRISPR immunity against bacteriophages**Author:** Rafael Custodio¹**Co-authors:** Edze Westra¹; Ellinor Alseth²; Maria Martignoni³; Pedro Moura-Alves⁴; Rachel Kuske³; Sam Brown³; Wenlong Zhu¹¹ *University of Exeter*² *The Arctic University of Norway*³ *Georgia Institute of Technology*⁴ *Universidade do Porto*

Bacteria–phage interactions are commonly studied in monoculture, yet bacteria in nature experience interspecific competition that may alter their antiviral defences. We investigated whether competition directly regulates CRISPR-Cas immunity in *Pseudomonas aeruginosa* PA14. Mathematical modelling predicted that competition-induced CRISPR regulation would cause rapid enrichment of CRISPR-immune cells, distinct from the delayed effects of ecological selection. We tested this prediction using transcriptional reporters and short-term spacer-acquisition assays following infection with phage DMS3vir in monoculture or co-culture with *Acinetobacter baumannii*, *Burkholderia cenocepacia*, or *Staphylococcus aureus*. Co-culture with *A. baumannii*, but not the other competitors, increased spacer acquisition approximately tenfold and strongly elevated CRISPR-Cas expression within three hours. CRISPR-Cas expression correlated positively with both short-term spacer acquisition and longer-term evolution of CRISPR-mediated phage resistance. Quorum sensing did not explain this response: interspecific competition suppressed Las signalling, and disruption of Las signalling further increased CRISPR-Cas expression during competition with *A. baumannii*. Instead, competition with *A. baumannii* induced a strong cellular SOS response, and experimentally imposed DNA damage with mitomycin C caused dose-dependent increases in CRISPR-Cas expression in the absence of competitors. Together, our results identify competition-induced stress as a physiological cue that primes bacterial adaptive immunity before selection acts. These findings connect interbacterial antagonism to phage defence and suggest that microbial community context can accelerate the emergence of CRISPR-mediated resistance during phage infection and therapy.

Poster-Session / 14

Poster #14: Molecular mechanisms of the synergistic interactions of Druantia III and ARMADA II in the antiphage defense of enterohaemorrhagic Escherichia coli strain EDL933**Author:** Huihui Li¹**Co-authors:** Stephanie Himpich²; Vu Van Loi³; Lena Grass²; Alexandros Iliopoulos²; Costanza Borelli³; Eberhard Klauck³; Tarek Hilal⁴; Bernhard Loll²; Markus C. Wahl²; Haike Antelmann³¹ *Freie Universität Berlin, Institute of Biology-Microbiology*² *Freie Universität Berlin, Laboratory of Structural Biochemistry*³ *Freie Universität Berlin, Institute of Biology-Microbiology*⁴ *Freie Universität Berlin, Research Center of Electron Microscopy and Core Facility BioSupraMol*

The EHEC O157:H7 outbreak strain EDL933 exhibits strong resistance against a broad range of phages and harbors three co-localized defense systems on O-island 172 (O172): Zorya II, Druantia III, and a ARMADA II system. To investigate the contribution and interplay of these systems, single and combined deletion mutants of these three Systems were generated in EDL933 and analyzed by EOP assays against the BASEL phage collection. Druantia III and ARMADA II provided broad-spectrum protection against most phage families, whereas Zorya II showed specific protection against Autographivirales. Notably, Druantia III and ARMADA II displayed strong synergistic interactions, resulting in enhanced phage resistance beyond the additive effects of the individual systems. To elucidate the molecular mechanism of ARMADA II, all four components were recombinantly produced and purified. Interaction studies revealed stable complex formation between two protein pairs, and cryo-EM enabled the structural characterization of a complex comprising a methyltransferase and a Snf2-type NTPase/helicase. Complementation with interaction-deficient variants demonstrated that complex formation is essential for antiviral activity. Moreover, a cofactor-bound structure revealed a unique conformational toggling mechanism of the NTPase/helicase relative to the methyltransferase, suggesting coordinated regulation of ARMADA II activity. Furthermore, global phage-host proteome and ArmC interactome analyses are underway to elucidate possible interactions among Druantia III and ARMADA II as well as host interactions of these systems. Together, our findings establish Druantia III and ARMADA II as key contributors to EHEC phage resistance and provide structural insights into the molecular mechanism of ARMADA II-mediated antiviral defense.

Poster-Session / 15**Poster #15: Guide amplification bootstraps immune specificity in a bacterial Argonaute****Authors:** Jack Bravo¹; Balwina Koopal¹; Kathrin Froschauer¹; Vojtech Orel¹; Daniel Williams-Jones¹¹ *Institute of Science and Technology Austria*

Bacteria face constant invasion by mobile genetic elements such as plasmids and phages, and many have evolved guide-directed nucleases related to eukaryotic Argonaute proteins to combat these threats. How such systems achieve specific recognition of foreign, supercoiled plasmid DNA without simply guessing based on guide sequence has been a longstanding puzzle. Here we describe a short prokaryotic Argonaute system that solves this problem in an unexpected way: rather than encoding specificity in the guides themselves, the system generates its own specificity dynamically, through a feedback process triggered by target recognition. This self-reinforcing behavior allows the system to home in on supercoiled plasmid targets while leaving the host chromosome untouched. We present structural, biochemical, and *in vivo* data that together reveal this mechanism, and discuss its implications for how specificity can emerge in nucleic-acid-guided immune systems more broadly, with intriguing parallels to amplification-based immunity in other domains of life.

Poster-Session / 18

Poster #18: Decoding the phage unknown with protein language models**Authors:** Simon Hackl¹; Mona Scheurenbrand¹; Kay Nieselt¹; Maik Wolfram-Schauerte^{None}¹ *University of Tübingen*

Bacteriophage genomes encode large numbers of proteins without functional annotation, collectively termed the phage “unknown.” This limits both mechanistic understanding of phage biology and the discovery of proteins with potential applications in biotechnology and therapy. Current annotation approaches rely primarily on sequence or structural homology and provide indispensable first evidence for newly sequenced phages. However, they often fail when similarity to characterized proteins is weak, leaving few experimentally testable hypotheses.

Here, we develop a machine-learning framework that predicts functional classes for phage proteins of unknown function. The model uses protein language model embeddings to assign proteins to one of 89 curated functional categories. It accurately recovers existing annotations in both a held-out test set and entirely unseen phages (F1-score > 90 %). Moreover, although genomic context is not included during training, predictions for proteins encoded within operons are functionally coherent, suggesting that the model captures biologically meaningful sequence features. Applied to the phage unknown, the framework produces confident functional-class predictions that increase the proportion of classifiable phage proteins to more than 60%. Analyses of the model phages T4 and phiKZ illustrate how these predictions can generate hypotheses for previously uncharacterized genes. A prototype web server integrates model outputs with sequence- and structure-based evidence and enables interactive visual inspection across the phage protein universe. Together, this framework expands functional annotation beyond detectable homology and supports targeted experimental characterization of the phage unknown.

Poster-Session / 19

Poster #19: Hidden Functional Diversity among Type V-A CRISPR Effectors**Author:** Oleg Dmytrenko¹¹ *Forschungszentrum Jülich*

Bacterial antiphage defenses, including CRISPR-Cas, are commonly classified according to their evolutionary relationships, presence of known functional domains, or genomic neighborhood. Yet, as viral predation drives rapid diversification, incomplete sequence sampling and fragmented knowledge of bacterial immunity often undermine our ability to reliably predict defense functions. For example, we have recently uncovered functional variation hidden among well-known type V-A CRISPR-Cas effectors. Unlike DNA-targeting Cas12a, these additional Cas12a2 and Cas12a3 clades target RNA. Further yet, their downstream nuclease activities and physiological outcomes differ strikingly. Activated Cas12a2 indiscriminately degrades ssRNA, ssDNA, and dsDNA. In bacteria, collateral DNA damage triggers an SOS response and growth arrest, preventing phage dissemination. We harnessed this property to selectively eliminate eukaryotic cells based on viral transcripts or oncogenic mutations. Cas12a3 instead recruits tRNAs through a novel loading domain and cleaves their conserved 3'-CCA tails. The resulting tRNA truncation arrests growth and limits phage replication. By designing synthetic reporters that mimic tRNAs, we expanded the multiplexing capacity of existing CRISPR-based diagnostic platforms for viral RNA detection. Together, these effectors show how evolutionary remodeling of a shared effector scaffold can redirect immunity from broad nucleic acid destruction to selective inactivation of the translation machinery, underscoring both the limits of predicting molecular function and persistent gaps in our knowledge of bacterial defenses. Looking ahead, we aim to systematically explore bacterial immunity across the evolutionary landscape to determine how variation in activation, regulation, molecular mechanism, and physiological outcome generates distinct antiviral strategies and new opportunities for biotechnology.

Poster-Session / 20

Poster #20: RNylation as a Potential Modulator of Translation in bacteriophage T4 Infection of Escherichia coli**Author:** Lydia Garrido García-Dorado¹**Co-authors:** Nurseda Yilmaz Demirel²; Katharina Höfer¹¹ *LIT Regensburg*² *Max Planck Institute for Terrestrial Microbiology, Marburg, Germany, International Max Planck Research School (IMPRS) "Principles of Microbial Life", Marburg, Germany*

RNylation is a novel discovered post-translational modification catalyzed by the bacteriophage T4 ADP-ribosyltransferase ModB in which NAD-capped RNAs are covalently attached to host ribosomal proteins. These ribosomal proteins play central roles in translation initiation and elongation. While RNylation has been shown to occur during bacteriophage T4 infection, its impact on the translational process remains largely unknown. Infections in *Escherichia coli* with bacteriophage T4 carrying a catalytically inactive ModB mutant result in delayed lysis, reduced burst size, and decreased phage adsorption (1). This suggests that ModB could play a crucial role in hijacking the translational apparatus of *E. coli*. Building on these observations, this study investigates how RNylation mediated by ModB during phage infection affects translation in *E. coli*.

To further explain the biological role of ModB, we aim to characterize its protein structure to gain mechanistic insight into its catalytic activity and substrate recognition.

Understanding how this modification affects translation will provide insight into phagehost interactions and will unravel novel strategies bacteriophage T4 use to manipulate *E. coli*.

Reference #1:

Wolfram-Schauerte M, Pozhydaieva N, Grawenhoff J, Welp LM, Silbern I, Wulf A, Billau FA, Glatter T, Urlaub H, Jäschke A, Höfer K. A viral ADP-ribosyltransferase attaches RNA chains to host proteins. *Nature*. 2023 Aug;620(7976):1054-1062. doi: 10.1038/s41586-023-06429-2. Epub 2023 Aug 16. PMID: 37587340; PMCID: PMC10468400.

Poster-Session / 21**Poster #21: Insights into plasmid elimination by a pAgo guided anti plasmid immune system****Author:** Kathrin Froschauer¹**Co-authors:** Balwina Koopal¹; Anastasiia Skuba¹; Vojtech Orel¹; Daniel Williams-Jones¹; Jack Bravo¹¹ *Institute of Science and Technology Austria*

Bacteria fight in an ongoing arms race against selfish mobile genetic elements, such as plasmids or phages, which drives the evolution of different defense systems against those invaders. Recently, a plethora of new defense systems have been identified and characterized, most of them protecting against phages. While plasmids can be of advantage for the bacteria by allowing adaptations to environmental stress (e.g., spread of antibiotic resistance genes) and driving evolution, they can become a significant burden to the bacterial host. Thus, it is not a surprise that in addition to anti phage also anti plasmid systems have been identified. We are currently investigating one of those systems consisting of a nuclease/helicase effector protein that is guided by a prokaryotic argonaut (pAgo).

We aim to understand how this system is specific for foreign plasmid DNA, how plasmid degradation is facilitated by the effector and pAgo pair and if further components are required to protect against plasmids.

Poster-Session / 24**Poster #24: Temperate versus virulent phage lifestyle impacts microdiversity in soil environments****Authors:** Thomas de Bruijn¹; Hilje Doekes¹; Anne Kupczok¹¹ *Wageningen University*

Bacteriophages are not only the most ubiquitous biological entity on earth, they also display remarkable genetic diversity across and within populations. While macrodiversity has been extensively studied, the drivers of microdiversity (intra-species genetic diversity) remain poorly understood, particularly in relation to phage lifestyle. The distinguishing ability of temperate phages to integrate themselves into the host genome has an unknown influence on the microdiversity present. This difference in microdiversity could impact the adaptability of phages to (a)biotic factors. To identify a possible association between microdiversity and lifestyle, we analysed 12 existing viromics datasets focusing on soil bacteriophages, including 43,171 viral genomes in total. We found that phages predicted to be temperate consistently exhibit higher microdiversity than their virulent counterparts in eight of 12 datasets. Of the remaining four datasets, one showed the opposite trend and three datasets did not show a significant trend at all. The detected pattern holds across multiple quality thresholds and lifestyle prediction methods. These findings suggest that lifestyle has a significant impact on genetic variation within phage populations, with potential implications for phage-host coevolution and environmental adaptability.

Poster-Session / 26

Poster #26: The Z-Project: Expanding the Bacteriophage Toolbox**Authors:** Clara Rolland¹; Johannes Wittmann¹**Co-authors:** Adèle Zrein²; Imen Nouioui¹; Meike Döppner¹; Moritz Rückwald¹; Stefanie Barbirz²; Stephanie Peter¹; Yvonne Mast¹¹ *Leibniz Institute DSMZ-German Collection of Microorganisms and Cell Cultures GmbH, Braunschweig*² *MSB Medical School, Hochschule für Gesundheit und Medizin, Berlin*

The Z-project of the DFG Priority Programme SPP 2330 provides standardized methodologies and infrastructure for bacteriophage research, including isolation, characterization, host-range determination, and infection kinetics. Collaborative projects with SPP partners aim to expand phage diversity by isolating novel bacteriophages from diverse bacterial hosts. These efforts resulted in the isolation of eleven phages infecting rare actinomycetes, including three *Lentzea* phages (two new genera) and a temperate *Actinomadura* phage, vB_AmeS_Stercus, whose endolysin shows potential against pathogenic *Actinomadura madurae* and *Actinomadura latina*. Five phages infecting *Kitasatospora* and two infecting *Salinispora* are currently being characterized. Additionally, 44 phages were isolated from *Citrobacter* and *Enterobacter* strains, including five infecting strains from both genera. *Citrobacter freundii* phage Cfr-14, a lytic myovirus of the *Pseudotevenvirus* genus, exhibits cross-infectivity with *Enterobacter cloacae* complex strains and demonstrates high infection efficiency. Infection kinetics revealed strain-dependent lysis and rapid, incomplete adsorption, suggesting limited receptor availability or receptor saturation. Ongoing studies aim to identify the phage receptor and further define its host range within *Enterobacterales*. Furthermore, we are actively looking for phages targeting clinically relevant oral pathogens. Three *Fusobacterium nucleatum* strains (DSM 15643, DSM 20482, DSM 19679) and several *Streptococcus* spp have been selected for phage isolation. Preliminary LPS analysis of *F. nucleatum* revealed distinct O-antigen structures, and potential phage particles were detected by dynamic light scattering (ZetaSizer) in enrichment approaches using DSM 19679, indicating promising leads. Collectively, these efforts expand available bacteriophage diversity, provide new models for phage–host interaction studies, and strengthen SPP 2330 bioresources, including the phage-dedicated database PhageDive.

Poster-Session / 28

Poster #28: Domain swapping reveals deep conservation of death-domain immune signaling**Author:** Gideon Cummings¹**Co-authors:** Marcus Taylor²; Tanita Wein¹; Finn Lobnow²¹ *Weizmann Institute of Science*² *Max Planck Institute for Infection Biology*

Death domains are central scaffolds of innate immune signaling in animals, where upon pathogen recognition they rapidly nucleate higher-order signalosomes that control inflammatory responses and programmed cell death. Homologous domains were recently identified in putative bacterial defense systems, where they occur in combination with diverse cell death effectors in a mix-and-match architecture. However, whether death domains share conserved mechanisms of assembly, signaling, and immune response execution across the tree of life remains unknown. We expressed several putative bacterial systems containing death domain homologues in *E. coli* and conducted infection assays. Our results show that these bacterial systems confer immunity against phage infection. Remarkably, anti-phage immunity remains functional in bacteria when bacterial and mammalian death-domain homologues are exchanged in chimeric constructs. Structure-guided mutagenesis demonstrated that residues conserved in bacterial and mammalian death domains, together with conserved effector active-site residues, are essential for immune function. These findings support a highly conserved mechanism of death-domain-mediated immune signaling that predates the emergence of eukaryotes. Our work therefore establishes bacterial death-domain systems as tractable models for discovering novel bacterial immune mechanisms and investigating the evolutionary conservation of supramolecular immune signaling across the tree of life.

Poster-Session / 29

Poster #29: Living together: molecular mechanisms of phage-bacterial coexistence in the gut**Authors:** Rodrigo Sanchez Martinez¹; Ana López-Moreno¹; Christina Ludwig²; Bärbel Stecher¹¹ *Chair of Intestinal Microbiome, Technical University of Munich, Freising, Germany*² *Bavarian Center for Biomolecular Mass Spectrometry (BayBioMS), Technical University of Munich, Freising, Germany*

Phages play a crucial role in maintaining gut microbiome homeostasis by regulating bacterial populations and modulating host metabolic and immune functions. Despite the remarkable stability of both bacterial and phage communities in the intestine, the mechanisms enabling their long-term coexistence remain poorly understood. This study aimed to identify the key ecological and molecular factors driving phage-bacterial coexistence in the gut environment.

To address this question, we used two well-established gut bacterial models, *Escherichia coli* and *Enterococcus faecalis*, together with three virulent phages infecting each species. Long-term in vitro coexistence assays were performed under different environmental conditions, including the presence of the defined microbial community Oligo-Mouse-Microbiota (OMM12). In parallel, comparative proteomic analyses were conducted to characterize bacterial responses associated with phage coexistence.

Long-term coexistence was observed for most phage-host pairs, with the presence of a microbial community enhancing phage persistence. Proteomic analyses revealed the upregulation of pathways involved in nutrient uptake and metabolism, particularly the transport and utilization of simple carbohydrates, suggesting that metabolic adaptation is a key mechanism supporting coexistence. In addition, some bacterial defense systems were differentially regulated, indicating a potential role in phage-bacterial coexistence. Ongoing validation using targeted knockout mutants is further supporting the contribution of these pathways to stable phage-host interactions.

Overall, this work provides new insights into the molecular and ecological mechanisms governing phage-bacterial coexistence in the gut and identifies potential targets for microbiome-based therapeutic strategies.

Poster-Session / 30

Poster #30: Phage-encoded single-guide RNAs subvert CRISPR-Cas9 immunity

Authors: Stephan Riesenberger¹; Nils Birkholz²; David Mayo-Muñoz²; Holly Wakelin²; Chris M. Brown²; Zasha Weinberg³; Rafael Pinilla-Redondo⁴; Peter C. Fineran²; Chase L. Beisel⁵

Co-authors: Maximilian Feussner³; Angela Migur⁵; Jinqun Li²

¹ *Max Planck Institute for Evolutionary Anthropology*

² *University of Otago*

³ *University of Leipzig*

⁴ *University of Copenhagen*

⁵ *Botnar Institute of Immune Engineering*

Bacteriophages are relentless innovators in the molecular arms race with bacteria. To evade adaptive immune systems like CRISPR-Cas—which utilize RNA-guided nucleases to cleave viral genomes—phages have evolved a vast array of counter-defences. However, despite this evolutionary creativity, the known anti-CRISPR (acr) repertoire remains almost exclusively composed of proteins. Here, we uncover a fundamentally distinct counter-defence strategy: phage-encoded viral single-guide RNAs (vsgRNAs) that disable type II CRISPR-Cas immunity through RNA-guided rewiring of Cas9 function. We show that vsgRNAs, encoded in *acr*-associated genomic contexts and predicted to inhibit diverse type II-A and II-C systems, act through a two-tier inhibitory mechanism. First, they capture the Cas9 apoenzyme and divert it from endogenous phage-targeting crRNAs. Second, by means of a truncated guide region, they redirect Cas9 to bind but not cleave its own promoter, thereby silencing *cas9* transcription. Strikingly, phages generated vsgRNAs by co-opting the same guide and promoter target site used by extended tracrRNAs that autoregulate *cas9* expression. In this way, vsgRNAs enable phages to simultaneously disable ongoing interference and restrict renewed production of the immune effector. We further show that phages similarly co-opted small CRISPR-associated RNAs (scaRNAs) targeting the *cas9* promoter. These findings reveal that phages hijack intrinsic CRISPR-Cas autoregulatory logic to repurpose a bacterial immune nuclease for RNA-mediated immune silencing, uncovering a previously unrecognized class of anti-CRISPR effectors and a new principle of prokaryotic virus–host conflict.

Poster-Session / 31

Poster #31: tRNA epitranscriptome plasticity optimizes phage infection in *E. coli***Author:** Luis Rivera-Montero¹**Co-authors:** Siobhan A. Cusack¹; Sebastián Riquelme-Barrios¹; Bibakhya Saikia¹; Pascal Giehr²; Stephanie Kaiser³; Kirsten Jung¹¹ Faculty of Biology, Microbiology, Ludwig-Maximilians Universität² Department of Chemistry, Ludwig-Maximilians Universität.³ Institute of Pharmaceutical Chemistry, Goethe Universität

The epitranscriptome is the collection of all chemical modifications in RNA molecules. Changes in the epitranscriptome alter the structure and functionality of RNAs, and thereby influence biological processes. The contribution of epitranscriptomic regulation to host–virus interactions in prokaryotes is poorly understood. To shed light on these effects, we performed time-resolved dual profiling of host and viral tRNA expression and modification patterns in an *Escherichia coli*–T5 phage infection model. Using direct RNA sequencing, proteomics, and mass spectrometry, we comprehensively analyzed the changes on tRNA and rRNA modifications and their relation with protein synthesis. Phage infection induced changes in host tRNA modifications. These modifications support optimal particle production and replication time. In parallel, viral tRNAs and tRNA-like structures are expressed and modified throughout infection. We observe that viral tRNA while having different modifications pattern from their cognate host tRNAs, substitute or complement the host tRNAs during infection and, together with their epitranscriptomic signature, support the protein synthesis requirements during the late stage of phage production. These findings reveal that the overall tRNA pool is adjusted at both the abundance and epitranscriptomic levels to optimize translation, highlighting RNA-based regulation and its modification as an additional layer of phage–host adaptation.

Poster-Session / 32

Poster #32: Mechanism of activation of the defense-associated reverse transcriptase DRT4 by a phage single-stranded DNA-binding protein

Authors: Monika Jasnauskaite¹; Augustas Cibulskis¹; Kristina Kazlauskaite¹; Aiste Skorupskaite¹; Jonas Juozapaitis¹; Algirdas Miksys¹; Audrone Ruksenaite¹; Migle Tomkuvienė¹; Lina Malinauskaite¹; Patrick Pausch¹

¹ Vilnius University

DRTs are widespread anti-phage defense systems, yet the molecular mechanisms underlying their activation remain poorly understood. Here, we investigate the mechanism of the type 4 defense-associated reverse transcriptase (DRT4), a member of the UG/Abi class 1 systems. Screening against the BASEL bacteriophage collection revealed that DRT4 provides broad protection against diverse phage families. Using phage escape mutants, we identified the Bas17-encoded single-stranded DNA-binding protein (SSB) as an activator of DRT4. Bacterial survival assays demonstrated that gp44 is sufficient to trigger DRT4-dependent growth arrest, indicating that gp44 directly activates the defense response.

CryoEM revealed that DRT4 forms a homohexameric complex and synthesizes covalently linked single-stranded DNA through a protein-priming mechanism. Biochemical and mass spectrometric analyses showed that the synthesized ssDNA is composed predominantly of adenine and guanine nucleotides, with the SSB altering its length distribution in vitro. AlphaFold3 predicted a direct interaction between SSB and the flexible C-terminal domain (CTD) of DRT4, which was supported by mutagenesis, phage infection assays, in vivo transformation assays, and in vitro reconstitution. Disruption of the predicted interaction interface or deletion of the CTD abolished anti-phage defense without impairing ssDNA synthesis, demonstrating that the CTD is required for productive activation rather than polymerase activity. Furthermore, the CTD-interaction was necessary for SSB-dependent stimulation of DRT4 RNase activity.

Together, these findings establish a mechanism in which a phage SSB protein activates DRT4 through interactions with its flexible CTD and ssDNA product, providing new mechanistic insight into reverse transcriptase-mediated bacterial immunity and expanding our understanding of UG/Abi defense systems.

Poster-Session / 33

Poster #33: Gasdermin-mediated phage defense as a model for intercellular immune signaling in *Streptomyces***Author:** Tal Livny¹**Co-authors:** Larissa Ernst²; Julia Frunzke²; Tanita Wein¹¹ *Weizmann Institute of Science*² *Forschungszentrum Jülich*

Gasdermins are pore-forming proteins that mediate programmed cell death in both eukaryotes and bacteria. In animals, pathogen-induced activation of gasdermins not only eliminates infected cells but also promotes the release of inflammatory signaling molecules that coordinate immune responses. In bacteria, gasdermins have recently been identified as anti-phage defense systems that abort infection through membrane pore formation, yet whether they also enable intercellular signaling remains unknown. We identified functional gasdermin homologs in *Streptomyces* species that provide defense against bacteriophage infection. As filamentous, multicellular bacteria that rely on extracellular signaling to coordinate development and stress responses, *Streptomyces* provide a unique model to investigate whether gasdermin activation is coupled to intercellular signaling during phage infection. We hypothesize that gasdermin pore formation facilitates the release of extracellular signaling molecules from infected filaments, allowing uninfected regions of the colony to detect phage attack and mount coordinated antiviral responses before infection spreads. Our work aims to determine whether bacterial gasdermins function not only as autonomous cell-death effectors but also as mediators of colony-wide immune coordination. This will provide insight into the evolution of innate immunity and reveal whether coordinated intercellular communication is a conserved feature of gasdermin-mediated defense across domains of life.

Poster-Session / 35

Poster #35: Identification of host factors involved in cystovirus replication**Authors:** Aatreyi Roy¹; Jens Hör²¹ *Helmholtz Institute for RNA-based Infection Research (HIRI), Helmholtz Centre for Infection Research (HZI), Würzburg, Germany*² *Helmholtz Institute for RNA-based Infection Research (HIRI), Helmholtz Centre for Infection Research (HZI), Würzburg, Germany; Institute of Molecular Infection Biology (IMIB), Würzburg, Germany*

RNA viruses have compact genomes that require them to hijack host factors for successful replication. This is also true for RNA phages with single-stranded genomes, for which it is well established that they co-opt various host factors involved in translation to facilitate their infection cycles. By contrast, host factors co-opted by cystoviruses, i.e., RNA phages with tri-segmented double-stranded genomes, remain largely unknown. To globally identify these factors, we employed high-throughput transposon sequencing to a panel of diverse cystoviruses under a variety of infection conditions. Our approach reveals both host factors that are commonly used by the studied phages as well as such that are uniquely co-opted by only single phages in our panel. We further highlight host genes that appear to be conditionally required for cystovirus replication and suggest the hitherto unknown receptors the phages use for adsorption. Overall, our results establish a global overview of host factors co-opted by cystoviruses under a variety of conditions, thus providing a framework for future studies on the underlying molecular mechanisms.

Poster-Session / 37

Poster #37: Unveiling the regulatory landscape of the double-stranded RNA phage $\phi 6$ using ribosome profiling**Authors:** Manuel Velasco Gomariz¹; Jens Hör²¹ HIRI-Würzburg² HIRI-Würzburg; IMIB-Würzburg

Regulation of gene expression is a key aspect of phage biology. DNA phages primarily rely on transcriptional regulatory mechanisms to modulate protein output both temporally and quantitatively. By contrast, phages with RNA genomes do not undergo a DNA stage during replication and therefore must rely on post-transcriptional regulation. These circuits, however, remain largely unexplored, particularly in phages with double-stranded RNA genomes.

To address this, we combine ribosome profiling and total RNA-seq to uncover the translational and transcriptional dynamics of the dsRNA phage $\phi 6$ during infection of its host *Pseudomonas savastanoi*. We determine the translational efficiency of each host and phage gene and identify potential regulatory events such as ribosome pausing. We further observe unexpected active translation within regions of both the phage genome and the host genome that are annotated as non-coding, thus suggesting the presence of novel ORFs.

This work reveals the sophisticated post-transcriptional regulatory network orchestrated by $\phi 6$ during infection, which appears to employ regulatory strategies reminiscent of ssRNA phages and eukaryotic RNA viruses. These findings expand our understanding of RNA phage biology and highlight the broader significance of post-transcriptional regulation, an emerging conceptual framework relevant for both RNA and DNA phages.

Poster-Session / 38

Poster #38: Establishment of phage-based biocontrol strategies against phytopathogens for sustainable plant disease management**Author:** Swati Sharma¹**Co-authors:** Borjana Arsova²; Julia Frunzke³; Sebastian H. Erdrich¹¹ IBG-1 and IBG-2² IBG 2³ IBG-1

In the search for sustainable agricultural practices, bacteriophages have emerged as promising modulators of plant–microbe interactions and biocontrol agents against bacterial plant pathogens. Phages are very host specific viruses of individual bacterial species, and as such can lead to targeted decrease of a known bacterial population.

Our initial work investigated the tripartite interaction between *Arabidopsis thaliana*, the bacterial pathogen *Xanthomonas campestris* pv. *campestris* (Xcc), and lytic bacteriophages Seregon. Using transcriptomic profiling combined with non-invasive phenotyping approaches, we show that single-phage application significantly reduces disease severity without fully eliminating the pathogen, leading to restoration of plant growth within 14 days post-inoculation. This recovery is associated with the modulation of plant immune responses, alongside a downregulation of bacterial virulence genes associated with the type III secretion system, effector proteins, and flagella biosynthesis genes. Notably, while Xcc rapidly evolved resistance to the phage under in-vitro conditions, the emergence of resistance in planta was comparatively delayed.

To address the limitations associated with single-phage treatment, we isolated and de-novo characterized an additional lytic phage targeting Xcc, confirming its suitability for biocontrol through genomic and morphological analyses. Additionally, to limit the emergence of bacterial resistance, we applied an experimental evolution approach to develop trained phages with improved infectivity. These evolved phages demonstrated a significant reduction of bacterial populations in planta, highlighting their potential for sustained pathogen suppression.

Together, our findings provide mechanistic insight into how bacteriophages reshape plant–pathogen interactions, highlighting their potential to enhance plant resilience.

Poster-Session / 39

Poster #39: Novel insight into the infection and egress dynamics of an Inovirus in *Shewanella oneidensis* MR-1**Authors:** Dorian Fischer¹; Kai Thormann¹¹ *Justus-Liebig-University*

Inoviruses are widely occurring filamentous phages; the best-known members are the Ff phages, including F1, Fd, and M13, which have been studied extensively to investigate fundamental aspects of molecular biology. Outside the Ff group, however, knowledge of Inovirus infection and egress mechanisms remains scarce.

We recently isolated a novel phage, *Shewanella* phage Dolos, belonging to the family Inoviridae. We found that Dolos's infection and extrusion process does not follow the dogmatic regimen of Ff phages: Dolos binds to the type IV MSHA pilus filament as its primary recognition structure to attach and invade the cell. After Dolos reaches the cell surface via pilus retraction, the MSHA pilus detaches from the cell body, resulting in decreased biofilm formation. Additionally, all characterized phages of this type strictly require the TolA protein of the TolQRA system to enter the host cytoplasm. We found that Dolos infects in the absence of TolA, the first example of an Inovirus infecting independently of the TolQRA system. Initial experiments suggest that MshF, an MshA biogenesis protein and ortholog of PilN, is required for host entry. Inovirus egress, an extrusion across the host cell envelope, usually requires adhesion zones between the inner and outer membranes. While Ff phages encode all components necessary for egress, Dolos does not encode an outer membrane export protein; instead, it uses the same structure for exit as for entry, utilizing the host's MshL secretin for virion export. Thus, Dolos is a valuable model system challenging the paradigm of Inovirus biology.

Poster-Session / 40

Poster #40: The temporal regulation of crAss-like phage phi14:2 vRNAP across its transcription cycle

Authors: Aleksandra Vysokikh¹; Amra Besirovic¹; Baturay Altay¹; Junchao Cheng^{None}; Leena Putzeys²; Maarten Boon²; Maria Sokolova¹; Nandika Sanjog Sahani¹; Rob Lavigne²

¹ *Max Planck Institute of Biochemistry, Martinsried, 82152, Germany*

² *Department of Biosystems, Laboratory of Gene Technology, KU Leuven, Leuven, 3001, Belgium*

Successful bacteriophage infection requires temporal regulations of both host and viral transcriptional machineries. To proceed through its infection cycle, crAss-like phage phi14:2 employs a virion encapsidated RNA polymerase (vRNAP) for its early genes and re-directs the host RNAP for the middle and probably late genes. The vRNAP is an evolutionarily divergent single-subunit RNAP that does not resemble T7-like RNAPs. Although the vRNAP is structurally characterized, the molecular mechanisms of its productive transcription cycle and its regulatory elements remain unknown.

Our transcriptomic analysis shows that the phage early genes are further separated into a pre-early and an early class where transcription of the pre-early genes requires only the virion packaged proteins. ONT-Cappable-seq identifies a TTS, and not a TSS that separates these temporal classes. Thus, pre-early genes probably encode an anti-terminator to facilitate vRNAP read-through over the TTS. To map out the proteins involved in phage transcriptional regulation, we performed AP-MS with both the vRNAP and the host *Cellulophaga baltica* RNAP. We successfully recovered temporally resolved interactomes of both RNAPs across the infection cycle. We show that the phage replication organizer gp022, a homolog of phage λ O protein, interacts with the vRNAP at later timepoints of infection. We successfully purified a transcriptional active gp066-gp022 complex and obtained a 3.92Å Cryo-EM density map, suggesting a functional coupling of phage transcription and genome replication. We also report that gp106, a phage middle protein, can regulate both the host and viral RNAPs. The in-vitro transcription activity of the vRNAP is inhibited by gp106.

Poster-Session / 41

Poster #41: pDolos: A plasmid-based phage satellite of the *Shewanella* inovirus Dolos**Authors:** Daniel Gohlus^{None}; Dorian Fischer¹; Kai Thormann¹; Nicole Schmid¹¹ *Justus-Liebig-University Giessen*

Phages are parasites that depend on their bacterial hosts for replication. Interestingly, these parasites can be hijacked by mobile genetic elements known as phage satellites. Phage satellites require the presence of a helper phage for lateral gene transfer.

Here, we present a *Shewanella oneidensis* MR-1 plasmid, named pDolos, which functions as a phage satellite for the inovirus Dolos. pDolos has a severe effect on its helper phage: The presence of pDolos decreases the number of Dolos plaque forming units by a factor of approximately 10, and furthermore, the number of phage satellite particles exceeds the number of phage particles by a factor of 250. qPCR analysis revealed that the reproduction rate of both Dolos and pDolos change during an infection. The reproduction of Dolos starts high but decreases over time, while pDolos reproduction peaks at later stages of the infection.

To determine the effect of the phage and its phage satellite on the host, we performed a proteomic analysis. Furthermore, pDolos appears to reduce the abundance of the replication protein from its helper phage at later stages of the infection, indicating a late takeover by the satellite. Further studies on the interactions between Dolos, pDolos and their host MR-1 suggest suppressed induction of the Prophage LambdaSo by phage Dolos. In addition, the presence of pDolos reduces the number of structural proteins from the *Shewanella* prophage MuSo1. Thus, *S. oneidensis*, Dolos and pDolos form an intricate ménage-à-trois balanced between mutual benefits and drawbacks.

Poster-Session / 42

Poster #42: Spatial regulation of bacterial antiphage defense systems within biofilms**Authors:** Shazeb Ahmad¹; Jens Hör²**Co-authors:** Laura Cecchino¹; Antoine-Emmanuel Saliba²¹ *Institute of Molecular Infection Biology (IMIB), University of Würzburg*² *Helmholtz Institute for RNA-based Infection Research (HIRI)*

Bacterial communities organized in biofilms exhibit distinct immune properties that enhance resistance against bacteriophage predation. Key players in the bacterial immune response are antiphage defense systems that restrict phage replication. However, the regulation and function of these defense systems within biofilms remain poorly understood, as most studies rely on liquid cultures that do not reflect native physiological conditions. Here, we hypothesize that bacterial communities regulate endogenous defense systems in a spatial and cell-specific manner. To investigate this, we use *Pseudomonas aeruginosa* biofilms as a model system in combination with BiofilmEx, an advanced spatial transcriptomics imaging technique that is based on expansion microscopy. We visualize and quantify transcript distributions across millions of sessile cells in three dimensions at single-cell resolution, enabling comprehensive mapping of defense system expression within intact biofilms. Together, this work will advance our understanding of bacterial immunity in structured communities and expand the methodological toolkit for biofilm research.

Poster-Session / 45

Poster #45: Regulation of Transcription by Jumbo Phage AR9 Encoding Two Multisubunit RNA Polymerases**Author:** Bhavna Menon¹**Co-authors:** Dennis Beuke¹; Tetiana Lavrenko¹; Philip Hartout Hartout²; Luis Wyss²; Barbara Steigenberger³; Karsten Borgwardt²; Maria Sokolova¹¹ *Research group Bacteriophages, Max Planck Institute of Biochemistry, Munich, Germany*² *Research Department Machine Learning and Systems Biology, Max Planck Institute of Biochemistry, Munich, Germany*³ *Mass Spectrometry Core Facility, Max Planck Institute of Biochemistry, Munich, Germany*

Group 1 jumbo phages, despite infecting diverse bacterial hosts, are unified by their multisubunit RNA polymerases (RNAPs): the virion RNA polymerase (vRNAP) and the non-virion RNA polymerase (nvRNAP). These polymerases are homologous to bacterial RNAP. Although the nvRNAP promoter recognition mechanism has been characterized, that of the vRNAP remains unknown.

Here we report the first purification of vRNAP of AR9, a Group 1 jumbo phage infecting *Bacillus subtilis*. This demonstrates that both AR9 RNAPs core subunits can assemble into functional complexes, without additional factors. Further, the purified vRNAP supports RNA primer extension on promoter-independent RNA–DNA scaffold templates.

Additionally, using AlphaFold predictions, we identified three candidate regulators of the vRNAP, including a putative sigma-like protein. Similar to gp226, the promoter recognition subunit of the AR9 nvRNAP, this protein also contains an N-terminal domain with three α -helices corresponding to σ region 2, the most conserved region of bacterial sigma factors. Unlike canonical σ^{70} -family of sigma factors, this protein binds DNA directly and specifically recognizes uracil-containing early promoters. Furthermore, homologs of this sigma-like protein are conserved across Group 1 jumbo phages, suggesting a conserved role in transcription regulation.

We further established crosslinking mass spectrometry workflow for *B. subtilis* infected with the AR9 phage, revealing interactions between the phage and the host transcription machinery and identifying the AR9 protein gp046 as a potential regulator of *B. subtilis* RNAP. Together, these findings provide insights into alternative strategies of transcription regulation, highlighting the diversity of phage transcription systems.

Reference #1:

Lavysh D, Sokolova M, et al., (2017) Transcription Profiling of *Bacillus subtilis* Cells Infected with AR9, a Giant Phage Encoding Two Multisubunit RNA Polymerases. *mBio*

Reference #2:

Sokolova, M., I. Misovetc, et al., (2020) “Multisubunit RNA Polymerases of Jumbo Bacteriophages” *Viruses*

Reference #3:

O'Reilly, F. J., A. Graziadei, et al., (2023) “Protein complexes in cells by AI-assisted structural proteomics” *Mol Syst Biol*

Poster-Session / 46

Poster #46: Pyocin-Mediated Growth Suppression and Bacterial Regrowth Dynamics**Authors:** Belinda Loh^{None}; Rayhaan Pais¹; Sebastian Leptihn²¹ *Fraunhofer IZI*² *Health and Medical University*

Tailocins are phage tail-like bacteriocins produced by bacteria to kill closely related strains and represent an evolutionarily repurposed mechanism of interbacterial competition. In *Pseudomonas aeruginosa*, these structures, termed pyocins, comprise morphologically distinct R- and F-type particles. While their bactericidal activity is well established, the dynamics of bacterial populations following exposure to different pyocins remain less well characterized.

Here, we investigated three distinct pyocins and their activity against clinical multidrug-resistant *P. aeruginosa* isolates. Pyocins were induced, purified and structurally characterized by transmission electron microscopy, and bactericidal activity was assessed using soft-agar overlays and bacterial growth kinetics. Distinct R1-, R5- and F-type pyocins exhibited potent antibacterial activity, with the extent and duration of bacterial growth suppression dependent on pyocin exposure. Despite strong initial growth suppression, bacterial growth was subsequently observed, prompting us to investigate how repeated and combined exposure to distinct pyocins influences subsequent growth dynamics, including persister cells and the emergence of resistance.

Poster-Session / 47

Poster #47: Phage-encoded ribosome-targeting factors in bacterial takeover**Author:** Fatoumata Sow¹**Co-authors:** Jörg Vogel²; Milan Gerovac¹; Valentin Rech³¹ *Complexes in Phage-infected Cells (CPIC), Helmholtz Centre for Infection Research, Brunswick, Germany*² *RNA Biology of Bacterial Infections (RABI), Helmholtz Institute for RNA-based Infection Research, Würzburg, Germany*³ *Institute for Molecular Infection Biology (IMIB), Julius-Maximilians-Universität Würzburg (JMU), Würzburg, Germany*

Bacteria employ a variety of strategies to defend themselves against bacteriophage infections. Bypassing these strategies is crucial for determining the fate of the bacteriophage replication and efficiency in therapeutic setups. Through co-fractionation in gradients, we identified the Φ KZ-encoded factor Φ KZ014 as a novel interaction partner with the protein production machinery [1]. This factor is unique in that it binds to the 5S rRNA of the large ribosomal subunit, and it is one of the earliest phage proteins expressed upon infection. In a clinical *Pseudomonas aeruginosa* isolate, Φ KZ014 is essential for phage replication. The relationship between translation and host takeover remains largely unexplored in general. We hypothesise that the role of Φ KZ014 in the host's translation machinery may be connected to the circumvention of bacterial defence or stress response and surveillance mechanisms. Elucidating the relevant host factors and determining how they are affected by Φ KZ014 is essential to understanding its broader role in orchestrating host takeover.

Reference #1:

1. Gerovac, M. et al. Phage proteins target and co-opt host ribosomes immediately upon infection. *Nat.A Microbiol.* 9, 787–800 (2024).

Poster-Session / 48

Poster #48: Quantitative and Biophysical Analysis of Phage-Induced Outer Membrane Vesicles in Salmonella

Authors: Shirin Aminian¹; Salvatore Chiantia²; Nina Bröker³; Stefanie Barbirz¹

¹ Department of Medicine, MSB Medical School Berlin, Berlin, Germany

² Institute for Biochemistry and Biology, University of Potsdam, Potsdam, Germany

³ Department of Medicine, HMU Health and Medical University Potsdam, Potsdam, Germany

Bacterial outer membrane vesicles (OMVs) influence phage-host interactions by acting as decoys that adsorb bacteriophages and reduce the number of infectious particles. This study investigates the molecular and biophysical mechanisms underlying OMV formation in *Salmonella enterica* serovar Typhimurium during bacteriophage infection.

The podovirus P22 is used as a model phage to examine the vesicular response of *S. Typhimurium* under controlled infection conditions. OMVs and phage particles are characterized using laser-scattering techniques and confocal fluorescence microscopy. Differential fluorescent labelling is applied to distinguish bacterial vesicles from phage particles, quantify particle populations, and assess changes in vesicle abundance, size distribution, and spatial association during infection. *In vitro* phage-OMV interactions are compared with observations obtained from growing bacterial cultures exposed to P22.

Fluorescent labelling enables discrimination between OMV and phage populations and provides a basis for quantitative analysis of their interactions. Ongoing measurements will determine whether phage exposure alters OMV production and identify particle profiles associated with phage binding to the bacterial outer membrane. Integration of *in vitro* and *in vivo* data will reveal whether changes in vesicle formation correlate with phage challenge and reduced infective phage abundance.

OMVs are hypothesized to function as an inducible decoy mechanism that modulates phage populations and protects *Salmonella* cells during infection. This work will clarify the contribution of OMVs to phage infection in Gram-negative bacteria and improve understanding of phage-host dynamics. The findings may also inform bacteriophage therapy by identifying OMVs as factors that influence phage availability, treatment efficacy, and microbiome-level interactions.

Reference #1:

Stephan, M. S., Broeker, N. K., Saragliadis, A., Roos, N., Linke, D., & Barbirz, S. (2020). In vitro Analysis of O-Antigen-Specific Bacteriophage P22 Inactivation by Salmonella Outer Membrane Vesicles. *Frontiers in Microbiology*, 11. <https://doi.org/10.3389/fmicb.2020.510638>

Poster-Session / 49

Poster #49: Drivers and Constraints of Prophage Persistence in Natural Communities**Author:** Karina Krammer¹**Co-authors:** Anne Kupczok²; Laura Reil³; Carolin Wendling¹¹ Max-von-Pettenkofer-Institut, LMU Medizin, Ludwig-Maximilians-Universität München² Bioinformatics Group, Wageningen University & Research, Wageningen, Netherlands³ Technische Universität München

Prophages are widespread in nature. If their costs outweigh their benefits, they are rapidly lost from bacterial populations in vitro [1]. However, the long-term persistence and co-evolutionary trajectories of functional prophages within a bacterial host inside natural microbial communities remain poorly understood.

To answer these questions, we conducted a longitudinal cohort study over 18 months to track the *in vivo* evolution of *Escherichia coli* lysogens repeatedly isolated from human fecal samples. Whole-genome sequencing of persisting *E. coli* lineages revealed an average of 7.4 prophages per strain, with half predicted to be complete or of high quality. Across all analyzed strains, prophages were neither lost nor acquired.

In theory, the persistence and evolution of a prophage is influenced by natural compatibility between both players and subsequent compensatory adaptation. To differentiate these mechanisms, we decoupled them experimentally. We first measured baseline compatibility by transferring isolated temperate phages from the cohort strains into lab *E. coli* strains and conducting fitness assays on these non-native lysogens. Next, we will quantify the extent of compensatory adaptation by experimentally evolving these non-native lysogens in gut microcosms.

Since prophages are common in bacteria, including pathogens, investigating such evolutionary dynamics clarifies their broader ecological and medical impact.

Reference #1:

Bailey, Z. M.; Igler, C.; Wendling, C. C. (2024): Prophage maintenance is determined by environment-dependent selective sweeps rather than mutational availability. In *Curr Biol* 34 (8), 1739-1749 e7. DOI: 10.1016/j.cub.2024.03.025.

Poster-Session / 50**Poster #50: The permanent replication cycle potential in Chimalliviridae****Author:** Milena Dziomba¹**Co-author:** Milan Gerovac¹¹ *Helmholtz Centre for Infection Research*

Until recently, phages were classified according to a binary system that distinguished clearly between lytic and lysogenic life cycles, either actively lysing the host cell or with the potential to integrate as prophages into the bacterial genome. However, recent studies challenge this dichotomy, suggesting that virulent phages with a strictly lytic lifecycle may maintain a permanent infection state within their host cells. Bacterial assemblies sequenced from single clones contained in about 0.1% of the cases virulent Chimalliviridae phages suggesting a permanent presence in bacterial isolates [1, 2]. These phages form nucleus-like compartments, thereby enabling them to operate largely independently of the host cellular machinery [3]. We hypothesize that nucleus-forming phages enter a state of permanent coexistence with the host, which is induced under adverse conditions and provides this phage family with an advantage over other phages that could impact also their therapeutic potential.

Reference #1:

Dougherty, P. E. et al. Persistent virulent phages exist in bacterial isolates. *Nat. Microbiol.* 11(1), 31-41 (2026). doi:10.1038/s41564-025-02207-0.

Reference #2:

Perfilyev, A., Gæde, A., Hooton, S. et al. Large-scale analysis of bacterial genomes reveals thousands of lytic phages. *Nat. Microbiol.* 11, 42-52 (2026). doi:10.1038/s41564-025-02203-4.

Reference #3:

Prichard, A. & Pogliano, J. The intricate organizational strategy of nucleus-forming phages. *Curr. Opin. Microbiol.* 79, 102457 (2024).

Poster-Session / 51

Poster #51: The role of the phage encoded auxiliary metabolic gene *pebS* during the infection of marine cyanobacteria**Author:** Nina Bäuerle¹**Co-author:** Nicole Frankenberg-Dinkel¹¹ RPTU

Some viruses infecting cyanobacteria carry auxiliary metabolic genes (AMGs) acquired from their hosts via horizontal gene transfer. They are thought to alter or supplement host metabolism, although their activity in the virocell during infection was not shown yet. Some AMGs in cyanophages (phages that infect cyanobacteria) exhibit notable similarities to host genes that function in the photosynthesis of cyanobacteria [1,2].

One such AMG frequently observed in cyanophages infecting *Prochlorococcus* and *Synechococcus* species is the gene encoding for the ferredoxin-dependent bilin reductase (FDBR) *PebS*. As demonstrated in vitro, *PebS* facilitates the four-electron reduction of biliverdin IX α to phycoerythrobilin (PEB) [3]. To date, no cyanobacteria have been identified to possess the *pebS* gene. Instead, they perform two subsequent two-electron reductions by the ferredoxin-dependent bilin reductases *PebA* and *PebB*. PEB is the major bilin found in the light-harvesting phycobilisomes of green light specialized cyanobacteria from the marine environment, like *Synechococcus* sp. WH8109. Interestingly, the majority of phages that carry *pebS* infect *Prochlorococcus* species that lack classical phycobilisomes.

The objective of this study is to examine the influence of the AMG *pebS* on the metabolism of *Synechococcus* sp. WH8109 and virus replication during the infection of the *pebS*-carrying cyanophage S-SSM7. Therefore, a knockout phage, lacking the *pebS* will be generated and the infection parameters of the wildtype and knockout phage will be compared under varying conditions.

Reference #1:

Rower, F., Nat. 459, 207-212 (2009)

Reference #2:

Breitbart, M., Nat. Microbiol.3, 754-766 (2018)

Reference #3:

Dammeyer, T., Curr. Biol. 18, 442-448 (2008)

Poster-Session / 52

Poster #52: Systematic studies of bacteriophage confinement in glycan-based biofilms of *Erwinia amylovora***Author:** Antonia Nowicki¹**Co-authors:** Nina Kristin Broeker¹; Anette Wensing²; Salvatore Chiantia³; Stefanie Barbirz¹¹ MSB Medical School Berlin² Julius Kühn Institut³ Universität Potsdam

Biofilms are a widespread microbial lifestyle in which cells are embedded in a self-produced extracellular polymeric substance (EPS) matrix composed of DNA, proteins, and glycans. In plant pathogens such as *Erwinia* spp., highly viscous, anionic polysaccharides dominate the EPS and act as effective diffusion barriers to bacteriophages, thereby influencing infection processes.

We investigated phage diffusion in glycan-based biofilms using tailed phages infecting *Erwinia* spp. In addition, polysaccharides from different *Erwinia* spp and *Pantoea* spp. biofilms were isolated, revealing pronounced biochemical diversity and variability in physical properties.

To quantitatively assess phage mobility, we tracked fluorescently labeled phages—representing both myoviruses and podoviruses—as well as similarly sized microspheres using single-particle tracking. Trajectory-based models identified distinct diffusion populations and enabled quantification of phage concentrations under diffusion-limited conditions across varying physicochemical environments. Notably, phage diffusion was strongly influenced by EPS concentration, pH, ionic strength, and phage-specific morphological properties, with myoviruses and podoviruses exhibiting distinct mobility patterns due to their distinct structural features and interaction dynamics with the biofilm matrix. Depolymerase-mediated degradation of biofilm polysaccharides proved to be a rapid and effective mechanism for modulating particle mobility, particularly enhancing the penetration of both phage types into dense biofilm regions. Furthermore, we link phage diffusion to host infection dynamics, revealing how myoviruses and podoviruses differentially navigate, penetrate, and infect bacterial hosts within dense, viscous biofilm environments—highlighting the importance of phage morphology in determining ecological success in complex microbial communities.

emphasized text

Reference #1:

Dunsing V, Irmscher T, Barbirz S, Chiantia S. Purely Polysaccharide-Based Biofilm Matrix Provides Size-Selective Diffusion Barriers for Nanoparticles and Bacteriophages. *Biomacromolecules*. 2019;20(10):3842-54.

Poster-Session / 53

Poster #53: DNA loaded outer membrane vesicles after bacteriophage treatment**Author:** Hannah-Maria Monks¹**Co-authors:** Nina Kristin Bröker¹; Stefanie Barbirz²¹ MSB Medicalschooll Berlin² MSB Medicalschooll Berlin; Universität Potsdam

Bacteria employ diverse defence mechanisms against bacteriophage infection, including the release of bacterial extracellular vesicles (BEVs). Gram-negative bacteria produce outer membrane vesicles (OMVs) composed of outer membrane lipids and proteins, including surface receptors such as the O-antigen of lipopolysaccharide (LPS). This study focuses on the interaction between *Salmonella* Typhimurium OMVs and the podovirus P22, a model phage that uses the O-antigen as its receptor. While OMVs can bind and neutralize phages, the fate of phage DNA and surface changes in OMVs is not well studied so far.

OMVs were isolated from *Salmonella* via explosive lysis and characterized using LPS and protein assays. Vesicle size was determined by dynamic light scattering. We analyzed DNA transfer using fluorescent ejection kinetics and electron microscopy.

In addition to DNA loaded OMVs, we observe phage particles that remain attached to the OMV surface. P22 binds to OMVs and injects its DNA into their lumen, generating DNA loaded vesicles. These OMVs exhibit distinct surface modifications after phage exposure, including alterations in O-antigen composition, and phage particles show divergent fates within the OMV batches.

Vesiculation occurs in response to stress caused by antimicrobial agents, toxic compounds or bacteriophage infection, and it contributes to intermicrobial communication. P22 uses the O-antigen of lipopolysaccharide as its receptor for DNA ejection. OMV phage interactions are particularly interesting: OMVs not only bind and inactivate phages, but they can also take up phage DNA. These findings highlight the important role of OMVs in influencing phage bacteria interactions.

Reference #1:

Mareike S Stephan, Nina K Broeker, Athanasios Saragliadis, Norbert Roos, Dirk Linke, and Stefanie Barbirz. In vitro analysis of O-antigen-specific bacteriophage P22 inactivation by *Salmonella* outer membrane vesicles. *Frontiers in Microbiology*, 11:510638, 2020.

Poster-Session / 55

Poster #55: Dissecting antiviral defense systems in Actinobacteria – from single cells to the community level**Author:** Patricia Blum¹**Co-author:** Julia Frunzke¹

¹ Forschungszentrum Jülich GmbH, Institute of Bio- and Geosciences, IBG-1: Biotechnology, Jülich, Germany; Heinrich-Heine-University Düsseldorf, Faculty of Mathematical and Natural Sciences, Institute of Microbial Interactions, Düsseldorf, Germany

Viruses infecting bacteria, so-called bacteriophages (phages for short) account for the most abundant biological entity on the planet and pose a constant threat for bacteria. Due to that omnipresent evolutionary pressure, bacteria evolved complex “immune systems” with a great diversity of antiviral defense mechanisms, such as abortive infection (Abi), restriction modification (RM) systems or CRISPR-Cas. In recent years, chemical antiviral strategies on a community level, mediated by small molecules and secondary metabolites of Actinobacteria, came into the focus of attention.(1) There are indications that the antiviral immune defense of bacteria is not limited to single cells, but rather plays a communal role in the so-termed “pan-immune system”(2).

Previous studies revealed that defense systems often cluster within “defense islands”. Based on these findings, bioinformatic prediction tools have been developed for the identification of new, yet uncharacterized defense systems. These tools show a particular bias toward Proteobacteria, and analysis across other bacterial phyla have revealed a notable “depletion” of so far known defense systems in other large phyla, such as the diverse phylum of Actinobacteria.(3) Here, we leveraged existing bioinformatics prediction tools to identify potential antiviral defense systems in the actinobacterial model strain *Corynebacterium glutamicum*. Using DefenseFinder and PADLOC, we identified overall 12 different systems with a putative role in antiviral defense.

Here, we investigate the role and potential co-regulation of selected defense systems during phage infection. Our initial screenings against a collection of 5 phages indicated that, in particular genomically-integrated (cryptic) prophages represent a hotspot for systems involved in antiviral defense.

Reference #1:

Georjon, H. & Bernheim, A. The highly diverse antiphage defence systems of bacteria. Nat. Rev. Microbiol. 21, 686–700 (2023).

Reference #2:

Bernheim, A. & Sorek, R. The pan-immune system of bacteria: antiviral defence as a community resource. Nat. Rev. Microbiol. 18, 113–119 (2020).

Reference #3:

Georjon, H., Tesson, F., Shomar, H. & Bernheim, A. Genomic characterization of the antiviral arsenal of Actinobacteria: This article is part of the Microbial Evolution collection. Microbiology 169, (2023).

Poster-Session / 56

Poster #56: Stepwise R-Loop Unwinding and Cas3-Mediated Target Degradation in a Multiprotein CRISPR–Cas System**Author:** Inga Songailiene¹**Co-authors:** Tomas Sinkunas²; Toma Liegute²; Lisa Schmidt³; Viktoras Ragozius²; Algirdas Miksys²; Lina Malinauskaite²; Virginijus Siksnys²¹ *University of Copenhagen*² *Vilnius University*³ *University of Heidelberg*

crRNA-guided effector complexes (ECs) of CRISPR–Cas systems mediate the cleavage or silencing of invading phage genetic material. Class 1 CRISPR–Cas systems, which rely on multiprotein effector assemblies, are the most abundant CRISPR systems in nature [1] and have been widely adapted for biotechnological applications, including genome engineering and transcriptional silencing [2]. In type I CRISPR systems, the crRNA-guided Cascade complex recruits the Cas3 nuclease–helicase, which drives processive and unidirectional (3'→5') degradation of target DNA [3].

Here, we investigate the molecular mechanism of CRISPR interference by resolving four Cryo-EM structures capturing intermediate steps of target recognition and stepwise R-loop propagation. Together with complementary biochemical analysis, these structural snapshots reveal how R-loop formation induces the conformational rearrangements required to expose the Cas3-binding interface and initiate target degradation. Mutational analyses identify key Cascade–Cas3 interactions that are essential for interference, while other contacts appear to be functionally dispensable. Furthermore, we examine the contribution of these interactions to primed adaptation and show that this process requires less stringent Cascade–Cas3 coordination than target interference.

Reference #1:

Makarova, K. S., et al. (2025). “An updated evolutionary classification of CRISPR–Cas systems including rare variants.” *Nature Microbiology*, 10(12), 3346–3361.

Reference #2:

Liu, T. Y., Doudna, J. A. (2020). Chemistry of Class 1 CRISPR-Cas effectors: Binding, editing, and regulation. *Journal of Biological Chemistry*, 295(42), 14473–14487.

Reference #3:

Šinkūnas, T., et al. (2013). “In vitro reconstitution of Cascade-mediated CRISPR immunity in *Streptococcus thermophilus*.” *The EMBO Journal*, 32(3), 385–394.

Poster-Session / 59

Poster #59: Sulfate-Reducing Bacteria and Their Viral Fingerprints in the Gut Microbiome: Uncovering Adaptation and Disease Markers in Inflammatory Bowel Disease**Author:** Anna Penzkofer¹**Co-authors:** Carolin Wendling¹; Jan Karpa²¹ Max-von-Pettenkofer-Institut, LMU Medizin, Ludwig-Maximilians-Universität München² Max-von-Pettenkofer-Institut, LMU Medizin, Ludwig-Maximilians-Universität München & Institute of Virology (VIRO), Helmholtz Munich, Germany

Inflammatory bowel diseases (IBD) arise from complex interactions between host genetics, immune dysregulation, and the gut microbiome. Sulfate-reducing bacteria, particularly the Desulfovibrionaceae family, are opportunistic pathobionts that are enriched in patients with IBD and contribute to epithelial damage by producing hydrogen sulfide and other metabolites. Like most gut microbes, these bacteria originate from environmental habitats and subsequently colonized and adapted to the gut. A critical open question is how these bacteria rewired their genetic and metabolic pathways to persist in such a complex and competitive ecosystem.

This project will use comparative genomics to investigate the evolution, gut adaptation, and pathogenic potential of Desulfovibrionaceae found within the human gut. Initially, we analysed publicly available genomes of the genus *Desulfovibrio* using an established bioinformatics pipeline to detect, filter, taxonomically classify, and characterise their prophages. The analysis showed numerous host-associated prophage clusters, whereby human-derived *Desulfovibrio* genomes contain more, generally shorter, prophages than environmental isolates. This indicates niche-specific pressures that select for diverse phage-host combinations.

To improve our understanding of bacterial adaptation and to support the development of novel diagnostic markers and therapeutic strategies for IBD, we will extend this analysis to the Desulfovibrionaceae family. We will focus on genetic differences and similarities between bacterial isolates from IBD patients and healthy controls and aim to identify prophage-associated signatures linked to IBD as well as to characterise their contribution to the gut environment by analysing the transcriptome, proteome, and metabolome.

Poster-Session / 60

Poster #60: Antibiotics and diet modulate phage-bacteria interactions in the gut microbiome**Authors:** Chloé Feltin¹; Karina Ilchenko¹**Co-author:** Eugen Pfeifer¹¹ Université Paris-Saclay, INRAE, AgroParisTech, MICALIS

The gut microbiome is vital for human health. While most studies focus on bacteria, roles of gut phages remain poorly understood. Our team aim to decipher how phage-bacteria interactions are shaped by diet and antibiotics, strong modulators of gut bacterial composition. To achieve this, we integrate metagenomics with longitudinal samples of *in-vitro* gut models and clinical cohorts.

Previously, we analyzed the impact of 3rd-generation cephalosporins on a healthy cohort. Beyond a 15–20% reduction in diversity (bacteria and phages), the treatment triggered the dominance of specific bacterial species reported to encode resistance genes. Interestingly, we also detected blooms of virulent phages. We propose that “Kill the Winner” dynamics generate abundant phages that act as perturbation markers. Consistently, we found dominant phages to be significantly enriched in a cohort undergoing chronic antibiotic treatment in comparison to healthy individuals. To explore those ecological dynamics, we will conduct *in-vitro* studies, where sampling frequency and host factors do not limit resolution.

We are now analysing a dataset from a collaborative project using advanced *in vitro* gut models to study diet effects. As 70–80% of gut bacteria carry prophages, we hypothesize that diet components modulate microbiota and prophage dynamics. Across >400 bacterial species, we identified 197 high-quality prophages (>90% completeness), of which approximately 50% were found active in at least one condition, many showing diet-associated activation patterns.

Collectively, our findings highlight that virulent and temperate gut phages shape microbiota diversity and resilience, paving the way for targeted interventions while underscoring the need for well-designed follow-up studies.

Poster-Session / 61

Poster #61: Towards the human gut microbiome: expanding phage-plasmid detection with tyPPing**Author:** KARINA ILCHENKO¹**Co-authors:** EDUARDO ROCHA²; EUGEN PFEIFER¹¹ *Université Paris-Saclay, INRAE, AgroParisTech, MICALIS, France*² *Institut Pasteur, Université de Paris Cité, CNRS UMR3525, Microbial Evolutionary Genomics, France*

Phage-plasmids (P-Ps) are temperate phages that replicate as plasmids during lysogeny while producing infectious virions upon induction. They are widespread and often carry important accessory functions, including virulence factors and antibiotic resistance genes. However, existing computational tools define them often either as phages or plasmids, limiting our understanding of their diversity and ecological roles.

To address this, we previously developed tyPPing [1], a rapid and accurate pipeline for detecting distinct P-P types (e.g. P1, N15, SSU5) in both complete and draft genomes. tyPPing uses curated protein profiles, conservation patterns, and size ranges to predict P-Ps with confidence scores ranging from low-to-high. Moreover, we demonstrated experimentally that high confidence matches correspond to functional phages.

Nonetheless, despite being capable to detect P-Ps infecting clinically relevant pathogens (predominantly Enterobacteriaceae), tyPPing has been limited in detecting P-Ps in other environments, such as the human gut. Phages, and especially prophages, are increasingly recognized as shapers of the bacterial landscape in the gut microbiome. We therefore sought to extend tyPPing's detection range, and screened gut phage databases for P-Ps. We identified >1400 confident matches accounting for 2.6% of high-quality sequences (up to 8.4% when considering all matches). This high prevalence suggests a significant ecological function for P-Ps in the gut. Collectively, through the development of tyPPing 2.0, we aim to further elucidate the diversity and functions of P-Ps.

Reference #1:

Ilchenko K, Bonnin RA, Rocha EPC, Pfeifer E. Efficient detection and typing of phage-plasmids. *mBio*. 2026;17(3):e0300025. doi:10.1128/mbio.03000-25

Poster-Session / 62

Poster #62: Phage P2-encoded Orf30 protein as a novel regulator of virion formation**Author:** Claudia Campobasso¹**Co-author:** Katharina Höfer¹¹ *LIT - Leibniz Institute for Immunotherapy*

Phage P2 is a temperate bacteriophage infecting *Escherichia coli* and the helper phage of the satellite phage P4. Despite decades of research, several highly conserved P2 genes remain uncharacterized. Among these, orf30 is constitutively expressed, has its own promoter, and is highly conserved among P2-like phages, yet its biological role has remained unknown. This study aimed to generate, for the first time, a P2 Δ orf30 mutant and investigate its function.

The lambda-red recombination system was optimised to overcome P2 Old protein interference, previously reported to hinder P2 engineering. An *E. coli* lysogen carrying a P2 Δ orf30 mutant was generated, and its plaque-forming ability was compared with the WT strain. Comparative genomic analyses assessed the conservation of orf30 and its distribution among *E. coli* genomes carrying P2- and P4-like phages.

The modified lambda-red system enabled construction of the mutant, which remained viable but exhibited a fourfold reduction in plaque-forming efficiency compared with the WT. Comparative genomic analysis showed that orf30 is highly conserved among P2-like prophages and present in 96% of genomes harbouring both P2- and P4-like phages, suggesting that P4 satellites are predominantly associated with P2 phages carrying the orf30 region.

This work establishes an effective strategy for engineering P2 phages and identifies Orf30 as a conserved factor contributing to efficient phage propagation. The conservation of orf30, together with prevalence in P2/P4 helper-satellite systems and the reduced plaque-forming efficiency of the Δ orf30 mutant, identifies Orf30 as a promising candidate for understanding the molecular mechanisms regulating P2 propagation and helper-satellite interactions.

Poster-Session / 63

Poster #63: Differences in regulatory genes drive divergent evolutionary outcomes under phage challenge**Authors:** Ariel Morales Araujo¹; Lavisha Parab²**Co-authors:** Abigail Dustour³; Carolin Wendling⁴; Dominik Refardt⁵; Jan Karpa⁶; Monica Goldade³; Sophie Baruth⁷¹ *Max von Pettenkofer Institutue - LMU*² *Max von Pettenkofer Institutue - LMU)*³ *ETH*⁴ *ETH, Helmholtz Munich, Max von Pettenkofer Institutue - LMU)*⁵ *ZHAW*⁶ *Helmholtz Munich, Max von Pettenkofer Institutue - LMU)*⁷ *ETH, University of Bonn*

Temperate phages use a bistable genetic switch to balance two different modes of transmission: lytic replication leads to horizontal transmission whereas lysogenic persistence allows vertical transmission. Dynamics of this switch are increasingly recognized as an evolvable trait shaped by the architecture of the underlying regulatory network, but the extent to which differences in its genetic architecture influence evolutionary outcomes remains unclear. Here we compare two closely related temperate phages, lambda and HK629, which share conserved structural genes, integration site, and host receptor usage but show significantly different infection outcomes in clonal bacterial populations. While infection with lambda resulted in a high level of lysogen formation, infection with HK629 led to rapid emergence of phage-resistant non-lysogens and low levels of lysogen formation. Whole-genome sequencing further revealed substantial amplification of the HK629 prophage locus, consistent with a 100-fold higher level of spontaneous prophage induction of HK629 lysogens compared to lambda lysogens. This suggests that closely related temperate phages can encode fundamentally different regulatory strategies that alter the stability and ultimately the fitness consequences of lysogeny.

Poster-Session / 65

Poster #65: Fun/Z is an abortive immunity system related to archaeal and eukaryotic replication component**Author:** Olha Dorozh¹**Co-authors:** Anton Derzhaev¹; Evgenia Novikova²; Dmitrii Lukianov²; Vadim Molodtsov¹; Artem Isaev¹¹ *Center for Molecular and Cellular Biology, Skolkovo Institute of Science and Technology, Moscow, Russia*² *Moscow State University (MSU), Moscow, Russia*

Temperate bacteriophages can benefit by protecting their hosts against other phages. The P2-like group of phages encodes a diversity of compact immunity modules. We reveal that the Fun/Z defense system of prophage P2 is related to ORC/Cdc6 domains involved in archaeal and eukaryotic DNA replication. In contrast to related STAND immunity ATPases, Fun/Z lacks predicted sensor or effector domains yet provides abortive defense. In vitro, Fun/Z exhibits DNA binding activity with specificity for ssDNA.

ORC proteins are single-turnover ATPases, and ATP binding activates the protein to promote DNA recognition. Interestingly, Fun/Z contains a modified Walker B motif ("DG"), implying slow ATP hydrolysis. Mutations in Walker A or B abolish defense, whereas restoring the canonical "DE" motif or introducing an ATP-trap ("DQ") mutation—expected to stabilize an activated conformation—makes Fun/Z expression highly toxic. Metabolic labeling revealed that these toxic mutants inhibit DNA replication. Together with its DNA-binding activity and homology to ORC proteins, this suggests that Fun/Z activation could interfere with replication initiation.

A search for Fun/Z escapers identified Vequintavirinae phage Msk10 with genome duplications. Analysis of the duplicated region uncovered Fzad (Fun/Z anti-defense) as the first characterized Fun/Z inhibitor, highlighting the importance of gene dosage effects in anti-defense mechanisms.

The work was supported by a Russian Scientific Foundation grant (224-74-10089).

Poster-Session / 66

Poster #66: Tin: A Minimalist Abortive PD-(D/E)XK Nuclease Fusing Sensor and Effector Functions for Anti-Phage Defense**Authors:** Anton Derzhaev¹; Artem Isaev¹; Chunyi Hu²**Co-authors:** Konstantin Severinov³; Kristina Potekhina¹; Natalia Morozova¹; Vadim Molodtsov⁴¹ *Center for Bio- and Medical Technologies, Moscow, Russia*² *National University of Singapore, Singapore*³ *Institute of Gene Biology, Russian Academy of Sciences, Moscow, Russia*⁴ *Research Institute of Molecular and Cellular Medicine, RUDN University, Moscow, Russia*

Bacterial abortive infection (Abi) systems typically require multi-component complexes to detect phages and execute cell death. We characterize Tin, a 253-amino-acid protein encoded by prophage P2, as one of the smallest known Abi proteins that mediate phage sensing and abortive response. We demonstrate that Tin protects against T4-like phages by directly recognizing the phage SSB protein gp32. Structural studies reveal that gp32 binds a dimeric core of Tin, stabilizing an activated conformation of the PD-(D/E)XK catalytic motif. Notably, Tin sensory loop integrates into and completes a nuclease domain after gp32 binding - a unique mechanism enabling sensor-effector fusion in such a compact protein. Upon activation, Tin non-discriminately degrades host and phage DNA, and demonstrates an activity against glucosyl-hydroxymethylcytosine-modified T4 genome, challenging the paradigm that such modifications universally block nucleases. Bioinformatic analysis links Tin to type I restriction-modification systems and NucC, underscoring its place among defense systems. This work explains how a regulation of the abortive nuclease can emerge via insertion of a short sensory loop interacting with the phage trigger.

Poster-Session / 67

Poster #67: Need for speed: early lysogen formation following rapid lysis drives temperate phage persistence**Author:** Lavisha Parab¹**Co-authors:** Amandine Dupuis¹; Carolin Wendling¹; Claudia Igler²; Monica Goldade³; Sophie Baruth³¹ *Ludwig Maximilians Universität (LMU) Munich*² *University of Manchester, UK*³ *ETH Zürich*

Infection by temperate phages leads bacteria down different evolutionary routes, each with specific fitness trade-offs. Lysogeny provides immunity against similar phages through superinfection exclusion but comes with costs such as genomic maintenance of the prophage and risk of future cell lysis. In contrast, lytic activity favours surface receptor modifications that confer phage resistance at the expense of receptor function. To investigate which phage traits favour the evolutionary preference for lysogeny over resistance, we tracked the dynamics of naïve *E. coli* K-12 populations when exposed to either of four temperate phages. Populations rapidly diversified into lysogens and non-lysogens, with non-lysogens almost entirely outcompeting lysogens in half the cases. Whole-genome sequencing and phenotypic screening confirmed that non-lysogens acquired phage resistance via receptor modifications. We initially hypothesised that prophages only persist when costs associated with receptor modifications outweigh those of prophage maintenance. Contrary to expectation, lysogens are as fit as or fitter than resistant mutants in competition assays and growth rate measurements, with no significant differences across phages. To identify the underlying viral drivers, we quantified differences in latent period, burst size, spontaneous induction rate, and adsorption rate. We demonstrate that significantly faster lysis times and higher burst sizes favour lysogen formation and persistence. Using mathematical modelling, we integrate how these empirical parameters predict long-term bacterial population dynamics in the presence of temperate phages. Our study highlights how early phage infection dynamics shape long-term bacterial population structure, underscoring how initial epidemiological parameters dictate the trajectory of bacteria-phage co-evolution.

Poster-Session / 69

Poster #69: Characterization of a new NAD⁺-processing domain involved in antiphage defense and shared across the tree of life**Author:** Wendy LE MOUËLLIC^{None}**Co-authors:** Virginie Borges-Cardoso¹; Julie Baltenneck¹; Jessie Kulsuptrakul¹; Lucie Etienne¹; François Rousset¹¹ CIRI, Centre International de Recherche en Infectiologie, INSERM U1111, CNRS UMR5308, Université Claude Bernard Lyon 1, Ecole Normale Supérieure de Lyon, Lyon, France

Just as eukaryotic cells activate multiple immune pathways to counter viral infection, bacteria employ diverse strategies to resist bacteriophage attack. For a long time, restriction-modification and CRISPR-Cas systems were the main antiphage systems described in bacteria. As these are absent from Eukaryotes, bacterial and eukaryotic immunities have historically remained two distinct fields of investigation. Yet this view has been challenged by the discovery of more than 250 types of bacterial defense systems, some of which share striking homology with known eukaryotic immune proteins or domains, such as cGAS receptor or TIR domains. Most of these conserved immune players were identified in bacterial immunity thanks to our knowledge of eukaryotic immunity. However, the considerable expansion of known antiphage systems raises the possibility that discoveries made in bacterial immunity may help uncover new immune actors in the eukaryotic world.

In this context, we report a novel protein domain found in diverse bacterial proteins with a predicted immune function. We show that this protein domain mediates anti-phage immunity in *E. coli* against different phage families. Activation of this domain upon phage infection triggers NAD⁺ depletion, thereby leading to abortive infection. Strikingly, this domain is found in diverse NLR-like proteins in multiple eukaryotic species, including humans. Remarkably, we show that the corresponding domain from the human TEP1 protein also exhibits NADase activity *in vitro*.

Together these findings identify a new NAD⁺-processing domain involved in antiphage defense and suggest a potentially conserved immune role for this domain in Eukaryotes, which we are currently investigating.

Poster-Session / 70

Poster #70: Exploring potential mechanisms of programmed cell death in *Legionella* phage defense: Interplay of NAD(H) depletion and the effector phospholipase PlaB**Authors:** Markosovo Marjaya¹; Antje Flieger¹**Co-authors:** Philipp Auraß¹; Christina Lang¹¹ Robert Koch Institut

Legionella pneumophila, an intracellular pathogen of human lungs and environmental protozoa, possesses a diverse array of virulence factors shaped by its niche. One such factor, the highly active phospholipase A PlaB, localizes to the bacterial outer membrane and contributes to virulence in host cells. Here, we propose an additional function for PlaB as an intrinsic cell death effector involved in phage defense.

Our findings reveal an intriguing regulatory mechanism in which intracellular NAD(H) controls PlaB activity. At physiological NAD(H) levels, inactive PlaB tetramers are stabilized by NAD(H) binding. Upon NAD(H) depletion, PlaB dissociates into active dimers, unleashing potent phospholipase activity. We hypothesize that activated PlaB targets bacterial membranes from within the cytosol, triggering autolysis and thereby limiting phage replication through an abortive infection strategy.

Consistent with NAD(H) being central for regulation of PlaB's activity, gene co-occurrence analyses show that PlaB can be found with genes encoding NAD(H)-consuming enzymes, such as RES-Xre toxin-antitoxin systems and SIR2- and TIR-domain proteins, suggesting a coordinated regulatory network. Supporting this observation, co-expression of PlaB with such enzymes in *E. coli* and *L. pneumophila* leads to rapid, PlaB-dependent cell death under low NAD(H) conditions.

These findings support a model in which NAD(H) serves as a molecular switch controlling PlaB activity and identify PlaB as a candidate effector linking NAD(H) depletion to altruistic cell death and abortive infection mechanism during possible phage infection. This work expands the functional repertoire of PlaB beyond virulence and suggests a possible implication in phage defense in *L. pneumophila*.

Reference #1:

Diwo M, Michel W, Aurass P, Kuhle-Keindorf K, Pippel J, Krausze J, Wamp S, Lang C, Blankenfeldt W, Flieger A. NAD(H)-mediated tetramerization controls the activity of *Legionella pneumophila* phospholipase PlaB. *Proc Natl Acad Sci U S A*. 2021 Jun 8;118(23):e2017046118. doi: 10.1073/pnas.2017046118. PMID: 34074754; PMCID: PMC8201859

Reference #2:

Kuhle K, Krausze J, Curth U, Rössle M, Heuner K, Lang C, Flieger A. Oligomerization inhibits *Legionella pneumophila* PlaB phospholipase A activity. *J Biol Chem*. 2014 Jul 4;289(27):18657-66. doi: 10.1074/jbc.M114.573196. Epub 2014 May 8. Erratum in: *J Biol Chem*. 2014 Jul 4;289(27):18667. PMID: 24811180; PMCID: PMC4081910

Reference #3:

Poster-Session / 71

Poster #71: New insights into the Mrr-domain-associated prokaryotic Argonaute systems SPARMA and SPARMTA

Authors: Ane Sanz Andres¹; Balwina Koopal²; Daan Swarts¹; Donata Dakinevičienė³; Jack Bravo²; Jinte Jespers¹; Mindaugas Zaremba³; Simon Lindhoud¹; Theophile Niault¹

¹ *Wageningen University & Research*

² *Institute of Science and Technology Austria*

³ *Vilnius University*

Argonautes are nucleic acid-guided proteins that use short oligonucleotides to recognize complementary nucleic acids. In eukaryotes, they mediate RNA interference, whereas the more diverse prokaryotic Argonautes (pAgos) protect their hosts against invading nucleic acids by either directly neutralizing the invader or triggering programmed cell death. Initially, research on pAgos focused on a subset of the “long” pAgos, specifically those with a domain architecture similar to that of eukaryotic Argonautes. More recently, attention has shifted to more divergent pAgos, including the so-called “short” pAgos, which lack the N-terminal lobe and instead associate with an APAZ-domain-containing protein fused to one of several functional domains. Interestingly, despite this variation, most short pAgo systems characterized to date oligomerize in some way upon activation. Our work presents structural insights into two divergent short pAgo systems associated with an Mrr domain. Although both are associated with this domain, they oligomerize in different ways upon activation, leading to the cleavage and removal of invading nucleic acids.

Poster-Session / 72

Poster #72: Investigating the mechanism of mRNA export from the early phage infection compartment of jumbo phage PhiKZ**Authors:** Shafagh Moradian¹; Leandro Buhlmann¹; Jörg Vogel²¹ Institute of Molecular Infection Biology (IMIB), University of Würzburg, Würzburg, Germany² Institute of Molecular Infection Biology (IMIB), University of Würzburg, Würzburg, Germany Helmholtz Institute for RNA-based Infection Research (HIRI), Helmholtz Centre for Infection Research (HZI), Würzburg, Germany

Early during infection of the opportunistic pathogen *Pseudomonas aeruginosa*, the jumbo bacteriophage PhiKZ forms a membrane-bound Early Phage Infection (EPI) compartment. This compartment spatially separates early viral transcription from the bacterial cytoplasm, while translation remains dependent on host ribosomes outside the EPI. How newly synthesised viral mRNA molecules are exported across the EPI membrane for cytoplasmic translation remains unknown.

To investigate the mechanism of viral mRNA export, we utilise a workflow combining programmable antisense oligonucleotides (ASOs) to inhibit the translation of candidate proteins potentially involved in export with single-molecule fluorescence *in situ* hybridisation (smFISH) to visualise and characterise early phage transcripts at single-cell resolution. We aim not only to determine how perturbing candidate factors affects phage infection dynamics and EPI maturation, but also to elucidate the PhiKZ machinery responsible for early mRNA export.

Reference #1:

Gerovac M, Buhlmann L, Zhu Y, et al. Programmable antisense oligomers for phage functional genomics. *Nature*. 2025;646:1195–1203. doi:10.1038/s41586-025-09499-6

Poster-Session / 73

Poster #73: Deoxydinucleotides are potential mediators of bacteria–phage interactions**Author:** Sofia Mortensen¹**Co-author:** Holger Sondermann²¹ DESY² DESY, Kiel University

Deoxynucleotides are emerging as important mediators of bacterial genotoxic stress response and bacteria–phage interactions. Recent studies suggest that single-stranded DNA, particularly deoxydinucleotides, may function as signaling molecules that activate bacterial defense systems. These discoveries include our identification of a novel family of DEDD nucleases, termed deoxydinucleases (diDNases), which specifically hydrolyze single-stranded DNA dinucleotides and are associated with phage defense islands in Actinobacteria and Clostridia. DiDNases represent a distinct evolutionary branch of dinucleases. Unlike related enzymes involved in RNA degradation, they exhibit strict specificity for DNA dinucleotides, enabled by conserved structural features that exclude RNA substrates. Their inability to complement canonical diribonucleotide-processing enzymes further supports a specialized physiological function. Together, these findings point to a previously unrecognized role for short DNA in bacterial immunity.

Poster-Session / 75

Poster #75: Who starts, who follows: reading assembly grammar in bacterial and metazoan death domains**Author:** Finn Lobnow¹**Co-authors:** Gideon Cummings²; Tanita Wein²; Marcus Taylor¹¹ *Max Planck Institute for Infection Biology*² *Weizmann Institute of Science*

Accurate cell signaling requires information to be moved through a cascade of signaling effectors in a directional and controlled manner. Death domains orchestrate metazoan signalosome formation through ordered, often heterotypic assembly.

However, whether these assembly rules are universal has remained unclear. The recent discovery of bacterial death domain homologs provides a valuable opportunity and we find that bacterial and metazoan death domains share the same assembly and directionality grammar: Each system has a nucleator to start complex formation and a dependent domain that requires the base created by its seeding partner. Recent work shows that bacterial death domains (bDDs) indeed mediate phage defense in various systems featuring TIR, PNP, and CHAT domains as effectors (in collaboration with Gideon Cummings, Wein Lab). Using TIRF microscopy at single molecule resolution, we characterize 11 bDDs from single- and dual-bDD systems regarding which domains can self-assemble and recruit their partner domains. One example is a dual-bDD system from *Ca. Magnetaquicoccus inordinatus*: The effector-associated bDDs assemble on a base of receptor-associated bDDs and only this direction enables the complex to form. Notably, bDD systems appear to differ in how they restrain constitutive activation, pointing to distinct regulatory architectures.

Poster-Session / 76

Poster #76: Regulation of the lysis-lysogeny switch in temperate actinobacteriophages of *Corynebacterium glutamicum* ATCC 13032**Author:** Samy Mechrouki¹**Co-authors:** Biel Badia Roigé¹; Julia Frunzke¹; Ombeline Rossier²¹ *Institute of Bio- and Geosciences, IBG1, Forschungszentrum Jülich, Jülich, Germany*² *Institute for Integrative Biology of the Cell, University Paris Saclay, Paris, France*

Prophages are ubiquitous elements of bacterial genomes (1). As major contributors to bacterial genetic diversity, prophages can impact host fitness and confer new phenotypes as they often encode defense systems or virulence factors (2). Intriguingly, many bacteria harbor multiple prophages – so-called polylysogens – making them valuable models to study phage-host and phage-phage interactions.

The actinobacterium *Corynebacterium glutamicum* ATCC 13032 harbors two cryptic prophages and the mobile genetic element CGP3. The ICE element CGP3 comprises nearly 6% (~200 kbp) of the genome and provides broad immunity against phage infection (3).

Here, we investigated the novel temperate phage CyranoPS infecting *C. glutamicum*. Interestingly, the attachment site of CyranoPS was found to be located within the phage repressor gene resulting in repressor truncation upon chromosomal integration. Expression of the full-length versus truncated variant of the repressor revealed that the truncated variant is indeed the active form of the protein. Using a reporter system, the master repressor was shown to promote lysogenic gene expression, while repressing the lytic promoter.

We further observed high spontaneous induction of CyranoPS yielding phage titers of up to 1E9 PFU/ml in the culture supernatant. This spontaneous induction was, however, significantly reduced in the presence of the cryptic ICE element CGP3. By mutational analysis and targeted overexpression of candidate genes, we identified two CGP3 encoded genes with a previously unrecognized role in inter-prophage competition. Together, these findings reveal how regulatory crosstalk between co-resident mobile genetic elements can modulate the lysis-lysogeny decision and thereby shape phage-host infection dynamics.

Reference #1:

Touchon et al. (2016) ISME J, doi.org/10.1038/ismej.2016.47

Reference #2:

Pfeifer et al. (2022) Curr Opin Microbiol, doi.org/10.1016/j.mib.2022.102230

Reference #3:

Badia Roigé et al. (2026) preprint, doi.org/10.21203/rs.3.rs-9446490/v1

Poster-Session / 78

Poster #78: Adaptive trajectories of bacteriophages: Evolutionary dynamics across contrasting environments**Author:** Thea Brunsch^{None}**Co-author:** Bas E. Dutilh¹¹ *Friedrich-Schiller University Jena*

Bacteriophages are among the fastest-evolving biological entities, yet the early dynamics and constraints of their adaptive trajectories remain incompletely understood. This PhD project will investigate long-term phage adaptation in a controlled experimental evolution system using a *Pseudomonas* strain and its lytic phage, with automated serial passage enabling standardized propagation across many replicate populations and dense temporal sampling of evolutionary change.

Phage populations will be evolved under three contrasting selection regimes: differences in infection dynamics (fast- vs. slow-burst phages), spatially structured growth in biofilm-like environments, and elevated-salinity physicochemical stress. Time-resolved whole-genome sequencing will be combined with phenotypic assays (adsorption, lysis time, burst size, environmental persistence, host range, killing efficacy) to determine which early, low-frequency mutations rise to dominance, which are transient, and how genotypic change maps onto phenotype.

We hypothesize that adaptation will repeatedly target host-interaction genes, particularly tail fiber and baseplate proteins, with regime-specific shifts: biofilm selection favoring altered adsorption and recognition strategies for heterogeneous surface-associated cells, and salinity acting primarily through demographic and ecological effects on host growth and phage persistence rather than mutation supply alone. We will further test whether long-term adaptation broadens, maintains, or contracts host range, and whether such shifts trade off against phage stability or ancestral-host fitness.

By tracing evolutionary routes from early mutations to complex adaptive outcomes across environments, this project establishes a predictive framework for phage adaptability, host-phage coevolution, and the general predictability of viral evolution.

Poster-Session / 80**Poster #80: 3D spatial analysis of fluorescence foci from z-stack microscopy using cell reconstruction****Author:** Lasse Hallenga¹**Co-authors:** Marc Erhardt¹; Philipp Popp¹¹ *Humboldt-Universität zu Berlin*

Bacteria encode diverse anti-phage defense systems, yet how their components are spatially organized during infection remains poorly understood. Three-dimensional multichannel fluorescence microscopy can resolve the spatial relationship between phages and fluorescently labelled defense components. However, this information is often lost during analysis when z-stacks are collapsed into maximum-intensity projections, obscuring their relationships to each other and to cellular structures.

We present an implemented workflow for geometry-aware analysis of infection- and defense-associated fluorescence in individual bacterial cells. The workflow combines 2D cell segmentation, three-dimensional reconstruction of cell volumes using radially symmetry, and foci detection on individual z-slices. Slice-wise detections are linked into 3D foci using spatial parameter.

These measurements enable us to address questions central to phage-host interactions: how bacterial immune systems are coordinated in space and time, where are phage-associated activities accumulated, whether defense components are recruited to infection sites, and how host-response signals co-localize relative to the cell envelope. This three-dimensional analysis aims to offer insights of spatially resolved, single-cell dynamics of phage infection and organization of bacterial antiviral defenses.

Reference #1:

Popp, P. et al. Molecular basis of the Druantia anti-phage defense system. *BioRxiv* (2026)

Reference #2:

Popp, P. et al. Structure and mechanism of the Zorya anti-phage defence system. *Nature* (2024)

Reference #3:

Baybay EK, et al. Pomegranate: 2D segmentation and 3D reconstruction for fission yeast and other radially symmetric cells. *Scientific Reports*. (2020).

Poster-Session / 82

Poster #82: Too Early or Too Late? Trade-Offs in the Timing and Specificity of Bacterial Defence Systems Against Mobile Genetic Elements**Author:** Paritosh Bedekar¹**Co-authors:** Edze Westra¹; Mario Recker¹; Stineke van Houte¹¹ *University of Exeter*

Horizontal Gene Transfer (HGT) is a key element of bacterial adaptation and evolution, enabling the acquisition of genes that influence critical traits, such as antibiotic resistance. HGT is mediated by Mobile Genetic Elements (MGEs) like plasmids, transposons and bacteriophages. While MGEs can be beneficial, some, such as bacteriophages, are harmful, prompting bacteria to evolve diverse anti-phage defence systems (DSs). To date over two hundred DSs have been identified, and a single cell, on average, has five DSs. This remarkable diversity has sparked an interest in trade-offs associated with DSs, such as the balance between effective protection and associated costs.

Here we focus on a trade-off involving the timing of activation of a DS and the ability to discriminate between MGEs. DSs that sense early infection signals, such as foreign DNA, can neutralise MGEs before they harm the host. However, these signals are often shared among diverse MGEs, making it difficult to distinguish between beneficial and harmful elements. On the other hand, DSs that detect later-stage signals, such as phage proteins, enable more acute discrimination but may activate too late to prevent cellular damage.

Using an Individual Based Model (IBM), we explore how ecological and evolutionary factors influence the fitness of early-acting versus late-acting DSs. We examine conditions under which one strategy outcompetes the other, and whether mixed strategies emerge. Our results offer insights into the evolutionary pressures that shape DS diversity, elucidating how bacteria navigate the complex landscape of HGT, and its implications in bacterial adaptation and evolution.

Poster-Session / 83

Poster #83: Multicellular defense in *Streptomyces*: a collective shield against phage predation**Author:** Moses Kabuu¹**Co-authors:** Astrid Wirtz²; Larissa Ernst¹; Julia Frunzke¹¹ Forschungszentrum Jülich GmbH and Heinrich-Heine-University Düsseldorf² Forschungszentrum Jülich GmbH

Bacteriophages predation exerts strong selective pressure on bacteria, driving the evolution of diverse antiphage defense systems. While most characterized defense systems act intracellularly post-infection¹, it is critical to explore defense strategies that function prior to infection considering that phage-host interactions occur within complex natural environments. Such pre-infection mechanisms may elucidate how bacterial populations collectively respond to phage predation at the community level.

Streptomyces characterized by a complex developmental lifestyle², and prolific production of specialized metabolites and proteins some of which possess antiphage properties³, provide a compelling model. We hypothesize that these multicellular bacteria evolved strategies to inactivate free phages either pre-infection or after bursts of infections. Our findings demonstrate that a burst of phage infection in a *Streptomyces* culture was frequently followed by regrowth of phage-resistant mycelium coinciding with a significant drop in the titer of infectious phage particles. This regrowth was accompanied by a decrease in the pH of the culture supernatant and elevated levels of organic acids. Furthermore, screening of spent media revealed the presence of secreted bioactive compounds inactivating extracellular phage particles, with a notable proteolytic activity that degrades phage particles.

Notably, the antiphage potential of this repertoire has only been recently recognized; with phage infection also identified as an elicitor of specialized metabolism. Taken together, these findings highlight the multifaceted defense strategies employed by *Streptomyces* to protect the community.

Reference #1:

1. Georjon, H., & Bernheim, A. (2023). The highly diverse antiphage defence systems of bacteria. *Nature Reviews Microbiology*, 21(10), 686-700. <https://doi:10.1038/s41579-023-00934-x>

Reference #2:

1. Luthe, T., Kever, L., Hänsch, S., Hardy, A., Tschowri, N., Weidtkamp-Peters, S., & Frunzke, J. (2023). *Streptomyces* development is involved in the efficient containment of viral infections. *MicroLife*, 4, uqad002. <https://doi:10.1093/femsml/uqad002>

Reference #3:

1. Hardy, A., Kever, L., & Frunzke, J. (2023). Antiphage small molecules produced by bacteria—beyond protein-mediated defenses. *Trends in microbiology*, 31(1), 92-106. <https://doi:10.1016/j.tim.2022.08.001>

Poster-Session / 84

Poster #84: Systematic characterization of prophage integration sites in Staphylococci**Author:** Neysa Rodriguez-Murillo¹**Co-authors:** Simon Heilbronner¹; David Gerlach¹¹ Ludwig-Maximilians-Universität München

Staphylococci - among them the important human pathogen *S. aureus* - represent key constituents of the nasal and skin microbiome (1). Temperate bacteriophages are major drivers of staphylococcal evolution, leading to the spread of virulence factors and resistance genes (2,3). The effects of lysogeny on the fitness of Staphylococcal species have not been explored in a systematic manner, especially for commensal non-*S. aureus*-staphylococci. In this work, a bioinformatic pipeline called StaPhI was developed to systematically assess prophage insertion sites across several staphylococcal species. The first species to be explored was the major skin commensal *Staphylococcus epidermidis* by analyzing over 150 high-quality genomes from various sources. We identified 8 new *attB* sites and corresponding integrases, of which a sizable number are shared across staphylococci, highlighting potential avenues of cross-species horizontal gene transfer (HGT). Our results showed that *S. epidermidis* prophages integrases are highly selective for their *attB* sequence, even when they have an alternative insertion site that also contains the same *attB*. Here, temperate prophages showed diverse integration strategies ranging from targeting intergenic regions (10%), redundant genes (50%), or essential genes (40%). In the latest case, as an example of host-virus mutualisms, prophages avoid disrupting the open reading frames of essential genes by introducing a stop codon in the same position as the wild-type protein. Our results provide the first steps to a systematic understanding of mechanisms of cross-species HGT in staphylococcal communities.

Reference #1:

Lindsay, J. A. (2014). *Staphylococcus aureus* genomics and the impact of horizontal gene transfer. International Journal of Medical Microbiology, 304(2), 103-109. <https://doi.org/10.1016/j.ijmm.2013.11.010>

Reference #2:

Gerlach, D., Guo, Y., De Castro, C. et al. Methicillin-resistant *Staphylococcus aureus* alters cell wall glycosylation to evade immunity. Nature 563, 705–709 (2018). <https://doi.org/10.1038/s41586-018-0730-x>

Reference #3:

Chen et al., Genome hypermobility by lateral transduction. Science 62,207-212(2018). <https://doi.org/10.1126/science.aat5867>

Poster-Session / 86

Poster #86: A widespread protein family inhibits both restriction-modification and CRISPR-Cas defenses**Authors:** Thomas Gaudin¹; Chase Beisel²¹ *HIRI*² *Botnar Institute*

Mobile genetic elements and their bacterial hosts are in a never-ending evolution dance, giving rise to a multitude of defense and evasion systems. For one given defense system, it is possible to find multiple escape effectors encoded by the bacteriophages, blocking, hiding or mimicking a crucial element for an efficient immune response. Some of those effectors can even block multiple defense system at once, but no anti-defense protein has been shown so far to be able to inhibit two evolutionary unrelated defense systems. Recently, an anti-CRISPR protein called AcrIC11, has been characterized as a type I-C CRISPR-Cas system. Interestingly, by sequence and structural prediction analysis, AcrIC11 have similarities to the broadly distributed anti-restriction protein ArdB, raising the question whether these anti-defense proteins can cross inhibit both defenses.

A set of ArdB orthologues has been determined by sequence similarities, to be tested against different type I CRISPR systems and type I restriction modification (RM) systems. So far, we have shown that ArdB orthologues inhibits also different RM systems, but also type I-C CRISPR system under TXTL condition. Mutational analysis of both ArdB and Cas3 revealed that the C-terminal region of ArdB is needed for inhibition, and that mutating close to the nuclease pocket of Cas3 restore degradation activity in the presence of ArdB. Finally, pull downs of ArdB indicate interaction with the HsdR subunit of type I RM system. These results highlight for the first time the existence of a crosstalk between anti-CRISPR and anti-restriction.

Reference #1:

Tesson, F., Huiting, E., Wei, L., Ren, J., Johnson, M., Planel, R., et al. (2025) Exploring the diversity of anti-defense systems across prokaryotes, phages and mobile genetic elements. *Nucleic Acids Research* 53: gkae1171.

Reference #2:

Wimmer, F., Englert, F., Wandera, K.G., Alkhnbashi, O.S., Collins, S.P., Backofen, R., and Beisel, C.L. (2024) Interrogating two extensively self-targeting Type I CRISPR-Cas systems in *Xanthomonas albilineans* reveals distinct anti-CRISPR proteins that block DNA degradation. *Nucleic Acids Research* 52: 769–783.

Poster-Session / 87

Poster #87: Transcriptional Reprogramming by Cyanophage Syn9: Investigating Host–Phage Interactions in *Synechococcus* WH8109**Author:** Caroline Jelen¹**Co-authors:** Claudia Steglich²; Dunja Ferring-Appel³; Frank Stein³; Mandy Rettel³; Matthias W. Hentze³¹ *Universität Freiburg*² *University of Freiburg, Germany*³ *European Molecular Biology Laboratory, Heidelberg, Germany*

The marine cyanobacterium *Synechococcus* WH8109 is a globally widespread and ecologically important model organism that contributes significantly to primary production in ocean environments, making it a valuable system for studying cyanobacterial ecology and virus–host interactions. Its cyanophage, Syn9, is a T4-like virus that infects *Synechococcus* and is used to study the molecular mechanisms underlying phage-driven reprogramming of the host.

One key aspect involved in manipulation of the host's transcriptional machinery during phage infection is the regulation and hijacking of host transcriptional regulators, such as RNA-binding proteins (RBPs). Using the complex capture approach, we identified several putative phage RBPs, which were subsequently cloned into *Synechococcus* for functional characterization. Following expression verification and growth assays across different clones, we aim to identify their RNA targets by CLIP-seq. In parallel, we are isolating Syn9 virion particles to determine whether RNA is packaged into the capsid, and if so, what type of RNA it is.

Poster-Session / 88

Poster #88: Characterization of the Methanosarcina Spherical Virus**Author:** Tim Hoff¹**Co-authors:** Finn Gehlert¹; Katrin Weidenbach¹; Ruth A. Schmitz¹¹ *Institute of General Microbiology, Kiel University*

Archaeal viruses remain among the most enigmatic and least explored components of microbiology. In recent years, several novel archaeal viruses have been isolated and characterized, revealing remarkable diversity not only in morphology but also in genome architecture (Prangishvili et al., 2017). One such example is the Methanosarcina spherical virus (MetSV), a lytic member of the Tectiviridae family that specifically infects the methanoarchaeal model organism *Methanosarcina mazei* (Weidenbach et al., 2017). The linear genome of MetSV is approximately 10.5 kbp in length and encodes 23 predicted ORFs, the majority of which have no assigned function. Recent genome re-annotation using established bioinformatic tools and databases has allowed functional predictions for several genes, including the type B DNA polymerase (MetSV_ORF07) and its adjacent predicted additional DNA polymerase subunit MetSV_ORF8 (Gehlert et al., 2023). Aiming to study functionality of the viral DNA polymerase, its composition, structure and requirement for virus replication, we started biochemical characterization of the virus proteins and performed a genetic approach using the recently established genetic system for MetSV (Gehlert et al., 2023). Initial findings are presented regarding (i) the characterization of individually expressed ORF7 and ORF8 alongside the fusion protein (ORF7–ORF8), as well as (ii) the phenotypic effects of the corresponding single (ORF8) and double (ORF7+8) viral deletion mutations.

Reference #1:

Gehlert, F. O., Weidenbach, K., Barüske, B., Hallack, D., Repnik, U., & Schmitz, R. A. (2023). Newly Established Genetic System for Functional Analysis of MetSV. *International Journal of Molecular Sciences*, 24(13), 11163. <https://doi.org/10.3390/IJMS241311163/S1>

Reference #2:

Prangishvili, D., Bamford, D., Forterre, P. et al. The enigmatic archaeal virosphere. *Nat Rev Microbiol* 15, 724–739 (2017). <https://doi.org/10.1038/nrmicro.2017.125>

Reference #3:

Weidenbach, K., Nickel, L., Neve, H., Alkhnbashi, O. S., Künzel, S., Kupczok, A., Bauersachs, T., Cassidy, L., Tholey, A., Backofen, R., & Schmitz, R. A. (2017). Methanosarcina Spherical Virus, a Novel Archaeal Lytic Virus Targeting Methanosarcina Strains. *Journal of Virology*, 91(22), 955–972. <https://doi.org/10.1128/JVI.00955-17>

Poster-Session / 93

Poster #93: The role of phage Thanatos ADP-ribosyl-transferases Alt1 and Alt2 in disarming and preparing the host**Author:** Alina Kemmler¹**Co-authors:** Athanasios Typas²; Daniel Gohlus¹; Isabelle Becher²; Kai Thormann¹; Mikhail Savitski²; Nicole Schmid¹; Tara Bartolec²¹ *Institute for Microbiology and Molecular Biology, Justus-Liebig-University Giessen, Germany*² *European Molecular Biology Laboratory, Molecular Systems Biology Unit, Heidelberg, Germany*

ADP-ribosyl-transferases (ARTs) are versatile enzymes found across prokaryotes, eukaryotes, and viruses. Genomic analyses have shown that ARTs are also widespread among bacteriophages. However, their functions in phage infection remain poorly understood. These enzymes modify host proteins through ADP-ribosylation or RNylation, particularly targeting proteins involved in transcription and translation, thereby contributing to host cell reprogramming. Here, we investigate two ARTs, Alt1 and Alt2, encoded by the *Shewanella* phage Thanatos. Both enzymes are injected directly by the phages and actively modify host proteins immediately after infection. We show that the Thanatos ARTs are highly promiscuous: They modify more than 500 host and phage proteins within the first minutes after phage infection, before their activity is terminated by automodification. Primary targets appear to be the host transcription and translation machinery. In addition, potential components of bacterial anti-phage defense systems are targeted by the ARTs. Notably, Alt1 and Alt2 partially restore the infectivity of *E. coli* phages T5 and T7 when they are co-expressed with a dGTPase, a known anti-phage defense factor. Together, our findings support a role for Alt1 and Alt2 as early infection effectors that can not only counteract host defense mechanisms but also prepare the host cell for phage replication.

Poster-Session / 94**Poster #94: The role of phages in microbial communities****Authors:** Gisela T. Rodríguez-Sánchez¹; Jeroen Meijer¹; Torsten Schubert¹; Bas E. Dutilh¹¹ *Balance of the Microverse, Friedrich-Schiller-University Jena*

Phages are abundant in natural microbial communities, yet their full role in shaping community evolution, diversity, function, and dynamics remains unresolved. A particularly case is their potential to influence multistate microbial communities—communities that assemble in alternative stable states under identical conditions. Recent evidence suggests that exposure of microbial communities to phage cocktails (and other mobile genetic elements), may promote or constrain switching among such states. We aim to clarify these phage-associated community transitions. To achieve this, we will experimentally enrich microbial communities under controlled conditions to generate multistate systems. We will then manipulate these communities with defined phage treatments to test how phage diversity and identity affect the likelihood, direction, and stability of state transitions. By linking phage perturbations to community-level responses, our work will provide empirical evidence for how bacteria–phage interactions shape community stability, transitions and evolution, advancing our understanding of microbial community dynamics.

Poster-Session / 95

Poster #95: Universal host contaminant protein identification and quantification for any production host by LC-MS/MS**Author:** Florian Sigloch¹**Co-authors:** Michelle Batsch¹; Tobias Welz¹; Michael Forchheim¹¹ PolyQuant GmbH**Objective**

Both the Eu. Ph. Chapter 5.13 and the EMA concept paper on manufacturing of phage products demand controlling host-cell derived impurities, e.g. host-cell proteins (HCPs), endo- and exotoxins, as well as prophages. Here, we present a universal method for HCP control of phage products by LC-MS/MS.

Methods

E. coli phage T7 was spiked with EcoCAT protein standards and measured via untargeted LC-MS/MS. HCPs were detected and quantified a) relative to the phage proteins, b) relative to the spiked standard, and c) relative to an *E. coli* lysate.

Results

In a semi-purified T7 sample, we detected over 1,250 *E. coli* host proteins. 13 HCPs were directly quantified by using the internal EcoCAT standards. By correlation of all HCP signals to these 13 datapoints, we estimated the absolute quantification of all 1,250 *E. coli* host proteins and calculated the total HCP protein abundance.

Conclusion

LC-MS/MS-based HCP testing enables highly sensitive and specific HCP detection independent of the host strain or organism. By using internal or external standards, total HCP levels can be calculated similar to the results of an ELISA. Additionally, LC-MS/MS based methods deliver quantitative results on single protein level. These additional data facilitate method development and risk assessment for therapeutic phages.

Poster-Session / 96**Poster #96: Structural and functional characterisation of a membrane associated anti-phage defence system****Author:** Nicole Rutbeek¹**Co-authors:** Haidai Hu¹; Nicholas M. I. Taylor¹¹ *University of Copenhagen*

Bacteria have evolved a number of diverse strategies to evade and resist phage infection, however the detailed mechanisms for many of these remain to be understood. In particular, fewer membrane associated anti-phage defence systems have been described in detail, especially ones functioning through a non-abortive mechanism. Here we investigate a two-protein defence system where one protein is predicted to be membrane associated and the other lacks any homology with known domains or existing structures. Using cryo-electron microscopy we show the predicted transmembrane protein forms a dimer, lacking any canonical membrane-spanning helices. Additionally, we obtained a high-resolution map of the two proteins in complex, revealing extensive conformational changes relative to the homodimer. Finally, we show a structure of the defence system bound to a nucleic acid, which allows us to speculate on the overall mechanism of direct defence of this previously undescribed system.

Poster-Session / 97

Poster #97: Cryo-EM structure of the Escherichia coli siphophage Bas24**Author:** Migle Sarpilo¹**Co-authors:** Marta Siborova²; Nicholas M. I. Taylor²¹ *University of Copenhagen, Taylor Lab*² *University of Copenhagen*

Escherichia coli siphophage Bas24 is a previously uncharacterized bacteriophage belonging to the subfamily Queuovirinae. To determine its structure and describe its main structural features, the Bas24 virion was analysed using single-particle cryo-electron microscopy (cryo-EM). The virion structure and morphology were resolved, with the prolate capsid reconstructed to 3.07 Å resolution. The portal, neck, tail tube, baseplate, adapter, and lateral tail fibres were resolved to 2.90, 2.89, 2.98, 2.93, 3.60, and 4.28 Å resolution, respectively. The baseplate exhibits C3 symmetry and consists of three lateral tail fibres and a central tail fibre. The lateral tail fibres interact with the primary receptor, while the central tail fibre interacts with the secondary receptor, LamB. This study presents the first structural characterization of the Queuovirinae phage Bas24. The resolved virion architecture reveals a prolate capsid and a C3-symmetric baseplate with three lateral tail fibres and a central tail fibre, expanding current knowledge of siphophage diversity and providing a foundation for future functional and comparative studies

Poster-Session / 98

Poster #98: Exploring flagellotropic phage adsorption by cryoEM**Author:** James Hodgkinson-Bean¹**Co-authors:** Ivo Hendriks²; Marc Erhardt³; Nicholas M.I. Taylor¹; Nicole Rutbeek¹; Philipp F. Popp³; Tillman H. Pape⁴¹ *Department of Drug Design and Pharmacology, University of Copenhagen*² *Center for protein research, University of Copenhagen*³ *Institute of Biology/Molecular Microbiology, Humboldt-Universität zu Berlin, Berlin*⁴ *Core Facility for Integrated Bioimaging (CFIB), Copenhagen University*

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 ϕ . These model phages 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 determine the flagella binding complex (FBC) produced by our target phage. Due to the unique structure of the complex, we were able to reconstruct the complex to near-atomic resolution (2.9-3.5 Å), identifying component proteins. The FBC is very large, composed of more than 150 protein chains that form a pseudo-symmetric structure weighing ~3.8 MDa. We were also able to resolve interactions between parts of the apparatus and the flagellum itself. Together with previous observations, these results allowed us to develop a testable hypothesis for the mechanism of phage–flagellum adsorption. The model accounts for previous observations regarding adsorption kinetics, including the requirement for active flagellar rotation during adsorption. These findings provide new insights into a remarkably complex flagellum binding complex, representing a significant advance in our understanding of flagellotropic phage infection.

Poster-Session / 99

Poster #99: A diverged ubiquitination pathway mediates antiviral defense in bacteria

Authors: Huijuan Li¹; Marta Siborova²; Radvilas Bendorys³; David Mayo-Muñoz⁴; Johannes Anton Kühn⁵; Victor Klein De Sousa⁶; Ruilaing Zhao⁷; Mario Rodríguez Mestre⁵; Leise Riber⁴; Monika Jasnauskaitė³; Gytis Dudas³; Leighton Payne⁵; Sarah Camara-Wilpert⁵; Patrick Pausch³; Nicholas M. I. Taylor⁶; Søren J. Sørensen⁵; Rafael Pinilla-Redondo⁵

¹ *ction of Microbiology, University of Copenhagen*

² *ILF, University of Copenhagen*

³ *LSC-EMBL, Life Sciences Center*

⁴ *Section for Microbial Ecology and Biotechnology, University of Copenhagen*

⁵ *Section of Microbiology, University of Copenhagen*

⁶ *Novo Nordisk Foundation Center for Protein Research, University of Copenhagen*

⁷ *Section of Microbiology, University of Cop*

Ubiquitination is a central regulatory mechanism in eukaryotic cell biology, governing processes ranging from protein turnover to antiviral defence. In bacteria, conjugation of ubiquitin-like proteins (Ubls) is emerging as a core function of diverse antiviral systems, including systems with evolutionary links to the human antiviral ISG15 pathway. Recent work has uncovered a diverged bacterial ubiquitination system with altered conjugation chemistry, but its biological function has remained unclear. Here, we show that this non-canonical ubiquitination pathway provides robust defence against diverse bacteriophages. Combining genetic, bioinformatic and biochemical analyses, we demonstrate that Ubl modification of phage lateral tail fibres impairs virion assembly and infectivity. Together, our findings establish the immune function of a diverged ubiquitination pathway and reveal covalent remodelling of viral structural proteins as a conserved antiviral strategy deployed across chemically diverse ubiquitination systems and domains of life.

Poster-Session / 100

Poster #100: Balancing of immune activation and suppression by N4-like *Pseudomonas aeruginosa* phages.**Authors:** Iana Fedorova¹; Yourun Yue²; Yue Feng²; Joseph Bondy-Denomy³¹ HZI² State Key Laboratory of Green Biomanufacturing, College of Life Science and Technology, Beijing University of Chemical Technology³ UCSF

Signaling-based anti-bacteriophage systems such as CBASS and Thoeris synthesize infection-triggered nucleotide signals that activate anti-phage effectors. However, the phage features sensed by these systems and the mechanisms phages use to evade signaling immunity remain poorly understood. Here, studying clinically relevant N4-like *Pseudomonas aeruginosa* phages from the Migulavirinae family, we show that closely related phages encode subtle allelic variation in side tail fiber proteins that determine sensitivity to type II Thoeris. In parallel, these same phages encode an “anti-defense hotspot” that contains three adjacent genes, each sufficient to facilitate phage evasion of both CBASS and Thoeris defenses, counter balancing the activating proteins. Comparative analysis of this anti-signaling hotspot across the broader family of related N4-like phages uncovered a new Thoeris anti-defense RyRTad protein that sponges NAD-derived molecules (e.g. gcADPR) and exhibits sequence and structural similarity to a poorly characterized nucleotide-binding region of the human ryanodine receptor. Together, these findings reveal how the balance between immune activation and antagonism shifts phage outcomes and reveal a surprising similarity between a phage molecular sponge and a human ryanodine receptor.

Poster-Session / 102**Poster #102: Exploring auxiliary metabolic genes (AMGs) from cyanophage Syn9**

Authors: Miguel Fernandez¹; Nicole Frankenberg-Dinkel¹

¹ RPTU

Auxiliary Metabolic Genes (AMGs) are found in the genomes of many phages that infect cyanobacteria. While their activities in the virocell during infection still need to be elucidated, it is thought that they are involved in reprogramming the host

metabolism rather than directly affecting viral replication.

AMGs often encode proteins involved in photosynthesis and light-harvesting and seem to have originated from a bacterial host.

In order to investigate the function of AMGs in the bacterial host during infection, we use *Synechococcus* WH8109 as a host and the phage Syn9, a T4-like Myovirus, to study different aspects of infection. One of the fairly abundant AMGs in cyanophage genomes is *cpeT*. This gene has sequence similarities to so-called phycobiliprotein lyases, proteins that in the host cell serve as assembly factors for the light harvesting phycobilisome.

In summary, our objective is to elucidate the role of AMGs from cyanophages and understand their impact on the interaction with the host.

Reference #1:

Gasper R, Schwach J, Hartmann J, Holtkamp A, Wiethaus J, Riedel N, Hofmann E, Frankenberg-Dinkel N. Biol Chem., 2017 292(8):3089-3098

Reference #2:

D. Lindell, J.D. Jaffe, Z.I. Johnson, G.M. Church, S.W. Chisholm. Nature, 2005, 438(7064), 86-89.

Poster-Session / 107

Poster #107: An Lsr2-like Xenogeneic Silencer Confers Immunity Against AT-Rich Bacteriophage Infection

Author: Biel Badia Roigé¹

Co-authors: Nadiia Pozhydaieva²; Ombeline Ombeline³; Konstantinos Kalogeropoulos⁴; Karen L. Maxwell⁵; Stan J. J. Brouns⁶; Eugen Pfeifer⁷; Julia Frunzke⁸

¹ IBG1

² Department of Bionanoscience, Delft University of Technology, The Netherlands

³ Université Paris-Saclay, CEA, CNRS, Institute for Integrative Biology of the Cell (I2BC), 91198, Gif-sur-Yvette, France

⁴ Center for Translational Protein Design, Technical University of Denmark, Søtofts Plads, DK-2800, Kongens Lyngby, Denmark

⁵ Department of Biochemistry, University of Toronto, Toronto, Ontario, Canada

⁶ Kavli Institute of Nanoscience, 2629 HZ Delft, The Netherlands

⁷ Université Paris-Saclay, INRAE, AgroParisTech, MICALIS, 78350 Jouy-en-Josas, France

⁸ HHU Düsseldorf, Faculty of Mathematics and Natural Sciences Institute of Microbial Interactions, Heinrich-Heine-University Düsseldorf, Düsseldorf, Germany

In Actinomycetota, Lsr2-like xenogeneic silencer proteins are widespread nucleoid-associated proteins that preferentially bind AT-rich foreign sequences, oligomerize across promoters and intergenic regions, and hinder transcription. While they function as a global regulator for virulence genes or biofilm promoting genes has been described in the past, its role during bacteriophage infection, one of the most direct encounters with foreign DNA, remain mainly unexplored. In *Corynebacterium glutamicum*, the Lsr2-like protein CgpS is encoded within CGP3, a 187 kb mobile genetic element rich in AT-sequences, and is known to silence transcription of foreign DNA within this region (1). In a recent study, we demonstrate that the CGP3-encoded Lsr2-like silencer CgpS functions as an antiviral factor by restricting infection by the AT-rich bacteriophage JeanGrey (2). CgpS binds and oligomerizes on phage-derived AT-rich sequences, repressing early phage transcription and thereby preventing productive replication. The redistribution of CgpS delays the phage program development and enables a coordinated activation of host defense pathways, including the LexA-regulated SOS response and cell division regulators. To place these observations in a broader evolutionary context, we extended our analysis by examining the distribution and genomic regions of Lsr2 homologues across actinobacterial lineages.

Our findings expand the known functional landscape of xenogeneic silencers and introduce chromatin-like silencing as a regulatory layer in bacterial antiviral defense, underscoring the remarkable functional diversity of this ancient protein family.

Reference #1:

Pfeifer, E. et al (2016) doi: 10.1093/nar/gkw692

Reference #2:

Badia Roigé, B. et al (2026) - in review. doi: 10.21203/rs.3.rs-9446490/v1

Poster-Session / 108

Poster #108: Identification of a non-WTA bacteriophage receptor in *Staphylococcus aureus***Author:** Janes Krusche¹**Co-authors:** Andreas Peschel¹; Tjark Schneider²¹ University of Tübingen² Infection Biology; Interfaculty Institute of Microbiology and Infection Medicine Tübingen (IMIT); University of Tübingen

Phages are major vehicles for horizontal gene transfer in the human pathogen *Staphylococcus aureus* and promising agents for the treatment of antibiotic resistant infections. All currently known *Staphylococcus* phages use wall teichoic acid (WTA), a bacterial cell wall glycopolymer, as their receptor, such that loss of WTA can lead to phage escape. We found that *Silviavirus* Remus can bind and infect *S. aureus* even in the absence of WTA, albeit with decreased efficiency, indicating that the phage uses an additional receptor. Analysis on phage resistant mutants of *S. aureus* revealed disruptions of the cell-wall bound peptidoglycan transglycosylase IsaA. Clean deletion of IsaA in a WTA-negative background confirmed the extracellular enzyme as the additional *S. aureus* *Silviavirus* receptor, which was further confirmed via detection of direct binding between phage Remus and IsaA. These findings illuminate the molecular specificities of *S. aureus*-phage interactions with important implications for future phage therapies.

Poster-Session / 122**Poster #122: Zorya-mediated defence results in distinct cellular outcomes following phage infection****Author:** Adhvaidhi Kuntran¹**Co-authors:** Lasse Hallenga¹; Philipp Popp¹; Marc Erhardt¹¹ *Humboldt University Berlin*

The Zorya defence system is a multi-protein antiviral complex that detects phage infection at the bacterial membrane and restricts viral replication. Although the three Zorya subtypes share homologous membrane-spanning components, they encode distinct cytoplasmic effector proteins, suggesting mechanistic divergence between subtypes.

Here, we investigate the anti-phage defence profile of Zorya using plaque assay, live-cell time-lapse microscopy and fluorescence imaging coupled with automated image analysis. We observe distinct infection phenotypes following phage infection, with the outcome of Zorya-mediated defence varying according to the infecting phage.

Using reporter strains, that enable to track the fate of the injected phage DNA and host DNA, we investigate the roles of Zorya subtype-specific effector proteins in mediating anti-phage defence.

Our findings reveal both shared and distinct features of Zorya-mediated immunity and indicate that the conserved membrane-associated machinery is coupled to subtype-specific effector activities that determine the outcome of antiviral defence.

Poster-Session / 123

Poster #123: ATP-dependent oligomerization triggers tRNA degradation by Schlafen-fused prokaryotic Argonaute proteins**Author:** Thomson Hallmark¹**Co-authors:** Jurre Steens¹; Simon Lindhoud¹; Adrie Westphal¹; John van der Oost¹; Daan C. Swarts¹¹ Wageningen University & Research

Organisms from all domains of life are under constant attack from selfish genetic elements including viruses, plasmids, and transposons. To defend against these invaders, a myriad of immune systems has evolved. Argonautes (Agos) are a heavily diversified family of immune proteins that are conserved from prokaryotes to eukaryotes. In general, Agos use small nucleic acid guides to recognize invader sequences, after which immunity is conferred through one of various mechanisms. Specific prokaryotic Agos (pAgos) are fused to Schlafen (Slfn) proteins, another immune protein found in all domains of life. In other systems, Schlafen proteins can cleave tRNA to restrict viral replication. However, the importance of the association of Slfn and pAgos proteins has remained unclear.

Here, we show that Slfn-pAgos form a novel major clade of pAgos that act as immune systems: Slfn-pAgo from *Runella zaea* (RzAgo) defends *E. coli* from several phages of the BASEL collection. *In vitro* biochemical experiments reveal that, upon RNA-guided DNA targeting by RzAgo, its Slfn domain is catalytically activated through ATP-dependent oligomerization, which results in tRNA cleavage. Combined, these results provide a foundational understanding of the mechanisms by which Slfn-pAgo systems provide immunity and further highlights the conservation of immune mechanisms across different domains of life.

Viral impact on microbial communities / 115**Temperate bacteriophage populations in the human gut: culturomics and experimental insights****Author:** Jeremy J. Barr¹¹ *Monash University*

While often overlooked, the most abundant entity within the gut are the viruses that infect bacteria, called bacteriophages (phages). Large-scale metagenomic and data mining efforts have uncovered an expansive diversity of phages within the human gut. However, the functional understanding of phage-host interactions and their impacts within this complex ecosystem have been limited due to a lack of cultured isolates for experimental validation. In recent work, we characterise 182 active prophages originating from 252 diverse human gut bacterial isolates using diverse induction conditions, including chemical cues, metabolites, starvation, and human cell derivatives. We found that active Bacteroidota prophages were among the most prevalent members of the gut virome, with extensive use of diversity generating retroelements and exhibiting broad host ranges. Moreover, active polylysogeny was present in 58% of studied gut lysogens and led to coordinated prophage induction. Transcriptomic analysis of selected isolates revealed fundamental information on life cycles of gut phages, including the constitutive expression of defence and counter-defence genes across both polylysogenic phages and their hosts. In this presentation, I will summarise our recent and unpublished work into the study and use of experimentally validated gut prophages, providing key insights into their diversity, biology, genetics, and function within these complex microbial communities. More broadly, this presentation will highlight the importance of experimental validation alongside genomic based computational prediction to enable further functional understanding of these commensal viruses within the human gut.

Viral impact on microbial communities / 101**No Escape – The ARTs of host takeover****Author:** Kai Thormann¹¹ *Justus Liebig University Giessen*

Lytic phages and their bacterial hosts pursue fundamentally opposing strategies: while phages aim to maximize their replication and progeny production, bacterial hosts seek to restrict or prevent phage propagation. To counteract host defense systems and make optimal use of host resources, many phages encode dedicated effector proteins. Upon infection, *Shewanella* phage Thanatos directly injects two ADP-ribosyltransferases (ARTs), Alt1 and Alt2, into the host cell. We found that both are highly active and promiscuous enzymes that ADP-ribosylate more than 500 host proteins within minutes of infection before undergoing self-inactivation. Primary targets include the ribosome and RNA polymerase complexes, suggesting that these major cellular machineries are rapidly reprogrammed for subsequent phage production. In addition, several potential phage defense systems are targeted. While neither ART is essential for Thanatos infection, together they increase phage yield by approximately 10-fold. Notably, we found that the Thanatos ART effectors also suppress the emergence of host escape mutants. Thus, the Thanatos ARTs represent a novel class of phage effectors that promote both the immediate and long-term success of phage proliferation.

Viral impact on microbial communities / 4**The Enemy of My Enemy Carries a Colicin: Engineering phages to redirect evolutionary trajectories of uropathogenic *E. coli*****Authors:** Sebastian A. Bernauer¹; Jiemin Du¹; Matthew Dunne¹; Martin J. Loessner¹; Enea Maffei¹**Co-authors:** Janik Mutter¹; Cassandra I. Matter¹; Leandro Sà Zanetti¹; Lisa Furter¹; Fatih Abdula²; Lorenz Leitner²; Shawna McCallin²; Thomas M. Kessler²¹ *ETH Zurich*² *Balgrist University Hospital*

Bacterial populations are shaped by antagonistic interactions, including phage predation and inter-bacterial competition mediated by toxins such as colicins. However, phages are usually used as natural bacterial killers rather than as programmable tools. Here, using uropathogenic *Escherichia coli* as a model, we show that broad-host-range phages can report phage-host interactions and constrain resistance emergence by delivering antibacterial toxins.

First, we isolated two naturally broad-host-range phages infecting uropathogenic *E. coli* and engineered them using CRISPR-assisted methods. NanoLuc reporter versions enabled susceptibility testing within ca. 6 h and collectively identified infection of 70% of an in-house uropathogenic *E. coli* collection ($n = 60$), with reporter activity closely matching plaquing host range.

Second, we engineered colicin-producing phages that couple viral predation to colicin-mediated bystander killing. Compared with wild-type phages, they reduced the emergence of resistant bacteria both in rich medium and in human urine, suggesting that phage-delivered toxins can reshape resistance dynamics in an infection-relevant environment.

Together, our results show that phages can be redesigned beyond strain-specific antimicrobials into tools that report infection networks and connect viral predation to interbacterial competition to limit resistance emergence.

Viral impact on microbial communities / 22**Targeted Antimicrobial Peptide Discovery and Phage Cocktail Development Against ESKAPE Pathogens****Authors:** Torben Sanders^{None}; Julia Ammann¹**Co-authors:** Kawtar Tiamani¹; Mohammadali Khan Mirzaei¹; Li Deng¹¹ *Helmholtz Munich*

Treatment options are being limited by the increasing threat of antimicrobial resistance among ESKAPE pathogens, with bacteriophages and phage-derived peptides being regarded as promising alternatives to conventional antibiotics.

Viral tagging, a culture-independent method, was used to identify wastewater-derived phages that selectively infect ESKAPE pathogens. After metagenomic assembly and viral discovery, the resulting viral genomes were screened for potential antimicrobial peptides (AMPs) using three distinct machine learning algorithms. Three candidate AMPs were synthesised and tested against 18 clinical ESKAPE isolates. In parallel, lytic phages were isolated using culture-based methods and formulated into species-specific and broad-spectrum ESKAPE phage cocktails.

Two novel AMPs demonstrated antibacterial activity, with MIC values as low as 4 μM against *Enterobacter cloacae* (AMP1), 5.3 μM against *Enterococcus faecium* (AMP1) and 10.6 μM against *Acinetobacter baumannii* (AMP3). The species-specific phage cocktails covered up to 91% of the tested strains, and the ESKAPE cocktail exhibited comparable or greater bacterial inhibition than the individual phages for most strains.

These findings demonstrate that integrating viral tagging with culture-based phage isolation expands access to host-specific phage diversity and enables the discovery of both phage-derived antimicrobial peptides and therapeutic phages against multidrug-resistant ESKAPE pathogens.

Viral impact on microbial communities / 6**PhaDRiM - Phage induced Strain Replacement in Microbiomes****Author:** Marla Gaissmaier¹¹ *Technical University of Munich*

Within the last decades microbiome research became more and more relevant to the field of public health. It vastly contributed to understanding the pathogenesis of major human diseases but also generated knowledge on the emergence and spread of antibiotic resistances (ABR). Nevertheless, new insights always open up more questions. An important one is: How can we use this knowledge to specifically eliminate harmful or ABR bacteria from the human gut without harming or disrupting overall microbiota composition?

To address this question, we focus on a new strategy termed “phage induced strain replacement within microbiomes”, which aims at combining phage- and probiotics-based therapy within one treatment. The idea behind this approach is to pose a fitness disadvantage on the targeted strain by a strain-specific phage cocktail and introduce a second, closely related, but phage-resistant competitor that competes for the same niche within the gut environment as the target strain. Ideally, this approach leads to strain replacement and elimination of the targeted strain without disturbing the overall gut microbiota composition. We focus on the three species *Escherichia coli*, *Enterococcus faecalis* and *Phocaeicola vulgatus* (formerly *Bacteroides vulgatus*). These species were chosen due to their clinical relevance, genetic diversity and high prevalence in the human gut. Currently, we are experimenting with a range of different environmental conditions in vitro and in vivo to further evaluate the potential efficiency of strain replacement as a new therapeutic approach to combat ABR and chronic intestinal diseases.

Viral impact on microbial communities / 116**Genetic perturbation of gut bacteria with engineered phage vectors and CRISPR-Cas systems****Author:** David Bikard¹¹ *Institut Pasteur*

The crucial role of the gut microbiome in human health has driven a need to understand bacterial function within their complex native ecosystem. CRISPR-Cas screens have become a powerful method to systematically assess the importance of bacterial genes for growth in different conditions, and are being adapted to the study of gut bacteria. We performed screens in different *E. coli* strains colonizing the mouse gut, revealing complex trophic interactions and adaptation strategies. We further investigate the use of phages as delivery vectors to directly modify target bacteria within the gut. Work performed with Eligo Bioscience demonstrated the feasibility of inactivating target genes by base editing, directly in the gut of mice. I will further discuss various strategies to overcome remaining challenges associated with DNA delivery to bacteria in the complex environment of the gut microbiome.

Viral impact on microbial communities / 90

Exploring virus dynamics in a *Nanobdellati* Co-culture system

Author: Sabine Schwarzer¹

Co-authors: Hiroyuki D. Sakai²; Satoshi Nakagawa³; Milad Reyhani⁴; Shigeru Shimamura⁵; Matthew D. Johnson⁴; Yuya Tsukamoto⁶; Michiru Shimizu⁷; Ken Takai⁸; Takuro Nunoura⁵; Norio Kurosawa⁹; Moriya Ohkuma¹⁰; Thomas Hackl¹¹; Debnath Ghosal⁴; Tessa E.F. Quax¹

¹ *Biology of Archaea and Viruses, Groningen Biomolecular Sciences and Biotechnology Institute, University of Groningen, Groningen, The Netherlands.*

² *Japan Collection of Microorganisms, RIKEN BioResource Research Center, Tsukuba, Ibaraki 305-0074, Japan.; Department of Science and Engineering for Sustainable Innovation, Faculty of Science and Engineering, Soka University, Hachioji, Tokyo 192-8577, Japan.; Research Institute for Geo-Resources and Environment, Geological Survey of Japan (GSJ), National Institute of Advanced Industrial Science and Technology (AIST), Tsukuba, 305-8567, Japan*

³ *Division of Applied Biosciences, Graduate School of Agriculture, Kyoto University, Kyoto 606-8502, Japan.; Institute for Extra-cutting-edge Science and Technology Avant-garde Research (X-star), Japan Agency for Marine-Earth Science and Technology (JAMSTEC), Yokosuka 237-0061, Japan.; Exploratory Research Center on Life and Living Systems (ExCELLS), National Institute of Natural Sciences, Okazaki, Aichi 444-8787, Japan.*

⁴ *Department of Biochemistry and Pharmacology, Bio21 Molecular Science and Biotechnology Institute, The University of Melbourne, Melbourne, VIC, Australia.; ARC Centre for Cryo-electron Microscopy of Membrane Proteins, Bio21 Molecular Science and Biotechnology Institute, University of Melbourne, Parkville, VIC 3010, Australia*

⁵ *Institute for Extra-cutting-edge Science and Technology Avant-garde Research (X-star), Japan Agency for Marine-Earth Science and Technology (JAMSTEC), Yokosuka 237-0061, Japan.*

⁶ *Department of Earth Science, Tohoku University, Sendai, Japan.*

⁷ *Japan Collection of Microorganisms, RIKEN BioResource Research Center, Tsukuba, Ibaraki 305-0074, Japan*

⁸ *Institute for Extra-cutting-edge Science and Technology Avant-garde Research (X-star), Japan Agency for Marine-Earth Science and Technology (JAMSTEC), Yokosuka 237-0061, Japan.; Exploratory Research Center on Life and Living Systems (ExCELLS), National Institute of Natural Sciences, Okazaki, Aichi 444-8787, Japan.*

⁹ *Department of Science and Engineering for Sustainable Innovation, Faculty of Science and Engineering, Soka University, Hachioji, Tokyo 192-8577, Japan.*

¹⁰ *Japan Collection of Microorganisms, RIKEN BioResource Research Center, Tsukuba, Ibaraki 305-0074, Japan.*

¹¹ *Eco-Evolutionary Bioinformatics, Groningen Institute for Evolutionary Life Sciences (GELIEFS), University of Groningen, Groningen, The Netherlands.*

Archaea are infected by structurally diverse viruses that are important drivers of microbial evolution. However, archaeal virus infections are typically studied in binary host-virus systems, while the influence of additional microbial partners on infection dynamics remains poorly understood. The archaeal kingdom *Nanobdellati* (formerly DPANN) comprises ultra-small symbionts with reduced genomes and cell sizes, yet their ecology and interactions with viruses remain largely unexplored because of limited cultivability.

In this study, we explored the interactions between the ultra-small archaeon YN4 of the order *Nanobdellales*, its archaeal host *Metallosphaera hakonensis* YN4HA, and the virus *Metallosphaera* Turreted Icosahedral Virus 4 (MTIV4), using a newly established, stable tripartite co-culture system. We combined physiological, genomic, transcriptomic, glycoproteomic, cryo-electron tomographic, and live-cell imaging approaches to characterise the three partners. Cryo-electron tomography revealed that the *Nanobdellales* ectosymbiont forms a direct connection with its host through a cone-shaped attachment organelle. Although MTIV4 infects only YN4HA and impairs host growth, the presence of the YN4 symbiont substantially mitigated this virus-induced growth inhibition. Our results show that an obligate symbiont can confer protection against viral infection, suggesting that the host-symbiont relationship may shift from a parasitic interaction towards a defensive partnership. Glycoanalysis further suggests that remodelling of host cell-surface glycans, triggered by the YN4 symbiont, may contribute to this protection.

This study provides the first in-depth exploration of a tripartite host-symbiont-virus system in archaea and highlights the importance of considering microbial communities when studying viral ecology and evolution.

Reference #1:

A Tripartite Co-culture System Reveals Defensive Mutualism Between a Nanobdellati Symbiont and Its Host

Hiroyuki D Sakai, Sabine Schwarzer, Satoshi Nakagawa, Milad Reyhani, Shigeru Shimamura, Matthew D Johnson, Yuya Tsukamoto, Michiru Shimizu, Ken Takai, Takuro Nunoura, Norio Kurosawa, Moriya Ohkuma, Thomas Hackl, Debnath Ghosal, Tessa Quax

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Viral impact on microbial communities / 58

Viral Take-over of Host Photosynthetic Electron Transport: Investigating the role of Cyanophage-encoded Plastocyanin**Author:** Nicola Sekularac¹**Co-authors:** Miguel Fernandez Contreras¹; Ran Tahan²; Laura Mages³; Antonia Ebberts¹; Eugenio Pérez Patallo¹; Susanne Zehner¹; Antonio Pierik⁴; Debbie Lindell²; Dennis Nürnberg³; Nicole Frankenberg-Dinkel¹¹ Department of Microbiology, RPTU, Kaiserslautern, Germany² Faculty for Biology, Technion Israel Institute of Technology, Haifa, Israel³ Department of Physics, Freie Universität Berlin, Germany⁴ Department of Biochemistry, RPTU, Kaiserslautern, Germany

Marine picocyanobacteria are the most abundant primary producers in the world's oceans. They are infected by specific viruses, cyanophages. So-called auxiliary metabolic genes (AMGs) encoded in the genome of cyanophages are believed to facilitate the takeover of the cyanobacterial cell by maintaining host metabolism during infection.[1] A commonly occurring AMG is the plastocyanin-like gene *petE*. [2] Plastocyanin (PetE) is found in most photosynthetic organisms and it ferries electrons from the cytochrome *b6f* complex to photosystem I (PSI). [3] We hypothesize that phage PetE is one of many tools cyanophages use to hijack cyanobacterial cells for viral replication and we investigated its role during infection.

We demonstrated that phage *petE* encodes for a functional plastocyanin. This viral gene is advantageous during infection as its deletion causes a clear delay in host cell lysis. Phylogenetic analysis showed that phage PetE has conserved amino acid alterations and clusters separately from its cyanobacterial counterparts. Furthermore, *in silico* prediction suggested that phage PetE has different properties compared to host PetE, and biochemical studies employing recombinant protein confirmed that phage and host PetE differ in midpoint redox potential and isoelectric point. In addition, the rate of *in vitro* PSI reduction by phage PetE was significantly slower than host PetE.

Our results suggest that the main interaction partner of phage PetE may not be PSI. Instead, we postulate that phage plastocyanin redirects electrons towards the respiratory cytochrome terminal oxidase. Thus, it contributes to the proton motive force, and helps meeting the high ATP demand required by phage genome replication.

Reference #1:

D. Lindell, J.D. et al. Nature, 2005, 438(7064), 86-89.

Reference #2:

Puxty, R. J., et al (2015. Photosynthesis Research, 126(1), 71-97.

Reference #3:

E.L. Gross. Photosynthesis Research, 1993, 37(2), 103-116.

Viral impact on microbial communities / 8**Metabolic reconstitution in the NAD⁺ battlefield: phages and bacteria in a molecular arms race****Authors:** Ilya Osterman¹; Rotem Sorek²¹ *European Molecular Biology Laboratory Hamburg*² *Weizmann Institute of Science*

Bacteria defend against phage infection through a variety of antiphage defense systems. Many of these systems deplete cellular NAD⁺ in response to infection by cleaving it into nicotinamide and ADP-ribose or related metabolites. We previously showed that a substantial fraction of phages encode the NAD⁺ reconstitution pathway 1 (NARP1), which restores NAD⁺ from its degradation products and counteracts NAD-depleting immunity.

Recently, we described the next stage of this metabolic arms race. We show that the aRES family of antiphage defense systems degrades NAD⁺ into nicotinamide and ADPR-1''-phosphate, a product that cannot be recycled by canonical NARP1. In response, phages have evolved an expanded NAD⁺ reconstitution pathway, NARP1-AP, which encodes a phosphatase that converts ADPR-1''-phosphate into ADP-ribose, thereby restoring NAD⁺ biosynthesis and overcoming aRES-mediated defense.

Our findings demonstrate how reciprocal innovation in NAD metabolism drives the co-evolution of bacterial defense and phage counterdefense, illustrating metabolic reconstitution as a powerful strategy in the phage–bacteria arms race.

Reference #1:

Osterman, I. et al. Phages reconstitute NAD⁺ to counter bacterial immunity. *Nature* 634, 1160–1167 (2024).

Reference #2:

Osterman, I. et al. Bacterial defense via RES-mediated NAD⁺ depletion is countered by phage phosphatases. *Cell Host & Microbe* (2026).

Viral impact on microbial communities / 117**Understanding the role of bacterial anti-phage defences to inform phage therapy****Author:** Stineke van Houte¹¹ *University of Exeter*

Bacteriophages are a major force in shaping microbial evolution and microbial community composition. In response, bacteria have evolved a wide repertoire of anti-phage defence systems. Most defence systems have been characterised in isolation and against only one or a few phages. As a result, we lack a quantitative and comparative understanding of defence range and the determinants of phage susceptibility.

In my talk I will discuss the evolutionary ecology of anti-phage defences, using *Pseudomonas aeruginosa* and its phages as a model system. I will talk about recent unpublished work aimed to understand the relative importance of these defences and the extent to which they can explain bacterial immunity to phage infections across clinical isolate collections.

Novel anti-viral defence systems / 111**Phage-induced membrane remodelling is a vulnerability targeted by bacterial immunity****Author:** Karen Maxwell¹**Co-authors:** Landon Getz¹; Vivian Liu¹; Alexander Keszei¹; Sam Fairburn¹; Gregory Cole¹; Trevor Moraes¹; Michael Norris¹¹ *University of Toronto*

Nucleus-forming jumbo phages establish infection by first enclosing their genome within an early phage infection (EPI) vesicle derived from the bacterial inner membrane. We discovered a bacterial immune system that targets this early membrane-bound stage of infection. This defence protein rapidly localizes to the site of phage infection and disrupts EPI vesicle morphology. In vitro, this defence protein can remodel bacterial lipid membranes, producing extensive membrane tubulation. Together, our findings reveal that bacterial immunity can directly target the membrane architecture required for viral replication.

Novel anti-viral defence systems / 34**ApeA cleaves genomic RNA to defend against RNA phage infection**

Authors: Arina Drobysheva¹; Manuel Velasco Gomariz²; Shazeb Ahmad¹; Sarah Reichardt²; Jens Hör²

¹ *Institute of Molecular Infection Biology, University of Würzburg, Würzburg, Germany*

² *Helmholtz Institute for RNA-based Infection Research (HIRI), Helmholtz Centre for Infection Research (HZI), Würzburg, Germany*

To protect themselves against viral infection, bacteria encode a vast diversity of antiphage defense systems. However, the mechanisms of action of most of these systems have exclusively been studied using phages with DNA genomes as the models, while phages with RNA genomes remain understudied. Here, we investigated how the defense system ApeA confers resistance against RNA phage infection. We show that two ApeA homologs, Ec1ApeA and Ps2ApeA, protect against a variety of single-stranded RNA phages. Focusing on Ec1ApeA, we find that it senses infection through a conserved pocket that binds an RNA structure in the phage genome. This activates the HEPN (higher eukaryotes and prokaryotes nucleotide-binding) RNase domain of Ec1ApeA which consequently cleaves the phage genomic RNA to restrict replication. In contrast to many other described defense systems, Ec1ApeA activity directly stops viral replication without inducing cell death, establishing ApeA as a non-abortive defense system that protects against RNA phages. Our results add to the increasingly diverse targets of antiviral HEPN RNases and provide insights into the understudied field of RNA phage defense.

Novel anti-viral defence systems / 54

Deoxydinucleotides activate the bacterial anti-phage defense system ApeA

Authors: Jonas Juozapaitis¹; Arūnas Šilanskas²; Audronė Rukšėnaitė²; Ema Ežerskytė²; Rugilė Puteikienė²; Domantas Vareika²; Lidija Truncaitė³; Giedrė Tamulaitienė²; Inga Songailienė²; Virginijus Šikšnys²; Giedrius Sasnauskas²

¹ EMBL Partnership Institute for Genome Editing Technologies and Institute of Biotechnology, Life Sciences Center, Vilnius University, Vilnius, LT-10257, Lithuania

² Institute of Biotechnology, Life Sciences Center, Vilnius University, Vilnius, LT-10257, Lithuania

³ Institute of Biochemistry, Life Sciences Center, Vilnius University, Vilnius, LT-10257, Lithuania

Bacteria carry many antiviral defense systems, most of which rely on toxic effectors that must stay inactive until infection. How infection is actually sensed is still unknown for most of them. Here we focus on ApeA, a single-protein system with a HEPN RNase domain. Testing six homologs against a large E. coli phage panel revealed distinct, homolog-specific protection profiles. Protection fails at high MOI, suggesting abortive infection, and RNA-seq of infected cells showed ApeA cutting host tRNAs inside the anticodon loop – the cell shuts down its own translation to strand the phage.

Cryo-EM of five homologs revealed unexpectedly large doughnut-shaped decamers, with HEPN active sites lining a tRNA-sized central channel and a conserved pocket sitting far from the catalytic center. In Ec2ApeA that pocket holds a 5'-phosphorylated deoxydinucleotide ending in guanosine (pdNG). Mutating the pocket or the oligomerization interface abolishes defense, and a specific loop shifts upon ligand binding, apparently relaying the signal across the ring. Deoxydinucleotides trigger tRNA cleavage in vitro; depleting them in cells shuts down ApeA toxicity.

We propose Ec2ApeA is sensing debris of its own genome, chewed up by phage nucleases – a new class of immune activator.

Novel anti-viral defence systems / 109

Bacterial multicellular behaviour in antiviral defense**Author:** Julia Frunzke¹**Co-authors:** Tom Luthe²; Larissa Ernst¹; Moses Kabuu¹; Samy Mechrouki¹; Bente Rackow³¹ *Forschungszentrum Jülich*² *Ruhr University Bochum*³ *Bio-Rad Laboratories*

Phage defense is commonly studied at the level of individual cells and intracellular immune systems. However, bacterial populations often display multicellular organization, developmental differentiation, and extensive chemical interactions that can influence the outcome of phage infection.

Using *Streptomyces* as a model, we examine antiviral mechanisms that operate at the population level. Developmental transitions can generate transiently phage-resistant cell states and restrict phage propagation within structured populations (1,2). Secreted secondary metabolites provide an additional layer of defense. In the first funding period, we have demonstrated that aminoglycoside antibiotics inhibit early stages of phage infection and that modifications through resistance mechanisms retain the antiviral properties of the molecule (2,3). DNA-intercalating antiphage molecules further illustrate how chemical defense can alter infection trajectories. For daunorubicin-sensitive phages, inhibition after first-step genome transfer of T5-like phages permits continued pre-early gene expression but prevents productive infection, resulting in abortive infection through “mutual destruction” of both phage and host (5). Related phenotypes occur across taxonomically distinct phages and chemically different DNA-intercalating compounds. Moreover, the DNA-intercalating anthracycline daunorubicin can synergize with downstream nucleic acid-targeting defense systems, demonstrating that chemical and canonical immunity can act cooperatively in a context-dependent manner.

Together, these findings show how developmental state, spatial organization, and extracellular metabolites contribute to multilayered antiviral defense in bacteria.

Reference #1:

Luthe T, Kever L, Hänsch S, et al. *Streptomyces* development is involved in the efficient containment of viral infections. *microLife*. 2023;4:uqad002. doi: 10.1093/femsml/uqad002.

Reference #2:

Kever L, et al. Aminoglycoside antibiotics inhibit phage infection by blocking an early step of the infection cycle. *mBio*. 2022;13:e00783-22. doi: 10.1128/mbio.00783-22.

Reference #3:

3) Ernst L, Gätgens C, Rackow B, et al. DNA-intercalating antiphage molecules trigger abortive infection through mutual destruction and synergize with bacterial immunity. *Proceedings of the National Academy of Sciences of the United States of America*. 2026;123(23):e2602073123. doi: 10.1073/pnas.2602073123

Novel anti-viral defence systems / 110**Using LC-MS/MS for identification and quantification of host contaminant protein during phage production****Author:** Stefan Miller¹**Co-authors:** Michael Forchheim¹; Tobias Welz¹; Michelle Batsch¹; Florian Christoph Sigloch¹¹ *PolyQuant GmbH*

Typical recombinant protein production often relies on standard ELISA assays for controlling product quality and safety. Phages however, require specific production hosts. Regulatory institutions demand phages to be controlled for presence of host-cell derived impurities, e.g. host-cell proteins (HCPs), endo- and exotoxins, as well as prophages.

LC-MS/MS-based HCP testing enables highly sensitive and specific HCP detection and identification independent of the host strain or organism. Data for *E.coli* phage T7 show, that LC-MS/MS data facilitate further purification development and risk assessment for therapeutic phages. In combination with a QconCAT-based internal isotope-labelled reference standard EcoCAT, total HCP levels can be quantified like with ELISA measurement.

Novel anti-viral defence systems / 23**Horizontal Gene Transfer Shapes Bacterial Defense in *Vibrio cholerae*****Author:** Melanie Blokesch¹¹ *Swiss Federal Institute of Technology Lausanne (EPFL)*

Horizontal gene transfer has played a central role in the evolution of *Vibrio cholerae*, transforming an environmental bacterium into the causative agent of cholera through the acquisition of key virulence determinants. Recent work has revealed that the same evolutionary process has also shaped the bacterium's arsenal of defense systems against bacteriophages and plasmids. In this talk, I will show how pandemic *V. cholerae* lineages have acquired diverse antiphage and antiplasmid defense systems through mobile genetic elements, including pathogenicity islands and prophages, and discuss how these systems may have contributed to the evolutionary success of the distinct seventh-pandemic El Tor lineage. I will then present our recent discovery that natural competence enables the horizontal acquisition of chromosomal integron cassettes, allowing *V. cholerae* to rapidly sample and express novel defense systems from lysed bystander cells. Together, these findings establish horizontal gene transfer not only as a driver of virulence evolution but also as a key mechanism underlying the diversification, dissemination, and continual renewal of bacterial immunity.

Reference #1:

Righi L., Stutzmann S., Bader L., Lemopoulos A., Blokesch M. (2026) Competence-mediated DNA uptake diversifies *Vibrio cholerae* sedentary chromosomal integrons. *Science*, 392:194-201.

Reference #2:

Adams D.W., Jaskólska M., Lemopoulos A., Stutzmann S., Righi L., Bader L., Blokesch M. (2025) West African South American pandemic *Vibrio cholerae* encodes multiple distinct phage defence systems. *Nat. Microbiol.*, 10:1352–1365.

Reference #3:

Jaskólska M., Adams D.W., Blokesch M. (2022) Two defence systems eliminate plasmids from seventh-pandemic *Vibrio cholerae*. *Nature*, 604:323-329.

Novel anti-viral defence systems / 17

Sulfonamide antibiotic rescues bacteria from phage-induced collapse**Author:** Juliana Geromiller¹**Co-authors:** Philipp Popp²; Marc Erhardt²; Ana Rita Brochado¹¹ *Tübingen University*² *Humboldt University Berlin*

Phage-antibiotic combinations are a promising strategy to combat antimicrobial-resistant pathogens, but we currently lack mechanistic understanding of underlying bacterial responses. Here, we characterize the interaction between the antifolate antibiotic sulfamethoxazole and phage T4 in *Escherichia coli*.

We combine quantitative with molecular and phage biology approaches, namely checkerboard assays, time-lapse microscopy and one-step growth curves, to characterize phage-sulfamethoxazole interactions in *E. coli*.

Sulfamethoxazole protects bacterial cultures from rapid T4-induced collapse, even at subinhibitory concentrations. Checkerboard assays show similar effects with phage T2, but not phages T5 or T7. Preliminary single-cell time-lapse microscopy shows delayed cell lysis, suggesting slower propagation of infective progeny, which was confirmed by time-resolved phage titer assays. After introducing the sulfamethoxazole resistance gene *sul2* into *E. coli*, the protection of the drug is completely abolished indicating that the on-target antifolate effect is driving the suppression of T4-induced collapse.

Our results identify a previously uncharacterized antagonistic interaction between sulfamethoxazole and T4, showing how this antibiotic can impact phage replication, even at subinhibitory concentrations. Ongoing experiments include a large genetic screen and dual-transcriptomics and quantitative proteomics to identify host factors underlying the molecular mechanism of this interaction.

Reference #1:

Theuretzbacher U et al (2020): The global preclinical antibacterial pipeline. *Nat Rev Microbiol* // 10.1038/s41579-019-0288-0

Reference #2:

Fungo G et al (2023): “Two Is Better Than One”: The Multifactorial Nature of Phage-Antibiotic Combinatorial Treatments Against ESKAPE-Induced Infections. *PHAGE* // 10.1089/phage.2023.0007

Reference #3:

Brochado AR et al (2018): Species-specific activity of antibacterial drug combinations. *Nature* // 10.1038/s41586-018-0278-9

Novel anti-viral defence systems / 68

Zorya-mediated defence results in distinct cellular outcomes following phage infection**Author:** Adhvaidhi Kuntran¹**Co-authors:** Lasse Hallenga¹; Philipp Popp¹; Marc Erhardt²¹ 1. *Institute of Biology/Molecular Microbiology, Humboldt-Universität zu Berlin, Berlin, Germany*² 1. *Institute of Biology/Molecular Microbiology, Humboldt-Universität zu Berlin, Berlin, Germany* 2. *Max Planck Unit for the Science of Pathogens, Berlin, Germany*

The Zorya defence system is a multi-protein antiviral complex that detects phage infection at the bacterial membrane and restricts viral replication. Although the three Zorya subtypes share homologous membrane-spanning components, they encode distinct cytoplasmic effector proteins, suggesting mechanistic divergence between subtypes.

Here, we investigate the anti-phage defence profile of Zorya using plaque assay, live-cell time-lapse microscopy and fluorescence imaging coupled with automated image analysis. We observe distinct infection phenotypes following phage infection, with the outcome of Zorya-mediated defence varying according to the infecting phage.

Using reporter strains, that enable to track the fate of the injected phage DNA and host DNA, we investigate the roles of Zorya subtype-specific effector proteins in mediating anti-phage defence.

Our findings reveal both shared and distinct features of Zorya-mediated immunity and indicate that the conserved membrane-associated machinery is coupled to subtype-specific effector activities that determine the outcome of antiviral defence.

Novel anti-viral defence systems / 121**Hidden Functional Diversity among Type V-A CRISPR Effectors****Author:** Oleg Dmytrenko¹¹ *Forschungszentrum Jülich*

Bacterial antiphage defenses, including CRISPR-Cas, are commonly classified according to their evolutionary relationships, presence of known functional domains, or genomic neighborhood. Yet, as viral predation drives rapid diversification, incomplete sequence sampling and fragmented knowledge of bacterial immunity often undermine our ability to reliably predict defense functions. For example, we have recently uncovered functional variation hidden among well-known type V-A CRISPR-Cas effectors. Unlike DNA-targeting Cas12a, these additional Cas12a2 and Cas12a3 clades target RNA. Further yet, their downstream nuclease activities and physiological outcomes differ strikingly. Activated Cas12a2 indiscriminately degrades ssRNA, ssDNA, and dsDNA. In bacteria, collateral DNA damage triggers an SOS response and growth arrest, preventing phage dissemination. We harnessed this property to selectively eliminate eukaryotic cells based on viral transcripts or oncogenic mutations. Cas12a3 instead recruits tRNAs through a novel loading domain and cleaves their conserved 3'-CCA tails. The resulting tRNA truncation arrests growth and limits phage replication. By designing synthetic reporters that mimic tRNAs, we expanded the multiplexing capacity of existing CRISPR-based diagnostic platforms for viral RNA detection. Together, these effectors show how evolutionary remodeling of a shared effector scaffold can redirect immunity from broad nucleic acid destruction to selective inactivation of the translation machinery, underscoring both the limits of predicting molecular function and persistent gaps in our knowledge of bacterial defenses. Looking ahead, we aim to systematically explore bacterial immunity across the evolutionary landscape to determine how variation in activation, regulation, molecular mechanism, and physiological outcome generates distinct antiviral strategies and new opportunities for biotechnology.

Novel anti-viral defence systems / 120**From genetic conflicts to molecular innovation****Author:** Sanne Klompe¹¹ *ETH Zurich*

Genetic conflict arises when distinct genetic elements within an organism pursue divergent evolutionary interests. These conflicting forces drive continuous cycles of adaptation and counter-adaptation that reshape microbial genomes and generate new molecular functions. In prokaryotes, this conflict is visible in the dynamic interplay between mobile elements such as transposons and bacteriophages, and the host genome. Bacteria deploy diverse mechanisms to combat foreign genetic elements, ranging from nucleic acid targeting and restriction to abortive infection and dormancy. To overcome these host barriers, mobile elements continuously evolve strategies to evade, subvert, and even repurpose host defense machinery for their own propagation. Far from being simple genetic parasites, mobile elements can shuttle functional cargo genes, including anti-phage defense systems, thereby facilitating their horizontal dissemination among bacterial lineages. The co-evolutionary interplay between phages, transposons, and host defense systems thus acts as a major engine for biological innovation, yielding intricate multi-protein complexes and versatile enzymatic tools. Understanding the mechanisms that emerge from these conflicts offers new perspectives on genome evolution while informing new strategies for biotechnology and synthetic biology.

Novel anti-viral defence systems / 118**Diverse responses of an archaeal CBASS****Author:** Uri Gophna¹**Co-authors:** Deepak K. Choudhary¹; Himani Singla¹; Hadar Shukrun¹; Dana Vassover¹; Leah Reshef¹¹ Tel Aviv University

Many bacterial cyclic-oligonucleotide-based antiphage signalling systems (CBASS) defend against viral infections by depleting cellular NAD⁺ levels, eventually leading to dormancy or death. This abortive infection strategy is beneficial in stopping fast lytic infections, as cells die before spreading the virus to neighbouring cells. However, in chronic viral infections, which often occur in archaea, abortive infection could be detrimental, as the cost of immunity may outweigh that of infection. We study an archaeal CBASS that is expressed in the model species *Haloferax volcanii* DS2 and *Haloferax gibbonsii* LR2-5. We show that the system provides protection to *H. gibbonsii* against a lytic tailed haloarchaeal virus HFTV1 by depleting NAD⁺, and causing premature lysis. H-CBASS2 is also activated to a lower degree during infection with the chronic, non-lytic, virus HFPV-1, and promotes virus clearance after several passages without killing host cells. Cells that cleared the HFPV-1 infection became substantially more resistant to subsequent infections, due to accumulated mutations in envelope-associated proteins. Cell death by extreme NAD⁺ depletion only occurred after a very long incubation with HFPV-1. These findings suggest that the magnitude of H-CBASS2 response is somehow tuned to the infection type and can benefit the host during non-lytic infections, potentially explaining why such systems are relatively common in archaea.

Novel anti-viral defence systems / 103**The ancient evolution of gasdermin-mediated immunity****Author:** Tanita Wein¹¹ *Weizmann Institute of Science*

Bacteria encode a remarkable diversity of immune systems, including systems that trigger cell death to halt phage infection. Gasdermins, which are best known as executors of inflammatory cell death in animals, are emerging as widespread effectors of bacterial anti-phage defense. Here, we uncover novel bacterial gasdermins and explore how these potent pore-forming proteins have diversified across evolution. By combining comparative genomics with functional and structural analyses, we reveal diverse mechanisms that keep bacterial gasdermins repressed and enable their rapid activation during immune defense. These systems expose both unexpected regulatory strategies and striking evolutionary connections to gasdermin cell death pathways across the tree of life. Our findings reveal bacteria as a rich reservoir of gasdermin diversity and provide a window into how regulated pore-forming cell death has evolved over billions of years.

Novel anti-viral defence systems / 74

Dozens of phage proteins block retron defense by targeting its toxicity on the ribosome

Authors: Federico Corona¹; Jacob Bobonis¹; Alessio L. J. Yang¹; Tara Bartolec¹; Sophie Moggridge¹; Joseph Dobbs¹; Wenhao Xu¹; Panagiotis Spatharas¹; Carlos G. P. Voogdt¹; Khalimat Murtazaliev²; Dimitris Papagiannidis¹; Isabelle Becher¹; Michael Knopp¹; Elisabetta Cacace¹; Vallo Varik¹; Nicolai Karcher¹; Julia Mahamid¹; Robert Finn²; Mikhail M. Savitski¹; Athanasios Typas¹

¹ *European Molecular Biology Laboratory (EMBL), Molecular Systems Biology Unit, Heidelberg, Germany*

² *EMBL-EBI, Hinxton, United Kingdom*

Retron-Sen2 from *S. Typhimurium* encodes a tripartite toxin-antitoxin (TA) system that defends bacteria against phage via abortive infection. Its toxic effector, RcaT, is a predicted ribosyltransferase neutralized by the msDNA-reverse transcriptase complex, with msDNA acting as a sensor for infection. RcaT retrons represent the most prevalent retron family, and other families likely share similar architecture and activation principles. Yet despite growing understanding of retron families generally, RcaT's mode of action, and more broadly how phages circumvent retrons, remains unclear.

To explore the diversity of phage-encoded anti-defense mechanisms, we used metagenomic phage DNA libraries and a forward genetics screen to identify proteins blocking RcaT toxicity. We found over 50 distinct proteins, most lacking obvious homology to known proteins. Many inhibit divergent RcaT homologues and frequently occur as blocks within phage genomes.

To understand how these blockers function, we first resolved RcaT's mechanism: it arrests translation termination by ADP-ribosylating conserved histidines on release factors (RF) 1 and 2, inactivating them and causing ribosome stalling and collisions at the termination codon. Some phage blockers mimic host proteins involved in this toxicity (such as RFs and ribosomal protein L7, RcaT's binding target) while others reverse ADP-ribosylation. However, most blockers act through unidentified mechanisms, and many bind the ribosome directly. This suggests phages preferentially target the conserved component of the toxicity mechanism (the ribosome) rather than the effector itself: a strategy that could let small phage genomes counter hundreds of bacterial defense systems, enabling broadly acting anti-defenses and convergent evolution in the phage-bacteria arms race.

Novel anti-viral defence systems / 119**Bacteria-inspired exploration of immune functions in eukaryotes****Author:** Rotem Sorek¹¹ *Weizmann Institute of Science*

A flurry of recent discoveries showed that defense islands in the microbial pan-genome contains hundreds of defense systems, the functions of many of which are still unexplored. The talk will present progress in understanding the mechanisms of action of new defense systems, will highlight cases in which bacterial defense from phage gave rise to key components in the eukaryotic innate immune system, and will demonstrate how phages evolved to overcome bacterial immunity.

Novel anti-viral defence systems / 57**A novel signaling system originating from a core metabolic enzyme****Authors:** Anna Kanevskaya¹; Francois Rousset²¹ *INSERM, CIRI*² *CIRI, INSERM, UCBL1, CNRS, ENS de Lyon*

Bacteria employ diverse antiphage systems that produce second messengers in response to infection, activating effector proteins to block phage propagation. In several defense systems, enzymes originally discovered in cellular processes have been repurposed to synthesize signaling molecules. Here, we discovered and characterized a two-gene system where the signaling enzyme is derived from ubiquitous housekeeping proteins involved in riboflavin biosynthesis. This enzyme constitutively produces a precursor of the second messenger which is likely matured into the final product by phage proteins during infection. Our results suggest that this molecule is then recognized by a transmembrane effector, thereby promoting oligomerization and subsequent pore formation, leading to cell death to prevent phage replication. Together, these findings reveal another unexpected example of the evolutionary transition of a core metabolic enzyme into a component of bacterial immunity.

Novel anti-viral defence systems / 85

Hidden Amun defensive proteins within defense islands broaden protectivity against phage families**Author:** Marcel Sprenger¹**Co-authors:** Ferenc Kanitz¹; Tamara Matthyssen²; Anna-Sophie Giese²; Sebastian Krautwurst¹; Markus Lake-mayer²; Kai Papenfort¹¹ *Friedrich Schiller University, Institute of Microbiology, 07745 Jena, Germany*² *Friedrich Schiller University, Institute for Organic Chemistry and Macromolecular Chemistry (IOMC), 07743 Jena, Germany*

The ongoing arms race between phages and bacteria is a biological struggle for survival where bacteria develop defense mechanisms to resist phage infections. *Vibrio cholerae* interacts with numerous different lytic and lysogenic phages and strains from the current cholera pandemic acquired over 30 different antiviral defense systems. This project aims to investigate the presence of new single-component defense factors in well-known defense islands.

Using differential RNA-seq and ribosome profiling, we identified a previously unrecognized short open reading frame (sORF) within the 5' UTR of the CBASS defense system in *V. cholerae*, encoding an 82-amino-acid protein. We therefore named the sORF for the Egyptian deity Amun. Both systems, Amun and CBASS, are transcribed from the same promoter and confer resistance to different phage families, increasing the protective potential of this genomic region. Amun homologs are widespread across more than 2,000 bacterial genomes and are often linked to operons with known or predicted antiviral roles, making Amun a valuable tool for identifying previously uncharacterized antiviral defense systems. While phage genome replication and viral transcription are only moderately impaired by Amun, host cell lysis and the release of progeny phages are markedly suppressed. Transcriptomic data indicated an altered cAMP response upon viral entry in cells expressing Amun. By manipulating the host cAMP production, we showed that Amun-mediated protection required cAMP. Taken together, our data suggest that a small, single-component antiviral effector expands the protective capacity of a defense island.

Novel anti-viral defence systems / 89

A compact Druantia defense clears phage infections via single-stranded DNA recognition and directional duplex unwinding**Authors:** Chase Beisel¹; Markus Wahl²; Stephanie Himpich²; Thomas Gaudin³¹ *Botnar Institue*² *Freie Universität Berlin*³ *HIRI*

Bacteria encode diverse anti-phage defense systems triggered by invader-specific molecular cues. Here, we report that the compact type III-A Druantia system recognizes exposed single-stranded DNA to drive phage clearance. Using representative systems from *Escherichia coli*, we show that the two encoded proteins, DruE and DruH, together clear restriction-sensitive or recombination-prone phages without affecting cell growth or viability. DruE dimerizes and engages DNA at exposed single-stranded regions to unwind DNA with 3'-to-5' directionality, resorting to unique molecular lock, wedge, and clamp elements that aid strand separation and processive translocation. DruH is a monomer in isolation and indirectly interacts with DruE and other host proteins under uninfected conditions, with an infection resulting in the dissociation of the complex. Taken together, our results reveal that exposed single-stranded DNA can trigger bacterial immunity through the directional helicase activity of type III-A Druantia.

Novel anti-viral defence systems / 16

Processing of forked DNA activates a helicase-nuclease immune system**Author:** Luuk Loeff^{None}**Co-authors:** Martin Jinek¹; Christelle Chanez¹; Pilar Bobadilla Ugarte²¹ *University of Zurich*² *Leiden University Medical Center*

Innate immune systems detect molecular signatures of infection to initiate antiviral defence, yet the identity of pathogen-associated signals that distinguish phage from host nucleic acids remains incompletely understood. While recent work has shown that nucleic acid structures can act as triggers for bacterial defense systems, how these structural signals are coupled with immune activation remain unclear. Here we show that forked DNA structures activate a helicase-nuclease immune complex in type III Druantia through a processing-dependent mechanism. Using cryo-electron microscopy and biochemical reconstitution, we find that the exonuclease DruH processes 3' DNA termini to generate 5' overhangs that recruit and activate the helicase-nuclease DruE at duplex-single-stranded DNA junctions. Structural analysis of the DruE-DruH complex reveals how substrate-dependent assembly remodels an autoinhibited helicase dimer into an active DNA degradation complex. Functional assays demonstrate that coordinated nuclease and helicase activities enable efficient degradation of forked DNA substrates and mediate phage defense without detectable host toxicity. Together, our findings define a mechanism in which enzymatic processing of replication-associated DNA structures licenses immune activation, providing a framework for how nucleic acid architecture is coupled to effector activation in bacterial immunity.

Index of Authors

- Abdula, Fatih, 87
 Ahmad, Shazeb, 37, 97
 Alseth, Ellinor, 16
 Alshech, , 9
 Altay, Baturay, 5, 35
 Aminian, Shirin, 41
 Ammann, Julia, 88
 Antelmann, Haike, 17
 Anton Kühn, Johannes, 78
 Arsova, Borjana, 33
 Auraß, Philipp, 58
 Avissar, , 9
 Azemi, Najiba, 8
 Azulay, Gil, 13

 Badia Roigé, Biel, 63, 81
 Baltenneck, Julie, 57
 Barbirz, Stefanie, 24, 41, 45, 46
 Barr, Jeremy J., 85
 Bartolec, Tara, 72, 108
 Bartolec, Tara Katarina, 3
 Baruth, Sophie, 53, 56
 Batsch, Michelle, 74, 100
 Becher, Isabelle, 72, 108
 Bedekar, Paritosh, 66
 Beisel, Chase, 69, 112
 Beisel, Chase L., 27
 Bendorys, Radvilas, 78
 Bernauer, Sebastian A., 87
 Besirovic, Amra, 35
 Beuke, Dennis, 38
 Biderman, Einat, 13
 Bikard, David, 90
 Birkholz, Nils, 27
 Blokesch, Melanie, 101
 Blum, Patricia, 47
 Bobadilla Ugarte, Pilar, 113
 Bobonis, Jacob, 108
 Bondy-Denomy, Joseph, 79
 Boon, Maarten, 35
 Borelli, Costanza, 17
 Borges-Cardoso, Virginie, 57
 Borgwardt, Karsten, 38
 Borovok, Ilya, 13
 Bravo, Jack, 18, 22, 59
 Brochado, Ana Rita, 102
 Broeker, Nina Kristin, 45
 Brouns, Stan J. J., 81
 Brown, Chris M., 27
 Brown, Sam, 16
 Brunsch, Thea, 64
 Bröker, Nina, 41

 Bröker, Nina Kristin, 46
 Buhlmann, Leandro, 11, 60
 Buianova, Elizaveta, 5
 Bäuerle, Nina, 44

 Cacace, Elisabetta, 108
 Camara-Wilpert, Sarah, 78
 Campobasso, Claudia, 52
 Cecchino, Laura, 37
 Chanez, Christelle, 113
 Cheng, Junchao, 35
 Chiantia, Salvatore, 41, 45
 Choudhary, Deepak K., 106
 Cibulskis, Augustas, 29
 Cohen, Yonatan, 13
 Cole, Gregory, 96
 Commichau, Fabian M., 8
 Corona, Federico, 108
 Cummings, Gideon, 25, 62
 Cusack, Siobhan A., 28
 Custodio, Rafael, 16

 Dakinevičienė, Donata, 59
 Das, Avijit, 13
 de Bruijn, Thomas, 23
 Deng, Li, 88
 Derzhaev, Anton, 54, 55
 Dmytrenko, Oleg, 20, 104
 Dobbs, Joseph, 108
 Doekes, Hilje, 23
 Dorozh, Olha, 54
 Drobysheva, Arina, 97
 Du, Jiemin, 87
 Dudas, Gytis, 78
 Dunne, Matthew, 87
 Dupuis, Amandine, 56
 Dustour, Abigail, 53
 Dutilh, Bas, 5
 Dutilh, Bas E., 64, 73
 Dziomba, Milena, 43
 Döppner, Meike, 24

 Ebbers, Antonia, 93
 Erdrich, Sebastian H., 33
 Erhardt, Marc, 65, 77, 83, 102, 103
 Ernst, Larissa, 30, 67, 99
 Etienne, Lucie, 57
 Ežerskytė, Ema, 98

 Fairburn, Sam, 96
 Fedorova, Iana, 79
 Feltin, Chloé, 50

- Feng, Yue, 79
 Fernandez Contreras, Miguel, 93
 Fernandez, Miguel, 80
 Ferring-Appel, Dunja, 70
 Feussner, Maximilian, 27
 Fineran, Peter C., 27
 Finn, Robert, 108
 Fischer, Dorian, 34, 36
 Flieger, Antje, 58
 Fofanov, Mikhail, 5
 Forchheim, Michael, 74, 100
 Frankenberg-Dinkel, Nicole, 44, 80, 93
 Froschauer, Kathrin, 18, 22
 Frunzke, Julia, 30, 33, 47, 63, 67, 81, 99
 Furter, Lisa, 87

 Gaissmaier, Marla, 89
 Garrido García-Dorado, Lydia, 21
 Gaudin, Thomas, 69, 112
 Gehlert, Finn, 71
 Gerlach, David, 68
 Geromiller, Juliana, 102
 Gerovac, Milan, 5, 10, 11, 40, 43
 Getz, Landon, 96
 Ghosal, Debnath, 91
 Giehr, Pascal, 28
 Giese, Anna-Sophie, 111
 Gohlus, Daniel, 36, 72
 Goldade, Monica, 53, 56
 Gophna, Uri, 106
 Grass, Lena, 17
 Gutman, , 9

 Hackl, Simon, 19
 Hackl, Thomas, 91
 Hadary, Romi, 14
 Hallenga, Lasse, 65, 83, 103
 Hallmark, Thomson, 84
 Hartout, Philip Hartout, 38
 Hashuel, Rachel, 9
 Hatfull, Graham F., 2
 Heilbronner, Simon, 68
 Hendriks, Ivo, 77
 Hentze, Matthias W., 70
 Herskovits, Anat, 13
 Hilal, Tarek, 17
 Himpich, Stephanie, 17, 112
 Hodgkinson-Bean, James, 77
 Hoff, Tim, 71
 Hu, Chunyi, 55
 Hu, Haidai, 75
 Höfer, Katharina, 4, 21, 52
 Hör, Jens, 31, 32, 37, 97

 Iglar, Claudia, 56
 ILCHENKO, KARINA, 51
 Ilchenko, Karina, 50
 Iliopoulos, Alexandros, 17
 Isaev, Artem, 54, 55

 Jasnauskaite, Monika, 29
 Jasnauskaitė, Monika, 78
 Jelen, Caroline, 70
 Jespers, Jinte, 59
 Jinek, Martin, 113
 Johnson, Matthew D., 91

 Jung, Kirsten, 28
 Juozapaitis, Jonas, 29, 98

 Kabuu, Moses, 67, 99
 Kaiser, Stephanie, 28
 Kalogeropoulos, Konstantinos, 81
 Kanevskaya, Anna, 110
 Kanitz, Ferenc, 111
 Karcher, Nicolai, 108
 Karpa, Jan, 49, 53
 Kazlauskaite, Kristina, 29
 Kemmler, Alina, 72
 Kessler, Thomas M., 87
 Keszei, Alexander, 96
 Khan Mirzaei, Mohammadali, 88
 Klauck, Eberhard, 17
 Klein De Sousa, Victor, 78
 Klompe, Sanne, 105
 Knopp, Michael, 108
 Koenigsfeld, Dan, 13
 Kohm, Katharina, 8
 Koopal, Balwina, 18, 22, 59
 Krammer, Karina, 42
 Krautwurst, Sebastian, 111
 Krusche, Janes, 82
 Kulsuptrakul, Jessie, 57
 Kuntran, Adhvaidhi, 83, 103
 Kupczok, Anne, 23, 42
 Kurosawa, Norio, 91
 Kuske, Rachel, 16

 Lakemayer, Markus, 111
 Lang, Christina, 58
 Lavigne, Rob, 6, 35
 Lavrenko, Tetiana, 38
 LE MOUELLIC, Wendy, 57
 Leitner, Lorenz, 87
 Leptihn, Sebastian, 15, 39
 Li, Huihui, 17
 Li, Huijuan, 78
 Li, Jinqun, 27
 Liegute, Toma, 48
 Lindell, Debbie, 93
 Lindhoud, Simon, 59, 84
 Lisitzin, Polina, 13
 Litvak, Yael, 9
 Liu, Vivian, 96
 Livny, Tal, 30
 Lobnow, Finn, 25, 62
 Loeff, Luuk, 113
 Loessner, Martin J., 87
 Loh, Belinda, 15, 39
 Loi, Vu Van, 17
 Loll, Bernhard, 17
 Lood, Cedric, 5
 Ludwig, Christina, 26
 Lukianov, Dmitrii, 54
 Luthe, Tom, 99
 López-Moreno, Ana, 26

 Maffei, Enea, 87
 Mages, Laura, 93
 Mahamid, Julia, 108
 Malinauskaite, Lina, 29, 48
 Marjaya, Markosovo, 58
 Martignoni, Maria, 16

- Martin, Hanna, 12
 Mast, Yvonne, 24
 Matter, Cassandra I., 87
 Matthyssen, Tamara, 111
 Maxwell, Karen, 96
 Maxwell, Karen L., 81
 Mayo-Muñoz, David, 27, 78
 McCallin, Shawna, 87
 Mechrouki, Samy, 63, 99
 Meijer, Jeroen, 73
 Melki, , 9
 Menon, Bhavna, 38
 Migur, Angela, 27
 Miksys, Algirdas, 29, 48
 Miller, Stefan, 100
 Moggridge, Sophie, 108
 Molodtsov, Vadim, 54, 55
 Monks, Hannah-Maria, 46
 Moradian, Shafagh, 60
 Moraes, Trevor, 96
 Morales Araujo, Ariel, 53
 Morozova, Natalia, 55
 Mortensen, Sofia, 61
 Moura-Alves, Pedro, 16
 Murtazaliev, Khalimat, 108
 Mutter, Janik, 87
 Müller, Lena Sarah, 3
- Nakagawa, Satoshi, 91
 Niaux, Theophile, 59
 Nieselt, Kay, 19
 Norris, Michael, 96
 Nouiou, Imen, 24
 Novikova, Evgenia, 54
 Nowicki, Antonia, 45
 Nunoura, Takuro, 91
 Nürnberg, Dennis, 93
- Ohkuma, Moriya, 91
 Ombeline, Ombeline, 81
 Orel, Vojtech, 18, 22
 Osterman, Ilya, 94
- Pais, Rayhaan, 39
 Papagiannidis, Dimitris, 108
 Pape, Tillman H., 77
 Papenfort, Kai, 111
 Parab, Lavisha, 53, 56
 Pausch, Patrick, 29, 78
 Payne, Leighton, 78
 Penzkofer, Anna, 49
 Peschel, Andreas, 82
 Peter, Stephanie, 24
 PFEIFER, EUGEN, 51
 Pfeifer, Eugen, 50, 81
 Pierik, Antonio, 93
 Pinilla-Redondo, Rafael, 27, 78
 Popp, Philipp, 65, 83, 102, 103
 Popp, Philipp F., 77
 Potekhina, Kristina, 55
 Pozhydaieva, Nadiia, 81
 Puteikienė, Rugilė, 98
 Putzeys, Leena, 35
 Pérez Patallo, Eugenio, 93
- Quax, Tessa, 12
- Quax, Tessa E.F., 91
- Rabinovich, Lev, 13
 Rachimi, , 9
 Rackow, Bente, 99
 Ragozius, Viktoras, 48
 Rappsilber, Juri, 8
 Rech, Valentin, 40
 Recker, Mario, 66
 Refardt, Dominik, 53
 Reichardt, Sarah, 97
 Reil, Laura, 42
 Reshef, Leah, 106
 Rettel, Mandy, 70
 Reyhani, Milad, 91
 Riber, Leise, 78
 Riesenberger, Stephan, 27
 Riquelme-Barrios, Sebastián, 28
 Rivera-Montero, Luis, 28
 ROCHA, EDUARDO, 51
 Rodríguez-Murillo, Neysa, 68
 Rodríguez Mestre, Mario, 78
 Rodríguez-Sánchez, Gisela T., 73
 Roitman, , 9
 Rolland, Clara, 24
 Rossier, Ombeline, 63
 Rousset, Francois, 110
 Rousset, François, 57
 Roy, Aatreya, 31
 Ruksenaite, Audrone, 29
 Rukšėnaitė, Audronė, 98
 Rutbeek, Nicole, 75, 77
 Rückwald, Moritz, 24
- Sahani, Nandika Sanjog, 5
 Saikia, Bibakhya, 28
 Sakai, Hiroyuki D., 91
 Saliba, Antoine-Emmanuel, 37
 Sanchez Martinez, Rodrigo, 26
 Sanders, Torben, 88
 Sanjog Sahani, Nandika, 35
 Sanz Andres, Ane, 59
 Sarpilo, Migle, 76
 Sasnauskas, Giedrius, 98
 Savitski, Mikhail, 3, 72
 Savitski, Mikhail M., 108
 Scheurenbrand, Mona, 19
 Schmid, Nicole, 36, 72
 Schmidt, Lisa, 48
 Schmitz, Ruth A., 71
 Schneider, Tjark, 82
 Schubert, Torsten, 73
 Schwarzer, Sabine, 12, 91
 Sekularac, Nicola, 93
 Selpiev, Zhanybek, 15
 Severinov, Konstantin, 55
 Sharma, Swati, 33
 Shimamura, Shigeru, 91
 Shimizu, Michiru, 91
 Shukrun, Hadar, 106
 Siborova, Marta, 76, 78
 Sigal, Nadejda, 13
 Sigloch, Florian, 74
 Sigloch, Florian Christoph, 100
 Siksny, Virginijus, 48
 Singla, Himani, 106

- Sinkunas, Tomas, 48
 Skorupskaite, Aiste, 29
 Skuba, Anastasiia, 22
 Sokolova, Maria, 5, 35, 38
 Sondermann, Holger, 61
 Songailiene, Inga, 48
 Songailienė, Inga, 98
 Sorek, Rotem, 94, 109
 Sow, Fatoumata, 40
 Spatharas, Panagiotis, 108
 Sprenger, Marcel, 111
 Stadnyuk, Olga, 13
 Stecher, Bärbel, 26
 Steens, Jurre, 84
 Steglich, Claudia, 70
 Steigenberger, Barbara, 5, 38
 Stein, Frank, 70
 Stokar-Avihail, Avigail, 3
 Swarts, Daan, 59
 Swarts, Daan C., 84
 Sà Zanetti, Leandro, 87
 Sørensen, Søren J., 78

 Tahan, Ran, 93
 Takai, Ken, 91
 Taktuk, , 9
 Tamulaitienė, Giedrė, 98
 Tang, Huanzhang, 5
 Taylor, Marcus, 25, 62
 Taylor, Nicholas M. I., 75, 76, 78
 Taylor, Nicholas M.I., 77
 Thormann, Kai, 34, 36, 72, 86
 Tiamani, Kawtar, 88
 Tomkuvienė, Miglė, 29
 Truncaitė, Lidija, 98
 Tsukamoto, Yuya, 91
 Typas, Athanasios, 3, 72, 108
 Tzarum, , 9
 Tzohar Rabinovich, Keren, 13

 van der Oost, John, 84
 van Houte, Stineke, 66, 95
 Vareika, Domantas, 98

 Varik, Vallo, 108
 Vassover, Dana, 106
 Velasco Gomariz, Manuel, 32, 97
 Vogel, Jörg, 5, 7, 11, 40, 60
 Voogdt, Carlos G. P., 108
 Vysokikh, Aleksandra, 35

 Wahl, Markus, 112
 Wahl, Markus C., 17
 Wakelin, Holly, 27
 Warneke, Robert, 8
 Wasserzug Pash, , 9
 Weidenbach, Katrin, 71
 Wein, Tanita, 25, 30, 62, 107
 Weinberg, Zasha, 27
 Wellins, Tamara, 9
 Welz, Tobias, 74, 100
 Wendling, Carolin, 42, 49, 53, 56
 Wensing, Anette, 45
 Westphal, Adrie, 84
 Westra, Edze, 16, 66
 Williams-Jones, Daniel, 18, 22
 Wirtz, Astrid, 67
 Wittmann, Johannes, 24
 Wolfram-Schauerte, Maik, 19
 Wyss, Luis, 38

 Xu, Wenhao, 108

 Yang, Alessio L. J., 108
 Yechezkel, , 9
 Yilmaz Demirel, Nurseda, 21
 Yue, Yourun, 79

 Zarembo, Mindaugas, 59
 Zarivach, Raz, 13
 Zehner, Susanne, 93
 Zhao, Ruilaing, 78
 Zhu, Wenlong, 16
 Zrein, Adèle, 24

 Šikšnys, Virginijus, 98
 Šilanskas, Arūnas, 98