National Institute of Virology and Bacteriology

NIVB MEETING 2024

BOOK of ABSTRACTS

30th SEPTEMBER – 2nd OCTOBER 2024

Kutná Hora, Czech Republic

The third annual meeting of the National Institute of Virology and Bacteriology (NIVB)

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Dear Colleagues in Virology and Bacteriology,

It is our pleasure to welcome you to the NIVB Meeting 2024, the third annual meeting of the National Institute of Virology and Bacteriology (NIVB), held in the historic town of Kutná Hora from September 30th to October 2nd, 2024. This meeting serves as an invaluable opportunity to reconnect with participating teams, engage in stimulating scientific discussions, foster collaborations, and showcase the advancements made over the past year to the International Scientific Advisory Board.

Thanks to the support from the Czech Economic Recovery Plan, our collective efforts have led to significant achievements, including the publication of numerous high-impact papers and the organization of several successful networking workshops. The progress made over the past year is a testament to the growing synergy among the 30 research teams from 8 Czech institutions that are part of the NIVB. This meeting in Kutná Hora is a celebration of these achievements and a platform for reflecting on the strides we've made.

The primary mission of the NIVB is to enhance collaboration among our diverse research teams, bridging the gaps between different institutions, disciplines, and regions. As a communication hub, the NIVB facilitates the vital exchanges necessary for advancing our shared goals.

We thank all the participants who contributed to the meeting with 30 oral presentations and 64 posters. The first NIVB meeting in 2022 successfully initiated a tradition of annual gatherings dedicated to virology and bacteriology, and NIVB 2024 proudly continues this tradition, striving to keep the scientific community informed about the latest developments, trends, and challenges in our fields.

We are looking forward to seeing you at the NIVB Meeting 2024.

Zdeněk Hostomský, Robert Vácha, Iva Pichová, and Šárka Šímová

INSTITUTE OF ORGANIC CHEMISTRY AND BIOCHEMISTRY OF THE CAS

L-01
NUCLEOSIDE BORONIC ACIDS

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Advances in the field of boron chemistry have allowed this element to be used in a range of new materials, including applications for medicine. Boron-based drugs have now been developed as therapeutic agents for cancer, and for viral, bacterial, and fungal infections. Bortezomib (Velcade®) was the first boronic acid-containing drug approved in 2003 as a proteasome inhibitor for the treatment of multiple myeloma. Several other boron-based compounds are in clinical trials, illustrating the promise of this class of chemical in drug discovery[1,2]. The boronic acid group is capable of forming bonds that are substantially stronger than a typical hydrogen bond (~75 *vs.* ~20–30 kJ/mol)[3]. The boronate group has previously been shown to bind in a mode where it can associate with multiple amino acids all at the one time (*e.g.* penicillin-binding protein)[4].

Bacteria resistance can often be associated with the overexpression of different classes of β-lactamases by Gram negative bacteria, and the design of novel potential inhibitors of this enzyme is a very useful strategy. The capacity of boronic acids as inhibitors of β-lactamase is related with their ability to connect with the serine residue of this enzyme. Studies about selective and potent boronic acid compounds are being made to discover novel compounds that can be administrated in combination with a β-lactam antibiotic agent. A very recent drug, vaborbactam, was approved by FDA in 2017 and by EMA in 2018, acting as a β-lactamase inhibitor[5].

Here we present design, synthesis and evaluation of nucleoside boronic acid analogues, and first data on synergy with β-lactam antibiotics on selected resistant bacterial strains. For the preparation of pyrrolidine nucleoside boronic acid **1**, the key step was the reaction of N-acryloyl group of the properly protected starting compound with bis-(pinacolato)diboron[6], whereas in the case of ribonucleoside boronic acid analogue **2**, the key reaction was the NaH-mediated alkylation of 5′-hydroxyl of protected ribonucleoside with pinacol iodo-methaneboronate. Preliminary data from the assays suggest possible activity as carbapenemase-3 inhibitors, confirming the importance of this line of our research and giving it high priority, extending the collaboration to obtain crystallographic data and eventually, *in silico* modeling.

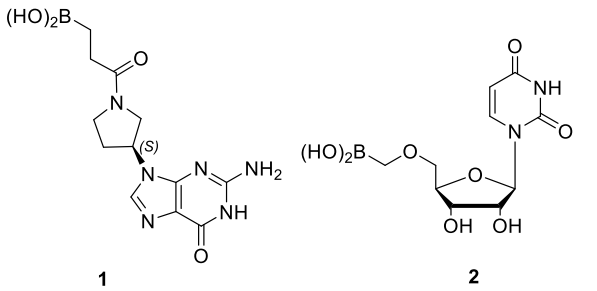

Fig. 1. **Structures of pyrrolidine and ribonucleoside boronic acids 1 and 2.**

Acknowledgement
The work was supported by the project National Institute of Virology and Bacteriology (Programme EXCELES, ID Project No. LX22NPO5103) – Funded by the European Union – NextGenerationEU.

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L-02
HOST-PATHOGEN INTERACTION DURING HEPATITIS B VIRUS INFECTION

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The hepatitis B virus (HBV) is a human pathogen that causes acute and chronic liver disease. Although vaccination can prevent new HBV infections, there is still a need for improved therapies for chronically infected patients who are at increased risk of liver cancer. To elucidate the mechanisms of viral replication and pathogenesis, it is crucial to understand the interaction between HBV and the host cell.

In this study, we used label-free differential proteomics to investigate HBV-induced changes in human hepatocytes *in vitro* and in their corresponding culture fluids. Uzing data-independent acquisition mass spectrometry, we quantified over 5,900 proteins in both naïve and HBV-infected cells, identifying differentially abundant proteins and affected cellular pathways. To distinguish specific responses to productive HBV infection from nonspecific effects of the viral inoculum, we applied the selective HBV entry inhibitor bulevirtide (BLV) and showed that most proteomic changes following *in vitro* infection are due to non-viral components of the HBV stock.

We further explored the interplay between host responses and viral replication, focusing on the role of the secretory carrier membrane protein (SCAMP) family in HBV infection. Specifically, HBV infection in HepG2-NTCP cells led to the upregulation of SCAMP3, a key regulator of membrane trafficking and recycling of the epidermal growth factor receptor (EGFR)[1], which also acts as a co-receptor for HBV entry[2]. We hypothesized that SCAMP3 may play a role in HBV entry into host cells.

To test this hypothesis, we silenced SCAMP proteins in HepG2-NTCP cells and primary human hepatocytes (PHH), two commonly used cell culture systems, and subsequently analyzed markers of viral infection. Our results showed that SCAMP3 silencing significantly reduced viral RNA production and viral antigen secretion after HBV infection. However, we observed no effect of SCAMP3 silencing or overexpression in HepG2.2.15 cells – a subclone of HepG2 cells that stably express the hepatitis B virus. This differential effect suggests that SCAMP3 is likely involved in the early stages of viral infection, possibly influencing HBV replication through mechanisms related to cellular entry or the regulation of transcription from viral covalently closed circular DNA (cccDNA). In addition, SCAMP4 silencing also inhibited HBV replication, highlighting the importance of SCAMP proteins in the HBV life cycle.

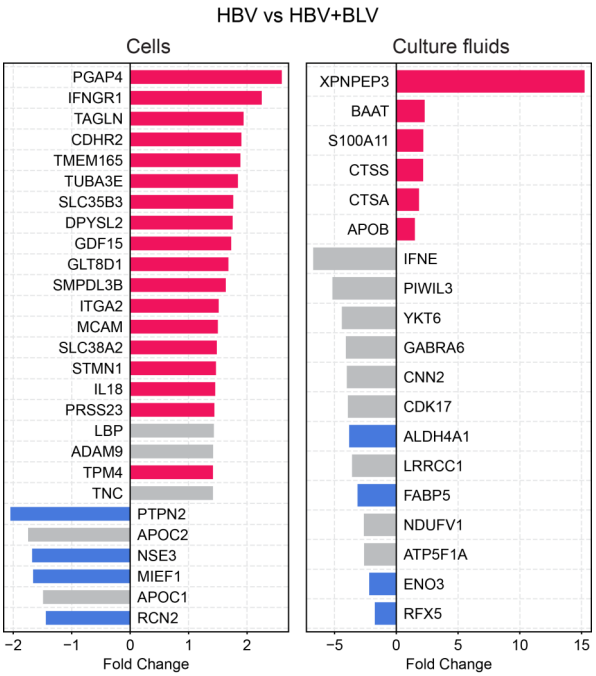

Fig. 1. **Host proteins differentially abundant in the proteome and secretome of PHH in response to productive HBV infection.** Upregulated (pink) and downregulated (blue) proteins resulting from pairwise analysis comparing HBV-infected samples and those infected in the presence of BLV (HBV vs. HBV+BLV) are shown for cell lysates and culture fluids. To differentiate the effect of BLV treatment, proteins differentially abundant in the comparison of untreated cells vs. cells treated with BLV are indicated in gray.

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The work was supported by the project National Institute of Virology and Bacteriology (Programme EXCELES, ID Project No. LX22NPO5103) – Funded by the European Union – NextGenerationEU.

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L-03
ELUCIDATION OF ENZYMATIC MECHANISM AND STRUCTURE OF NTAYA NS5 ENZYME

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Flaviviruses are single-stranded positive-sense RNA (+RNA) viruses that cause several (re)emerging diseases, including Yellow fever, Dengue, and West Nile fevers[1]. The Zika epidemic exemplified their potential danger when a relatively harmless virus known since the 1950s became a deadly pathogen[2]. The NS5 protein (non-structural protein 5) is essential for viral replication, comprising an N-terminal methyltransferase (MTase) domain and a C-terminal RNAdependent RNA polymerase (RdRp) domain. This protein plays a key role in RNA replication and the installation of the 5' RNA cap[3]. In our study, we structurally and biochemically analyzed the Ntaya virus MTase and RdRp domains, comparing their properties with those of other flaviviral NS5 proteins. While the enzymatic centers are highly conserved across *Flaviviridae* family, suggesting the potential for developing broad-spectrum of antiviral drugs targeting all flaviviruses, significant differences were observed in the enzymatic activites of the isolated MTase domains[4].

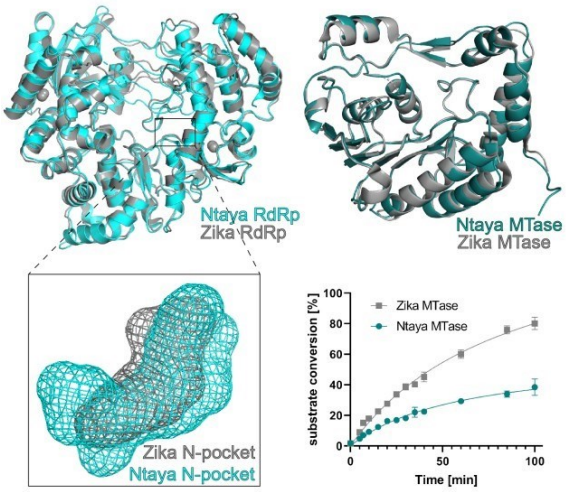

Fig. 1. **Flavivirus polymerases and MTases:** Same same but different

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L-04
INHIBITORS OF SARS-COV-2, DENGUE, AND MPOX METHYLTRANSFERASES

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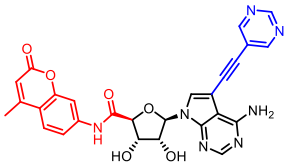
Viral methyltransferases (MTases) are critical enzymes that facilitate the replication and survival of various viruses by modifying their RNA. These modifications are essential for the stability, translation, and evasion of host immune responses. Among these enzymes, the non-structural protein 14 (nsp14) from SARS-CoV-2, the virus responsible for the COVID-19 pandemic, plays a dual role as an exoribonuclease and a MTase. The MTase activity of nsp14 is crucial for the methylation of *N*7 position of viral RNA cap[1,2]. Similarly, the NS5 protein from Dengue virus (DENV) is a multifunctional enzyme with both RNA-dependent RNA polymerase and MTase activities. The MTase function of NS5 is responsible for the methylation of the viral RNA cap both at *N*7 position of the GTP and 2'-*O* of the first viral RNA nucleotide. This ability to methylate the viral cap at both of these positions is quite specific to Flavivirus NS5 proteins, whereas in both human cells and most other viruses it is mediated by two different proteins/enzymes.[2] In the case of Mpox (formerly known as monkeypox), the VP39 protein serves as a viral 2'-*O*-methyltransferase. VP39 is involved in the methylation of viral mRNA, which helps the virus to efficiently replicate and evade the host's immune defenses.



HK370

Fig. 1. **Example of a suitably modified bisubstrate inhibitor nsp14 from SARS-CoV-2**

In our lecture, we will discuss inhibitors of three specific MTases that could potentially serve as antiviral agents against severe diseases caused by these viruses.

In the first part of our work, we focused on developing new inhibitors for the nsp14 *N*7 MTase from SARS-CoV-2. Using a combination of methodologies, including structure-based ligand design, and parallel synthesis, we aimed to design and create several series of inhibitors derived from SAH. These inhibitors were designed to lack the amino acid portion of the SAH molecule while functioning as bisubstrate nasp14 inhibitors. As an example of our intensive efforts, we will demonstrate the approach used for the design and synthesis of new inhibitors that feature variously substituted amide functions at the 5' position of the sugar moiety (Fig. 1). Some of these compounds showed significant inhibitory activity against nsp14 in enzyme assays and also exhibited antiviral activity in our cell-based experiments.[4-7] Through this example, we aim to illustrate the correlation between the inhibitory activity of these derivatives and their predicted affinities from docking experiments.

The second part of our presentation will be dedicated to the development of suitable assays for testing new inhibitors of the NS5 MTase from DENV. We successfully established three different assays for evaluating new inhibitors of this MTase, two of which were binding assays, and the third was functional. The first assay was based on fluorescence polarization (FP), for which we prepared several fluorescent probes. This method, which we currently use routinely and have adapted for high-throughput screening (HTS), was originally described by Samrat and co-authors.[8] The second method, which in many aspects surpasses the FP method and can significantly complement its results, although not applicable for HTS, is monitoring binding through ^{15}N DENV3 NS5 MTase domain NMR titration. The final technique was based on ECHO-MS, which allows for the monitoring of methylation reaction efficiency in various MTases by tracking the formation of SAH. We adapted this method for DENV in an HTS mode. For the purposes of this study, we also prepared a compound library mimicking both the amino acid portion of SAH and methylated RNA, and we resolved the crystal structure of one obtained derivative using X-ray crystallography.

In the final part of the lecture, we will return to the highly relevant topic of Mpox MTase VP39 inhibitors, where we have demonstrated their ability to effectively inhibit the replication of these viruses in cell cultures.[9] We will explore the possibilities of new derivatives that could exhibit significantly enhanced activity against Mpox MTase and the aspects that could enable the use of such derivatives in clinical practice.

Acknowledgement
The work was supported by the project National Institute of Virology and Bacteriology (Programme EXCELES, ID Project No. LX22NPO5103) – Funded by the European Union – NextGenerationEU.

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L-05

IDENTIFICATION AND CHARACTERIZATION OF POLYMERASE INHIBITORS OF L-PROTEIN OF RIFT VALLEY FEVER VIRUS

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Rift Valley fever virus (RVFV) is a pathogenic arbovirus from the family *Phenuiviridae*, causing severe disease in both humans and domesticated animals. Outbreaks of this mosquito-borne virus can have devastating effect on the livestock, which exhibit unusual sensitivity to the infection, with possible losses reaching hundreds of millions USD[1]. Currently, there is no approved treatment ot prevention against the human RVFV infections, although attenuated vaccines are available for veterinary use. Nevertheless, their safety and effectivity is dubious. Like other viruses of the former *Bunyavirales* family (now *Elliovirales* and *Hareavirales*), replication mechanism of the RVFV is mediated by its polymerase – the L protein. The 250 kDa large protein is responsible for most of the virus replication and transcription[2]; it contains the endonuclease domain, the RNA-dependent RNA-polymerase domain and the cap-binding domain. This organization is very similar to the linear composition of the heterotrimeric polymerase complex PA-PB1-PB2 of the influenza virus[3]. Similarly to influenza, the process of viral transcription is initiated by a cap-snatching mechanism, during which the host mRNA is cleaved by the L protein endonuclease domain[4]. As the L protein is heavily conserved across the members of the former *Bunyavirales* family and, although sequentially different, it is structurally and functionally closely similar to the RNA polymerase complex of the influenza A virus[5], we believe its domains are viable targets for development of novel antivirals**.**

We have succeesfully established two strains of the Rift Valley fever virus in the BSL-3 laboratory – the wild type ZH-548 strain as well as the attenuated MP-12 strain used in the veterinary vaccines. Using these viruses, we have identified several polymerase inhibitors of the RVFV L-protein and characterized their efficacy against live virus *in vitro.* Several of these compounds were identified during screening of the library of the polymerase inhibitors from the Radim Nencka's group. Furthermore, we have validated these hits using the RVFV minigenome system based on the luciferase reporters. Using our discovered hits, we have generated mutant variants of the available virus strains – non-pathogenic MP-12 strain as well as wild type ZH-548. These resistant variants have greatly increased or complete resistance to their respective inhibitors. We have identified and described multiple mutations providing the resistance to the selected inhibitors. We want to further structurally characterize the mutations and the dynamic between the resistant virus and its insect hosts will be further studied by the research group of Petr Volf from the laboratory of vector biology.

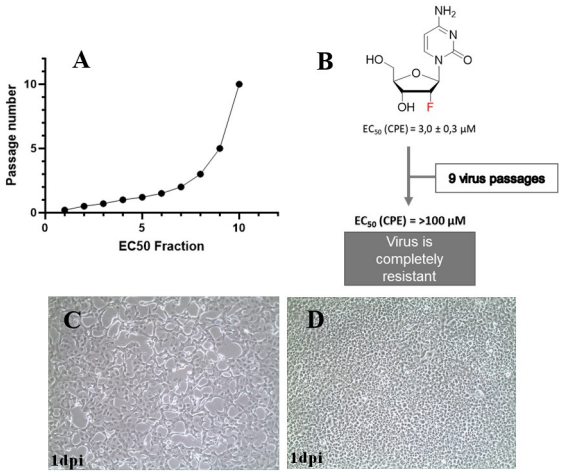

Fig. 1. **Rift Valley fever virus strain ZH-548 was treated with increasing concentration of the selected inhibitor for sequential passages (A). After reaching 10x concentration of the inhibitor added to the medium, the resistance of the virus was assayed using cythopatic effect reduction assay (B). Our resistant viruses displayed strong cytopathic effect as early as 1dpi (C) compared to the control (D).**

Acknowledgement
This work was supported by the project National Institute of Virology and Bacteriology (Programme EXCELES, ID Project No. LX22NPO5103) – Funded by the European Union – NextGenerationEU.

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L-06
HIV-1 UNCOATING AND BEYOND

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Human immunodeficiency virus type 1 (HIV-1) is an enveloped retrovirus responsible for the global AIDS pandemic. Within the virion lies a conical core containing two copies of single-stranded positive-sense genomic RNA. Upon entry into a host cell through membrane fusion, the RNA genome is reverse transcribed into viral double-stranded DNA (vDNA). This vDNA is then imported into the nucleus and integrated into the host genome, establishing a permanent infection. HIV-1 can infect non-dividing cells, necessitating mechanisms to traverse the nuclear membrane without compromising its integrity[1].

Reverse transcription, catalyzed by HIV-1 reverse transcriptase (RT), occurs within the conical core composed of HIV-1 capsid protein (CA) hexamers and pentamers. The core must uncoat to release the vDNA before integration, which is catalyzed by HIV-1 integrase (IN) within the preintegration complex (PIC). The composition of the PIC remains elusive, and the spatio-temporal coordination of uncoating and integration, as well as the role of CA, has recently undergone reevaluation[2].

In this study, we performed a CA-based pull-down assay on cellular components from HIV-1 infected MT-4 cells at various early post-infection time points. Quantitative SWATH MS/MS analysis was employed to identify CA-interacting proteins, resulting in the identification of over 1000 proteins, including 20 helicases. Two cellular RNA helicases, DDX1 and DDX3, were selected for further investigation.

We assessed the impact of helicase knockdown on HIV-1 replication in the Jurkat E6 cell line and primary blood mononuclear cells from three healthy donors. Knockdown of both helicases significantly reduced newly released virions, as quantified by RT assay.

To differentiate the roles of these helicases in early and late stages of the HIV-1 life cycle, e.g. nuclear export of viral RNAs[3], we conducted single-round infection assays and quantified integrated HIV-1 genomes in Jurkat E6 cells at 24 h.p.i. using digital droplet PCR. The reduced number of integrated HIV-1 genomes indicated the involvement of these helicases in pre-integration steps (Fig. 1).

Co-immunoprecipitation confirmed direct interaction between HIV-1 CA and DDX3, whereas no such interaction was observed with DDX1.

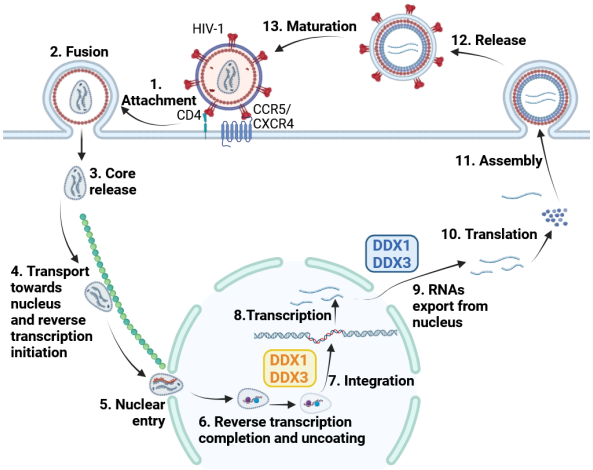

Fig. 1. **HIV-1 life cycle with depicted steps of DDX1 and DDX3 action. The blue box represents their published role in the viral RNAs export and the yellow box represents their role in HIV-1 uncoating and integration suggested by our findings.** Created with BioRender.com.

Despite the availability of antiretroviral drugs, HIV-1 remains a significant public health challenge due to its ability to establish untreatable infections following integration into the host DNA. Understanding the steps preceding provirus integration is critical for developing new interventions. The capsid protein plays a crucial role in pre-integration processes, including immune evasion, nuclear transport, and nuclear pore passage. The events between viral genome uncoating and integration remain unclear, presenting a window of opportunity for targeting cellular helicases such as DDX3.

Acknowledgement
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P-01

DESIGN AND SYNTHESIS OF NOVEL MOLECULAR PROBES TO STUDY CYTOSOLIC PROTEINS IN RIFAMYCIN-RESISTANT BACTERIA

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Rifamycin-derived antibiotics are highly effective inhibitors of prokaryotic DNA-dependent RNA polymerase (RNAP) and are used to treat various bacterial infections, including tuberculosis. However, bacteria can develop resistance to these antibiotics, and several modifications of the rifamycin structure that prevent RNAP binding have already been discovered. Recently, a photo-crosslinking probe with a biotin handle based on rifamycin B was used to identify a specific protein that displaces the antibiotic from RNAP[1].

Although rifamycin B has a free carboxylic group that could potentially be used for derivatization through amide coupling, no commercially available antibiotics are based on it due to its poor activity and low stability when exposed to air and light[2]. These factors also likely affect the price and availability of rifamycin B, resulting in syntheses with small amounts of this very expensive and unstable compound, which have proven to be challenging.

Rifampin and rifapentine are both derivatives of the readily available 3-formylrifamycin SV, which is a stable and relatively inexpensive chemical. Both drugs are derivatized at the formyl group by *N*-alkyl-*N'*-aminopiperazines to form hydrazones.

In this work, we present newly designed rifamycin-biotin probes for identifying rifamycin-binding cytosolic proteins. The structure of these probes varies by type of photo-crosslinking component (PCC) used (no PCC, diaziridine based PCC or benzophenone based PCC) and employs a well-proven hydrazone-based linking moiety to rifamycin. These probes have the potential to enhance our understanding of how bacterial resistance to rifamycin antibiotics develops.

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P-02

TRANSCRIPTOMIC ANALYSIS OF HEPATITIS B AND HEPATITIS D VIRUS INFECTIONS IN PRIMARY HUMAN HEPATOCYTES

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Hepatitis B virus (HBV) causes both acute and chronic liver disease, often leading to cirrhosis and liver cancer. Hepatitis D virus (HDV) is a satellite virus that depends on HBV for replication and can cause more severe liver damage when co-infection occurs. Together, HBV and HDV represent a significant global health challenge due to their complex interaction and impact on the progression of liver disease. By studying the gene expression profiles of hepatocytes infected with HBV, HDV, or superinfected with both viruses, we identified specific genes and pathways that are altered by infection. By using the HBV and HDV entry inhibitor bulevirtide to block infection in reference cells, we were able to distinguish transcriptomic changes caused by the *in vitro* infection process from true responses induced by both viruses. We show that HBV remains undetected by innate immune signaling and causes only minor changes in the host transcriptome. In contrast, both HDV infection and HBV/HDV superinfection induce a strong antiviral response. This transcriptomic analysis in primary human hepatocytes, which closely mimics *in vivo* liver conditions, helps to elucidate the host cellular response to viral replication and potential biomarkers of disease progression.

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P-03
PHENOTYPIC CHARACTERIZATION OF THE HYPOXANTHINE-GUANINE PHOSPHORIBOSYLTRANSFERASE IN MYCOBACTERIA

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Hypoxanthine-guanine phosphoribosyltransferase (HGPRT) is a purine salvage enzyme that catalyses the formation of guanosine and inosine 5'-monophosphates from their respective bases and phosphoribosyl pyrophosphate, thereby recycling free bases into the metabolic cycle. Random saturation mutagenesis experiments have previously shown that HGPRT is essential for the growth of *Mycobacterium tuberculosis* (Mtb). The reason why this enzyme, which is dispensable for many bacterial species, is essential for Mtb is not known.

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P-04
STRUCTURAL INSIGHT INTO THE ATP-MEDIATED INHIBITION OF MYCOBACTERIAL GMP REDUCTASE

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GMP reductase (GMPR) catalyzes conversion of GMP to IMP, a key metabolite in the biosynthesis of all purine nucleotides. This reaction allows mycobacteria and most other organisms to use guanine nucleotides to produce adenin nucleotides without the need for *de novo* synthesis.

In our studies of purine metabolism in mycobacteria, we use *Mycobacterium smegmatis* (Msm) as a model for the infectious *Mycobacterium tuberculosis* (Mtb), the causative agent of human tuberculosis. Previously[1], we showed that the enzymatic activity of Msm GMPR is allosterically regulated by ATP and GTP. While ATP inhibits the enzymatic activity of Msm GMPR, GTP counteracts this inhibition, and thus restores the activity of Msm GMPR.

Here, we present a molecular explanation for the allosteric regulation of Msm GMPR by ATP and GTP at the molecular level, based on crystal and cryoEM structures of Msm GMPR in complex with ATP and GTP.

Msm GMPR forms tetramers with four-fold axis, which further assemble into octamers with D4 symmetry. The two tetramers in the octamer adopt either a compressed or an extended conformation. ATP and GTP compete for a binding site located at the interface of the two tetramers. In the compressed octamer, the position of the tetramers prevents essential conformational changes of the active site. ATP stabilizes the compressed conformation, thereby inhibiting the activity of Msm GMPR. GTP prevents ATP binding and stabilizes the extended conformation, allowing the enzyme to remain active.

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P-05
STRUCTURAL BASIS FOR RNA-CAP RECOGNITION AND METHYLATION BY THE MPOX METHYLTRANSFERASE VP39

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Mpox, formerly known as monkeypox, is a zoonotic disease caused by the mpox virus (MPXV), which has gained attention due to its rapid and widespread transmission, with reports from more than 100 countries. The virus belongs to the Orthopoxvirus genus, which also includes variola virus and vaccinia virus. In poxviruses, the RNA cap is crucial for the translation and stability of viral mRNAs and also for immune evasion[1]. This study presents the crystal structure of the mpox 2′-*O*-methyltransfarase VP39 in complex with a short cap-0 RNA. The RNA substrate binds to the protein without causing any significant changes to its overall fold and is held in place by a combination of electrostatic interactions, π-π stacking and hydrogen bonding. The structure also explains the mpox VP39 preference for a guanine base at the first position; it reveals that guanine forms a hydrogen bond that an adenine would not be able to form[2].

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P-06
INFLUENCE OF SARS-COV-2 NSP14 METHYLTRANSFERASE INHIBITORS ON VIRAL REPLICATION

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RNA methyltransferases (MTases) belongs among the key components of multiprotein replication machinery of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). The methyltransferases, nsp10/16 and nsp14, are crucial for the formation of the viral RNA cap[1]. Without this RNA cap, the viral RNA becomes vulnerable to innate immune responses, leading to RNA degradation and initiating cellular antiviral activities.

Viral methyltransferases present valuable targets for the development of antiviral therapies[2,3,4]. Therefore, the establishment of a robust screening methodology for identifying potential inhibitors is important. In our study, we employed a previously developed *in vitro* assay coupled with the ECHO-MS system to measure nsp14 methyltransferase activity[5] to identify promising compounds synthetized in collaborating laboratory. Now we present results from experiments on infected Vero-6 and Caco-2 cells, in which selected inhibitors were tested for their effects on viral RNA replication using qRT-PCR and confocal microscopy.

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P-07

NOVEL INHIBITORS OF THE METHYLTRANSFERASE METTL3-METTL14 COMPLEX

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N6-methyladenosine (m6A) is abundant and reversible internal RNA modification, influencing transcript fate and function in uninfected and virus-infected human cells. Addition of m^6A mark by the nuclear RNA methyltransferase-like 3 (METTL3) occurs cotranscriptionally. However, the genomes of some cytoplasmic RNA viruses, namely SARS-CoV-2 and a human β-coronavirus HCoV-OC43 are also m^6A-modified. It has been demonstrated that the viral polymerase RdRp interacts with METTL3 and regulates its sumoylation and ubiquitination to affect its expression and localization[1]. Therefore, host m^6A pathway components control β-coronavirus replication.

In human, the m6A modification is catalyzed by a heterodimer methyltransferase complex, which includes METTL3 and METTL14. METTL3 primarily functions as catalytically active subunit, containing co-factor *S*-adenosylmethionine (SAM) binding pocket while METTL14 serves as an RNA-binding platform. Upregulation of METTL3-METTL14 complex has been recently linked to aberrant gene expression and protein synthesis, leading to developmental defects and cancer progression[2]. Targeting of host m^6A pathway illustrates the therapeutic potential for treatment of several diseases and at the same time represents a new way of how to restrict β-coronavirus replication. Here, we present crystal structure of this complex with a high-resolution (1.8 Å) view of the catalytic site of METTL3 occupied by a co-factor competitive inhibitor PD-2082. This inhibitor is structurally based on previously reported STM2457[3] currently used for the research of acute myeloid leukaemia. This preliminary crystal structure provides valuable structural insights for further rational design of the METTL3 inhibitors as potential drugs

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P-08

NSP14 FROM SARS-CoV-2 AND VP39 FROM MPOX VIRUS: VIRAL METHYLTRANSFERASES AS TARGETS FOR POTENTIAL ANTIVIRALS

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Methyltransferases are enzymes that play vital roles in viral replication and immune evasion, making them key targets for antiviral therapies. This study examines two important viral methyltransferases: the nonstructural protein 14 (nsp14) from SARS-CoV-2 and VP39 from the Mpox virus.

SARS-CoV-2, responsible for COVID-19, relies on nsp14, a dual-function enzyme with both 3'-5' exonuclease and N7-methyltransferase. The N7-methyltransferase domain of nsp14 is essential for capping viral RNA, a mechanism that protects the virus from host immune recognition and facilitates efficient translation of viral proteins. Inhibiting the methyltransferase function of nsp14 could interfere with the formation of the viral RNA cap structure, thereby hindering viral replication and enhancing the host's antiviral response[1].

Similarly, the Mpox virus, an emerging zoonotic pathogen, utilizes VP39 as its viral mRNA cap-specific methyltransferase. VP39 is responsible for the 2'-*O*-methylation of the viral mRNA cap, a modification crucial for the stability and translation of viral transcripts. Targeting VP39 could destabilize viral mRNA, impairing the synthesis and replication of viral proteins[2].

This work delves into the structural and functional aspects of these methyltransferases, highlighting our recent progress in developing inhibitors targeting nsp14 and VP39 (ref.[3,4]). It discusses the potential of these inhibitors as antiviral agents, focusing on their mechanisms of action and the challenges associated with their therapeutic application. By exploring the inhibition of these previously untargeted viral methyltransferases, this work enhances our understanding of their roles in viral pathogenesis and contributes to the development of innovative antiviral strategies.

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P-09

DISCOVERY OF HIGHLY POTENT SARS-COV-2 NSP14 METHYLTRANSFERASE INHIBITORS BASED ON ADENOSINE 5′-CARBOXAMIDES

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The COVID-19 pandemic, caused by SARS-CoV-2, has accelerated the development of advanced antiviral strategies. The coronaviral methyltransferases nsp14 and nsp16, essential for RNA capping, have emerged as promising drug targets[1]. Our research is focused on the design and evaluation of small-molecule inhibitors targeting nsp14.

We designed and synthesized a series of adenosine 5′-carboxamide derivatives as potential nsp14 inhibitors and identified coumarin analogs to be particularly effective. To further enhance inhibitory activity, we synthetised a series of 7-deazaadenosine analogs featuring arylalkynyl moiety at the *C*7 position, strategically designed to target a lateral cavity above the SAM-binding site[2,3]. Structural modifications revealed the critical role of the 5′-carboxyl moiety in maintaining inhibitory activity. Analogs bearing a methylated amide, reversed amidic linker, sulfonamide, sulfide, or sulfone showed significant decrease or complete loss of the inhibitory activity against nsp14.

Notably, some compounds demonstrated high selectivity, favorable pharmacokinetic properties, and exhibited moderate antiviral activity in cell-based assays. Overall, this work provides a strong foundation for the development of nsp14 inhibitors as a novel therapeutic approach for combating COVID-19 and other coronavirus-related diseases, addressing the urgent global need for effective antiviral solutions[4].

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P-10

NOVEL C-NUCLEOSIDES: SYNTHESIS AND BIOLOGICAL EVALUATION FOR ANTIVIRAL POTENTIAL

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Nucleosides and nucleotides form a cornerstone of medicinal chemistry, playing a critical role in the treatment of viral diseases, microbial infections, and cancer. When the typical C-N bond between the nucleobase and the sugar is replaced by a C-C bond, the resulting molecules are known as C-nucleosides. This structural modification significantly enhances their stability, rendering them resistant to degradation by nucleoside phosphorylases. A notable example of a C-nucleoside is Remdesivir[1], initially developed for filoviruses like Ebola, which later demonstrated broad-spectrum antiviral efficacy against paramyxoviruses, pneumoviruses, flaviviruses, and coronaviruses[2].

This research presents a novel class of C-nucleosides, detailing their design, synthesis, and biological evaluation.

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P-11

IDENTIFICATION AND CHARACTERIZATION OF INHIBITORS OF RNA-DEPENDENT RNA POLYMERASES OF THE *BUNYAVIRICETES*

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Bunyaviricetes are rapidly emerging arboviruses causing disease in both humans and animals. Many of them are considered by World Health Organization (WHO) as pathogens with pandemic potential. Prime examples are Rift Valley Fever virus (RVFV), Crimean-Congo Haemorrhagic Fever virus (CCHFV) and quite newly described Yezo virus (YEZV). They are negative single stranded viruses with segmented genome.

The entire replication and transcription machinery is provided by the viral RNA-dependent RNA polymerase (also called L-protein) and is occurring in the cytoplasm. For the transcription the L-protein utilize cap-snatching mechanism, whole mechanism of action is strikingly similar to the influenza A polymerase. We plan to use our previous experience on influenza A and apply it to the current thread that *Bunyaviricetes* represents. Despite the growing burden of these viruses on both human and animal health, surprisingly small number of therapeutics are available for treatment of these potentially deadly viruses. Prevention is also still not possible since no vaccine against these viruses is licensed for human use.

Our project aims to develop and screen the small molecule inhibitors of the polymerases of the RVFV, CCHFV and YEZV. Our scope of work includes the production of recombinant proteins in *E. coli* and baculovirus expression system, including production of full-length L-protein of RVFV and endonuclease domain. Furthermore, we are focused on characterization of the protein-ligand interactions using the isothermal titration calorimetry, and X-ray crystallography, since there are still no structures of these proteins. Our expertise also includes the inhibitor testing against their respective targets using various *in vitro* methods, minigenome reverse genetics systems and work on living viruses. We were currently able to characterize interesting hit and managed to crystallize apo structure of the endonuclease domain of RVFV.

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P-12

MOLECULAR INSIGHTS INTO HOST FACTOR ROLES IN THE REPLICATION OF THE RIFT VALLEY FEVER AND INFLUENZA VIRUSES

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Rift Valley Fever virus (RVFV), an RNA virus from the *Phenuiviridae* family, is recognized by the World Health Organization (WHO) as a pathogen with significant pandemic potential. Like many other RNA viruses, RVFV replication occurs entirely in the cytoplasm, away from the nuclear environment. Despite its importance, the host factors involved in RVFV replication, particularly those that interact with the viral RNA-dependent RNA polymerase (RdRp), remain largely unknown. Influenza virus, RNA virus from *Orthomyxoviridae* family, poses a severe threat to global public health, causing widespread respiratory illness. Although some host proteins that interact with the viral polymerase are known, many aspects of their role in viral replication are still not fully understood. Among the identified host factors, the influenza virus polymerase subunits PA and PB1 are known to interact with RanBP5, a karyopherin that facilitates nuclear import. Additionally, the PB2 subunit has been shown to interact with JAK1, a kinase involved in the host immune response, highlighting the complex interplay between viral replication machinery and host cellular processes.

This research focuses on the proteomic identification and characterization of host factors interacting with RdRp of both RVFV and influenza virus. As a novel approach to identify RVFV host factors, MicroID2 will be utilized for proximity proteomics. Selected host factors will be produced as recombinant proteins in bacteria or using baculovirus expression system. The interactions between these host factors and viral polymerase will be analyzed using advanced biophysical techniques, including surface plasmon resonance (SPR). Furthermore, the structural basis of these interactions will be explored through X-ray crystallography or cryo-electron microscopy, providing detailed insights into the molecular mechanisms of viral replication. Understanding these host-virus interactions at a structural level may reveal new targets for antiviral therapies.

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P-13
ARTIFICIAL ZYMOGENS OF THE SARS-COV-2 MAIN PROTEASE FOR DRUG EVALUATION

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The main protease (M^{pro}) of SARS-CoV-2 (also known as 3CL protease or NSP5) is an important drug target. Several M^{pro} inhibitors are now in clinical use. Paxlovid, approved by the Food and Drug Administration and the European Medicines Agency, contains nirmatrelvir (a covalent reversible inhibitor of M^{pro}) as well as the pharmacokinetic booster ritonavir. Simnotrelvir, a covalent reversible M^{pro} inhibitor boosted by ritonavir, is sold in China under the brand name Xiannuoxin. Ensitrelvir, a non-covalent protease inhibitor, is approved in Japan under the brand name Xocova.

M^{pro} is synthesized as part of the viral polyproteins pp1a and pp1ab. To be fully active, M^{pro} must be autocatalytically released from the polyprotein. Once a homodimer is formed, M^{pro} then cleaves viral polyproteins into functional proteins involved in the synthesis of viral RNA. The interplay between autoprocessing and dimerization is still not fully understood. Improving knowledge in this area may inform the design of innovative drugs targeting the first irreversible steps of the maturation cascade.

We designed variants of M^{pro} zymogens, including those defective in autoprocessing. We employed several experimental setups involving expression in *Escherichia coli*, biochemical markers using purified variants of zymogens, and a mammalian fluorescent cell-based reporter. We found differences in the susceptibility of *cis*- and *trans*-cleavage of the zymogen to inhibition using both commercially available and our newly designed small molecules. Our model zymogens exhibited a tendency toward activation rather than inhibition under specific conditions.

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P-14
BIOINFORMATIC ANALYSIS OF aGPCRs' EXPRESSION UPON VIRAL INFECTION

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Adhesion G protein-coupled receptors (aGPCRs) are known for their crucial roles in neurodevelopment, immune defense, and cancer. However, their role during viral infections remains largely unexplored. Currently, we recognize 33 human aGPCRs, mostly orphan receptors with unknown functions.

This study aims to identify specific aGPCRs implicated in mammalian cell infection by various viruses. We conducted bioinformatic analyses of existing RNA-seq datasets from Influenza A infection in Calu-3 cells (GSE162494), Monkeypox virus infection in NHEK-Ad keratinocytes and iPS cells-derived colon organoids (GSE219036), and HIV-1 infection of dendritic cells (DC) (GSE189747). RNA-seq analysis revealed significant changes in aGPCRs' expression in response to Influenza A infection in Calu-3 cells, uncovering multiple promising candidates including ADGRB2 (BAI2), ADGRD1 (GPR133), ADGRF3 (GPR113), ADGRF4 (GPR115), ADGRF5 (GPR116), ADGRG3 (GPR97) and ADGRG6 (GPR126). Analysis of Monkeypox virus infection showed cell-specific effects on aGPCRs' expression, emphasizing the importance of aligning RNA-seq analyses with related cell model studies. Additionally, the analysis revealed consistent expression changes among different viral strains (Clade I, Clade IIa and Clade IIb members) in NHEK-Ad keratinocytes in genes such as CELSR2, CELSR3, ADGRF4 (GPR115), ADGRG1 (GPR56), and ADGRL2 (LPHN2). Furthermore, RNA-seq analyses of HIV-1 infection in dendritic cells identified ADGRL3 (LPHN3) as a potent candidate for further study, with elevated expression observed across all tested DC subtypes. Commonly altered candidates following infection with both Influenza A and Monkeypox viruses include ADGRB1 (BAI1), ADGRB2 (BAI2), ADGRG3 (GPR97), ADGRF3 (GPR113), and ADGRF4 (GPR115). Notably, ADGRD2 (GPR144) and ADGRG1 (GPR56) were found to be affected by Monkeypox and HIV-1 infections, while ADGRD1 (GPR133) and ADGRE1 (EMR1) showed altered expression in response to both HIV-1 and Influenza A infections. These findings provide insights into the complex interplay between viral infections and aGPCRs' expression, highlighting potential targets for further research.

Acknowledgement
This work was funded by the Ministry of Education, Youth and Sports of the Czech Republic, INTER-COST, Grant No. LTC20065 and by the National Institute Virology and Bacteriology (Programme EXCELES, Project No. LX22NPO5103) – Funded by the European Union – NextGenerationEU. We thank COST Action CA18240.

P-15

THE HEPATITIS B VIRUS HIJACKS HOST UBE2O E2/E3 UBIQUITIN LIGASE FOR PRODUCTIVE REPLICATION

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Hepatitis B virus is a blood-borne virus that establishes chronic liver infection, associated with potential complications of cirrhosis or hepatocellular carcinoma (HCC). Currently, cumulative evidence indicates that the host ubiquitin pathway plays vital roles in HBV replication, particularly in recognition of viral nucleocapsids by ESCRT (endosomal sorting complex required for transport) components and their recruitment into multivesicular bodies (MVBs) for virus assembly and egress.

Here, we characterized the association of host hybrid E2/E3 ubiquitin ligase, UBE2O, with viral core protein (HBc). Co-immunoprecipitation analysis with wt and various HBc deletion mutants demonstrated that HBc interacted with UBE2O via its C-terminal domain. Co-expression of HBc and UBE2O resulted in mono-ubiquitination of hypophosphorylated HBc. The role of UBE2O in viral lifecycle was investigated in HBV-infected HepG2-NTCP cells as well as primary human hepatocytes (PHH) upon downregulation of endogenous UBE2O. The knock-down of UBE2O expression led to suppression of HBV replication as estimated by the levels of intracellular HBV DNA, pgRNA and encapsidated pgRNA. Furthermore, HBV core and UBE2O have been found to co-localize within the late endosomal compartments. The immunofluorescence confocal microscopy and proximity ligation assays revealed that both UBE2O and viral capsids accumulated in CD63-positive endosomes. Co-staining of UBE2O and ubiquitin resulted in a strong overlapping signal suggesting accumulation of UBE2O and ubiquitinated proteins within the same cellular compartments and further supporting the link between UBE2O and endosomal pathways.

In summary, this is the first report to demonstrate that UBE2O is a key regulatory protein of the host endosomal secretory pathway, which is hijacked by the virus for productive replication. Based on these findings, UBE2O may be a valuable target for HBV control.

Acknowledgement
This work was funded by the ERDF/ESF project ChemBioDrug CZ.02.1.01/0.0/0.0/16_019/0000729 and by the National Institute of Virology and Bacteriology (Programme EXCELES, Project No. LX22NPO5103) – Funded by the European Union – NextGenerationEU.

BIOLOGY CENTRE CAS

L-07
GUANINE QUADRUPLEXES IN THE RNA GENOME OF THE TICK-BORNE ENCEPHALITIS VIRUS: THEIR ROLE AS A NEW ANTIVIRAL TARGET AND IN VIRUS BIOLOGY

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Guanine quadruplexes (G4s) are non-canonical secondary structures in nucleic acids[1,2]. This study identified seven potential G4-forming sequences within the RNA genome of the tick-borne encephalitis virus (TBEV), specifically located in the C, NS1, NS4b, and NS5 genes, as well as the 3′-UTR (ref.[3]). Biophysical methods confirmed the ability of five of these sequences to form stable monomolecular G4 structures. All the G4s observed were parallel-stranded.

Among these, the G4 structure derived from the NS4b/NS5 region (TBEV-5) was identified as the most promising due to its strong phylogenetic conservation and favorable G4 properties. This particular G4 exhibited robust interactions with selected small molecule-based G4 ligands, which were subsequently shown to have low cytotoxicity and potent antiviral efficacy against TBEV in cell-based systems. G4 ligands, including pyridostatin, carboxypyridostatin, the bisquinolinium derivative PhenDC3, the porphyrins NMM and TMPyP4, and the plant alkaloid berberine, were found to suppress the expression of the viral surface E protein and reduce viral RNA copy numbers in virus-infected PS cell cultures[3].

Notably, pyridostatin and berberine demonstrated significant antiviral effects only during the late phase of the TBEV life cycle. In contrast, NMM also exhibited inhibitory activity during the early steps of TBEV replication (entry/fusion), which was attributed to the NMM-mediated photodynamic inactivation of TBEV virions, rendering them non-infectious[4]. These findings indicate that even well-characterized G4-binding ligands can impact different stages of the viral replication cycle and may target multiple viral and host molecular structures beyond G4s alone. Consequently, the observed antiviral effects of such compounds may result from their combined inhibitory actions at various levels of the viral replication process[3].

The role of G4 structures in the TBEV life cycle was further investigated through site-directed mutagenesis and reverse genetics. Several TBEV mutants with either stabilized or destabilized TBEV-5 quadruplex sequences were constructed and analyzed for their biological properties. The mutant with a highly stabilized TBEV-5 quadruplex (the positive mutant) displayed increased sensitivity to G4-binding ligands, significantly reduced replication fitness, and altered plaque morphology compared to the wild-type virus. Mechanistically, the altered phenotype of the TBEV-5 mutant can be explained by G4-mediated inhibition of viral RNA synthesis, as demonstrated by an *in vitro* flaviviral RdRp stalling assay[3].

In contrast, TBEV mutants with destabilized TBEV-5 quadruplexes (the negative and super-negative mutants) showed no phenotypic differences compared to the wild-type virus. Moreover, the super-negative mutant rapidly reverted to the wild-type genotype. These findings suggest that there is a stability threshold for G4 sequences in the TBEV RNA genome, and disruption of G4 stability either leads to significant changes in viral phenotype or results in a swift reversion to the optimal level of G4 stability[3].

Overall, the data indicate that G4 structures are crucial elements influencing the TBEV genome's architecture, play key regulatory roles in TBEV replication, and represent promising targets for novel small molecule-based therapeutics.

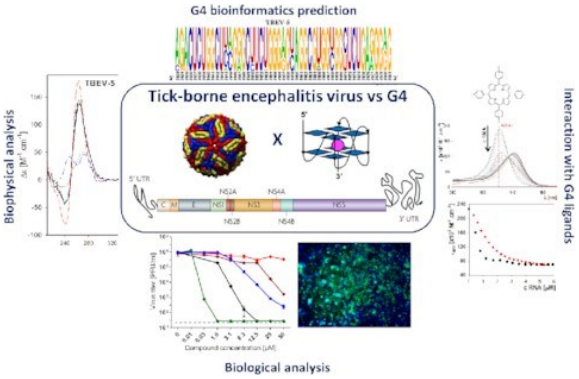

Fig. 1. **General scheme of the study using a combination of multiple methodological approaches to investigate TBEV G4s (ref.[3])**

Acknowledgement
The work was supported by the project National Institute of Virology and Bacteriology (Programme EXCELES, ID Project No. LX22NPO5103) – Funded by the European Union – NextGenerationEU.

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P-16
DETECTION OF TICK-BORNE ENCEPHALITIS VIRUS RNA IN PATIENT SAMPLES ACROSS DIFFERENT INFECTION STAGES

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Tick-borne encephalitis (TBE) is an important neuroviral disease in Europe and Asia, characterised by a biphasic progression: an initial viremic phase with nonspecific symptoms, followed by a neurological phase[1]. This study evaluated the effectiveness of detecting TBE virus (TBEV) RNA in various clinical samples from 1,125 patients in South Bohemia, Czech Republic, a highly endemic region for TBE. The samples included blood, serum, cerebrospinal fluid (CSF), and urine. The detection of TBEV RNA was most successful in serum during early infection but decreased as the IgG antibody response developed. Rarely, RNA was detectable in serum even after seroconversion.

The detection in CSF and urine was infrequent, with only one positive CSF and one positive urine sample among the 30 and 52 samples tested, respectively. Furthermore, the study highlighted the diagnostic challenges in immunocompromised patients, where delayed antibody responses hinder timely diagnosis. In such cases, PCR could have significantly shortened the diagnostic timeline.

Additionally, five patients initially not diagnosed with TBE were retrospectively found to have TBEV RNA, indicating possible underdiagnosis, particularly in mild or atypical presentations. These findings underscore the value of early RNA detection in improving diagnosis and treatment outcomes.

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The work was supported by the project National Institute of Virology and Bacteriology (Programme EXCELES, ID Project No. LX22NPO5103) – Funded by the European Union – NextGenerationEU.

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P-17
QUASISPECIES IN TICK-BORNE ENCEPHALITIS VIRUS – UTILISING LONG-READ NEXT-GENERATION SEQUENCING TO DETERMINE POPULATION DIVERSITY AND GENOMIC PLASTICITY IN VIRAL EVOLUTION

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Quasispecies theory has been widely adapted to study the evolutionary biology of RNA viruses, where large population sizes, small genomes and high rates of error-prone replication make the impact of selection on these dynamically heterogeneous viral populations more evident than in other viral / organismal systems[1]. For members of the *Flaviviridae*, a family of single-stranded positive-sense RNA viruses containing a number of disease-causing arboviruses including, amongst others the mosquito-vectored Dengue and West Nile viruses, a growing-body of literature employing quasispecies theory is beginning to unravel the underlying processes and evolutionary dynamics of these viruses[2].

Utilising Tick-Borne Encephalitis Virus (TBEV) as our primary model we are developing protocols which apply long-read next generation sequencing methodologies to study viral quasispecies diversity at the whole-genome level, furthering our ability to understand the effects of distant mutations in combination and to better measure genomic plasticity in flaviviruses. Here we present preliminary findings of quasispecies diversity in TBEV and discuss further developments in expanding the use of Flaviviruses to study fundamental viral evolutionary proceses.

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UNIVERSITY OF CHEMISTRY AND TECHNOLOGY PRAGUE

L-08
THE USE OF PATHOGEN DETECTION IN WASTEWATER AS A NEW TOOL IN THE HANDS OF EPIDEMIOLOGISTS

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The contamination of drinking water and its connection to improper sanitation (i.e. sewage collection and treatment systems) has been the core of modern epidemiology since the groundbreaking work by John Snow in 1849 (ref.[1]). In his study, John Snow showed how the contamination of one well by leakages from cesspools caused cholera outbreak in an entire neighbourhood (Albion Terrace, London) using that well as a source of drinking water. Ever since, sewage has been seen as the major transporting medium that allows the spread of human pathogens from household to household.

With the powerful modern tools for trace chemical analysis and molecular techniques, urban wastewater became extremely rich source of information for epidemiologists in many different fields such as the abuse of illicit drugs, use of antibiotics, spread of antibiotic resistant bacteria, and the spread of human pathogens during epidemics outbreaks[2].

Even though the term "Wastewater-based epidemiology" (WBE) has been around at least since 2014, the COVID-19 pandemics became the "eye opener" showing how powerfull tool may WBE be. Since the pioneering work of Medema et al. in 2020[3], there has been thousands of papers published only on the topic of COVID-19 epidemics. WBE has been used also for other diseases such as polio or monkeypox[4] and wastewatere survilance has become established part of national counterepidemic systems. As shown by many studies, it can be used on various levels to trace epidemic sources, track the spread of new virus variants or to explain the overall development of epidemics (Fig. 1). Most recently, in the revised directive for urban wastewater treatment, the European Union requires wastewater survailance program to be established by all member states.

This contribution aims to give a comprehensive view of the oportunities offered by WBE in all relevant fields. It will show the current state-of-the-art in WBE and review the most important international iniciatives that promote the use of WBE for the protection of public health worldwide.

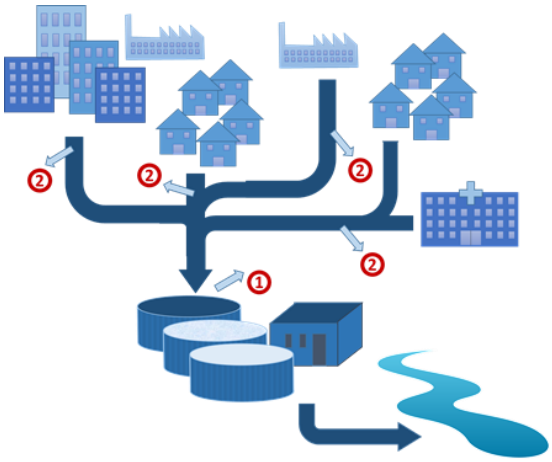

Fig. 1. **Illustration of wastewater sampling (1) to monitor the general development of epidemics on city level and (2) the identification of epidemics sources or hotspots at the level of different facilities such as schools, hospitals, or nursery houses or various neighbourhoods**

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P-18

FLUORINATED SELENOESTERS AS A NEW POTENTIAL ADJUVANT FOR THE THERAPY OF AMINOGLYCOSIDE-RESISTANT MRSA INFECTIONS

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According to estimates, as early as 2050, around 10 million people should die annually due to multi-resistant infections. As new antibiotics are only rarely approved and resistance genes to existing antibiotics are spreading rapidly, it is necessary to look for alternative approaches. One of the methods that has recently received attention is adjuvant therapy. It is based on the classic antibiotic treatment; however, it uses the synergistic action of an antibiotic with another non-toxic substance - an adjuvant that blocks the mechanism of resistance. The effectiveness of this therapy is therefore mainly against already resistant strains of bacteria.

High-throughput testing of potential adjuvants revealed synergistic action of selenoester compounds with gentamicin against clinical isolates of MRSA. Nanopore sequencing confirmed the presence of three determinants of resistance to aminoglycosides – the bifunctional enzyme AAC/APH (aminoglycoside-acetyl/phosphotransferase), aadD (adenyltransferase) and aph(3')-IIIa (phosphotransferase).

The genes of these three enzymes of the relevant clinical isolate were cloned into vectors and produced in *E. coli*. Specificity to different aminoglycosides was determined in the prepared genetically modified strains and it was also predicted that selenoesters restore sensitivity to gentamicin by inhibiting the AAC/APH enzyme. This enzyme was subsequently isolated and used to verify whether selenoesters actually function as AAC/APH inhibitors. SAR study was performed to reveal the key structural features of effective adjuvants and the mechanism of action of the best of them was subsequently studied. Additionally, studies of the separate AAC/APH domains have shown that selenoesters inhibit both activities of the bifunctional enzyme. This property, together with a good effect *in vivo*, make selenoesters promising adjuvants for aminoglycoside therapy.

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P-19

CAN CALMODULIN INTERACTION WITH MASON-PFIZER MONKEY VIRUS MATRIX PROTEIN AFFECT THE LATE-PHASE LIFE CYCLE?

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The N-terminal domain of the retroviral structural polyprotein Gag, named matrix protein (MA), plays a key role in targeting viral components to the plasma membrane (PM). MA also enables interaction with the PM through its bipartite motif, which consists of an N-terminal myristoyl moiety and a highly basic region (HBR). An interaction between HIV-1 MA and the ubiquitous cellular protein calmodulin (CaM) has been described in the region encompassing the HBR[1]. CaM concentration is elevated in HIV-1 protein-producing cells[2]. The interaction of CaM with HIV-1 MA was found to trigger a myristoyl (myr) switch that facilitates membrane binding[3].

We extended this investigation to the Mason-Pfizer monkey virus (M-PMV) and showed that a similar CaM-MA interaction occurs in this retrovirus, despite its distinct late-phase life cycle mechanism. Unlike HIV-1, which assembles at the plasma membrane, M-PMV assembles in the cytoplasm. Our findings show that the recombinant MA protein interacts with CaM in a Ca^{2+} dependent manner and that, like HIV-1, this interaction triggers a myr switch. CaM-MA interaction was further studied *in vivo* by co-immunoprecipitation of transfected HEK 293T cell lysates.

We are currently focusing on elucidating the interaction interface between the CaM and MA domains and what prevents the myr switch to occur at intracytoplasmic membranes. A deeper understanding of the role of CaM interaction with retroviral structural proteins would enhance our understanding of retroviral life cycle mechanisms at the molecular level.

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P-20
TARGETING SARS-COV-2 EXORIBONUCLEASE: EVALUATION OF PHYTOCHEMICALS FOR ANTIVIRAL THERAPY

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The nsp14-nsp10 exoribonuclease (ExoN) complex plays a pivotal role in maintaining the fidelity of RNA synthesis of SARS-CoV-2. Furthermore, its enzymatic activity is often associated with resistance to nucleoside analogues, which can be excised from the nascent RNA strand in a manner resembling the removal of incorrectly incorporated nucleotides. Therefore, ExoN represents a vital target for the development of adjunctive therapies for the treatment of COVID-19 (ref.[1]).

In the presented study, we focused on hydroxylated phytochemicals, such as simple phenolics, flavonoids and naphtoquinones, which have been previously indicated as potential inhibitors of the SARS-CoV-2 ExoN through computational studies[2–4]. Initially, we produced individual nsp10 and nsp14 capable of reconstituting a functional ExoN complex. Subsequently, we developed methodologies for the assessment of enzyme activity and small-molecule binding utilising nano-differential scanning fluorimetry, which were validated through the testing of aurin tricarboxylic acid – a known ExoN inhibitor[5]. These methods were then employed for the analysis of the inhibitory potency of a selected series of phytochemical compounds. The majority of the compounds had no significant effect. However, three compounds exhibited varying degrees of inhibitory activity. Moreover, Subsequent evaluation of the most potent compound, Shikonin, indicated a novel mode of action involving direct binding to nsp14. In conclusion, our results elucidate the *in vitro* potency of phytochemicals against SARS-CoV-2 ExoN and further advance the drug development process by identifying new inhibitor compounds.

Acknowledgement
The work was supported by the project National Institute of Virology and Bacteriology (Programme EXCELES, ID Project No. LX22NPO5103) – Funded by the European Union – NextGenerationEU.

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MASARYK UNIVERSITY

L-09
CARPE PILI! HUNTING STRATEGY, STRUCTURE, AND REPLICATION OF *P. AERUGINOSA* PHAGE JBD30

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Phages are the most abundant biological entities on Earth[1], but our understanding of many aspects of their lifecycles is still incomplete. Here, we have used cryo-electron and fluorescence microscopy to analyze the replication cycle of the siphophage Casadabanvirus JBD30. Phage JBD30 is a temperate phage that infects the bacterium *Pseudomonas aeruginosa,* a human pathogen known for causing infections that are often difficult to treat due to its multidrug resistance[2,3].

The virion of phage JBD30 consists of an icosahedral capsid, connector, long flexible non-contractile tail, and a baseplate equipped with the tail fibres. These tail fibres attach to *P. aeruginosa* type IV pilus. Type IV pili are thin filaments growing from the cell poles that the bacteria repeatedly extend and retract to move over surfaces[4]. The retraction of the pilus brings the phage to the bacterial cell surface. The interaction between the baseplate and the pilus orients the JBD30 virion so that the tripod of receptor-binding proteins faces the bacterial surface, ensuring proper orientation of the baseplate for cell attachment and genome ejection. Subsequently, the tripod of receptor-binding proteins attaches to the outer bacterial membrane and slides aside, triggering the opening of the baseplate, leading to the release of three copies of the tape-measure protein. The release of the tail tape measure proteins triggers DNA ejection. For replication, phage DNA redistributes from the cell poles throughout the cytoplasm.

Capsids of JBD30 progeny are assembled as procapsids, which expand by 7% in diameter upon filling with newly synthesized dsDNA. The DNA-filled heads are then joined with 180-nm-long tails. The tails assemble independently by polymerization of major tail proteins around the tape measure protein into hexameric discs. The bending of the tail tube is allowed by the flexible loops of the major tail protein that mediate the contacts between successive tail discs. The JBD30 replication cycle ends with host cell lysis and the release of phage progeny. The first virions occur at 80 min post-infection, and the titer peaks at 100 min post-infection.

The combination of cryo-electron tomography and fluorescent microscopy has enabled the characterization of the replication cycle of phage JBD30, shedding light on its intricate mechanisms of host cell recognition, genome delivery, and progeny particle formation. It is likely that the structural features and replication mechanisms described here for phage JBD30 are conserved among siphophages that utilize bacterial type IV pili for initial cell attachment.

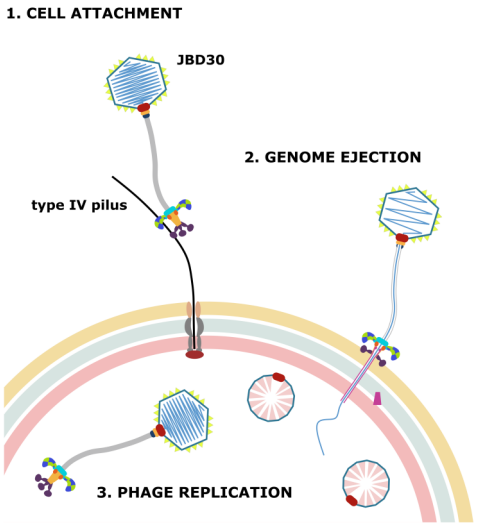

Fig. 1. **Replication of phage JBD30 in *P. aeruginosa* cell**

Acknowledgement
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L-10
CONTROLLING BIOMOLECULAR CONDENSATES FOR VIRAL APPLICATIONS

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The precise organization of biomolecular condensates is required for viral replication[1]. Various cellular components make up these condensates, including proteins, molecules, and nucleic acids. The individual interactions of these components and their local concentrations dictate the internal organization. Viruses can create condensates of their own or introduce new components to pre-existing ones in the host cell[2]. Biophysical understanding of this process allows for the rational design of condensate control mechanisms.

For viral applications, there are three areas of interest. The first is maturation, where the liquid-like properties are arrested due to strong interactions, trapping components within the condensate. The second is dissolution where condensates are unable to form a dense phase. The third is compositional control, where individual components can be removed from condensates. Here we show computational approaches that can define the interaction network within condensates and allow us to control them.

We focus on leveraging interactions between multi-domain proteins in condensates. Intrinsically disordered regions facilitate separation into a protein dense and dilute phase[3]. However, globular regions can also enhance phase separation and are easier targets for drug delivery. Initial work was done on the two-component system of RECQL5 and RNA-Polymerase II (RNAPII). These two proteins interact during transcription, a process which can be hijacked by viruses[4]. We isolated the three distinct domains of RECQL5 responsible for the condensate formation, both *in vitro* and *in silico*. A schematic description can be seen in Figure 1. Three types of interaction are present (1) globular-globular (2) disordered-disordered (3) globular-disordered. Each interaction exhibits control over different organization stages. The disordered interactions localize RECQL5, the globular-disordered interactions recruit RNAPII, and the globular interactions slow transcription.

So far, we have interrupted these mechanisms through domain removal. However, we next move towards using peptides and drug molecules to exercise condensate control. We further extend these tools and methods to 228 other multi-domain proteins involved in genomic maintenance[5].

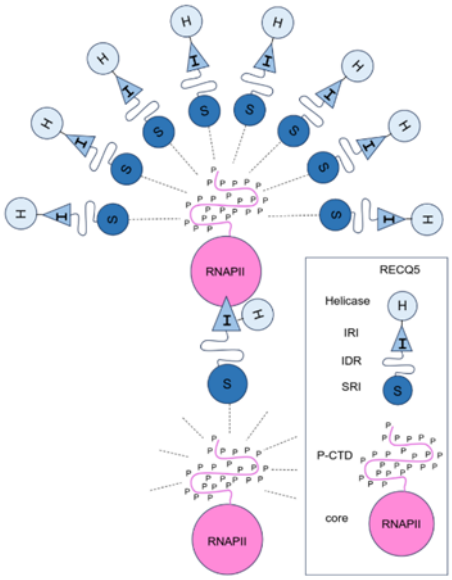

Fig. 1. **Schematic depiction of interaction network between RNA-Polymerase II (RNAPII) and RECQL5.** Each colored region represents a different globular domain.

Acknowledgement
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L-11
INVOLVEMENT OF A NOVEL RIBOSOME ASSOCIATED FACTOR IN TRANSLATION REGULATION

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Ribosome inactivation is a vital adaptive mechanism that allows the cells to conserve resources under stress by controlling protein synthesis[1–3]. Although ribosome-interacting proteins in facilitating ribosome dimerization and hibernation are well studied in bacteria and eukaryotes[4–8], similar mechanisms in archaea remain poorly understood. Here we present high-resolution cryo-electron microscopy structures of an archaeal 30S ribosomal dimer stabilized by a ribosome dimerization factor (aRDF) from *Pyrococcus furiosus*. Structures were resolved at 3.2 Å with a unique head-to-body architecture of the 30S ribosomal subunits, distinct from the dimerized ribosome structures observed in other domains of life. The aRDF protein interacts directly with the eS32 ribosomal protein, a key player in subunit association. Therefore, based on biochemical and structural evidence aRDF´s function is to halt the assembly of functional 70S ribosomes. These findings provide novel insights into ribosome inactivation mechanisms in archaea presenting aRDF as an archaeal ribosome anti-association factor that inhibits ribosome subunit joining for translational control purposes (Scheme 1).

Acknowledgement
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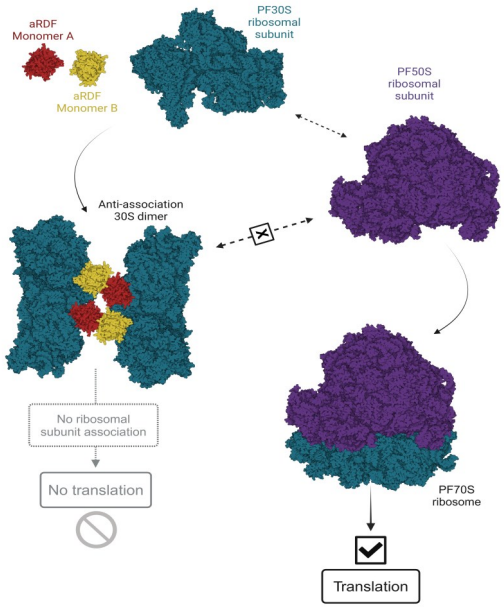

Scheme 1. **Illustration of the potential role of aRDF as an anti-association factor regulating ribosome assembly and translation in *P. furiosus***

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L-12
CEFTRIAXONE RESISTANCE OF *TREPONEMA PALLIDUM* INTRODUCED BY NATURAL *IN VITRO* MUTAGENESIS

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The incidence of syphilis, caused by *Treponema pallidum* subsp. *pallidum* (TPA), remains high despite the available causative antibiotic therapy. The syphilis treatment was for many years considered straightforward due to the universal susceptibility of TPA to penicillin antibiotics[1]. We identified mutations A1873G and G2122A in the TP0705 gene in a clinical TPA isolate obtained from ceftriaxone treatment failure case. The A1873G mutation was successfully introduced into 2 laboratory TPA strains, which resulted in partial resistance to ceftriaxone and penicillin G *in vitro*[2]. Analysis of available sequence data revealed that the majority of contemporary TPA SS14-like strains[3] harbor this mutation and thus are partially resistant to ceftriaxone and penicillin G representing potential risk of TPA strains accumulating additional mutations leading to increased degree of β-lactam antibiotics resistance. New strategies in the syphilis control are thus needed[3], therefore, extensive studies towards development of a multiplex syphilis vaccine are under way. The critical step of reliability of future vaccine[4] is the identification of suitable protein candidates along with the determination of antigenic diversity of these targets within TPA strains circulating in the human population. We analyzed TPA outer membrane determinants in 21 clinical samples preselected based on different MLST profile (Fig. 1) and revealed evolutionary processes consistent with adaptive evolution that in many bacterial pathogens operate in protein determinants involved in the host-pathogen interactions. Our findings underscore the importance of analyzing TPA clinical samples isolated from diverse geographical regions in order to understand TPA outer membrane protein (OMP) variability worldwide.

In addition to OMP variability, we tested potential differences in TPA physiology *in vitro* and revealed differences in generation times of individual TPA strains. Growth rates during *in vitro* co-cultivation experiments of seven *Treponema pallidum* subsp. *pallidum* (TPA) strains including DAL-1, Madras, Mexico A, Haiti B, SS14, Grady and Philadelphia 1 were assesed. During three week-long co-cultivation of 21 possible binary combinations (7×6/2), some TPA strains grew significantly faster compared to other strains. These differences among TPA strains might be responsible for variability of syphilis symptomatology observed in previous decades and centuries.

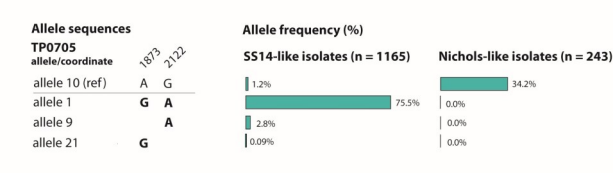

Fig. 1. ***In silico*** **analysis of TPA sequences containing 1873G variant at the TP0705 locus responsible for partial resistance to tested antibiotics**

Acknowledgement
The work was supported by the project National Institute of Virology and Bacteriology (Programme EXCELES, ID Project No. LX22NPO5103) – Funded by the European Union – NextGenerationEU.

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L-13
INSIGHTS INTO THE DIVERSITY OF TRANSDUCED STAPHYLOCOCCAL MOBILOME USING OXFORD NANOPORE SEQUENCING

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Staphylococci commonly cause nosocomial infections, often complicated by antibiotic resistance. *Staphylococcus aureus* is a known opportunistic pathogen, while coagulase-negative *Staphylococcus epidermidis* has been assumed as a human commensal. However, it has also currently become an increasing source of infections in hospital environments complicated by biofilm formation. The acquisition of mobile genetic elements (MGEs), which are the source of virulence and drug-resistance genes that can spread by intra- and inter-species horizontal gene transfer, is a major contributor to the evolution of staphylococci, leading to the emergence and evolution of antibiotic-resistant strains[1].

Temperate phages contribute to the pathogenic potential of lysogenic hosts and can mediate generalized, specialized, and lateral transduction of chromosomal and MGE sequences[2]. It can be triggered by phage infection or prophage excision caused by SOS response due to antimicrobials or under stress conditions. The subsequent spread of MGEs containing resistance or virulence genes, together with strong selection, leads to the emergence of more pathogenic bacteria in hospitals.

In addition to plasmids and prophages, genes for virulence and resistance factors are often carried by chromosomal islands that can be mobilized by helper temperate phages. Hence, they are called phage-inducible chromosomal islands (PICIs)[3]. PICIs can interact with the virion assembly of helper phages and alter the capsid architecture through interfering proteins, leading to the formation of small-headed virions (Fig. 1). This results in a reduction in the number of normal virions capable of carrying the whole helper phage genome. Infection by a small -headed virion containing a partial phage genome does not lead to a productive phage infection. In contrast, the capacity of such a particle is sufficient to carry a full-length PICI and can, therefore, be transferred to a new bacterial cell.

To date, no method has been able to effectively distinguish and compare individual packaged DNAs (genophores) in phage and phage-derived small-headed particles. At the same time, it was not possible to determine the ratio between genophores of different origins, even less how it differs when the lysogenic bacterial cell is exposed to different antimicrobial agents and their combinations.

Using long-read Nanopore sequencing in the *S. epidermidis* model, we determined the origin of the packaged DNAs and the proportion of different types of genophores, where phage and PICI sequences prevailed, while plasmid and chromosomal sequences were represented marginally. The ratios appeared to vary mainly depending on the helper phage and antibiotics used for induction. We have shown that contrary to antibiotics, treatment of a bacterial strain with a lytic phage does not increase the risk of MGE spreading. Nevertheless, the combination of lytic phage with an antibiotic was the most effective in eradicating the bacterium, while the amount of released MGEs was no higher than with antibiotics alone.

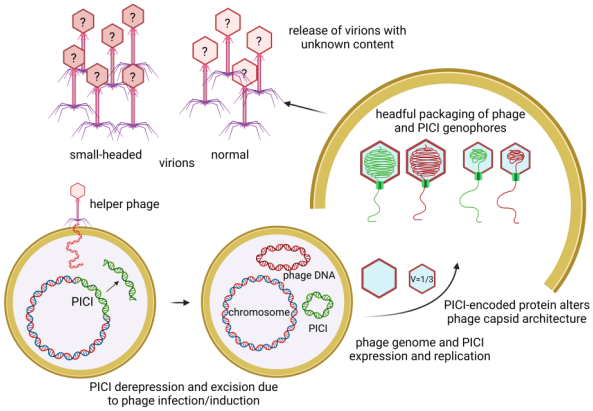

Fig. 1. **Mobilization of a phage-inducible chromosomal island (PICI) and its interaction with phage assembly and packaging machinery**

Our results provide a new approach to the study of the mobilome and allow us to assess the expected impact of different types of therapy of staphylococcal infections on the risk of the spread of virulence and resistance genes and thus, the emergence of new pathogenic strains.

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The work was supported by the project National Institute of Virology and Bacteriology (Programme EXCELES, ID Project No. LX22NPO5103) – Funded by the European Union – NextGenerationEU.

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L-14
TRANSLATION CONTROL AND CO-TRANSLATIONAL PROCESSES IN HEALTH AND DISEASE

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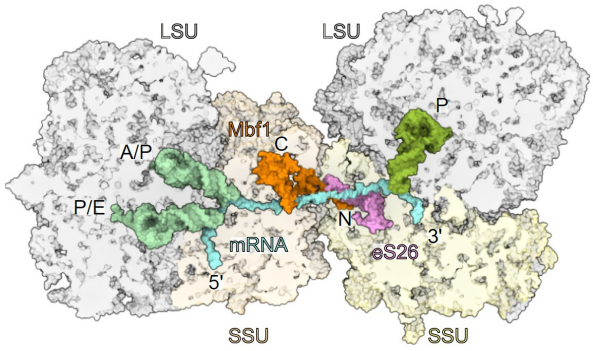
Co-translational quality control is triggered as a response to translational stalling events. Yet, different molecular mechanisms are employed for the recognition of these stalls and to trigger downstream rescue and quality control pathways. While the recognition of individual stalled ribosomes is poorly understood, the use of collided ribosomes as a proxy for the recognition of translation problems in the cell is conserved from bacteria to humans[1–3]. In eukaryotes, co-translational quality-control processes triggered by ribosome collisions accomplish several tasks and eventually trigger stress response signalling pathways[4].



Scheme 1. **Cut view of the Mbf1-bound disome structural model as a surface representation highlighting the translocation state of the stalled (right) and colliding (left) ribosome, the mRNA path, the Mbf1 and the eS26 of the stalled ribosome. Large ribosomal subunit (LSU) and small ribosomal subunit (SSU) positions are also indicated.**

As a key pathway affecting host translation the integrated stress response (ISR) is a highly conserved eukaryotic mechanism for integrating multiple signals to reprogram gene expression. These signals are conveyed by protein kinases that phosphorylate the α subunit of the initiation factor 2 (eIF2). Mammals have four known eIF2α kinases: GCN2, PERK, HRI, and PKR, which are activated in response to amino-acid starvation, ER stress, cytoplasmic protein misfolding and viral infection, respectively[5].

We examined the function of Mbf1 during induction of the ISR, and the role of ribosome binding in modulating this activity. We present a cryo-EM structure, in which Mbf1 acts as a bona fide ribosome collision sensor with its N terminus resolved and bound to the stalled ribosome. Moreover, we show that this region is important for Gcn2 activation, establishing Mbf1 as an integral component of the ISR.

To ensure the translation of their own mRNAs, viruses take control of the host protein synthesis machinery. Conversely, the host's innate immune defenses often target translation to disable the infected cell's protein synthesis apparatus. At the same time, innate defenses rely on protein production for their activation. As a result, the complex connections among the translation machinery, viruses, and innate immunity remain poorly understood. This applies especially in the field of translation control. Recent work has revealed that ribosome collisions trigger a series of quality control events and activate both a dedicated ribosome-associated protein quality control (RQC) and the Integrated Stress Response (ISR)[4]. Here, the mechanisms by which viruses avoid translational shutdown initiated by ribosomal collisions have only begun to emerge.

Viruses targeting the ISR response include the Epstein-Barr virus (EBV), which uses its EBV deubiquitinase (DUB)[6]. This viral DUB (vDUB) directly targets the initiation of RQC by countering the ubiquitination of the 40S by ZNF598. This ubiquitination could normally lead to activation of the ISR via GCN2, which inhibits global host translation. To fully understand this host-pathogen interaction, we set out to study wild-type and inactive vDUB mutants engaging stalled translating ribosomes by cryo-EM. Doing so could provide invaluable mechanistic insight into not only how the vDUB affects host quality control but also into the elusive mechanistic details of both ZNF598 and GCN1 action on collided ribosomes.

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P-21
USING LIGHT SHEET FLUORESCENCE MICROSCOPY TO IMAGE BACTERIAL BIOFILM DEVELOPMENT

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Bacterial biofilms are a significant global health problem due to their resistance to immune system, antibiotics and the resulting persistence of infections. *Staphylococcus aureus* and *Pseudomonas aeruginosa* are the two most common microorganisms found in cystic fibrosis lungs and their co-infection is associated with poor clinical outcomes. To understand the multi-step process of biofilm formation we developed a new imaging method that enables continuous imaging of biofilm development. We used light-sheet fluorescence microscope (LSFM) with an integrated microfluidic system to monitor biofilm growth in situ. To visualize the biofilm-forming cells, we modified *S.aureus* and *P.aeruginosa* to stably express fluorescent proteins. The main components of biofilm matrix, such as extracellular DNA and polysaccharide intercellular adhesins, were labelled by specific fluorescent dyes.

LSFM enabled us to detect the distinct stages of biofilm formation in single species as well as in mixed biofilms. In our conditions *S.aureus* generates robust biofilm, in contrast, *P.aeruginosa* biofilm has the shape of a thin layer with dispersed clusters of cells. *S.aureus* with *P.aeruginosa* can coexist and form two species biofilm which is gradually dominated by *P.aeruginosa*. LSFM image stacks recorded from different orientations were used for 3D reconstruction, enabling the identification of the distribution of biofilm matrix components in single-species biofilms, along with the spatial arrangement of *S.aureus* and *P.aeruginosa* in the dual-species biofilm. We also tested the presence of competitive interactions and adaptive processes between *S.aureus* and *P.aeruginosa*. This was achieved by introducing conditioned media from co-culture cultivation into *S.aureus* biofilm, leading to prompt biofilm dispersion.

LSFM with integrated microfluidic system represents a new tool for visualizing single species or mixed species biofilm formation. In addition, this system enables delivery of various agents into the mature biofilm to study their effect on bacteria by time-lapse imaging.

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P-22
ASYMMETRIC RECONSTRUCTIONS OF IMMATURE TICK-BORNE ENCEPHALITIS VIRUS PARTICLES REVEAL ASSEMBLY MECHANISM OF FLAVIVIRUSES

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Tick-borne encephalitis virus (TBEV), an enveloped virus belonging to the Flaviviridae family, causes severe central nervous system disease in humans. The virus has a smooth surface covered by envelope proteins (E-proteins), which, along with membrane proteins (M-proteins), are anchored in the viral lipid bilayer. During its life cycle, the immature, non-infectious virus undergoes a maturation process characterized by the proteolytic cleavage of prM and significant rearrangement of the envelope proteins on its surface.

We isolated immature TBEV particles from infected tissue culture cells and visualized their structure using cryo-electron microscopy. We solved the high-resolution structure of the E-protein-prM-protein complex, which forms the "spiky" surface of immature particles. Through combination of cryo-electron tomography and single-particle analysis, we demonstrated that TBEV immature particles are asymmetric. Assembly defects often disrupt the symmetric, icosahedral structure of the E-protein-prM-protein spikes on the particle surface. However, these irregularities do not impede the subsequent maturation process, resulting in mature particles with vacant patches in the "herringbone" pattern of the mature viral surface.

The findings shed additional light on the viral assembly of TBEV and its maturation process, which may be the subject of future antiviral medication development.

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P-23
GENOME ANCHORING, RETENTION, AND RELEASE BY NECK PROTEINS OF *HERELLEVIRIDAE* PHAGE 812

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The virion of Herelleviridae phage 812 is formed by an icosahedral capsid and a contractile tail joined together by neck proteins[1]. Despite the role of the neck proteins in virion assembly, DNA packaging, and regulation of genome release, their functions are not well characterized.

Here we show that the neck of phage 812 consists of portal, adaptor, stopper, and tail terminator proteins decorated on the outside by two types of cement proteins. A dodecameric DNA-binding site on the portal complex anchors a region of the genome inside the capsid, which directs the spooling of the packaged genome and may prevent an accidental escape of the DNA during the initial stages of genome packaging. The adaptor complex induces a local B-to-A form transition of the DNA in the neck channel that may serve to pause genome translocation. The gating loops of the stopper proteins prevent genome loss from fully packaged proheads by blocking the neck channel prior to the tail attachment. The binding of the tail terminator complex to the stopper complex induces opening of the gating loops and advancement of DNA into the tail. The structure of neck proteins is unchanged by tail sheath contraction. The expulsion of the tail tape measure protein rather than tail sheath rearrangement thus triggers genome release.

Our results explain how the active interplay between neck proteins and the genome directs DNA packaging, prevents premature genome release, and enables its ejection into the host cell.

Acknowledgement
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P-24
REVEALING THE REPLICATION CYCLE OF STAPHULOCOCCUS AUREUS PHAGE 3A USING CRYO-ELECTRON MICROSCOPY

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Phage 3A infects *Staphylococcus aureus*, an opportunistic human pathogen[1,2]. It has siphovirus morphology: prolate head composed of major capsid protein, about 310-330 nm long non-contractile tail terminated by a compact baseplate. The phage genome contains integrase and an *S. aureus* virulence factor; therefore, it belongs to temperate phages, which were up to date mostly studied regarding their lysis/lysogeny decision-making[3,4]. In our work we reveal major steps of phage 3A life-cycle *in vivo* using cryo-electron tomography of focused ion beam milled infected *S. aureus* cells.

We observed the early stages of page infection including the attachment of the baseplate to the peptidoglycan surface of the bacterial cell wall and the ejection of the phage genome into the cells. After infection the assembly of new heads starts on the inner surface of the plasma membrane and the early assembly intermediates (preheads) are gradually converted into late intermediates (proheads). The late intermediates are quickly filled with phage genomic DNA and finally, the tail/ baseplate complex is attached. Late phase of infection is characterized by presence of phage bundles bound by their tails.

Detailed understanding of the phage life-cycle is crucial for further use of phages in medical applications, such as "phage-therapy", against multiresitant strains of bacteria[5].

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P-25
UNDERSTANDING THE MECHANISMS OF GENOME REPLICATION AND TRANSCRIPTION IN RIFT VALLEY FEVER VIRUS

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The Rift Valley Fever Virus (RVFV) is a highly infectious zoonotic pathogen with mortality rates in severe cases reaching up to 30%. Endemic to parts of Africa and the Arabian Peninsula, RVFV poses a significant public health threat[1]. Given the absence of specific treatments, understanding the replication and transcription mechanisms is crucial for developing effective antiviral strategies. RNA replication and transcription in RVFV are catalysed by the multi-functional and monomeric viral RNA-dependent RNA polymerase, referred to as L-protein (LP)[1–3]. However, the mechanistic understanding of these processes remains incomplete[3].

This study aims to generate high-resolution cryo-EM structures of replication and transcription intermediates of LP at near-atomic scale. Currently, we are examining critical steps in replication, such as the accommodation of the viral promoter and the *de novo* prime-and-realign mechanism for initiation of replication. The formed pre-replication complexes on specific viral RNA fragments show dynamic behaviour of the cap-binding and endonuclease domains of the LP. The detailed understanding of viral replication and transcription *via* high-resolution cryo-EM structures could lead to promising opportunities for drug development.

Acknowledgement
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P-26
***IN VITRO* CULTIVATION OF *TREPONEMA PALLIDUM*: ISOLATION OF CONTEMPORARY STRAINS AND ESTABLISHMENT OF HUMAN CELLS FOR GROWTH OF THE SYPHILIS AGENT**

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Treponema pallidum subsp. *pallidum* is the causative agent of syphilis, a sexually transmitted human disease with over 6 million new syphilis cases per year worldwide[1].

T. pallidum is an obligatory human pathogen, which was considered to be uncultivable for decades. In 2018, Edmondson and colleagues published a revolutionary technique allowing *in vitro* cultivation of *T. pallidum* in laboratory conditions. *T. pallidum* multiplies in the presence of rabbit epithelial cells and modified complete medium at 34°C in the microaerobic atmosphere (5% CO_2 and 1.5% O_2). Long-term cultivation requires regular subcultures every 7 days[2].

For decades, only a handful of treponemal strains have been isolated and maintained *in vivo* using passages in rabbits. In this study, two contemporary *T. pallidum* strains (CI-7 and CI-10) originating directly from human clinical material (i.e., genital smear) were isolated and established using the *in vitro* cultivation system. Both *in vitro* cultures are propagated for more than 100 days, representing >50 treponemal generations.

While rabbit epithelial cells support the treponemal multiplication in the original *in vitro* system[2], cocultivation with human foreskin cells was established in our laboratory. In this system, strain DAL-1 is continuously cultivated for over one year.

Taken together, we established the protocol for isolation of contemporary *T. pallidum* strains without a need to propagate them in rabbits, and our improved cultivation protocol could be a useful model for the study of syphilis.

Acknowledgment
The work was supported by the project National Institute of Virology and Bacteriology (Programme EXCELES, ID Project No. LX22NPO5103) – Funded by the European Union – NextGenerationEU.

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P-27
PHAGE TRAINING: ENHANCING BACTERIOPHAGE EFFICACY IN ANTIBIOTIC-INFUSED ENVIRONMENTS

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Viruses of microbes (bacteriophages) are ubiquitous entities present in our environment. From the beginning of the previous century, humankind has been looking for their utilization in medicine[1]. The medicinal approach counts only with strictly lytic phages, which lyses the target pathogenic bacteria without adverse effects on the human host. So, the second most important criterion is the phage ability to lyse the selected bacterial strain. Unfortunately, the phage banks may not always contain suitable phages, and the isolation of the new lytic phages could be tedious and not fast enough for the patient. Another obstacle is that the lytic ability of the phage could change during the therapeutic process because of the emergence of phage resistance[2]. Phage training could be a solution for these situations[3]. It is a process of adaptive laboratory evolution, where the phages should acquire the ability to lyse the selected bacteria.

Our phage training approach is based on Phage-Antibiotic Synergy effect (PAS)[4]. The phage-antibiotic interactions could lead to an antagonism or synergy, where is the bacteria more efficiently eradicated. Both of these situations were used for the training of therapeutic phages. The resulting phages were tested for their efficacy against the parental strain of *Staphylococcus aureus* and the best clones were isolated and sequenced by Nanopore technology. Genome changes were evaluated in comparison to the parental phage. Also, the evolved bacteria were isolated, and tested for their genome stability and resistance to the parental phage. The identifiaction of these changes could reveal a new insight into the phage-antibiotic function and evolution.

Acknowledgement
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P-28
NOVEL PHAGE FROM THE GENUS TWORTVIRUS: A PROMISING TREATMENT FOR *STAPHYLOCOCCUS PSEUDINTERMEDIUS* PYODERMA

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Staphylococcus pseudintermedius is a commensal bacterium and an opportunistic pathogen found predominantly in dogs. The first methicillin-resistant strains of *S. pseudintermedius* (MRSP) were identified in 2007, and since then these multidrug-resistant strains have become increasingly prevalent. The treatment of such resistant bacteria is an increasing challenge for human and veterinary medicine. Phages are now being investigated as a potential therapeutic option[1].

In this study, we isolated and characterized a novel phage from the genus *Twortvirus* and investigated its morphology, growth characteristics, host range, and proteomic and genomic properties. The genome of *S. pseudintermedius* strains used for propagation was sequenced. In addition, veterinary clinical trials using phage lysates for the treatment of canine pyoderma were conducted by Bivoveta (project number PP14/2020).

The novel phage belongs to the family *Herelleviriade* and has a typical myovirus morphotype according to negative stain transmission electron microscopy. The length of the phage genome containing two LTR is 154 kbp with a G+C content of 30.7%. The whole genome sequence of the propagating S. pseudintermedius strain is 2.553 Mbp long and has a G+C content of 37.4%. The described polyvalent phage was able to lyse 72% of tested bacterial strains, including MRSP strains. In clinical trials, almost 74% of 60 dogs showed a significant reduction of clinical symptoms – healing of skin wounds and reduction of inflammation after pyoderma therapy.

A new phage from the genus *Twortvirus*, which has a broad spectrum of activity against animal staphylococci, has been described in detail for its potential in veterinary research. Initial results from clinical trials in the treatment of pyoderma in dogs are promising and suggest potential for future use.

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The work was supported by the project National Institute of Virology and Bacteriology (Programme EXCELES, ID Project No. LX22NPO5103) – Funded by the European Union – NextGenerationEU.

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P-29
FREEZE-DRIED SOLID DOSAGE FORM CONTAINING BACTERIOPHAGES AGAINST *PSEUDOMONAS AERUGINOSA* AND *STAPHYLOCOCCUS AUREUS*

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Incorporating therapeutic bacteriophages into solid dosage drug forms is a significant challenge during the implementation of phage therapy into practice because of many requirements for phage stability and dosage form quality.

This study aims to optimize the freeze-dried dosage form containing *Pseudomonas aeruginosa* and *Staphylococcus aureus* phages. We applied the set of two staphylococcal and two pseudomonal phages of podovirus (*Rosenblumvirus* and *Phikmvvirus*) and myovirus (*Kayvirus* and *Pbunavirus*) morphologies. Firstly, we optimized storage conditions with and without humidity regulation and determined the most suitable filler from gelatine, polyvinylpyrrolidone or maltodextrin. We compared the potency of bacteriophages alone and in a phage cocktail during long-term storage of variant dosage forms. All parameters were assessed according to the phage titer stability determined by standard spot assay.

The most stable bacteriophages with a maximal 10 % loss of initial titer for the freeze-dried form were staphylococcal podovirus of the genus *Rosenblumvirus* and pseudomonal podovirus of the genus *Phikmvvirus*, with maltodextrin as a filler stored with humidity regulation at 4 °C for twelve months. All freeze-dried phages were effective alone and in the cocktail for twelve months at room temperature and 4 °C with a maximal 53 % loss of initial titer in contrast with 37 °C when the phages lost their titer. In the freeze-dried form, the stability at room temperature was improved in the case of staphylococcal phages compared to the stability of purified liquid lysate.

This pilot study points out essential steps for the optimization of solid dosage forms containing bacteriophages, and it shows the possibilities of bacteriophage implementation in solid dosage forms for relevant effects, safer application and better patient compliance during phage therapy.

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P-30
PHAGE-INDUCIBLE CHROMOSOMAL ISLAND INTERFERENCE WITH PHAGES IN *STAPHYLOCOCCUS EPIDERMIDIS*

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Opportunistic pathogen *Staphylococcus epidermidis* serves as a reservoir for horizontally transferable genes for other pathogenic species, namely *Staphylococcus aureus*. This horizontal gene transfer is mediated mainly by temperate bacteriophages. Many frequently transferred genes are located on mobile genetic elements such as plasmids or phage-inducible chromosomal islands (PICIs). PICIs are widespread among staphylococci and usually encode one or more genes associated with virulence. For their transfer, PICIs hijack the temperate phage machinery for packaging their DNA to capsids composed of phage proteins and, at the same time, interfere with phage propagation. So far only one member of PICIs of *S. epidermidis* designated as $SeCI_{SE48}$ was characterized in detail[1].

The genome structure of $SeCI_{SE48}$ strongly resembles typical PICI, but unlike others, it does not encode any genes associated with virulence or antibiotic resistance. As $SeCI_{SE48}$ interferes with the phage life cycle, it prevents plaque formation by some phages. We focused on genes associated with phage interference and determined their effect on sensitivity to phages E72, 15, 27, 48, 456, 459, and AV01 and $SeCI_{SE48}$ transfer frequency by these phages. The introduction of the erythromycin resistance gene into $SeCI_{SE48}$ DNA and in -frame deletion in individual $SeCI_{SE48}$ genes allowed us to asses their role in interference with phages and transduction frequencies.

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P-31
CRISPR-CAS10 ENGINEERING OF *STAPHYLOCOCCUS AUREUS* BACTERIOPHAGES FOR BIOSENSING APPLICATIONS

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The increasing prevalence of antimicrobial-resistant bacterial pathogens, including multidrug-resistant strains of *Staphylococcus aureus*, has intensified the need for alternative therapeutic approaches. Particular attention is being paid to phage therapy, which utilizes viral particles infecting bacteria as a key component in the treatment process.

Significant advantages of bacteriophages as lytic agents include high specificity (which reduces the impact on natural microflora), their ability to penetrate biofilms, and the potential for phage property modification.

The CRISPR-Cas10 technology, a powerful tool for precise genetic engineering, enables targeted modifications of viral genomes by facilitating the selection of recombinant bacteriophages[1]. This method holds the potential for constructing viral particles with enhanced therapeutic properties, such as an expanded host range or improved lytic efficiency. The novel, unique characteristics also broaden the scope of their application in biotechnology.

The use of modified bacteriophages in combination with biosensing technologies opens up new possibilities for applications in diagnostics and environmental monitoring, as well as in research and development.

In our study, we focus on modifying the structural proteins of *S. aureus* therapeutic phages by adding a polyhistidine (His) tag to facilitate the oriented binding of particles to a biosensor chip. To monitor the binding kinetics and confirm particle attachment, we will use Bio-Layer Interferometry (BLI) and Surface Plasmon Resonance (SPR) biosensors.

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The work was supported by the project National Institute of Virology and Bacteriology (Programme EXCELES, ID Project No. LX22NPO5103) – Funded by the European Union – NextGenerationEU.

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L-15
A MULTIFACETED ROLE OF FILAMENTOUS HEMAGGLUTININ IN THE VIRULENCE OF PATHOGENIC BORDETELLA SPECIES

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Filamentous hemagglutinin (FHA), a major virulence factor of classical *Bordetella*e, is a rod-shaped molecule that plays an important role in the adherence of bacteria to ciliated epithelial cells of the upper respiratory tract and suppresses the host innate and adaptive immune response. FHA is translated as a 360-kDa FhaB precursor that is exported across the outer bacterial membrane by a two-partner secretion mechanism involving the outer membrane protein FhaC and shed into external environment as an N-terminal 'mature' 220-kDa FHA protein after processing by surface-exposed SphB1 protease. The remaining C-terminal 130-kDa FhaB prodomain is thought to regulate maturation process and rapidly degraded in the periplasm. We show here that both the extreme C terminus (ECT) of the FhaB prodomain and the mature FHA play the pivotal roles in the virulence of *B. pertussis*. The NMR-based structural analysis of ECT, a highly-conserved the C-terminal 100 residues of the FhaB precursor, revealed that the ECT polypeptide adopts a rigid structure with a 'pilin-like' protein fold. Deletion of the sequence encoding ECT (ΔECT) resulted in a significant decrease in bacterial colonization within the nasal cavity of infected mice, comparable to *B. pertussis* strain lacking the FhaB precursor (ΔFhaB). Intriguingly, the ΔECT strain exhibited a complete loss of its ability to bind cilia on human nasal epithelial cells grown at the air-liquid interface, emphasizing the indispensable role of ECT in the adherence of Bordetella cells to ciliated epithelial cells. Furthermore, we demonstrate the mature FHA confers resistance of *B. pertussis* to complement-mediated killing, highlighting its involvement in protection of bacterial cells against the host's innate immune response. Collectively, these results provide novel insights into FHA biology, unraveling its multifaceted role in the virulence of pathogenic *Bordetellae*.

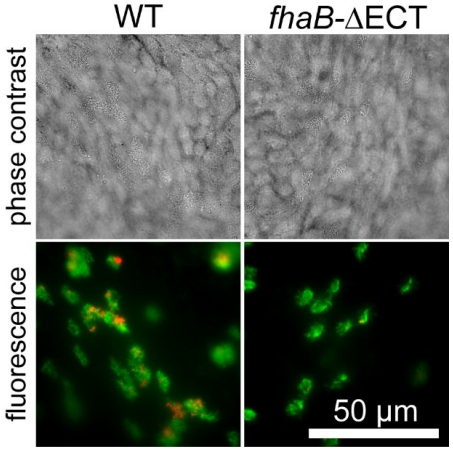

Fig. 1. **Fluorescence microscopy of the interaction of the wild-type and the fhaB-ΔECT mutant strains of *B. pertussis* with human nasal ciliated epithelial cells cultured at air-liquid interface (ALI).** Differentiated ALI cultures were infected with mScarlet-expressing *B. pertussis* cells (in red) and incubated at 37 °C for 18 h. Following fixation with 4 % (w/v) paraformaldehyde, the cells were permeabilized with 0.5 % (v/v) Triton-X100 and cilia of the ciliated cells were labeled with anti-acetylated tubulin antibody (in green)

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L-16
SWEET LIFE: WHAT THE ANTIBIOTIC RIFAMPICIN AND SUGARS HAVE IN COMMON

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The phosphoenolpyruvate phosphotransferase system (PTS) is a crucial metabolic pathway found universally in eubacteria but absent from eukaryotes, making it a prime target for antimicrobial agents. This system involves a series of proteins that undergo sequential phosphorylation to facilitate the simultaneous phosphorylation and uptake of various sugars.

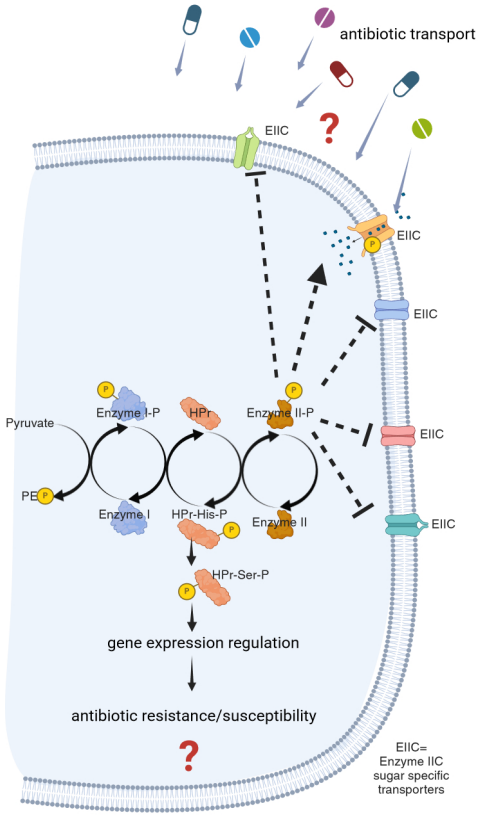

Fig. 1. **The scheme of the PTS system and its potential for antibiotic transport and gene expression regulation of bacterial response to antimicrobial stress (depicted as red question marks)**

PTS proteins and their structural homologues have been frequently linked to both sensitivity and resistance to various bactericidal compounds[1–4]. An active PTS is necessary for the effectiveness of some antimicrobial agents, while phosphate transfer can lead to harmful gain-of-function effects. Additionally, PTS proteins can transport certain agents or even phage DNA into the cell[5-7]. Consequently, overexpression of certain PTS components increases cellular sensitivity to some antimicrobials[2]. Furthermore, the PTS has been proposed as a mechanism for controlling outbreaks of *Enterococcus faecium*, a major cause of multi-drug resistant infections in hospitals[3].

Despite the known influence of PTS proteins on cellular responses to multiple antimicrobial agents, there is a lack of comprehensive studies systematically examining their role in antibiotic resistance. This project aims to uncover the unknown functions of PTS proteins in antibiotic resistance and clarify their contribution to the innate antibiotic resistance of bacteria with a focus on resistance to antibiotic targeting RNA polymerase- rifampicin.

Acknowledgement
This work was supported by the project National Institute of Virology and Bacteriology (Programme EXCELES, ID Project No. LX22NPO5103) – Funded by the European Union – NextGenerationEU.

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L-17

CLINCELIN: A REDESIGNED LINCOSAMIDE COMBATS RIBOSOME RESISTANCE MODIFICATION THROUGH ENHANCED BINDING AND STRUCTURAL FLEXIBILITY

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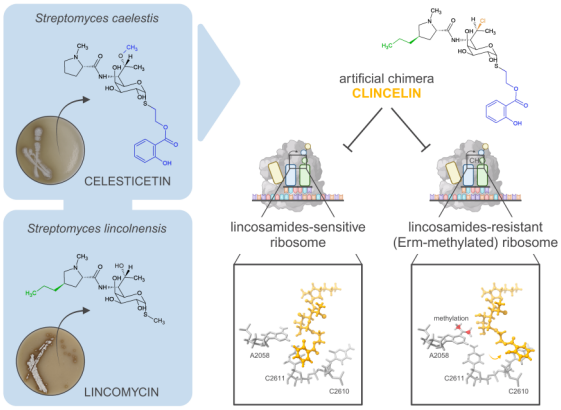
Lincosamides, an important class of antibiotics in human medicine, inhibit translation by binding to the catalytic centre of the ribosome. However, their efficacy is impaired if the ribosome bears widespread A2058 methylation conferred by the erm resistance gene, rendering all clinical lincosamides ineffective. In this study, we present clincelin, a novel chimeric compound structurally derived from the natural lincosamides celesticetin and lincomycin[1]. Rigorous *in vitro* tests show that clincelin has significantly higher antibacterial activity compared to the two parent compounds and the clinically used lincosamide clindamycin. Remarkably, clincelin retains its efficacy also against erm-mediated resistant strains. The cryo-EM analysis reveals the unique mechanism underlying clincelin's resistance evasion: Not only does it exhibit improved binding contacts with the ribosome, but also has a remarkable structural flexibility that allows different binding modes depending on the presence of Erm methylation. To the best of our knowledge, clincelin is the first antibiotic to exhibit such anti-resistance adaptation to overcome the resistance. Our detailed characterization paves the way for the development of next-generation lincosamides for clinical use and establishes a paradigm for overcoming antibiotic resistance through molecular design.



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P-32
DECIPHERING THE EARLY INNATE IMMUNE RESPONSE OF NASAL MUCOSA TO BORDETELLA PERTUSSIS INFECTION

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Bordetella pertussis is a strictly human pathogen that elicits a highly contagious respiratory illness known as pertussis, or whooping cough. Current mouse models enabled identification of many bacterial virulence factors and development of pertussis vaccines, but the mechanisms underlying the process of B. pertussis transmission during the catarrhal phase of pertussis disease remain largely unexplored due to lack of a convenient animal model. Recently, we have used immunodeficient MyD88 knock-out mice to achieve a human-like high level of nasal mucosa infection, which triggered rhinitis and catarrhal shedding of bacteria from mouse nasal cavity and transmission of the infection onto co-housed adult animals[1]. Here, we compared the early innate immune response of the conventional C57BL/6 mice and the MyD88-deficient mice upon intranasal challenge with B. pertussis. Flow cytometry analysis of cells from nasal tissue shows how multiple immune cell populations infiltrate the nasal mucosa after infection in both strains of mice. Single-cell RNA sequencing revealed that nasal mucosa response of conventional mice infected with B. pertussis is characterized by the expansion of a highly activated neutrophil subset characterized by an interferon-stimulated gene signature, which does not develop in the MyD88KO mice. A similar interferon-stimulated gene signature is also observed in the epithelial cells. Moreover, B. pertussis infection is associated with an upregulation of expression of genes encoding antimicrobial peptides (Lipocalin 2) and chemoattractant molecules (Lix and Cxcl10), as detected by qPCR analysis. These results open the way for a detailed understanding of innate immune responses involved in B. pertussis clearance from nasal mucosa of the host.

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P-33
LESS REACTOGENIC WHOLE-CELL PERTUSSIS VACCINE CONFERS PROTECTION FROM *B. PERTUSSIS* INFECTION

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Pertussis resurged over the last decade in most countries that replaced the traditional whole cell pertussis vaccines (wP) by the less reactogenic acellular pertussis vaccines (aP). The aP vaccines induce a Th2-polarized immune response and by a yet unknown mechanism hamper the induction of IL-17 and IFN□-secreting antigen-specific tissue-resident memory T cells (TRM) required for orchestration of clearance of B. pertussis from infected nasopharyngeal mucosa. The aP-induced pertussis toxin-neutralizing antibodies effectively prevent the life-threatening pertussis pneumonia in infants, but aP-elicited immunity fails to prevent infection of nasopharyngeal mucosa and transmission of B. pertussis. In contrast, the more reactogenic traditional wP vaccines, alike natural infection, elicit a broad antibody response and trigger a Th1/Th17-polarized T cell immunity with homing of protective TRM cells into airway mucosa. We tackled here the reactogenicity of the conventional wP vaccines by genetic modification of the Fim2 and Fim3-producing B. pertussis strains used for wP vaccine manufacturing. Mutations were introduced into the genomes of vaccine strains (i) to reduce the TLR4 signalling potency of the lipid A of B. pertussis lipooligosaccharide (ΔlgmB), (ii) to eliminate the enzymatic (immunosuppressive) activity of the pertussis toxin (PtxS1-R9K/E129G) and (iii) to ablate the production of the dermonecrotic toxin (Δdnt). Experimental alum-adjuvanted wP vaccines prepared from such triply modified (3M) bacteria exhibited a reduced pyrogenicity in rabbits and a reduced systemic toxicity in mice, while conferring a comparable protection from B. pertussis infection as the unmodified wP vaccine.

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P-34
NEUROTOXIC ACTIVITY OF *BORDETELLA* DERMONECROTIC TOXIN AT SUB-PICOMOLAR CONCENTRATIONS

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Lyzing Bordetella bacteria release a neurotropic dermonecrotic toxin (DNT) that is endocytosed into host cells and permanently activates the RhoA family GTPases by polyamination or deamidation of the glutamine residues in their switch II regions (e.g. Gln63 of RhoA). DNT was found to enable high level of bacterial colonization of nasal cavity of pigs by B. bronchiseptica and the capacity of DNT to inhibit differentiation of nasal turbinate bone osteoblasts accounts for atrophic rhinitis in infected pigs. However, it remains unknown
whether DNT plays any role in virulence of the human pathogen B. pertussis and in pathogenesis of the whooping cough disease.

We report a procedure for purification of large amounts of LPS-free recombinant DNT that exhibits a high biological activity on cells expressing DNT receptors, the Cav3.1 and Cav3.2 low voltage T-type calcium channels. These channels are highly expressed on neuronal cells, such as astrocytes and neurons. We show that very low amounts of DNT (≈fM) affect the function of primary rat neurons sub-cultured *in vitro*. DNT action destroys the long astrocyte protrusions and stops their feeding of neurons, causing progressive death of neurons and loss of their action potential transmission capacity. Intraperitoneal or intravenous administration of as little as 3 ng (18 fmol) of DNT then causes weight loss and neurological symptoms leading to death of mice. Progress in deciphering of the molecular basis of DNT action at the extremely low concentrations and the mapping of its cell-binding domains is reported.

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P-35
BACTERIAL EF-TU AND ITS ROLE IN TRANSCRIPTION AND ANTIBIOTIC RESISTANCE

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Elongation factor Tu (EF-Tu) is an essential and most abundant protein in bacteria. During translation, EF-Tu delivers amino acyl tRNAto the A site of the ribosome[1]. Exept for its role in translation, EF-Tu is involved in other cellular processes. It interacts with MreB, an actin-like cytoskeletal protein essential for the rod cell shape of *Bacillus subtilis*[2]. *Escherichia coli* EF-Tu can be hijacked by phages Qβ and MS2 and used as a subunit of its RNA replicase, functioning as a molecular chaperon and enabling the replicase to function[3,4]. Furthermore, EF-Tu increases resistance of the ribosomal apparatus to antibiotics tetracycline, streptomycin, spectinomycin, erythromycin[5]. Finally, the roles of EF-Tu in microbial patogenesis were also studied[6].

We have performed a set of experiments revealing an effect of EF-Tu on the transcription machinery. The results will be presented and discussed.

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P-36
SUBLANCIN AND ITS POTENTIAL TARGET IN GRAM-POSITIVE BACTERIA

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Bacteria are equipped with an arsenal of toxic weapons that they use against their competitors in the constant battle for nutrients and living space.

One of these weapons is sublancin, a 37 amino acid long glycopeptide secreted by *Bacillus subtilis* into its environment. Sublancin is encoded by the *sunA* gene that is localized together with other accessory genes (*e.g.*, sublancin exporter, glycosyltransferase and antitoxin) in the SPβ prophage region. Sublancin kills other Gram-positive bacterial species including pathogens (*e.g.*, *Bacillus cereus*, *Staphylococcus aureus* or *Streptococcus pneumoniae*), making sublancin an important substance with a potential as an effective antibiotic[1, 2].

It is already known that the phosphotransferase system (PTS) of the target cell is somehow involved in sensitivity to sublancin[3]. However, the sublancin target(s) and exact mechanism of action remain elusive.

This project characterizes the mode of action of sublancin. Using purified sublancin from *B. subtilis*, we investigated its killing ability/kinetic against different *B. subtilis* strains (wt, Δ*sunI*, Δ*ptsG*) by time-lapse microscopy. In parallel, using mass spectrometry and a tagged version of sublancin, we detected potential targets of this toxin in *B. subtilis* and *P. megaterium*. These targets were subsequently validated by a functional assay, identifying the cellular process that is blocked by the action of sublancin. The results will be presented and discussed.

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P-37
RIFAMPICIN: NOVEL MECHANISM OF ITS RESISTANCE

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Nowadays, antibiotic resistance has become a serious worldwide problem. Rifampicin (RIF) is a clinically important antibiotic, an essential component of the anti-tuberculosis therapy[1]. RIF targets bacterial DNA-dependent RNA polymerase (RNAP). It binds to the β subunit of RNAP in its DNA/RNA channel and sterically blocks the RNA exit channel, forcing RNA synthesis to stop at the stage of only several nucleotides transcribed[2].

Bacteria have developed various strategies of resistance against rifampicin. One of the strategies is decreasing the affinity of β for RIF by mutating the *rpoB* gene. Another strategy is enzymatic inactivation of the antibiotic by ADP-ribosyl transferases (Arrs), RIF glycosyltransferases (Rgt), RIF monooxygenases (Rox), and RIF phosphorylases (Rph)[3]. Alteration of cell permeability and active efflux can also confer rifampicin resistance[4].

Our goal was to reveal and describe novel mechanisms of rifampicin resistance in the model organism *Bacillus subtilis*. Via a proteomic approach we identified upregulated proteins after treatment with sub-inhibitory concentration of rifampicin compared to an untreated control. Phenotypic assays then showed that knock-outs in genes encoding selected protein candidates became more sensitive to rifampicin compared to the wild-type strain. The role of the selected protein candidates as well as regulation of their gene expression will be presented and discussed.

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P-38
MECHANISMS OF FIDAXOMICIN RESISTANCE IN BACTERIA

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Fidaxomicin (FDX) is a macrocyclic antibiotic that inhibits bacterial growth by targeting RNA polymerase, preventing movement of the clamp[1,2]. Fidaxomicin has a narrow-spectrum activity and is currently used in the treatment of *Clostridioides difficile* infections[3]. Mechanisms of FDX resistance are still largely unexplored.

To provide insights into these mechanisms, *Bacillus subtilis* that can encounter the producer of FDX, *Dactylosporangium aurantiacum*, in its natural habitat, soil, was exposed to sub-inhibitory concentration of FDX and protein levels were compared to an untreated control. Significantly upregulated proteins (FDX+) could potentially be involved in FDX resistance.

A total of 87 proteins were identified to be significantly upregulated. The phenotypes of respective knockout strains were evaluated for FDX susceptibility. A knock-out strain in gene X (not disclosed here) increased sensitivity to FDX. Complementation by X then reversed the phenotype. Further, the expression of X is induced by lower concentrations of FDX. The results will be presented and discussed.

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P-39
DECIPHERING THE SIGNALING FUNCTION OF ANTIBIOTIC RESPONSIVE ABCF ATPase LmrC

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Multidrug resistance compromises the efficacy of antibiotics. ABC Family F ATPases (ABCFs) are pivotal proteins that bind to ribosomes, playing a role in regulating translation and conferring antibiotic resistance to all major antibiotic groups that bind to the large ribosomal subunit (50S) through ribosome protection[1]. LmrC, a member of the antibiotic resistance subfamily 5 (ARE5) found in the lincomycin biosynthesis gene cluster of *Streptomyces lincolnensis*, has been revealed to possess a transduction function that responds to antibiotics, activating the transcription of *lmbU* gene encoding transcriptional regulator[2]. However, the specific mechanism by which LmrC transmits signals from antibiotics is currently unknown.

To elucidate the mechanism underlying LmrC-mediated signaling and its biological role, we use a combined *in vivo* and *in vitro* approach. A reporter assay is used to characterize the biological function of LmrC-mediated signaling. Simultaneously, the molecular mechanisms of LmrC-dependent transcriptional regulation of *lmbU* in response to antibiotics is investigated using single-molecule cryo-electron microscopy, ribosome toeprinting and coupled transcription-translation assays. Our results support the hypothesis that LmrC exerts its regulatory function *via* the 5' untranslated region of *lmbU* mRNA.

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P-40

IDENTIFICATION OF A NOVEL ERYTHROMYCIN RESISTANCE MECHANISM MEDIATED BY MRMA METHYLTRANSFERASE IN *C. DIFFICILE*

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Clostrioides difficile is one of the main causes of hospital-acquired diarrhea[1]. However, the accumulation of antimicrobial resistance in epidemic *C. difficile* lineages such as RT176 poses a significant risk for the spread of resistance determinants to other bacterial species targeted by these antibiotics. A comparative genomic analysis of one erythromycin-susceptible and six erythromycin-resistant *C. difficile* strains identified the novel resistance determinant carried by transposons Tn*6110* and Tn*7806* for which we propose the name *mrm*A (macrolide resistance methyltransferase A). We demonstrated that heterologous expression of the *mrm*A gene in *E. coli* confers resistance to erythromycin and to a lesser extent to streptogramin B, but not to other ribosome-targeting antibiotics.

MrmA encodes a putative SAM-radical 23S rRNA methyltransferase, similar to RlmN and Cfr[2]. RlmN is a housekeeping enzyme involved in translation fidelity and methylates nucleotide A2503 at carbon C2[3]. Cfr-mediated methylation at the same A2503, but at carbon C8, confers resistance to antibiotics targeting the peptidyltransferase center (PTC)[3]: phenicol, lincosamide, oxazolidinone, pleuromutilin and streptogramin A, with no effect on erythromycin activity. In contrast, resistance to erythromycin is generally conferred by a different Erm-family of 23S rRNA methyltransferases[3]. These Erm enzymes dimethylate nucleotide A2058 in the exit tunnel, resulting in resistance to macrolides, lincosamides and streptogramins B antibiotics.

We hypothesize that MrmA methylates a different adenine residue on the 23S rRNA compared to Cfr and RlmN, which selectively affects erythromycin binding without affecting oxazolidinones or lincosamides3. To elucidate the exact site of modification, we will use *in vitro* biochemical and structural biology approaches, which we will present in detail.

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P-41

THE INTERPLAY OF ANTIBIOTICS, RIBOSOMES, AND ABCF ATPASES IN FINE-TUNING GENE EXPRESSION

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Antibiotics that bind to the catalytic centre of the bacterial ribosome interact directly with the nascent peptide, leading to variability in the inhibitory effect that depends on the specific amino acid sequence of the nascent peptide[1]. However, this phenomenon, known as context-dependent translation inhibition, has not yet been studied with classes of antibiotics that inhibit translation initiation, such as lincosamides, pleuromutilins and streptogramins A (LSaP). Bacteria utilise the context-dependent inhibition of translation by ribosome-targeting antibiotics to modulate gene expression: Ribosome which is blocked only by specific antibiotic while translating regulatory uORFs in the 5'UTR of target mRNAs triggers conformational changes that allow transcription or translation. This mechanism, known as ribosome-mediated attenuation, plays a critical role in the control of antibiotic resistance genes encoding ABCF ATPases, which also regulate gene expression in response to antibiotics[2]. Our results suggest that context dependency and ribosome-mediated attenuation are important for ABCF-mediated antibiotic signalling. Using gene reporter assays and *in vitro* ribosome profiling techniques[3], we decipher the context dependency of LSaP antibiotics, their effects on the regulation of ABCF protein-encoding genes and how ABCF protein activity influences this regulation. We propose that this intricate interplay between antibiotic, ribosome, and ABCF protein represents a novel mechanism that fine-tunes bacterial gene expression in response to antibiotic and thereby maintain their ability to survive in the natural environment.

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CHARLES UNIVERSITY

L-18
NOVEL AUTOPHAGY INDUCER INHIBITS HEPATITIS B VIRUS S ANTIGEN SECRETION

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Chronic hepatitis B (CHB), a liver infection caused by the hepatitis B virus (HBV), affects more than 300 million people worldwide. If left untreated, CHB can progress to liver cirrhosis and hepatocellular carcinoma, resulting in over 800,000 deaths annually. While current therapies based on nucleot(s)ide analogues effectively slow disease progression and reduce viral load, they require lifelong adinistration. Pegylated interferon alpha therapy can achieve clearance in a small fraction of patients but is associated with significant side effects[1].

The primary goal of CHB treatment is to eliminate HBV DNA from liver cells, though this remains a considerable challenge. Recent therapeutic research is focused on targeting different stages of the viral life cycle, with a particular emphasis on reducing the secretion of the HBV surface antigen (HBsAg). HBsAg is a key factor in the disruption of immune system function in CHB. Lowering HBsAg levels could improve the efficacy of combination therapies[2].

HBsAg is a transmembrane protein produced in the endoplasmic reticulum (ER) and may be secreted via ER-phagy, a process involving the degradation of part of the ER by vacuoles or lysosomes. It is then transported to multivesicular bodies, where infectious virions assemble. These virions, along with empty HBsAg subviral particles, are subsequently released from the cell through exocytosis[3]. Disrupting the HBsAg trafficking pathway could potentially reduce the amount of secreted HBsAg.

In this study, we introduced a small-molecule compound that enhances proteasome activity and autophagy by triggering downstream events dependent on Nuclear Factor Erythroid 2-related factor 1 (NRF1), without inducing ER stress. Notably, this compound significantly reduced HBsAg secretion in both *in vitro* HBV infection models (Figure 1) and HBV-producing cell lines.

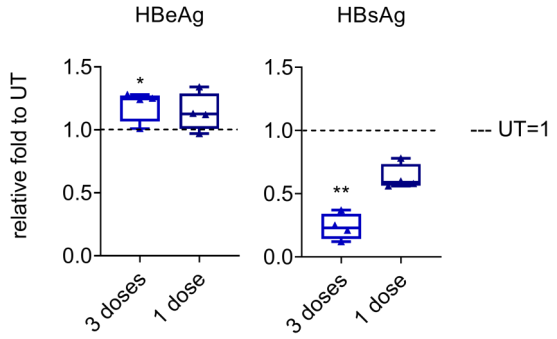

Fig. 1. **Effect of tested compound on secretec HBV antigens E (HBeAg) and S (HBsAg) by HBV-infected HepG2-NTCP cells.** Results represent relative fold change to untreated infected HepG2-NTCP (UT). N=4, Friedmann test with uncorrected Dunn|s post/hoc test for multiple comparissons: *=p≤0.05, **=p≤0.01

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L-19
MODULATION OF *LEISHMANIA* INFECTION IN SAND FLIES BY INSECT´S MIDGUT MICROBIOTA

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The midgut microbiota of insect vectors has been recognized as a critical factor influencing the transmission dynamics of vector-borne pathogens[1–3]. Sand flies (Diptera: Phlebotominae), tiny blood sucking insects, are best known for their role in the transmission of parasitic protists of the genus *Leishmania*, the causative agents of leishmaniases, a disease ranked among the top 10 neglected tropical diseases globally[4]. Within sand flies, bacteria of genus *Asaia* constitute an integral part of both the insect´s dietary intake and its midgut microbiome[3,5,6].

To investigate the interactions between *Asaia* bacteria and *Leishmania* protist within sand flies, we established a laboratory model to monitor the development of these bacteria and their impact on *Leishmania* infection and growth within the sand fly vector.

Two wild type *Asaia* species, *Asaia siamensis* and *A. krungthepensis*, were introduced to the midgut of *Phlebotomus duboscqi* females via sugar meals. These bacteria successfully colonized the sand fly midgut for up to 8 days and were vertically transmitted to the next generation through contamination of the egg surface. However, when sand flies infected with *Asaia* were subsequently exposed to *Leishmania major* promastigotes via membrane feeding of infected blood, no significant impact was observed on *Leishmania* infection parameters, namely infection rate, parasite load, localization of infection. (Fig. 1).

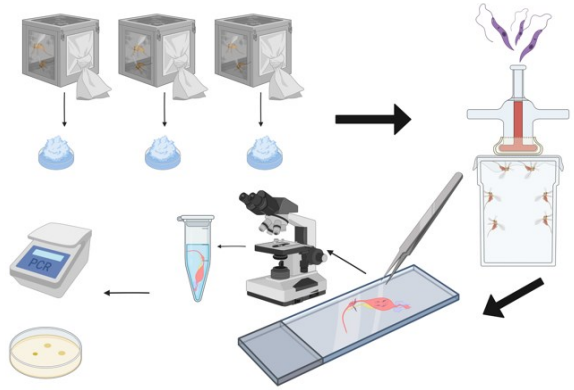

Fig. 1. **Experimental superinfection of *Leishmania major* and *Asaia* sp. in *P. duboscqi***

Furthermore, we evaluated an engineered strain of *Asaia* expressing a surface protein from *Wolbachia* (WSP)[7], given that *Wolbachia*-mediated activation of insect immunity has been shown to play a role in vectorial capacity[8]. This modified strain of *Asaia* also readily colonized the midgut of *P. duboscqi*. However, similarly to the wild-type strains, this modified bacterium did not appear to affect leishmania infection in sand flies.

In conclusion, our findings suggest that in *L. major* – *P. duboscqi* model, bacteria of genus *Asaia* do not significantly affect the course of *Leishmania* infection. Nevertheless, our experiments are ongoing, and further studies are needed to fully understand the complex interactions between the sand fly microbiome and pathogen transmission.

Acknowledgement
This work was supported by the project National Institute of Virology and Bacteriology (Programme EXCELES, ID Project No. LX22NPO5103) – Funded by the European Union –NextGenerationEU.

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L-20
INVESTIGATION OF BKPYV LIFE CYCLE, ACTIVATION OF INNATE IMMUNE RESPONSES AND MEMBRANE REMODELING IN MICROVASCULAR ENDOTHELIAL CELLS

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Polyomaviruses (PyVs) are small double-stranded DNA viruses that occur widely in nature. Among the 14 known human PyVs, three are particularly relevant: BK Polyomavirus (BKPyV), JC polyomavirus, and Merkel cell carcinoma Polyomavirus. BKPyV, with a global prevalence of approximately 80% in adults, typically causes asymptomatic primary infection. After initial infection, it disseminates via the bloodstream and establishes persistence in the urinary tract. Reactivation of BKPyV can occur in immunocompromised patients, leading to complications such as nephritis or graft loss[1-2]. Unfortunately, there are currently no specific antiviral treatments available for BKPyV infection.

Recently, for the first time, human microvascular endothelial cells from the bladder (HMVECs bd) have been proposed as viral reservoir cells due to their unique response to infection, involving interferon (IFN) production[1]. In this study, we aim to unravel the molecular details of BKPyV replication and the underlying activation of the IFN response in primary HMVECs bd.

We found that at early times post-infection BKPyV virions are located inside internalized monopinocytic vesicles and later can be detected in late endosomes, lysosomes, tubuloreticular structures, ER and vacuoles-like vesicles. Interestingly, we also noted abundant lipid droplets and remodeling of ER membranes, suggesting a viral-induced ER stress response.

We detected that the production of virus progeny starts at 36hpi while increased permeability of the cell membranes and peaks of virion release coincide with leakage of viral and cellular DNA to cytosol around 60hpi.

Leaked viral and cellular DNA colocalize with and activate cGAS leading to activation of STING and consequent transcription of IFN β and IFN-related genes, ISG56 and CXCL10. Importantly, the IFN response to BKPyV primarily results from IRF3 activation, not NF-kB. Overall, the IFN response is moderated compared to other stimuli.

The reduction of the IFN response by the cGAS inhibitor, G140 highlights the importance of the cGAS-STING pathway in the innate immune response of HVMECS bd to BKPyV.

Although the BKPyV life cycle in HBMVECs follows a similar pattern and kinetics to that of renal proximal endothelial cells (the primary target cells for the virus during reinfection), the formation of tubuloreticular structures, ER membrane remodeling, vacuole-like vesicles, and lipid droplets—presented here—are not well-documented yet as a feature of BKPyV infection.

Concerning the innate immune responses, our results here suggest that moderate activation of the innate immune response via the cGAS-STING pathway at a late stage of infection is a unique virus strategy that supports viral persistence. This approach ensures that some virus is produced, while simultaneously triggering an antiviral state in cells.

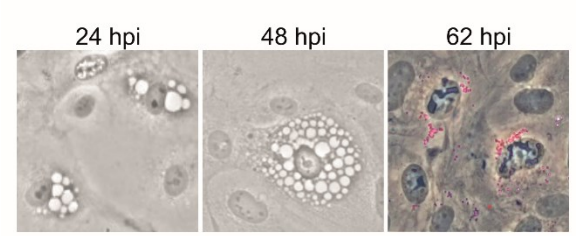

Fig. 1. **Formation of vacuoles like-vesicles and lipid droplets during BPyV infection of HMVECs bd.** Cell morphology was assessed by bright-field (BF) microscopy at 24, 48, and 62 hpi. At 62 hpi a stain for detection of neutral lipids (red) was used

Acknowledgement
The work was supported by the project National Institute of Virology and Bacteriology (Programme EXCELES, ID Project No. LX22NPO5103) – Funded by the European Union – NextGenerationEU.

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L-21

RELATIONSHIP BETWEEN YEAST VIRUS LIKE ELEMENTS AND POXVIRUSES AT THE TRANSCRIPTIONAL LEVEL

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Yeast cytoplasmic double-stranded DNA virus-like elements (VLEs, also known as linear plasmids) were found in a number of yeast species belonging to nine genera. The *Kluyveromyces lactis* pGKL1/2 plasmids, which serve as archetypes of yeast linear plasmids, are peculiar in many respects. Both plasmids are cytoplasmically localized, possess proteins covalently linked to their terminal inverted repeats and their compact genomes code for 15 genes in total including a killer toxin, two DNA polymerases, an RNA polymerase, a DNA helicase and a capping enzyme. Functions of most of the genes are putative and have not been assigned to them experimentally yet. We and others have found, that the RNA polymerase encoded by pGKL plasmids, as well as the capping enzyme and RNA helicase, are similar to the corresponding enzyme encoded by vaccinia virus (VACV). We have recently described unique structures of yeast VLEs mRNAs, we found that although these VLEs encode their own putative capping enzyme, only a few VLEs genes code for 5'-capped transcripts and that all of the VLE specific mRNAs are not 3'-polyadenylated. Surprisingly, the majority of VLE promoters give rise to uncapped mRNAs starting with short poly(A) sequences at their 5' ends that are not complementary to the plasmid DNA.

Because VLEs show a high similarity of their transcriptional apparatus with the transcriptional apparatus of poxviruses, we focused on the detailed analysis of poxviral mRNA molecules. Vaccinia virus is a prototypical poxvirus originally used for eradication of smallpox. Investigation into VACV mRNAs carried out almost half a century ago substantially contributed to the fundamental discovery of the 5' mRNA cap, a hallmark of all eukaryotic and many viral mRNAs. VACV research also facilitated the identification and understanding of the general mechanism of 5' mRNA cap synthesis. We characterized the VACV transcripts at the individual mRNA molecule level and found that vaccinia postreplicative mRNAs, containing nontemplated 5' poly(A) leaders, surprisingly lack the 5' cap structure *in vivo*. We showing that 5' cap occurrence in viral mRNAs gradually decreases in each successive gene time classes, in contrast to the reciprocal increase in 5' poly(A) leader lengths, and that these two variables are mutually negatively correlated. We also demonstrate that the initiator region element (INR) directly or indirectly influences both the frequency of 5' mRNA capping and the occurrence of 5' poly(A) leaders, including their lengths in postreplicative VACV mRNAs. Considering all the results together, we can speculate that the degree of 5' mRNA polyadenylation can directly affect the synthesis of the 5' cap by some hitherto unknown mechanism. This idea is further supported by our observation that 5' poly (A) leaders in m^7G cap-containing VACV late transcripts are significantly shorter than the 5' mRNA leaders, these lengths of which were calculated from the unbiased set of all VACV late mRNAs. Collectively, our results support the hypothesis that VACV transcription regulation ensures a gradual shift in viral mRNA translation initiation from a cap-dependent to cap-independent mechanism, which is accompanied by virus-induced modification of the host translation machinery.

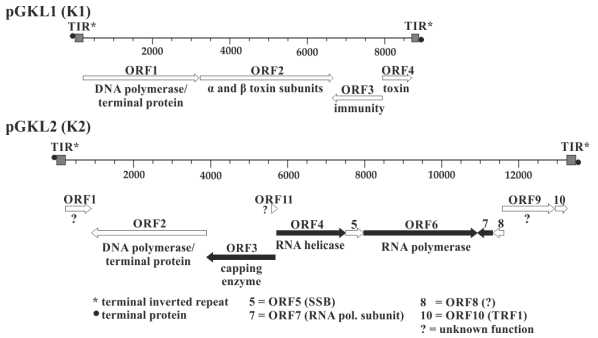

Fig. 1. **Genetic organization of pGKL VLEs (linear cytoplasmic plasmids) with indicated genes encoding components of the transcription apparatus**

Acknowledgement
The work was supported by the project National Institute of Virology and Bacteriology (Programme EXCELES, ID Project No. LX22NPO5103) – Funded by the European Union – NextGenerationEU and Project P JAC CZ.02.01.01/00/22_008/0004575 RNA for therapy.

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L-22
UNVEILING VIRAL DIVERSITY THROUGH WHOLE-VIROME SEQUENCING

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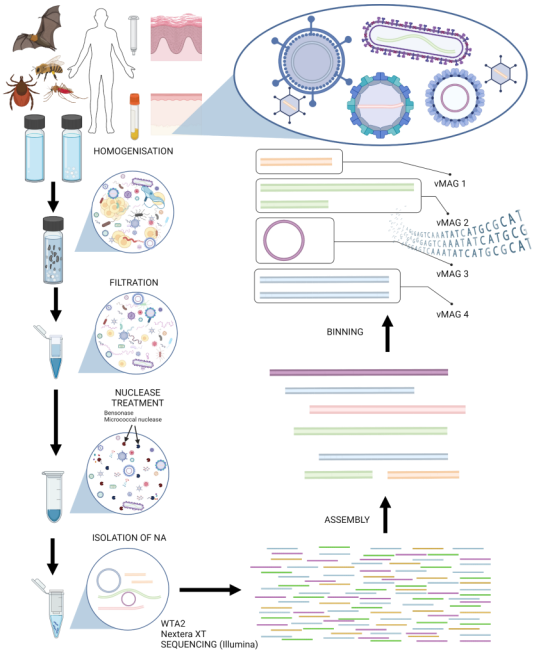
In the dynamic landscape of viral metagenomics, our research focuses on whole-virome sequencing using the Novel enrichment technique of viromes protocol (NetoVir protocol, 1), which we have adapted to diverse starting materials. The NetoVir protocol is a robust and fast approach for efficiently identifying DNA and RNA viruses without structural or genome preferences, ranging from small Picornaviridae to large viruses of the Mimiviridae family. The individual steps of the protocol allow for the enrichment of capsid-protected viruses, and through random nucleic acid amplification, sufficient reads can be obtained for subsequent bioinformatic analysis. Using a comprehensive bioinformatics, we can obtain information on a wide range of viruses without limiting our investigation to specific virus groups. This allows us to gain insight not only into known but also into unknown viruses.

Utilizing and modifying the NetoVir protocol for various samples (Scheme 1), we investigated the whole virome in samples obtained from humans and animals that either have a high economic impact on humans or are an important source of potential zoonotic infections.



Scheme 1. **Overview of sample preparation and analysis**

To characterise the full length of the newly discovered large DNA viruses, we have successfully combined bioinformatic analysis with classical laboratory techniques (2). Our focus includes the creation of viral Metagenome-Assembled Genomes (vMAGs), with a rigorous selection of high-quality sequences for further analysis, with an emphasis on completeness (complete or 50+% complete). Since viruses generally have much smaller genomes in comparison to cellular organisms, there are differences between non-viral and viral MAGs, especially in the number of contigs that make up the MAG. This feature provides an opportunity for consequent modification of the number of contigs using different computational analyses and to continue to complete the sequences using the wet lab approaches.

This interdisciplinary approach, combining wet-lab experiments and bioinformatics, provides a complex view of viral metagenomics and opens the way to a deeper understanding of viral diversity and evolution.

Acknowledgement
The work was supported by the project National Institute of Virology and Bacteriology (Programme EXCELES, ID Project No. LX22NPO5103) – Funded by the European Union – NextGenerationEU.

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L-23

THE COMPOSITION OF THE GUT VIROME IS ASSOCIATED WITH THE LATER DEVELOPMENT OF COELIAC DISEASE: RESULTS OF A PROSPECTIVE FOLLOW-UP OF TWO NEONATAL COHORTS

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Celiac disease (CD) develops in only a tiny fraction of individuals who carry susceptible HLA genotypes and consume gluten. This has prompted the search for environmental triggers or accelerators, including the realm of viruses. The objective of this study was to investigate whether gut virus exposure in early life differs between children later developing celiac disease, compared to tightly matched CD-free controls.

Two newborn cohorts preselected by HLA screening were investigated: the Norwegian MIDIA and Finnish DIPP. Cases of CD were identified by testing in late childhood, and stool samples from infancy were retrieved from the repositories. Each case of CD was matched to two CD-free controls by date and place of birth. Stool samples collected monthly between the age of 3 and 36 months were subjected to unbiased virome metagenomic sequencing. A total of 2043 viromes from 41 case-control trios were characterised. Previously unknown viruses were identified by cross-assembly and sequence-based classification. The association of the gut virome with the subsequent CD was evaluated by comparing results of standard machine learning techniques (partial least squares model and naive Bayes model) between actual data, and their variants with random permutations of the case-control labels.

In total, more than 9,000 previously unknown bacteriophages were newly classified and their genomes were annotated. The composition of the viromes differed significantly between children who later developed CD versus their matched controls (kappa statistics = 0.11, $P=10^{-14}$, Figure 1). This indicates an etiological involvement of phages or their bacterial hosts very early in the CD pathogenesis. Although the signal is clear, the magnitude of the effect is minute, and no single virus signature could be identified that would explain the association.

In a proof-of-concept study, we demonstrated that common gut bacteriophages significantly affect the risk of later non-infectious immunopathological disease, in a manner analogous to that observed for e.g. asthma.

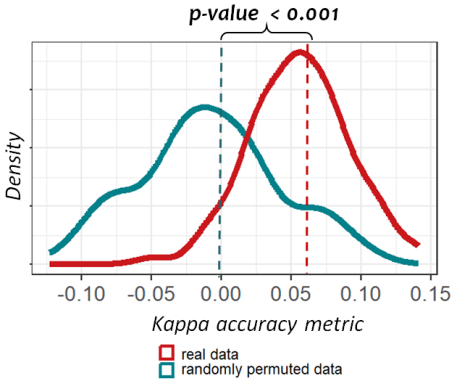

Fig. 1. **The accuracy metric of coeliac disease autoimmunity prediction by virome data as compared to permutations with random assignment of the case-control status**

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The work was supported by the project National Institute of Virology and Bacteriology (Programme EXCELES, ID Project No. LX22NPO5103) – Funded by the European Union – NextGenerationEU.

L-24
EPIDEMIOLOGY OF CARBAPENEMASE PRODUCING ENTEROBACTERALES IN THE CZECH REPUBLIC

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Carbapenemases are versatile β-lactamases capable of hydrolyzing carbapenem antibiotics. They are classified into Ambler classes A, B, and D. Class A carbapenemases, such as *Klebsiella pneumoniae* carbapenemase (KPC) and Guyana extended-spectrum beta-lactamase (GES), hydrolyze penicillins, cephalosporins, carbapenems, and aztreonam. Class B carbapenemases, defined by their metallo-beta-lactamase (MBL) structures, hydrolyze all beta-lactams except aztreonam. While class D carbapenemases, including oxyacillin-hydrolyzing carbapenemase (OXA) enzymes, have decreased hydrolyzing activity against carbapenems and penicillins.

In this study, we deliberate the emergence, gene transition, and continuous spread of carbapenemase-producing *Enterobacterales* in the Czech Republic, to develop a snapshot of the epidemic distribution in correlation to carbapenem resistance gene (CRG) repertoire, we explored the genomes of 791 different *Enterobacterales* collected between 2018-2023. Understanding the factors that cause CRE to spread so successfully in the Czech Republic is crucial to curtail its dissemination and prevent it from becoming nationally untreatable.

Fig. 1. **Carbapenemases distribution in the Czech Republic;** circles in orange, red, green, dark blue, yellow corresponds to NDM-4, NDM-5, NDM-1, KPC and OXA-48-Like respectively

Out of the 791 isolates sequenced, 212 were NDM producers with 68 NDM-4, 44 NDM-5 and 100 NDM-1. Moreover, 99/791 were KPC producers, 79 were OXA-48 like producers and 12 VIM-producers.

Regarding NDM-4; a major clonal outbreak has been detected in Plzen. 30/68 was identified as *E. hormaechei* ST 182. *C. freundii* ST100 also played a role in the outbreak but in smaller numbers. Nevertheless, even with the detection of this clonal outbreak, the gene was carried on an IncX3 plasmid that ahs been identified in the same hospital 7 years ago, and thus dissemination is mainly plasmid mediated.

NDM-5 was also identified as a clonal outbreak of *E. coli* ST 38 in Brno. The gene here is carried within the chromosom and the outnbreak is deemd as clonal.

NDM-1 corresponds to most of the cases with different outbreaks of: *K. pneumoniae* ST321 in Ceska Lipa (#9), *K. pneumoniae* (36 different STs) and *P. mirabilis* (#13) in Cesky Brod, *K. pneumoniae* (#8) in Ostrava and 50 *K. pneumoniae* (mainly ST11) in Prague. The data shows that all genes are carried on different plasmid types and sizes and rarely on chromosome indicating the involvment of different plasmids in the dessimination of this variant.

Regarding KPC, 54 isolates of different species and STs in Hradec Kralove with *E. Hormaechei* ST421 being the most abundant (#9) and 22 isolates of different species and STs in Prague. Again, the data shows that all genes are carried on different plasmid types and sizes and rarely on chromosome indicating the involvment of different plasmids in the dessimination of this variant.

Finally, OXA-48 were the most abundant in both Plzen and Prague where the data shows the dissemination is plasmid mediated by the well previously described IncL plasmid in the Czech Republic and the world.

In conclusion, the data shows that there are multiple factors that is mediating carbapenem resistance in the Czech Republic, ranging from clonal outbreaks such the *E. coli* NDM-5 in Brno to plasmid mediated like the NDM-4 IncX3 plasmid in Plzen and Prague. To a smaller extent, integrons are also playing a role such as In110 in the cases of VIMs. Continues monitoring is required to deal with rapid dessimination.

Acknowledgement
The work was supported by the project National Institute of Virology and Bacteriology (Programme EXCELES, ID Project No. LX22NPO5103) – Funded by the European Union – NextGenerationEU.

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L-25
STRUCTURAL INSIGHTS AND FUNCTIONAL IMPACT OF THE MMPL3 INHIBITOR C215

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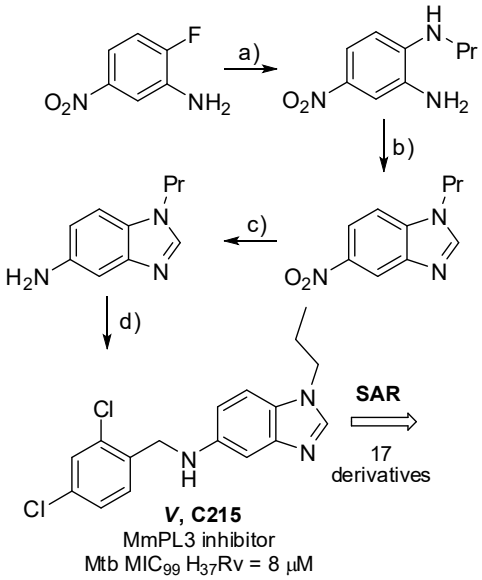
Tuberculosis TB is a globally prevalent infectious disease caused by *Mycobacterium tuberculosis* (*Mtb*). As one of the most common causes of death from a single infectious agent, TB poses significant health and economic burdens, particularly in Sub-Saharan Africa and South-East Asia. According to the World Health Organization (WHO) 2022 report, there were 10.6 million new cases of TB and approximately 1.6 million TB related deaths in 2021. Alarmingly, the same year saw 450,00 cases of drug resistant TB[1]. In 2015, the WHO launched the "End TB Strategy" to reduce new TB cases by 80 %, TB deaths by 90 %, and eliminate catastrophic costs for TB affected households by 2030[2]. However, the COVID-19 pandemic has complicated these goals.

While TB primarily affects the lungs, it can also target other tissues. Disrupting the synthesis of the lipid-rich cell wall of *Mtb* is an effective strategy for TB treatment. The MmPL3 enzyme (Mycobacterial membrane protein large 3) is a crucial transport protein in mycobacteria, including *Mtb*. MmPL3 is essential for the synthesis and transport of mycolic acids, which are key components of the mycobacterial cell wall. Inhibiting this enzyme disrupts cell wall synthesis, leading to the death of the *Mtb*[3].

C215, a benzimidazole derivative identified through whole cell screening, shows a good anti-TB activity (*Mtb* MIC_{99} $H_{37}Rv$ = 8 μM) and is effective against intracellularly residing *Mtb* (MIC *Mtb* in macrophages = 37.5 μM). Whole genome sequencing (WGS) of C215 resistant strains confirmed that C215 targets MmPL3[4]. However, data covering structure-activity relationships (SAR) and cytotoxic and metabolic profile of C215 still lacking. Additionally, C215 and its derivatives are not readily available, and no research has been conducted to expand the portfolio of C215 analogues or optimize them to develop new lead compounds. To address this, we developed a straightforward synthesis of C215 and its derivatives and established a basic pharmacological profile in order to identify potential lead candidate.



Scheme 1: a) *n*-propylamine, K_2CO_3,DMSO; b) PTSA monohydrate, TEOF, MeOH; c) H_2, Pd/C, EtOH; d) 2,4-dichlorobenzaldehyde, AcOH, $NaBH_3CN$, MeOH

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L-26
DECODING EVOLUTIONARY DYNAMICS AND BEYOND

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SARS-CoV-2 is continuously evolving to better adapt and stay ahead of the immune system's responses. Our goal is to uncover the evolutionary processes behind the changes in the Receptor Binding Domain (RBD), a hotspot for mutational activity. Our general approach involves mimicking natural evolutionary forces in an *in vitro* setting. However, alternative approaches must be taken to address some of the evolutionary pressures. The lecture will showcase various projects undertaken in the lab, viewed from a computational perspective and their applications beyond SARS-CoV-2 research.

1) Our main project focuses on analyzing the selective pressure exerted on the virus to maintain a strong binding to the ACE2 receptor. Using mutation libraries, next-generation sequencing, and analysis, we investigate how stringent this selection is and what opportunities it provides the virus for acquiring mutations that grant immune escape properties (Fig,1).

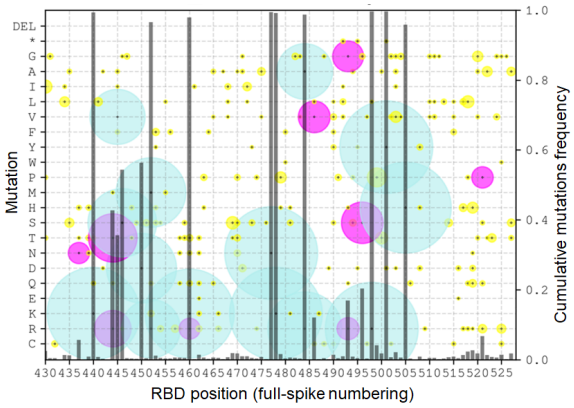

Fig. 1. **Analysis of RBD mutations and their frequencies in a library subjected to strong selection pressure for tighter binding than wild-type affinity.** Frequencies are represented by circles, with values below 0.05 shown in yellow, those above 0.05 in magenta, and those exceeding 0.3 in sky blue

2) The main computational effort involves the study of the fatty acid (FA) binding site of the SARS-COV-2 spike, employing Molecular Dynamics simulations. When the FA site is occupied by linoleic acid (LA), the spike is stabilized in its locked conformation[1]. However, it is postulated that the mutations found in the omicron variants, coupled with weak acidic conditions (pH=~4.5) destabilize the site, leading the spike to adapt the open conformation.

The study is split in two parts: The first evaluates the impact of single mutations found in the omicron variants on the binding of LA. By computing the ΔΔG values of mutations via Thermodynamic Integration (TI), mutations can be classified either as impactful on the binding of LA or not. The latter mutations can possibly contribute to the immune evasion mechanism of the virus. A total of 16 mutations are investigated and the dependence of the pH on their binding strengths is explored.

The second part involves large scale dynamics (>1 ms) of the whole SARS-COV-2 spike of the wild-type (WT) and models of the BA.1 and BA.2 omicron variants. The variants are modeled on the wild type (pdb:6zb5)[2], since no LA-bound omicron structure is available. Through non-equilibrium dynamics, the differences between the WT and the omicron variants, upon LA dissociation, are recorded and scrutinized.

3) The d-block metal-binding ability of the SMARCA5 protein, a divalent magnesium cation – dependent ATPase involved in host – virus interactions was studied. We established that the SMARCA5 sequence contains an i+4 dHis motif known to bind divalent metal cations[3], and proceeded to validate the hypothesis of an alternative binding site by IMAC chromatography and quantum mechanics simulations[4].

4) Another computationally-driven study involves the Orange Carotenoid Protein (OCP) a photoprotective protein found in cyanobacteria. Its photorelaxation process is currently under intense debate[5]. The computational effort involves the relaxed scan of a crucial dihedral angle[6] of the echinenone carotenoid embedded in OCP, in an effort to interpret the experimental time-resolved Raman spectra.

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P-42
MiRNA146a IS A KEY COMPONENT OF IMMUNNOSUPRESSIVE ENVIRONMENT OF HEPATOCYTES CHRONICALLY INFECTED WITH HBV

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Hepatitis B virus (HBV) causes acute hepatitis and can lead to chronic liver inflammation, often resulting in hepatocellular carcinoma. Known as a "stealth virus," HBV can evade recognition by the immune system. Plasmacytoid dendritic cells (pDCs), robust producers of type I and III interferons (IFNs),[1] which are crucial for HBV clearance[1]. However, miRNA146a, an immunosuppressive and proliferative miRNA in hepatocytes, modulates pDCs by silencing TRAF6 and IRAK1/4 proteins[2].

We hypothesize that HBV-infected hepatocytes produce miRNA146a within extracellular vesicles (EVs)[3], which can deliver miRNA146a into pDCs and inhibit their functions[4]. Indeed, the supernatant from HBV-producing cells contained higher levels of miRNA146a than supernatant from noninfected cells and inhibited TLR9 agonist-induced IFNα production by model pDC cell line Gen2.2. We further demonstrated the functional role of miRNA146a in IFNα downregulation by using a miRNA146a inhibitor. Supernatant collected from miRNA146a inhibitor-treated HBV-producing cells showed decreased levels of miRNA146a and did not inhibit IFNα production in Gen2.2 cells.

Since miRNA146a levels may be altered by HBV infection in patients and interfere with the immune responses, targeting this miRNA could be of significant therapeutic interest.

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P-43
MONOCYTE CELL DEATH INDUCED BY STING AGONISTS COMBINES APOPTOSIS, PYROPTOSIS AND CASPASE 8 ACTIVATION

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The cyclic GMP-AMP synthase – stimulator of interferon genes (cGAS-STING) pathway recognizes double-stranded DNA in cytoplasm. Activation of the pathway induces secretion of proinflammatory cytokines and subsequently regulates immune mechanisms[1]. The pathway can also be triggered by small molecule-based STING agonists[2]. As such, the cGAS-STING pathway is of therapeutic interest in the field of viral infections and cancer[1,2]. Importantly, activation of the cGAS-STING pathway can also lead to cell death regulated[3]. We previously demonstarted that STING agonists induce apoptosis in human monocytes[2]; however, the precise processes involved are still under investigation.

Therefore, we further analyzed the mechanisms underlying STING agonist-induced cell death in monocytes. We observed the activation of apoptotic caspases 3 and 7, and pyroptotic caspase 1, gasdermin D, and the secretion of interleukin 1β (IL1β) and IL18. However, we did not detect activation of the necroptotic RIP kinases and the pseudokinase MLKL. Interestingly, we observed active caspase 8, regulates not only apoptosis but also pyroptosis and necropotosis[4]. Furthermore, we detected a cleaved fragment of RIPK1 consistent with caspase 8-mediated cleavage, suggesting that caspase 8 may negatively regulate the necroptotic pathway. Additionally, STING agonists induced mitochondrial dysfunction, indicating a potential role for mitochondria in the regulation of cell death.

Taken together, our findings provide new insights into the effects of the cGAS-STING pathway in immune cells, highlighting its potential in therapeutic applications.

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P-44
SMALL-MOLECULE ACTIVATORS OF NRF1 TRANSCRIPTIONAL ACTIVITY PREVENT PROTEIN AGGREGATON

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Intracellular protein aggregation causes proteotoxic stress, underlying highly debilitating neurodegenerative disorders in parallel with decreased proteasome activity[1]. Nevertheless, under such stress conditions, the expression of proteasome subunits is upregulated by Nuclear Factor Erythroid 2-related factor 1 (NRF1), a transcription factor that is encoded by *NFE2L1*. Activating the NRF1 pathway could accordingly delay the onset of neurodegenerative and other disorders with impaired cell proteostasis. Here, we present a series of small-molecule compounds based on bis (phenylmethylen)cycloalkanones and their heterocyclic analogues, identified via targeted library screening, that can induce NRF1-dependent downstream events, such as proteasome synthesis, heat shock response, and autophagy, in both model cell lines and *Caenorhabditis elegans* strains. These compounds increase proteasome activity and decrease the size and number of protein aggregates without causing any cellular stress or inhibiting the ubiquitin-proteasome system (UPS). Therefore, our compounds represent a new promising therapeutic approach to various protein conformational diseases, including the most debilitating neurodegenerative diseases and viral diseases.

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P-45
TWO PATHOGENS IN ONE VECTOR

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Sand flies (Diptera: Phlebotominae) are tiny hematophagous insects that play a critical role in the circulation of various pathogens, including arboviruses such as the human pathogenic Toscana virus, and parasitic protists of the genus *Leishmania,* which cause leishmaniases. These pathogens often circulate in the same geographical areas, leading to the potential for co-infections or super-infections in both vectors and humans[1–4]. However, little is known about the outcomes of these possible interactions.

To explore whether these pathogens interact within their shared vector, *Phlebotomus tobbi*, we developed laboratory model to study co-infections with Toscana virus and *Leishmania infantum*. Our ongoing experiments aim to uncover any interactions between these pathogens, which could have important epidemiological implications for disease transmission. The results of this study will be presented at the conference in Kutná Hora.

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P-46
UNDERSTANDING THE INTERPLAY BETWEEN BK POLYOMAVIRUS AND CELULAR RESTRICTION FACTORS

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The most important human polyomavirus are the Merkel cell polyomavirus, the etiological agent of 80% of Merkel cell carcinoma, JCPyV that causes progressive multifocal leukoencephalopathy and BKPyV which causes nephropathy. After initial primary infection and dissemination in the organism, persistence of polyomavirus is, by not well-known mechanism. Local innate immune response and other cellular restriction factors such as macromolecular protein complexes such as the PML nuclear bodies (PML NB) can play a role in the outcome of the infection and promote viral persistency. Our reseach focus on the understanding the molecular mechanisms of innate immune response activation and modulation in response BKPyV.

For this we use two cellular models the human microvascular endothelial cells (HMEVC) from the bladder (bd) which respond to BKPyV by producing interferon (IFN), and the renal proximal tubular epithelial cells (RPTEC), which although do not respond to BKPyV infeciton by producing IFN, PML NBs are reorganized/modifeid during infection, the PML NB are less and larger.

To understand the role of viral replication in the IFN innate immune responses and in the PML NB remodeling induced by the virus, we designed, prepared and characterized a replication-defective BKPyV by introducing a point mutation in the LT helicase domain. The innate immunity response of cells to the mutant virus was studied by quantifying the expression of IFN-β and interferon stimulated genes and analyzing the phosphorylation of STING. In addition, the genomes of the mutant virus were visualized by fluorescence in situ hybridization and their distribution compared with the localization of PML NBs. Our results indicate that HMVECs bd can lauch moderate innate immune response to the nonreplicating virus although the response differ from the response to the wt virus. Furthermore, non-replicating BKPyV genomes associate with PML NBs, thus suggesting that replication is dispensable for interaction bwteeen PML and viral genomes. Further studies are being carried out to fully characterize the cell responses launched by both cell lines to the mutant virus.

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P-47
INTERACTOME ANALYSIS OF THE MOUSE POLYOMAVIRUS LARGE T ANTIGEN

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Polyomaviruses are small nonenveloped viruses that replicate in the nucleus of the host cell. The mouse polyomavirus (MPyV) large T antigen (LT) is the multifunctional protein expressed in the early phase of the viral infection. LT initiates viral replication, acts as an ATPase, helicase and transactivates late viral expression[1]. Polyomavirus LTs have been shown to interact with a number of cellular proteins such as the oncosuppressor pRB or p53. The interaction of cellular proteins with LT and other early viral antigens leads to reprogramming of the cell, its immortalization and transformation. The LT antigen causes genomic instability of the cell, activates DNA repair mechanisms and prolongs the cellular S-phase. This allows the virus to use host replication proteins for its own use[2]. Due to the multifunctional nature of the LT antigen, it is likely that not all LT interaction partners of mouse polyomavirus are known.

We performed screening of interaction partners of LT in mouse fibroblasts transfected with LT-expressing plasmid using mass spectrophotomether analysis. Selected hits were then confirmed in mouse fibroblasts infected with MPyV by using confical microscopy. We found co-localization of LT and several candidate interacting proteins, namely BAF57, Bag-2, MKK3, PCR1 and WDR48. We focused on the kinase MKK3, which is part of the p38 MAP kinase pathway. However, redusing of MKK3 expression by siRNA had no effect on the number of cells infected with MPyV or the amount of infectious progeny *in vitro*.

Since the members of the polyomaviridae family share most functional domains, studies of the interaction between the LT of the human Merkel cell polyomavirus and human BK polyomavirus and the proteins selected above will be investigated.

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P-48
EFFECT OF THE ADAR1 SIGNALLING PATHWAY ON HCV REPLICATION

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The hepatitis C virus (HCV) is a member of the Flaviviridae family, the genome of which consists of a +RNA molecule. It causes hepatitis C, which infects tens of millions of people worldwide. Although new direct-acting antivirals (DAAs) are highly effective in treating hepatitis C, a preventive vaccine against HCV has not yet been developed. This report examines the relationship between hepatitis C virus (HCV) and the double-stranded RNA editing enzyme adenosine deaminase 1 (ADAR1). As part of the innate immune response, ADAR1 catalyses the conversion of adenosine to inosine, which affects both the stability of the edited double-stranded RNA helix and the information encoded in the primary sequence of nucleotides.

In order to evaluate the impact of ADAR1 on HCV replication, an ADAR1 knockout cell line was generated from Huh7.5 hepatocellular carcinoma cells. The findings of preliminary experiments examining HCV replication in the Huh7.5 ADAR1 KO cell line will be presented.

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P-49
ASPH INHIBITION REVEALS DIFFERENTIAL GENE EXPRESSION PATTERNS IN HPV-POSITIVE AND HPV-NEGATIVE CELL LINES

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Cancer is associated with alterations in key cellular pathways, including the aspartate β-hydroxylase (ASPH) pathway, an oncogene upregulated in various carcinomas that plays a crucial role in tumor progression and metastasis[1]. To elucidate the mechanisms of ASPH oncogenicity, we examined the effects of ASPH inhibition in human papillomavirus (HPV)-positive (CRL-3240, HeLa) and HPV-negative (FaDu, MCF-7) cell lines. Inhibition of ASPH's catalytic activity using the small molecule inhibitor MO-I-1151 resulted in a significant reduction in proliferation, migration, and invasiveness of these tumor cells. Transcriptome analysis via bulk RNA sequencing revealed 2387 and 2350 differentially expressed genes in hypoxic and normoxic conditions, respectively, compared to controls. Notably, 1460 genes in hypoxia and 1325 in normoxia were significantly downregulated, particularly those involved in cell cycle regulation, DNA replication, and epithelial-mesenchymal transition (EMT). RT-qPCR validated the downregulation of key genes (IL7R, LY6D, LY6E, LY6K, TRIP13, SUV39H1, ELAVL2, WNT10B) in at least three cell lines. The interleukin 7 (IL7) receptor gene (IL7R) was consistently downregulated across all cell lines. IL7R, shared by the IL7 and thymic stromal lymphopoietin (TSLP) receptors, interacts with JAK1/3 and JAK1/2 through their intracellular domains[2,3]. Western blot analysis showed that MO-I-1151 or tofacitinib (a Janus kinase inhibitor) reduced key JAK/STAT pathway components, including JAK2/3, STAT5, BCL-2, MCL1, and cyclin D1 in CRL-3240 and HeLa cells, indicating that ASPH inhibition primarily targets the JAK/STAT pathway via IL7. These findings suggest the potential of targeting IL7/TSLP receptor signaling and ASPH activity as therapeutic strategies in cancer treatment.

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P-50
THE BIOMOLECULAR MARKERS FOR THE PERSONALIZED PREDICTION OF THE TREATMENT EFFECT IN PATIENTS WITH HEAD AND NECK CANCER: PILOT STUDY

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The clinical utility of liquid biopsies as diagnostic, predictive and prognostic biomarkers has been shown for several cancers. Our study analyses the dynamics of human papillomavirus (HPV) cell-free DNA (cfDNA) levels during the surveillance of patients with oropharyngeal tumours to improve patient management. Here, we present preliminary data on the evaluation of plasma collection methods, and their use in subsequent analyses of the dynamics of HPV cfDNA levels.

Plasma was collected the day before treatment and at frequent intervals during post-treatment follow-up. Two plasma collection methods were evaluated by chip electrophoresis. ctDNA was isolated from plasma and the quality of the extracted ctDNA was assessed. HPV cfDNA was amplified and tested by digital droplet PCR, which allows its absolute quantification.

Fifty-five paired samples from 20 patients were analysed. Twenty-six paired sample profiles of isolated cfDNA were compared by chip electrophoresis. The quality and quantity of HPV cfDNA varied more with the individual patient's condition than with the collection method or the time prior to sample processing. The amount of HPV cfDNA decreased after surgical treatment as well as after the start of radiotherapy.

In summary, we have confirmed the good performance of the plasma collection method, which allows easier sampling logistics for subsequent HPV cfDNA quantification. HPV cfDNA was detected in all patients and levels decreased to zero during treatment.

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P-51
THE DIFFERENCES IN VIROME FROM DISTINCT ANATOMICAL LOCATIONS IN PSORIASIS PATIENTS

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Psoriasis is a chronic inflammatory skin condition with a complex aetiology, where the role of the virome–viruses present in various biological niches remains poorly understood. This study leverages the NetoVIR protocol, an advanced viral enrichment and metagenomic sequencing method, to explore the viral communities associated with psoriasis.

Our research focuses on ten psoriasis patients, from whom we collect samples from three distinct sites: saliva, the psoriatic lesion, and non-psoriatic skin. The NetoVIR protocol allows for comprehensive virome profiling by efficiently capturing both known and novel viruses across these sites. Our primary objective is to conduct a comparative analysis of viral diversity between these sites, aiming to identify potential viral signatures or population shifts that may correlate with psoriatic pathology. Specifically, we seek to determine whether psoriatic lesions harbour a unique virome distinct from non-psoriatic skin and saliva, potentially implicating viral agents in the onset or exacerbation of psoriasis.

Preliminary findings reveal significant differences in viral communities across the three sampled sites. Notably, the virome of the psoriatic lesions exhibits distinct characteristics compared to non-psoriatic skin, while saliva presents its own unique viral profile. These findings suggest that site-specific viral populations may play a crucial role in the pathogenesis of psoriasis, opening new avenue for understanding this complex disorder and potentially informing future therapeutic strategies.

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P-52

FAECAL BACTERIOME AND METABOLOME PROFILES ASSOCIATED WITH DECREASED MUCOSAL INFLAMMATORY ACTIVITY UPON ANTI-TNF THERAPY IN PAEDIATRIC CROHN'S DISEASE

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Treatment with anti-TNFα antibodies (anti-TNF) modifies the dysbiotic faecal bacteriome in Crohn's disease (CD), but it is not yet clear whether these changes result from decreased mucosal inflammatory activity or if similar bacteriome reactions might be observed in gut-healthy subjects. Therefore, we explored faecal bacteriome and metabolome changes upon anti-TNF administration (and therapeutic response) in children with CD and compared these changes to anti-TNF-treated children with juvenile idiopathic arthritis (JIA).

Faecal samples were collected longitudinally before and during anti-TNF therapy from 111 children (CD=54, JIA=18, healthy=49). The bacteriome was analysed by massively parallel sequencing of the 16S rDNA (V4 region), and the faecal metabolome was analysed by 1H nuclear magnetic resonance. The response to treatment by mucosal healing was assessed by the MINI index three months after treatment initiation. We also tested several representative gut bacterial strains for in-vitro growth inhibition by infliximab.

The study‘s analysis revealed that in CD, bacterial community composition changed upon anti-TNF therapy, with three members of class Clostridia increasing and class Bacteroidia decreasing. Among faecal metabolites, glucose and glycerol levels increased, while isoleucine and uracil levels decreased. Some of these changes were associated with the therapeutic response (mucosal healing) post anti-TNF. No significant changes in bacteriome or metabolome were noted upon anti-TNF in JIA. Bacterial growth was not affected by infliximab in a disc diffusion test.

Our findings suggest gut mucosal healing is responsible for the bacteriome and metabolome changes observed in CD rather than any general effect of anti-TNF therapy.

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P-53

HERPES SIMPLEX VIRUS IS AN IMPORTANT LUNG PATHOGEN IN CRITICALLY ILL PATIENTS – TEN YEARS EXPERIENCE

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Herpes simplex virus (HSV) 1 and 2 are known aggressive human herpesvirus responsible especially for skin and mucous lesions and neuroinfections and rarely for organ complications (e.g. hepatitis and pneumonitis). We analysed the data from patients (pts) hospitalised at ICUs from Dept. of Anaesthesiology, Resuscitation and Intensive Care Medicine, Dept. of Pneumology and 3rd Dept. of Surgery (performing the lung transplant program) and tested for presence of HSV in lower respiratory tract samples (LRTs) and whole blood (WB) samples due to severe damage of the lung tissue.

Between 2012 and 2022, we have obtained 2682 LRTs (BALs, endotracheal tube aspirates and sputum) and 2614 WB samples from 1953 pts (735 women and 1217 men, median age at first testing was 60.4 yrs. range 0.04-94.85 yrs.). Extraction of nucleic acid was performer by Qiagen Blood Mini kits and QIAsymphony DSP DNA Mini Kit and MoBio extraction kit and Qiagen QIAamp Viral RNA Mini Kit. HSV was quantified by in house RQ-PCR assay.

HSV was detected in 461 samples (378 LRTs and 83 whole blood samples) from 281 pts (14.4%). HSV was detected in LRTs from 254 pts (18% of tested in LRTs) with median of 6,53E+04 HSV copies/ml (range 7.14E+04–1.68E+10) and in whole blood samples from 69 pts (4.7% of tested in WB; median 2.0E+03 HSV copies/ml; range 5.0E+02-3.62E+07). Proportion of positive pts varied between 2012 and 2022 (18.5%, 18.0%, 13.2%, 13.5%, 10.1%, 11.6%, 25.0%, 14.3%, 25.9% (2020), 9.7%, 12.4%).

During the years, HSV was detected in only 3.6% of the tested WB samples (range 0.6%-12.8%), but in approx. 16.6% (range 10.5%–44%) of LRTs. High HSV quantity in LRTs likely associated with HSV pneumonia (above 1.0E+05 copies/mL) was observed in 118 pts (8.36% of pts with tested LTRs) and acyclovir therapy was started; usually with good clinical response; such approach likely improved survival at the COVID-19 ICU (approx. about 20% comparing the similar units).

HSV is important lung pathogen which should be tested in ICU patients and subsequential aimed virostatic therapy likely improves the survival of such critically ill patients.

Acknowledgement
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P-54
CARBAPENEM-RESISTANT *PSEUDOMONAS AERUGINOSA* PREVAILING AMONG THE HOSPITALS IN THE CZECH REPUBLIC: THE EVOLUTION OF A REGIONAL MENACE

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Pseudomonas aeruginosa poses a significant threat to global health due to its intrinsic resistance to several antimicrobial agents and develops carbapenem resistance, most importantly via acquiring carbapenemases which have significant contribution necessitating the pivotal role of carbapenems in clinical management. The aim of this study is to investigate the prevalence of carbapenem-resistant *Pseudomonas aeruginosa* (CRPA) that exacerbates this challenge, demanding a comprehensive understanding of its genomic characteristics and clinical implications.

In this study, a non-representative collection of *P. aeruginosa* isolates was obtained from 50 hospitals across 37 cities in the Czech Republic between January and October 2022. 235 CRPA isolates identified through PCR screening, were subjected to whole genome sequencing (WGS) using Illumina. To assess the persistence of CRPA isolates, biofilm-forming potential was evaluated using a semi-quantitative microtiter plate method over three days. Concurrently, we investigated *in silico* database the prevalence of CRISPR/Cas systems as integral components of bacterial defense mechanisms. The predominant carbapenem-encoding genes identified were bla_{IMP} (47%), bla_{VIM} (36%), bla_{GES} (7%), and bla_{NDM} (9%) respectively. The genomic analysis further unveiled the presence of additional resistance genes. Diverse sequence types (ST) were observed among the isolates, highlighting the genetic heterogeneity of CRPA. Most represented STs were high-risk clones ST357 (n = 104), ST235 (n = 30), ST233 (n = 20), ST111 (n = 31), ST773 (n = 21), and ST395 (n = 9). Biofilms were measured and classified as strongly adhered. Interestingly on day three, biofilms showed the highest formation in 23% of the total isolates. 50 out of 68 representative isolates harbored CRISPR/Cas systems. Remarkably, 41 of these strains featured a type I-F system, while 5 isolates with the bla_{NDM} gene carried a type I-E system. Serum Bactericidal Assay (SBA) revealed that 28 out of 30 were resistant to serum killing after 3 hrs and only 2 were susceptible.

The findings emphasize the urgent need for continued quality monitoring, collaborative efforts, and novel research directions to mitigate the escalating morbidity and mortality associated with CRPA.

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P-55
ANALYSIS OF MICROBIAL POLYSACCHARIDES BY MALDI-TOF MASS SPECTROMETRY

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The introduction of MALDI-TOF (Matrix-Assisted Laser Desorption/Ionization Time-of-Flight) mass spectrometry (MS) has significantly changed the taxonomic identification of microbes in routine microbiological diagnostics. This technology enabled a significant reduction in the time needed to achieve taxonomic identification of bacteria and micromycetes and rapid identification of microbes from blood cultures and other clinical samples, such as urine. Despite the application of machine learning methods, subtyping to the subspecies or clone level cannot be achieved. The challenge is the identification of microbial polysaccharides, which can be used for subtyping microbes, but also the direct identification of microbes from clinical material. However, the analysis of polysaccharides is complicated by their molecular size and the inability to ionize with MALDI-TOF MS.

In our laboratory, we have developed a compound combining basic fuchsin and vanillin (see Figure 1), with which it is possible to derivatize mono- and oligosaccharides containing an aldehyde group at the end of the unit in linear form. This molecule can also be well ionized. Thus, no specific matrix for measurement is needed.

At the same time, we proposed modifications of this molecule. This research is a result of collaborative efforts, and we are excited to share our findings with the scientific community. Using MALDI-TOF MS and LC/MS (Liquid Chromatography/Mass Spectrometry), we demonstrated the ability of this molecule to bind mono-, di-, and oligosaccharides. Different serotypes of *Escherichia coli*, *Shigella* spp., and *Salmonella* spp. were chosen for the identification of bacterial polysaccharides. A procedure for extracting lipopolysaccharide from the cell wall of a bacterial cell and its acid hydrolysis was proposed, which enables effective fingerprinting of these bacteria.

Here, we present a novel method of analyzing microbes' cellular structures. This method can be used for typing and for direct identification from a clinical sample after the concentration of these structures in the clinical sample.

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The work was supported by the project National Institute of Virology and Bacteriology (Programme EXCELES, ID Project No. LX22NPO5103) – Funded by the European Union – NextGenerationEU. A national patent application Nr. PV2024-48 has been acknowledged.

P-56
MIMICKING VIRAL EVOLUTION BY YEAST SURFACE DISPLAY TO PREDICT NEW CORONAVIRUS-RELATED PANDEMICS

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SARS-CoV-2, the causative agent of the COVID-19 pandemic, emerged in Wuhan (China) at the end of December 2019. Since then, the virus has evolved profoundly with many variants. Although SARS-CoV-2 has posed major health and economic challenges, its control has clearly improved during the more than four years of the pandemic. However, the chance that a new coronavirus, with relatively similar characteristics to the SARS-CoV-2, emerges in the future remains high. For this reason, we have selected four coronaviruses closely related to SARS-CoV-2 (BANAL-52, BANAL-236, Guangdong-1 and GX-P5L) as well as the SARS-CoV-1, which caused several outbreaks between 2002 and 2004 in the human population. As both SARS-CoVs, the first three of these coronaviruses were found in bats, its most plausible original host, whereas GX-P5L was detected in pangolins, a suspected intermediate host. All these coronaviruses interact with the angiotensin-converting enzyme 2 (ACE2), the cell host receptor, through the receptor binding domain (RBD) of their spike (S) proteins. For this reason, RBDs were expressed in the yeast surface using a yeast surface display technique and, with the help of flow cytometry (FC), the affinities between the RBDs of these other five coronaviruses and the ACE2 receptor were estimated and compared with the one of SARS-CoV-2 RBD. Furthermore, five consecutive rounds of error-prone mutagenesis to generate libraries and select the highest affinity clones by fluorescent-activated cell sorting (FACS) were conducted. Finally, we used Sanger and Next-Generation Sequencing to reveal these increasing affinity RBD mutations, which should be taken into consideration in case these coronaviruses infect humans in the future.

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PALACKÝ UNIVERSITY OLOMOUC

L-27
NEW DIAGNOSTIC APPROACHES IN HPV ASSOCIATED DISESASES

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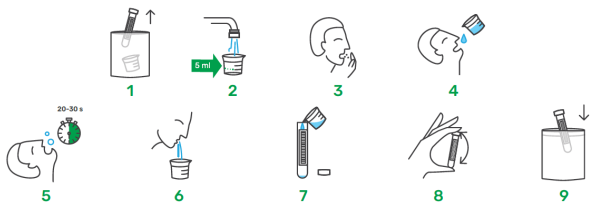
Human papillomavirus (HPV) is the most common sexually transmitted infection globally, with over 100 identified types. High-risk HPV is a significant causal factor in the development of several cancers, most notably cervical and oropharyngeal cancer. While the incidence of cervical cancer is declining in developed countries[1], the incidence of HPV-driven oropharyngeal cancer is on the rise[2].

Cervical cancer is nearly entirely preventable through vaccination and regular screening. In the Czech Republic, as with global trends, the incidence of cervical cancer is decreasing[3]. However, further reductions could be achieved by introducing primary HPV-based cervical cancer screening, offering self-sampling options to non-attendees, and increasing vaccination coverage.

In contrast to cervical cancer, the incidence of HPV-associated oropharyngeal cancer is highest in high-income countries, and no screening programs are currently available[4]. In several countries, including the UK and the USA, the incidence of HPV-associated oropharyngeal cancer has already surpassed that of cervical cancer. Although specific data attributing the proportion of oropharyngeal cancer cases to HPV in the Czech Republic is lacking, publicly accessible data on malignant tumor epidemiology suggests that in 2021, the overall incidence of oropharyngeal cancer exceeded that of cervical cancer [3]. The introduction of a screening program akin to cervical cancer screening would greatly enhance early detection of HPV- associated oropharyngeal cancer.



Scheme 1. **Schematic illustration of gargle lavage sampling procedure.** (1) Gargle lavage sampling set, (2) adding of 5 ml of tap water to the cup, (3) coughing, (4) pouring water into the mounth, (5) gargling for 30 seconds, (6) spitting of sampled water to the cup, (7) spilling to the tube, (8) shaking of the tube, (9) sending to the laboratory.

We have recently demonstrated that self-sampling (both cervicovaginal and oral) is highly accepted by the Czech population[5,6], and this method shows great promise for the prevention of both cervical and oropharyngeal cancers. Cervicovaginal self-sampling has already been integrated into cervical cancer screening programs in several countries[7]. Similarly, oral sampling/self-sampling appears to be a promising approach for oropharyngeal cancer screening. To advance this, we have developed a gargle lavage sampling kit (scheme 1) and optimized an HPV PCR detection method, along with a triage test for individuals who test positive. This workflow could be effectively utilized for HPV-driven oropharyngeal cancer screening.

Acknowledgement
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L-28
THE PREVALENCE OF ANTIBIOTIC RESISTANCE GENES AMONG POULTRY CHICKEN POPULATION

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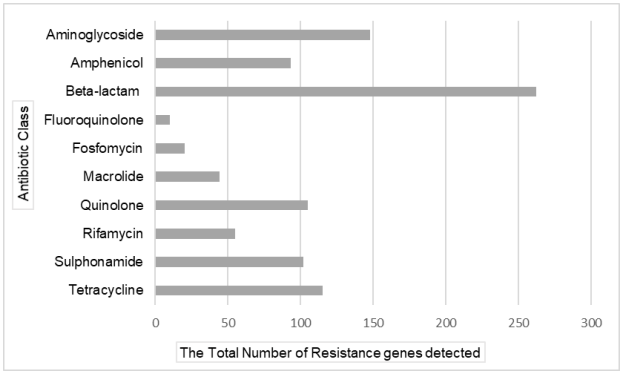
Antibiotics are widely used worldwide in poultry processing to improve production. It was proved that this action leads to higher resistance in chicken and human isolates. During the last decade, more antibiotic resistance was detected in the food industry[1]. There are many known ways of bacterial antibiotic resistance and specific enzyme production in one of them. The significant and crucial enzymes are beta-lactamases, especially ESBL (extended-spectrum beta-lactamases). They can hydrolyze penicillins, broad-spectrum cephalosporins, and monobactams. These enzymes are generally derived from CTX-M-, SHV- and TEM-type enzymes[2]. However, it is not only enzymatic inactivation by beta-lactamases but also changes in cell permeability, alterations in antibiotic binding sites, efflux pump overexpression, and significant changes in metabolic pathways[3].

This study analyzed one hundred forty-seven isolates of *Enterobacterales* and other gram-negative non-fermenting bacteria of animal origin resistant to cefotaxime, ceftazidime, or meropenem. Twenty-five bacterial species were identified among the samples. Isolates were collected in Moravian poultry farms. The PCR amplification and WGS (whole genome sequencing) were used for screening and sample characterization. The post-sequence analysis was performed using Geneious Prime software[4].



Graph 1. **The total number of resistance genes detected in examined isolates sorted by antibiotic class**

The most numerous group of resistance genes was beta-lactamase (*bla*) genes. We identified more than 49 beta-lactamase variants. They contained various combinations of AmpC variants, ESBL, and oxacillinases. The new variants of *blaL1*, *blaHugA-like*, *blaMUS-like*, and *blaPST* genes were detected. Furthermore, thirty-three putative genes encoding new unknown beta-lactamases were found. Besides beta-lactamases, we also detected aminoglycoside-modifying enzymes, chloramphenicol acetyltransferase, methylases, and *sul* and *tet* genes. Our genome analyses approved the co-occurrence of these resistance genes, confirming a multi-drug resistance profile to beta-lactams tetracyclines, aminoglycosides, macrolides, and sulfonamides.

The study aimed to determine the total occurrence of antibiotic resistance genes in bacteria from poultry farm. The still-growing antibiotic resistance in bacteria is a hospital problem that spreads among animals on farms and slaughterhouses. We assume that poultry farms could be a potential reservoir of ESBL-producing gram-negative bacteria in many countries[5]. The current epidemiology of beta-lactamase-producing bacteria in veterinary medicine has yet to be fully understood, as data on the presence and distribution of these genes are lacking, so continuous monitoring is essential. In addition, our results showed a match between PCR detection and WGS regarding the types of demonstrated beta-lactamases. Thanks to WGS, we could detect other resistance mechanisms unrelated to beta-lactamases.

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P-57
PET/CT IMAGING OF BORDETELLA PERTUSSIS INFECTION

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The interaction of *B. pertusis* (*BP*) with its human host results in inflammation, activation of the immune response and damage to host tissues. (1, 2) The use of siderophores and the haem utilisation systems of *BP* are genetically characterised and share key regulatory features. We investigated the imaging of *BP* by positron emission tomography (PET) using radiolabelled siderophore [^{68}Ga]Ga-desferrioxamine-B (DFO-B). We monitored *BP* infection in a mouse muscle model.

DFO-B was labelled with gallium-68. The radiochemical purity of complex (> 95%) was measured on RP-HPLC. We study *BP* – Wild type (WT), ΔbfeA (a mutant with deleted ferric-enterobactin receptor gene), ΔfauA (deleted alcaligin receptor gene), ΔbhuR (deleted heme receptor gene) and combination ΔbfeA+ΔfauA, ΔbfeA+ΔfauA+ΔbhuR. *In vitro* uptake of [^{68}Ga]Ga-DFO-B was evaluated in all pathogens, which were cultivated in Stainer-Scholte medium with - where is significantly reduced absorption of the ^{68}Ga-siderophore - and without ferrous sulfate heptahydrate. The heat-inactivated bacterial cultures displayed also diminished uptake of ^{68}Ga-siderophore. *In vivo* PET/CT imaging was performer 45 min after [^{68}Ga]Ga-DFO-B injection in *BP* infected Balb/c mice and 5 h post-infection. The infected left hind legs (WT) showed a radioactivity accumulation after injection. Right hind legs of infected mice injected with mutants and their combination showed also uptake of [^{68}Ga]Ga-DFO-B.

The [^{68}Ga]Ga-DFO-B.can be used for detection and monitoring of infections caused by *BP* and its mutants.

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P-58
EPIDEMIOLOGICAL AND GENETIC ANALYSIS OF METHICILLIN-RESISTANT *STAPHYLOCOCCUS AUREUS* IN UNIVERSITY HOSPITAL OLOMOUC

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Methicillin-resistant *Staphylococcus aureus* (MRSA) strains represent a significant medical challenge in terms of limited therapeutic options, increased morbidity and mortality, and elevated economic costs associated with treatment.

This study aims to conduct an epidemiological and genetic analysis of MRSA in the University Hospital Olomouc (UHO) for the period 2022–2023.

From September 1, 2022, to December 31, 2023, MRSA strains were collected from patients at UHO. Standard microbiological procedures were used to identify and confirm the MRSA phenotype. One MRSA isolate per patient was included in the study. Positive results were confirmed by the detection of the *mecA* gene. Selected virulence factors were identified using PCR methods. Molecular biological typing was performed on chosen isolates to determine strain clonality using pulsed-field gel electrophoresis (PFGE).

A total of 66 MRSA strains were isolated from patients at UHO. MRSA strains were predominantly isolated from the Third Department of Internal Medicine and the Department of Dermatology and Venereology. Most MRSA strains were recovered from wounds and upper respiratory tract samples. Antibiotic resistance of MRSA strains was as follows: erythromycin 91%, clindamycin 89%, ciprofloxacin 71%, co-trimoxazole 5%, vancomycin 0%, ceftaroline 0%, linezolid 0%, and tigecycline 0%. The presence of virulence factor genes and similarity comparison of chosen isolates will be presented in the poster.

Monitoring epidemiological trends and implementing effective control measures are crucial for limiting the spread of this serious resistance phenotype.

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P-59
DETECTION OF *MCR* GENES AND BETA-LACTAMASES IN ENTEROBACTERIA

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As early as 1945, Alexander Fleming warned of the inevitable emergence of antibiotic resistance, and this prediction proved accurate. The most feared pathogens in hospital settings are *Enterobacteriaceae*, *Pseudomonas* and *Acinetobacter*. These pathogens have developed resistance to carbapenems, beta-lactams and polymyxins, whose resistance genes are often carried together on plasmids, allowing horizontal spread of resistance across bacterial populations.

Mobile colistin resistance is of particular concern because colistin is becoming an increasingly crucial last line of defence against fatal infections caused by multidrug-resistant organisms.

This study detected *mcr* genes in 70 clinical isolates by PCR using 11 specific primer pairs designed in the bioinformatics program Geneious Prime. Based on WGS data, the overall prevalence of functional *mcr* genes in the clinical isolates studied was 10%. The *mcr-1*, *mcr-9* and *mcr-10* genes were detected with a prevalence of 1.4%, 2.9% and 5.7%, respectively. Furthermore, *in silico* analysis of the presence of beta-lactamases was performed in 70 sequenced clinical samples. All isolates contained at least one beta-lactamase, the most frequent being ACT (45.7%), MIR (27.1%), SHV (20%) and TEM (15.7%). In addition, a phylogenetic tree was constructed, and point mutation studies were performed on individual MCR enzymes to identify evolutionarily active sites.

Future measures should consider systematically monitoring colistin-resistant bacteria producing MCR enzymes in hospitals, farms, food and the environment.

Acknowledgement
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L-29
AVIAN INTERFERON REGULATORY FACTORS (ALMOST) RESOLVED

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Interferon regulatory factors (IRFs) are transcription factors that play a key role in anti-viral immunity response of vertebrates. After viral RNA is recognized by RIG-I-like receptors (RLR), IRF3 and IRF7 activate expression of IFN-β which is together with other type I interferons responsible for forming ISGF3 complex (formed by STAT1, STAT2 and IRF9). IRF9 protein has two domains, IRF-associated domain (IAD) that binds to STAT2 and DNA binding domain (DBD) that binds in the nucleus with interferon-stimulated response element (ISRE) in promotor of interferon-stimulated genes (ISGs) and activates their expression[1,2]. These pathways are well described in mammals and some other vertebrates but there is limited information available in birds. Moreover, IRF9 and IRF3 are thought to be missing in birds[3] even though both of the pathways are functional.

Using *in silico* comparative analysis and de novo assembly, we were able to annotate IRF3 in paleognath birds and IRF9 in multiple avian species across most avian orders that has conserved both of the important protein domains. We used duck fibroblast cell line as a model system for characterization of avian IRF9. We investigated the role of duck IRF9 (dIRF9) in type I IFN pathway and its role in regulation of ISGs expression after interferon stimulation. Using CRISPR-Cas9 we created dIRF9 knock out cell line. Our results show decrease in the expression of ISGs in the absence of dIRF9 that is partially restored after transfection with endogenous dIRF9 confirming importance of IRF9 in inducing avian ISGs expression following IFN stimulation. We also tested the effect of dIRF9 absence in protection against cytopathic effect of vesicular stomatitis virus (VSV). We observed decreased cytopathic effect inhibition in KO cell line after treatment with duck type I IFN and infection with VSV. We also showed that activation of the avian IFN pathway is consensus ISRE dependent by luciferase assay and both IRF9 and consensus sequence of ISRE is needed. Finally, we made ISGF3 protein complex prediction to verify binding of dIRF9 to dSTAT2 and ISRE.

Interestingly we were not able to annotate IRF9 in chicken or other galliform birds. Using chicken DF1 cells and tagged chicken STAT2 cloned in an expression plasmid we were able to determine its possible interactors after interferon stimulation using mass spectometry. According to the results of mass spectometry we then investigated a possible IRF9 substitutor in chicken. We were able to predict a correct structure of ISGF3 complex in chicken using Alphafold and our preliminary experimental results suggest that we indeed possibly found the missing protein of chicken ISGF3 complex.

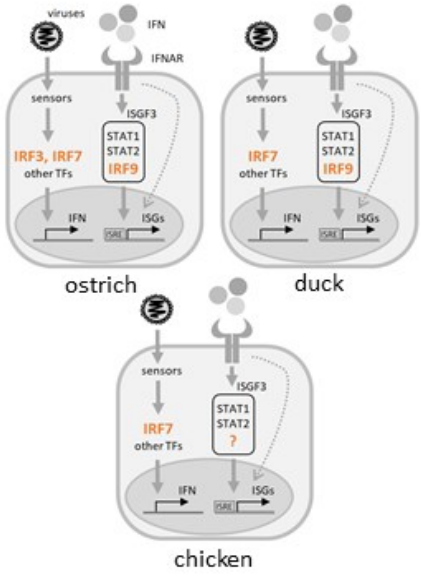

Fig. 1. **Schematic depiction of IFN induction and signaling in three avian species**

Acknowledgement
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L-30
IN SITU T-CELL RESPONSES TO INFLUENZA AND BORDETELLA PERTUSSIS INFECTIONS

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The upper respiratory tract (URT) is a site of entry for many public health-concerning pathogens such as SARS-CoV-2 (ref.[1]), the Influenza virus[2], or Bordetella pertussis[3]. Although the URT represents an important site for the initiation and transmission of the infection, understanding the site-specific immunity in the nasal tissue during different infections is limited. So far, most works focused on respiratory infections have been concerned only with the lower respiratory tract (LRT)[2]. The adaptive immune system of URT consists of nasal-associated lymphoid tissue, which is a highly organized lymphoid structure with T and B cell areas and dispersed Tissue-resident memory T cells (Trm). Trm cells are a subset of memory T cells, which reside in non-lymphoid tissues, where they can act as alarm sensors in the immune surveillance network or as cytotoxic cells. Due to their advantageous location, they can be part of the first line of defense against many infections. The mechanism of development and the diversity and function of Trm cells are not yet completely understood[4].

We hypothesize that different infections such as intracellular viral infection or extracellular bacterial infection give rise to phenotypically and functionally distinct $CD4^+$ and $CD8^+$ T-cell subsets. To address this hypothesis we utilized two murine infection models: viral infection (Influenza A) and bacterial infection (Bordetella pertussis). Samples from murine URT and LRT were analyzed by flow cytometry and T-cells were sorted for single-cell RNA sequencing (Fig. 1).

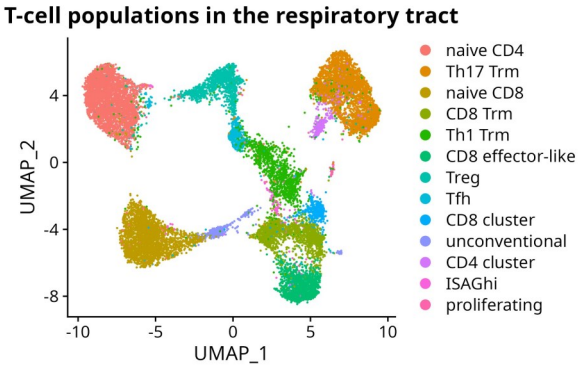

Fig. 1. **Uniform Manifold Approximation and Projection for Dimension Reduction (UMAP) analysis of single-cell RNA-seq samples from murine upper and lower respiratory tract isolated 45 days after Influenza or Bordetella pretussis infections**

We observed striking differences between both $CD4^+$ and $CD8^+$ T cell subsets after the infections. Particularly, we identified a unique subset of $CD8^+$ T cells arising after Bordetella pertussis infection. These cells were highly clonally expanded and obtained an atypical phenotype characterized by the expression of Granzyme K, transcription factor Eomes, checkpoint-inhibition receptors such as PD-1 or TIGIT as well as the IL-10 receptor. The origin, function, and specificity of these cells will be studied further.

Acknowledgement
The work was supported by the project National Institute of Virology and Bacteriology (Programme EXCELES, ID Project No. LX22NPO5103) – Funded by the European Union – NextGenerationEU.

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P-60
THE INTERPLAY OF RETROVIRAL PROTEINS FACILITATING CELL-CELL FUSION IN THE HUMAN PLACENTA

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The placenta is a transient yet crucial organ that facilitates the exchange of nutrients, gases, and waste products between the mother and the developing fetus. Central to this vital function is the multinucleated syncytiotrophoblast layer, whose formation depends on the expression of genes derived from ancient retroviruses. These genes include human *syncytin-1*, which encodes retroviral envelope glycoprotein (ENV) with a novel physiological function. Interaction between Syncytin-1 and its membrane receptor ASCT2 triggers the fusion of cytotrophoblast cells, forming the syncytiotrophoblast layer[1].

Additionally, the interaction between Syncytin-1 and ASCT2 is modulated by another placenta-specific, humanized ENV called Suppressyn. In contrast to Syncytin-1, Suppressyn is a soluble, secreted protein that lacks fusogenic properties, yet it binds ASCT2 as a receptor. Suppressyn has been shown to inhibit the Syncytin-1/ASCT2 interaction *in vitro*, suggesting a potential role in controlling cell-cell fusion in the human placenta[2]. Furthermore, Suppressyn has also been observed to block infection by other retroviruses that utilize ASCT2 as a receptor[3]. However, the precise role of Suppressyn in syncytiotrophoblast formation and the molecular mechanisms underlying its inhibitory effects remain unclear.

To elucidate the inhibitory mechanism of Suppressyn, we established a quantitative fusion assay in the HEK293T model. We have created stable cell lines expressing split fluorescent and split luciferase reporters, enabling us to measure cell-cell fusion levels through microscopy imaging and luminescence quantification. We also integrated a receptor-downregulation assay based on the luciferase activity to verify if Suppressyn inhibits cell fusion through competitive binding or ASCT2 downregulation. Using these quantitative assays, we want to investigate the complex interplay between ENV proteins and their receptors and their combined impact on cell-cell fusion in the human placenta.

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P-61
BREAKING THE DOGMA OF SYNCYTIN-1 RECEPTOR DUALISM

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Syncytin-1 is a physiologically important protein involved in the development of the human placenta. Expressed in cytotrophoblasts, Syncytin-1 facilitates the fusion of cellular membranes, leading to the formation of the syncytiotrophoblast, a semi-permeable layer that separates the embryonic and maternal bloodstreams. This fusogenic function is linked to Syncytin-1's origin as an envelope protein of an ancient retrovirus. Originally exposed on the viral membrane, Syncytin-1 attached to specific cellular receptors and mediated the fusion of viral and cellular membranes. This enabled the integration of the retroviral genome, including the *syncytin-1* gene, into the genome of our ancestors. Since then, Syncytin-1's capacity for membrane fusion has been further improved but still depends on priming by cellular receptors.

Two amino acid transporters, ASCT1 and ASCT2, are widely accepted as receptors for Syncytin-1[1,2]. Both are multi-membrane-spanning proteins with a trimeric organization. These complex properties make them challenging subjects for molecular biology studies, which are essential for a proper understanding of physiological and pathological processes in the human placenta.

Using a finely-tuned *in vitro* model system based on ASCT1/2 double-knockout clones, we observed that ectopically coexpressed ASCT1 and ASCT2 form heterotrimers. These heterotrimeric complexes are capable of priming Syncytin-1 for fusion. However, when expressed separately, only ASCT2 functions as a receptor for Syncytin-1, while ASCT1 alone lacks this ability, as confirmed by sensitive fluorescent and luminescent assays. After thorough verification of correct ectopic ASCT1 expression, localization, and transport function, we conclude that the only functional receptor for Syncytin-1 in the human placenta is ASCT2, but not ASCT1.

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P-62
LONG-TERM STABLE GENE EXPRESSION IN LAMINA-ASSOCIATED DOMAINS THROUGH TARGETED GENE DELIVERY

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Precise gene insertion into selected genomic loci is a key goal of genetic engineering, crucial for ensuring controlled and predictable gene expression. Traditionally, gene delivery has been achieved using retroviruses, which integrate into the host genome in a semi-random manner, often influenced by the local chromatin environment. While this approach can lead to efficient gene integration, the random nature of insertion can result in variable gene expression and potential disruptions to host gene function. In contrast, the CRISPR-Cas9 system offers a more precise method for gene delivery, allowing for targeted integration at specific genomic sites.

Our previous studies on various retroviruses revealed that different retroviruses exhibit varying sensitivities to the site of integration within the genome. Recently, we discovered that gammaretroviruses are only partially sensitive to the epigenomic environment and are capable of driving long-term gene expression across diverse epigenomic contexts, including within lamina-associated domains (LADs) —regions of the genome considered to form a repressive environment for gene transcription.

In this study, we aimed to achieve long-term stable gene expression following targeted gene insertion into LADs. To this end, we inserted gene cassettes equipped with gammaretroviral promoters via homology-directed repair guided by CRISPR-Cas9. We present evidence of successful gene delivery into LADs and demonstrate long-term stable gene expression post-insertion. Our findings show that, despite the traditionally repressive nature of LADs, stable and consistent gene expression can be maintained over extended periods. This challenges the conventional view of LADs as strictly repressive regions and highlights their potential as safe landing pads for therapeutic gene delivery.

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P-63
IDEIS: A TOOL TO IDENTIFY PTPRC/CD45 ISOFORMS FROM SINGLE-CELL TRANSCRIPTOMIC DATA

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Recently, robust single-cell RNA sequencing (scRNAseq) methods have emerged to study the gene expression at the single-cell and have soon become a broadly used tool in life sciences including immunology. Since then the number of specialized scRNAseq protocols has expanded and started to involve additional layers such as analysis of chromatin accessibility. However, a typical employed scRNAseq analysis pipelines quantify only the abundance of particular transcripts, without accounting for alternative splicing. In immunology, CD45, encoded by the *PTPRC* gene, is a well-established pan-leucocyte marker presenting alternative splicing variants, or isoforms, that define different immune cell subsets. While some of the isoforms can be detected in scRNAseq experiments by using isotype-specific DNA oligo-tagged anti-CD45 antibodies, such approach requires generation of an additional DNA library.

Here we present IDEIS, a user-friendly software that takes transcriptomic data from scRNAseq as an input and uses them to quantify CD45 isoforms. We demonstrate that software accurately quantifies canonical human CD45 isoforms in data sets generated by 10X Genomics 5' sequencing assays. Additionally, we validated the usage of IDEIS on murine data, revealing the specificity of *Ptprc* splicing pattern for murine leucocyte subsets.

Overall, IDEIS is a useful tool that might provide an alternative solution to approaches using anti-CD45 antibodies and allows to re-analyze *PTPRC* splicing pattern in already sequenced libraries.

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P-64

SINGLE-CELL ATLAS OF CD8+ T CELL DIFFERENTIATION REVEALS KEY PATHWAYS REGULATING EFFECTOR AND MEMORY CELL FORMATION AND UNVEILS CRITICAL IMPORTANCE OF TGF-B

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Upon activation, naive $CD8^+$ T cells differentiate into short-lived effector cells (SLECs), which provide immediate protection against infections, and long-lived memory cells, which retain immunological information long after pathogen clearance. Despite the crucial roles of these subsets in the development of effective anti-cancer therapies and vaccines, the molecular mechanisms driving the commitment to either SLEC or memory cell fates remain incompletely understood.

To address this gap, we generated a comprehensive single-cell multiomics atlas of $CD8^+$ T cells, profiling them across various steady-state and infection conditions, including multiple pathogens and time points. This resource enabled us to characterize the developmental trajectories of effector and memory cells from the earliest divisions following activation, and to identify key regulatory factors, such as *Nr4a3, Myc, Odc1, Satb1,* and *Zbtb20*. Targeted CRISPR knockouts of these genes in naive $CD8^+$ T cells showed that their absence significantly influences the differentiation towards either SLEC or memory cells *in vivo*. Furthermore, parallel single-cell RNA and ATAC sequencing from the first division post-activation uncovered a critical role for TGF-β signaling in SLEC formation. Consistent with this, knockout of TGF-β receptor subunits or downstream signaling components resulted in an increased propensity for memory cell formation *in vivo*.

Collectively, our findings demonstrate that the fate determination of $CD8^+$ T cells into effector versus memory lineages is established early after activation, is TGF-β-dependent, and can be modulated through targeted genetic interventions.

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P-65
ARE5 ABCF PROTEINS OF STREPTOMYCES COELICOLOR DECODE THE HORMETIC EFFECT OF LS_AP ANTIBIOTICS

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Antibiotics are essential for the treatment of bacterial infections as they suppress their growth. However, in low concentrations, antibiotics also act as signaling molecules that trigger various cellular responses *via* mechanisms that are not yet well understood. The catalytic center of the bacterial ribosome is targeted by many classes of clinically important antibiotics. ABCF proteins, a family of ribosome-binding ATPases, confer resistance to these antibiotics by rescuing stalled ribosomes. Our research has shown that ABCF proteins can also fine-tune gene expression to ensure bacterial adaptation under stress conditions[1].

The present study focuses on two closely related ABCF proteins from the ARE5 subfamily encoded in soil-dwelling *Streptomyces coelicolor*. Functional analysis revealed that they play different roles in decoding antibiotic signaling at the ribosome. The expression of both proteins is regulated by ribosome-mediated attenuation in response to antibiotics from the lincosamide, streptogramin A and pleuromutilin (LSaP) groups, which inhibit the early steps of bacterial translation. Both mediate the initiation of actinorhodin production in response to the presence of these antibiotics in the environment, but each protein responds to a different concentration range and type of LSaP antibiotics, making their expression mutually exclusive. Overall, our study has revealed a delicate interplay between two ABCF proteins, reflecting the hormetic effect of antibiotics that enables an adaptive response at low antibiotic concentrations and survival at high, inhibitory concentrations.

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