National Institute of Virology and Bacteriology

NIVB MEETING 2025

4th – 7th NOVEMBER 2025

Kutná Hora, Czech Republic

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

UNIVERSITY OF CHEMISTRY AND TECHNOLOGY PRAGUE

IOCB PRAGUE

MUNI

IMG

Palacký University Olomouc

MBU

BIOLOGY CENTRE ASCR

CHARLES UNIVERSITY

The project National Institute of Virology and Bacteriology (Programme EXCELES, ID Project No. LX22NPO5103) –Funded by the European Union – Next Generation EU.

Funded by the European Union NextGenerationEU

CZECH RECOVERY PLAN

MINISTRY OF EDUCATION, YOUTH AND SPORTS

Dear Colleagues in Virology and Bacteriology,

It is our pleasure to welcome you to the NIVB Meeting 2025, the fourth and final annual meeting of the National Institute of Virology and Bacteriology (NIVB), taking place once again in the historic town of Kutná Hora from 4th to 7th November 2025. This meeting marks not only an opportunity to reconnect with our colleagues, exchange ideas, and showcase scientific achievements, but also the culmination of a highly successful multi-year project.

Over the past four years, thanks to the support of the Czech Recovery Plan and the dedication of our teams, the NIVB has achieved remarkable results. Our 30 research teams from 8 Czech institutions have published numerous high-impact papers, organized workshops, and established strong inter-institutional collaborations. These achievements reflect the power of collective effort and the growing synergy within our scientific community.

The primary mission of the NIVB has been to enhance collaboration across disciplines and regions, providing a communication platform for sharing knowledge, fostering cooperation, and addressing key challenges in virology and bacteriology. This final meeting is a moment to reflect on the lasting impact of the NIVB project.

„We thank all participants for their contributions, including 30 oral presentations and 57 posters, highlighting the breadth and depth of research within the NIVB network. We hope the collaborations and networks fostered through the NIVB will continue to inspire scientific excellence in the years to come.

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

INSTITUTE OF ORGANIC CHEMISTRY AND BIOCHEMISTRY OF THE CAS

L-01
SYNTHESIS AND ANTIBACTERIAL ACTIVITY OF THE SECOND GENERATION LEGO-LPPO

VIKTOR MOJR[a], IVANA KÓŠIOVÁ[a], NITJAWAN SAHATSAPAN[a] , KATEŘINA BOGDANOVÁ[b], RENATA VEČEŘOVÁ[b], MILAN KOLÁŘ[b], DOMINIK REJMAN[a]*

[a] Institute of Organic Chemistry and Biochemistry, Czech Academy of Sciences v. v. i., Flemingovo nám. 2, 166 10 Prague 6, [b] Department of Microbiology, Faculty of Medicine and Dentistry, Palacký University Olomouc, Hněvotínská 3, 775 15 Olomouc, Czech Republic
rejman@uochb.cas.cz

Bacterial pathogens resistant to antibiotics (ATB) are becoming an increasingly serious global problem. Current ATB targeting bacterial biosynthetic processes are facing emerging resistant strains. An attractive target for the development of antibacterial compounds is the cytoplasmic membrane as the composition of bacterial and mammalian cell membranes differs, resulting in different biophysical properties[1]. In contrast to majority of classical antibiotics requiring metabolically active bacterial cells, membrane targeting antimicrobials are capable of also killing persistent (dormant) bacteria. Antimicrobial peptides (AMP) and host defense peptides (HDP) are examples of membrane-active compounds that represent the first line of defense in many multicellular organisms and possess a broad range of biological activities. However, their clinical usage is limited by their *in vivo* toxici-ty, stability, limited bioavailability, and large production costs.

We are developing lipophosphonoxins (LPPO) – antimicrobial compounds belonging to the class of small molecule membrane targeting agents (SMMTA, Figure 1). Their general structure consists of four modules: a nucleoside module (NM), a polar module (PM), a hydrophobic module (HM), and a phos-phonate connector module (CM). The first-generation LPPO (LPPO I)[2] demonstrated excellent bactericidal activity against various Gram-positive species. We have shown that at their bactericidal concentrations, LPPO act via the disruption of the cytoplasmic membrane[2]. The second-generation LPPO (LPPO II) have redesigned PM to bear more polar charges. LPPO II increased their efficacy to Gram-positive and extended bacteri-cidal activity to Gram-negative bacteria[3]. Their antibacterial activity, however, is abolished in the presence of serum albumins.

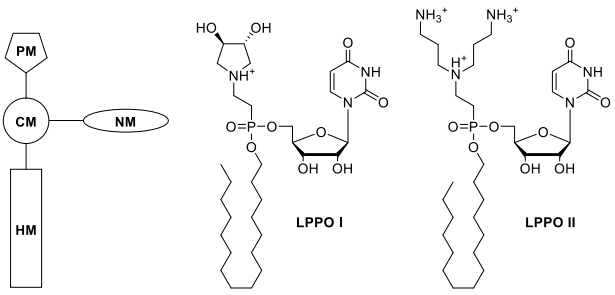


Fig. 1. **Schematic modular structure of LLPO (left) and examples of first two generations LPPO (right)**

By introducing LEGO-LPPO (Linker-Evolved-Group-Optimized-LPPO, Figure 2), modular configuration was modified to include two LPPO units symmetrically attached by CM at the ends of linker module (LM), NM was omitted. Redesign of molecular skeleton brought improvements in antimicrobial activity and eliminated interactions with serum albumins[4]. Because of chiral nature of phosphorus atoms in the connector modules the first generation LEGO-LPPO molecules are isolated as a mixture of three diastereoisomers. Here we present design and synthesis of new generation LEGO-LPPO-II based on connector modules with defined chirality.

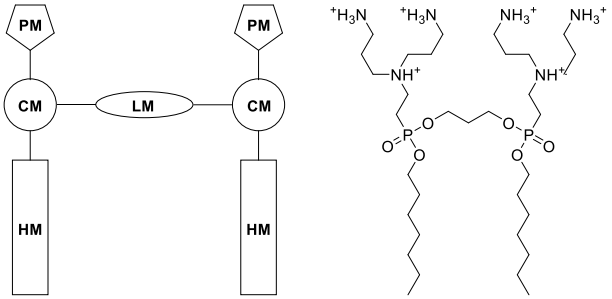


Fig. 2. **Schematic modular structure of LEGO-LPPO (left), example of LEGO-LPPO (right)**

In LEGO-LPPO-II), phosphonate-based CM of LEGO-LPPO I was replaced by glycerol moiety and the synthesis was planned from enantiomerically pure starting materials using enantiospecific reactions to obtain final products with defined chirality. These new SMMTA were prepared in both configura-tions and testing of their antibacterial and hemolytic activities and cytotoxicities are currently under way.

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 Czech Health Research Council (NW24-08-00073).

REFERENCES

1. Ghosh C., Haldar J. ChemMedChem, *10*, 1606 (2015).
2. Panova N., Zborníková E., Šimák O., Pohl R., Kolář M., Bogdanová K., Večeřová R., Seydlová G., Fišer R., Hadra-vová R., Šanderová H., Vítovská D., Šiková M., Látal T., Lovecká P., Barvík I., Krasný L., Rejman D. PLoS One *10*, No. e0145918 (2015).
3. Seydlová G., Pohl R., Zborníková E., Ehn M., Šimák O., Panova N., Kolář M., Bogdanová K., Večeřová R., Fišer R., Šanderová H., Vítovská D., Sudzinová P., Pospíšil J., Benada O., Křížek T., Sedlák D., Bartůněk P., Krasný L., Rejman D. J. Med. Chem. *60*, 6098 (2017).
4. Do Pham D. D., Mojr V., Helusová M., Mikušová G., Pohl R., Dávidová E., Šanderová H., Vítovská D., Bogdanová K., Večeřová R., Htoutou Sedláková M., Fišer R., Sudzinová P., Pospíšil J., Benada O., Křížek T., Galandáková A., Kolář M., Krásný L., Rejman D. J. Med. Chem. *65*, 10045 (2022).

L-02
STRUCTURAL BASIS FOR ALLOSTERIC REGULATION OF MYCOBACTERIAL GUANOSINE 5′-MONOPHOSPHATE REDUCTASE BY ATP AND GTP

MICHAL DOLEŽAL, ZDENĚK KNEJZLÍK, TOMÁŠ KOUBA, ANATOLIJ FILIMONĚNKO, HANA ŠVÁCHOVÁ, MARTIN KLÍMA, IVA PICHOVÁ

Institute of Organic Chemistry and Biochemistry of the Czech Academy of Sciences, Flemingovo náměstí 2, 166 10 Prague, Czech Republic
michal.dolezal@uochb.cas.cz

GMP reductase (GMPR) catalyzes NADPH-dependent conversion of GMP to IMP, a key metabolite in the biosynthesis of all purine nucleotides. This reaction allows mycobacteria and most other organisms to utilize guanine nucleotides for the production of adenine 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 tuberculosis in humans.

In a previously published study[1], we demonstrated 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 enzymatic activity.

Here, we combine X-ray crystallography, cryo-electron microscopy, and biochemical binding assays to elucidate the molecular basis of MsmGMPR regulation by ATP and GTP[2]. *Msm*GMPR forms tetramers with four-fold axis which further assemble into octamers with D4 symmetry. The two tetramers in the octamer adopt either compressed or extended conformation. ATP and GTP compete for a binding site located at the interface of the two tetramers. We show that ATP stabilizes a compressed conformation that inhibits the enzyme by restricting access to the active site and preventing NADPH binding. In contrast, GTP counteracts ATP binding, promoting an active conformation that enables catalysis.

Our results provide insight into how *Msm*GMPR senses and responds to the cellular purine nucleotide balance, revealing a novel mode of allosteric regulation by a CBS domain.

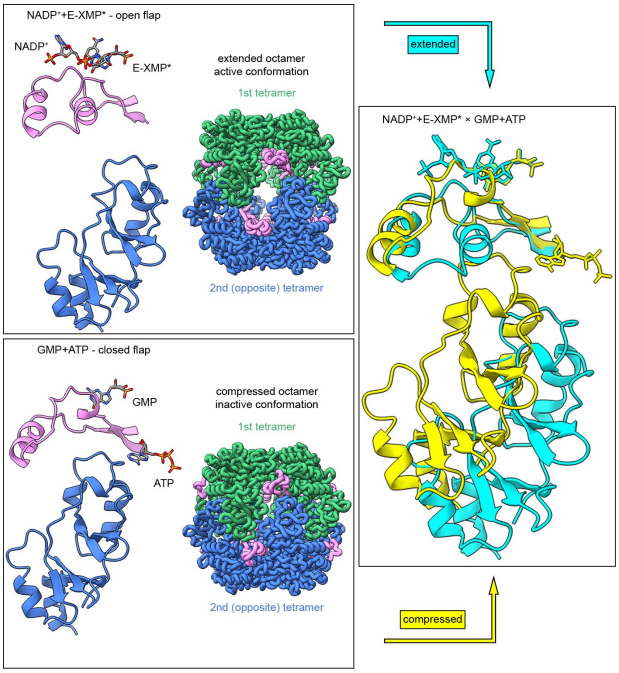


Fig. 1. **Conformational changes in the flap region of *Msm*GMPR induced by ligand binding**

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.

REFERENCES

1. Knejzlík Z., Doležal M., Herkommerová K., Clarova K., Klíma M., Dedola M., Zborníková E., Rejman D., Pichová I.: FEBS J. *289*, 5571 (2022).
2. Doležal M., Knejzlík Z., Kouba T., Filimoněnko A., Šváchová H., Klíma M., Pichová I.: Manuscript under revision (2025).

L-03
STRUCTURE AND INHIBITION OF DhhP, A c-di-AMP PHOSPHODIESTERASE OF BORRELIA BURGDORFERI

MARTIN KLIMA[a], MILAN DEJMEK[a], ADELA PALUSOVA[b], DOMINIKA CHALUPSKA[a], PETR PACHL[a], ANDREA HUSKOVA[a], JAKUB HRANICEK[c], KAREL CHALUPSKY[a], RADIM NENCKA[a], JAN PERNER[b], EVZEN BOURA[a]*

[a] Institute of Organic Chemistry and Biochemistry, Czech Academy of Sciences, v.v.i., Prague, [b] Institute of Parasitology, Biology Centre, Czech Academy of Sciences, v.v.i., Ceske Budejovice, [c] Faculty of Science, Charles University, Prague, Czech Republic
boura@uochb.cas.cz

Second messenger signaling through cyclic dinucleotides (CDNs) such as c-di-AMP, c-di-GMP, and cGAMP regulates critical processes in pathogenic bacteria, including virulence and survival[1]. In *Borrelia*, the causative agent of Lyme disease, c-di-AMP is synthesized from ATP by c-di-AMP synthase CdaA and degraded to AMP by phosphodiesterase DhhP (Fig. 1a). While altered expression of CdaA does not change intracellular concentrations of c-di-AMP, genetic inactivation of DhhP leads to its accumulation[2]. Thus, c-di-AMP levels in *Borrelia* are primarily regulated by DhhP. Genetic inactivation of DhhP in *Borrelia* is lethal both *in vitro* and within a mammalian host[2], making DhhP an attractive therapeutic target. The absence of DhhP homologues in humans suggests that specific DhhP inhibitors could exhibit high selectivity, minimizing potential off-target effects on human cyclic nucleotide metabolism.

In this study, we present the crystal structure of DhhP, revealing that the enzyme forms asymmetric dimers with coordinated open and closed conformations, suggesting an alternating mechanism for substrate processing (Fig. 1b-c). We demonstrate that DhhP contains a unique heterometallic binuclear active site with specifically positioned manganese and iron ions, challenging the prevailing paradigm of homo-bimetallic active centers in bacterial phosphodiesterases (Fig. 2).

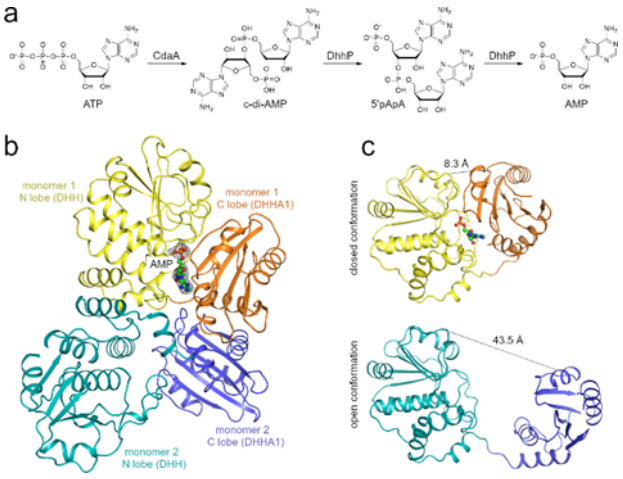


Fig. 1. **c-di-AMP metabolism and the DhhP overall structure**

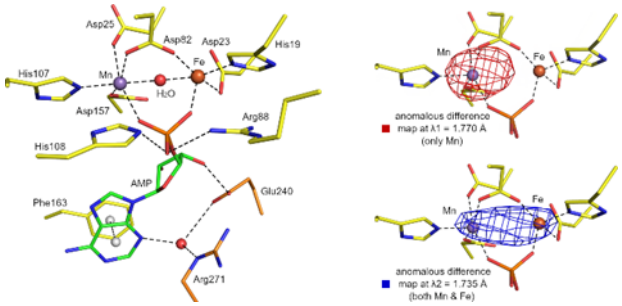


Fig. 2. **Detailed view of the heterobimetallic DhhP active site**

Additionally, we show that DhhP can be effectively inhibited by CDN analogs, in which phosphate linkages are substituted with vinylphosphonate groups. We demonstrated their ability to inhibit the growth of *B. burgdorferi* and disrupt spirochete morphology. These compounds represent the first reported inhibitors of any bacterial c-di-AMP specific phospho-diesterase and establish proof-of-concept for specific targeting of these enzymes and further research of c-di-AMP roles in bacterial cells.

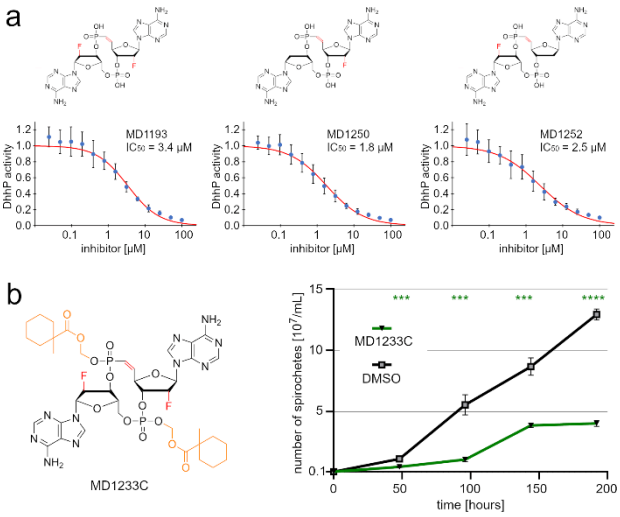


Fig. 3. **DhhP inhibition *in vitro* and in cultured *B. burgdorferi***

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 – Next Generation EU.

REFERENCES

1. Zarrella T. M., Bai G.: J. Bacteriol. 203 (2020).
2. Ye M., Zhang J. J., Fang X., Lawlis G. B., Troxell B., Zhou Y., Gomelsky M., Lou Y., Yang X. F.: Infect. Immun. *82*, 1840 (2014).

L-04
DESIGN AND SYNTHESIS OF NOVEL INHIBITORS OF VIRAL METHYLTRANSFERASES

MILAN ŠTEFEK[a,b], TOMÁŠ OTAVA[a], EVA ŽILECKA[a], DOMINIKA CHALUPSKÁ[a], KAREL CHALUPSKÝ[a], MICHALA ZGARBOVÁ[a], MARTIN KLÍMA[a], MILAN DEJMEK[a], HUGO KOCEK[a], EVŽEN BOUŘA[a], RADIM NENCKA[a]*

[a] *Institute of Organic Chemistry and Biochemistry of the Czech Academy of Sciences, Flemingovo náměstí 542/2, 160 00 Prague 6,* [b] *Department of Organic Chemistry, Faculty of Science, Charles University, 128 00 Prague, Czech Republic*
radim.nencka@uochb.cas.cz

Viral methyltransferases (MTases) are essential enzymes that secure efficient RNA translation and protect viral transcripts from host innate immunity by participating in the capping of their 5′ ends. These enzymes have emerged as highly attractive antiviral targets, and their inhibition offers a promising strategy to block viral replication[1]. Our research group has developed extensive expertise in the design and synthesis of nucleoside-based MTase inhibitors, particularly through modifications of the 7-deazapurine scaffold.

Since the 2020 outbreak of SARS-CoV-2, we have made significant contributions to the discovery of coronavirus MTase inhibitors, focusing on the N7-MTase non-structural protein 14 (nsp14). Using a structure-based approach, we designed 7-deazapurine analogues of *S*-adenosylhomocysteine (SAH) that engage a lateral cavity adjacent to the *S*-adenosylmethionine (SAM) binding site[2]. Follow-up work led to the design and synthesis of bisubstrate inhibitors of nsp14 by incorporating modifications into both the adenine base and the amino acid side chain (Fig.1, structure **A**)[3]. These analogues exploit interactions in the SAM-binding site as well as part of the adjacent RNA-binding region. Several lead compounds demonstrated nanomolar inhibitory activity in biochemical assays against SARS-CoV-2 nsp14 and exhibited excellent selectivity over human MTases. Structural studies confirmed the bisubstrate character of these inhibitors and revealed that bulky C7 substituents displace an ordered water network normally mediating SAM binding[4].

In parallel, work by our colleagues has established the mpox virus 2′-O-MTase VP39 as a promising antiviral target. Structural analyses demonstrated that C7-substituted SAH analogues bind to VP39 in a conserved fashion, while cellular assays showed that such inhibitors suppress mpox virus replication[5,6].

Building on this foundation, we developed a new series of VP39 inhibitors (Fig.1, structure **B**). Using the SAH template with canonical amino acid residues at the 5′-position, we explored C7 substitution with diverse branched substituents. Among the synthesized series, carboxamide-linked analogues showed promising inhibitory activity in enzymatic assays, providing a basis for further optimization.

In addition, while investigating suitable C7 substituents, we expanded the chemistry of this position to synthesize C7-sulfonamido-7-deazaadenosines, a structural type not previously reported. This strategy enabled efficient diversification and afforded sangivamycin derivatives with potent activity against Haspin kinase.

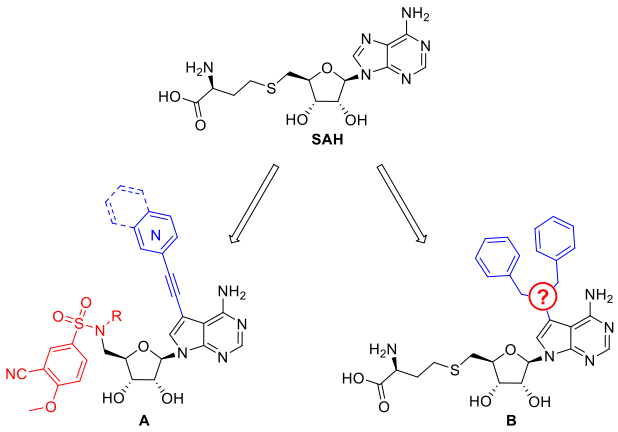


Fig 1. **Design of nsp14 MTase (A) and VP39 MTase (B) inhibitors derived from the structure of SAH**

Together, these results illustrate how targeted modifications at the 5′- and C7-positions of 7-deazapurines can yield inhibitors of viral MTases while also introducing a novel class of nucleosides with kinase activity. These outcomes broaden the scope of nucleoside-based medicinal chemistry and contribute to the wider collaborative effort on viral enzymes and related therapeutic targets.

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.

REFERENCES

1. Nencka, R., Silhan, J., Klima, M., Otava, T., Kocek, H., Krafcikova, P., Boura, E.: Nucleic Acids Res. *50*, 635 (2022).
2. Otava T., Sala M., Li F., Fanfrlik J., Devkota K., Perveen S., Chau I., Pakarian P., Hobza P., Vedadi M., Boura E., Nencka R.: ACS Infect. Dis. *7*, 2214 (2021).
3. Stefek M., Chalupska D., Chalupsky K., Zgarbova M., Dvorakova A., Krafcikova P., Li A. S. M., Sala M., Dejmek M., Otava T., Chaloupecka E., Kozak J., Kozic J., Vedadi M., Weber J., Mertlikova-Kaiserova H., Nencka R.: ACS Omega *8*, 27410 (2023).
4. Zilecka E., Klima M., Stefek M., Dejmek M., Nencka R., Boura E.: J. Struct. Biol. X *10*, 100109 (2024).
5. Silhan J., Klima M., Otava T., Skvara P., Chalupska D., Chalupsky K., Kozic J., Nencka R., Boura E.: Nat. Commun. *14*, 2259 (2023).
6. Zgarbova M., Otava T., Silhan J., Nencka R., Weber J., Boura E.: Antiviral Res. *218*, 105714 (2023).

L-05

RIFT VALLEY FEVER VIRUS POLYMERASE: FROM ANTIVIRALS DISCOVERY TO RESISTANCE SELECTION

MICHAL KRÁL[a,b]*, TOMÁŠ KOTAČKA[a,b], ANNA BLAHOŠOVÁ[a,c], VERONIKA LIŠČÁKOVÁ[a,b], JAN HODEK[a], AMIYARANJAN DAS[d,e], GABRIEL DEMO[d], MILAN KOŽÍŠEK[a]*

[a] *Institute of Organic Chemistry and Biochemistry of the Czech Academy of Sciences, Flemingovo n. 2, 166 10 Prague 6,* [b] *First Faculty of Medicine, Charles University, Kateřinská 1660/32, 121 08 Prague 2,* [c] *Faculty of Science, Charles University, Hlavova 8, 128 00 Prague 2,* [d] *Central European Institute of Technology, Masaryk University, Kamenice 753/5, 625 00 Brno,* [e] *National Centre for Biomolecular Research, Faculty of Science, Masaryk University, Kamenice 5, 625 00 Brno, Czech Republic*
michal.kral@uochb.cas.cz, milan.kozisek@uochb.cas.cz

Rift Valley fever virus (RVFV) is a mosquito-borne pathogen of the family *Phenuiviridae* that causes severe and often fatal disease in humans and domesticated animals. Outbreaks occur sporadically but can have devastating consequences for public health and agriculture. In livestock, RVFV infection results in high rates of abortion and mortality among young animals, with economic losses estimated in the hundreds of millions of U.S. dollars[1]. Despite this major impact, no approved treatment or prevention strategies are available for human use. Attenuated vaccines have been developed for veterinary application, yet their safety and efficacy remain uncertain, and the risk of reversion to a pathogenic form is an ongoing concern.

RVFV replication depends on the multifunctional L protein, a ~250 kDa viral polymerase that integrates three enzymatic activities: an endonuclease, an RNA-dependent RNA polymerase, and a cap-binding domain[2]. This organization closely mirrors the functional modules of the heterotrimeric influenza A virus polymerase (PA–PB1–PB2)[3]. Both viruses initiate transcription via a cap-snatching mechanism, whereby host mRNAs are cleaved by the endonuclease to prime viral RNA synthesis. The high level of conservation across bunyaviral polymerases further underscores the L protein as a promising target for therapeutic intervention.

To investigate this, we established two RVFV strains under biosafety level 3 conditions: the wild-type ZH-548 strain and the attenuated MP-12, which is used in experimental veterinary vaccines. With these systems, we screened and identified several small-molecule inhibitors of the L protein. Their antiviral efficacy was validated using live-virus assays.

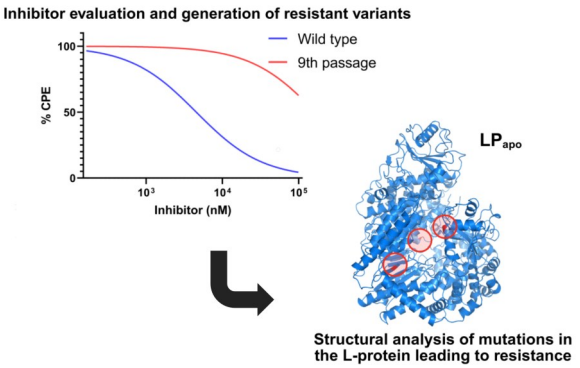
Resistance studies were performed by serial passaging of RVFV in the presence of these inhibitors. Resistant viral variants emerged, displaying either strongly increased or complete resistance to their respective compounds. Sequencing of the resistant strains revealed multiple non-synonymous mutations in the L protein.

In collaboration with the group of Gabriel Demo, we further explored the structural basis of resistance. Using cryo-electron microscopy, a 3.5 Å structure of the wild-type RVFV L protein was obtained in its apo form. Mapping of resistance-associated mutations onto this structure provided valuable mechanistic insight into how amino acid substitutions influence inhibitor binding, polymerase conformation, and overall enzymatic function. This structural framework not only clarifies the resistance mechanisms but also guides the rational design of next-generation inhibitors with improved efficacy and resilience to viral escape.

Beyond antiviral development, we also investigated the stability of the attenuated MP-12 vaccine strain. Reversion of attenuating mutations is a critical safety concern for all live-attenuated vaccines, as illustrated by the well-documented case of oral polio vaccine reversion. Our analysis of MP-12 focused on identifying mutations most susceptible to reversion, thereby assessing the long-term stability of the vaccine strain. Understanding these dynamics is essential for ensuring the safety profile of MP-12 and for informing the design of future live-attenuated vaccines with reduced reversion risk.

In collaboration with the group of Petr Volf, we plan to study the interactions between resistant RVFV variants and their insect vectors. Since vector competence plays a key role in shaping viral evolution and outbreak dynamics, such studies will provide important insights into how resistance mutations may affect transmission and fitness in natural hosts.

In summary, our study highlights the RVFV L protein as a central target for antiviral development, demonstrates the emergence and structural basis of resistance mutations, and provides critical data on the genetic stability of the MP-12 vaccine strain.



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 – Next Generation EU.

REFERENCES

1. Oymans et al.: PLoS Negl. Trop. Dis. *14*, e0007898 (2020).
2. Reguera et al.: PLoS Pathog. *6*, e1001101 (2010).
3. Olschewski et al.: Trends Microbiol. *28*, 293 (2020).

L-06
ELUCIDATING NEW ROLES OF UBIQUITIN LIGASES IN THE HBV LIFE CYCLE

VÁCLAV KROPÁČEK[a,b], BARBORA LUBYOVÁ[a], EVA TIKALOVÁ[a], VÁCLAV JANOVEC[a,c], BORIS RYABCHENKO[c], JAN HODEK[a], KRISTÝNA KRULOVÁ[a], HANA LANGEROVÁ[a], SANDRA HUÉRFANO[c], IVAN HIRSCH[a,c], JAN WEBER[a]*

[a] *Institute of Organic Chemistry and Biochemistry of CAS, Flemingovo nám. 2, 166 10 Prague,* [b] *Department of Genetics and Microbiology, Faculty of Science, Charles University, Viničná 5, 128 00 Prague,* [c] *Department of Genetics and Microbiology, Faculty of Science, Charles University, BIOCEV, Vestec, Czech Republic*
jan.weber@uochb.cas.cz

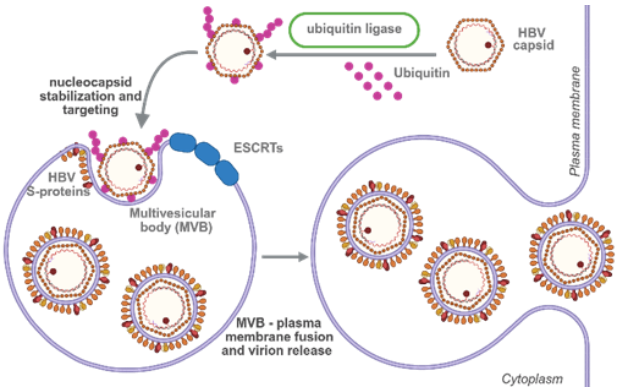


Fig. 1. **Role of ubiquitin ligases in HBV virion secretion.** Created in BioRender. Lubyova, B. (2025) https://BioRender.com/9ynwuqb

Ubiquitination, the covalent attachment of one or more ubiquitin moieties to a target protein catalyzed by E3 ubiquitin ligases, regulates several cellular processes. Depending on its topology and form, ubiquitination influences protein stability, half-life, subcellular localization, and secretion. These mechanisms can be exploited by viruses to facilitate their life cycle.

The hepatitis B virus (HBV) is an enveloped, hepatotropic virus that can cause chronic hepatitis B (CHB), a condition affecting more than 254 million people worldwide. HBV depends on host cell autophagy and secretory pathways for effective replication and the production of infectious viral progeny. Nedd4, an E3 ubiquitin ligase, polyubiquitinates the HBV capsid protein (HBc), enabling its recognition by the ESCRT-0 (endosomal sorting complexes required for transport) protein TSG101 and its subsequent sorting to the multivesicular body (MVB), thereby facilitating virion secretion[1].

Employing mass spectrometry, we searched for novel HBc-interacting host proteins and identified the E2/E3 hybrid enzyme UBE2O and the E3 ubiquitin ligase HUWE1[2]. UBE2O possesses dual activity as an E2 ubiquitin-conjugating enzyme and an E3 ligase. HUWE1, a HECT domain-containing E3 ligase, is implicated in DNA damage repair, the ubiquitin-proteasome system, and autophagy.

To investigate the role of UBE2O and HUWE1 in the HBV life cycle, we performed specific siRNA-mediated knockdowns in HBV-infected HepG2-NTCP cells. At 6 d.p.i., we measured viral parameters, including the levels of HBV RNA/DNA and secreted HBeAg using (RT-)qPCR and ELISA, respectively. Additionally, we analyzed the amount and phosphorylation status of intracellular nucleocapsids, as well as the quantities of secreted viral particles. Compared with HBV-infected cells transfected with a non-specific control siRNA, UBE2O depletion led to a significant decrease in the levels of HBV RNA/DNA and secreted HBeAg. Intracellular capsids and HBc were also significantly reduced upon UBE2O knockdown, suggesting a potential pro-viral role of UBE2O.

In contrast, HUWE1 depletion had no significant effect on HBV RNA/DNA levels or secreted HBeAg, but led to a marked accumulation of intracellular HBc/capsids. Notably, the downregulation of either UBE2O or HUWE1 significantly reduced virion secretion, while leaving naked capsid secretion unaffected.

Previously, we demonstrated that UBE2O catalyzes multi-monoubiquitination of HBc and viral nucleocapsids[3]. Mechanistic studies on a potential interaction between HBc/capsids and HUWE1, as well as HUWE1-mediated ubiquitination of HBc/capsids, are currently ongoing.

To further investigate the possible connection between UBE2O-mediated capsid monoubiquitination and the host endosomal secretion pathway, we used confocal microscopy and capsid co-immunoprecipitation. Both UBE2O and HBc colocalized with compartments positive for the MVB marker CD63. Subsequent experiments revealed an increased association between the ESCRT-0 component HGS and capsids upon UBE2O overexpression. Furthermore, an interaction between capsids and the ESCRT-II accessory protein EAP30 occurred exclusively in the presence of UBE2O.

Collectively, our data identify UBE2O as a key host factor supporting HBV replication and nucleocapsid formation, and indicate that both UBE2O and HUWE1 promote virion egress.

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.

REFERENCES

1. Rost M., Mann S., Lambert C., Doring T., Thome N., Prange R.: J. Biol. Chem. 281(39) (2006).
2. Langerová H., Lubyová B., Zábranský A., Hubálek M., Glendová K., Aillot L., Hodek J., Strunin D., Janovec V., Hirsch I., Weber J.: Cells 9(12) (2020).
3. Lubyova B., Tikalova E., Janovec V., Krulová K., Ryabchenko B., Huérfano S., Hirsch I., Weber J.: Czech Chem. Soc. Symp. Ser. *21*, 177 (2023).

P-01
NUCLEOSIDE AND NON-NUCLEOSIDE BORONIC ACIDS BASED ON PYRROLIDINE SKELETON

MAGDALENA PETROVÁ[a]*, RADEK POHL[a], JITKA VIKTOROVÁ[b], KATEŘINA BOGDANOVÁ[c], RENATA VEČEŘOVÁ[c], MILAN KOLÁŘ[c], MATTIA MORI[d], DOMINIK REJMAN[a]

[a]Institute of Organic Chemistry and Biochemistry, Czech Academy of Sciences v.v.i. Flemingovo nám. 2, 166 10 Praha 6, Czech Republic, [b]Department of Biochemistry and Microbiology, University of Chemistry and Technology, Technická 5, 166 28 Praha 6, Czech Republic, [c]Department of Microbiology, Faculty of Medicine and Dentistry, Palacký University Olomouc, Hněvotínská 3, 775 15 Olomouc, Czech Republic, [d]Department of Biotechnology, Chemistry and Pharmacy, University of Siena, via Aldo Moro, 2, 53100 Siena, Italy
petrova@uochb.cas.cz

Antibiotic resistance becomes one of the greatest global health challenges. Most widely used ATBs based on β-lactams are becoming inactive due to the increasing activity of β-lactamases which hydrolytically cleave the β-lactam ring. A successful strategy to overcome this is co-administration of the β-lactam ATB together with a β-lactamase inhibitor. Boronic acid in uncharged trigonal planar sp^2 form mimics the carbonyl carbon of β-lactam ring, whereas at pH above pKa it adopts tetrahedral sp^3 form as a transition-state analogue of the hydrolytic mechanism. This concept was proved by the first boronic acid Vaborbactam approved by FDA as a β-lactamase inhibitor, currently used in clinical practice[1].

In our lab, we have designed and synthesized ribonucleoside boronates and pyrrolidine boronates which show synergy with selected ATBs against KPC-3 producing *Klebsiela pneumoniae*, and *in vitro* assay on isolated recombinant *Klebsiella* KPC-3 revealed inhibitory effect of these compounds with K_i values comparable to Vaborbactam. *In silico* modelling on *E. coli* outer membrane indicated low membrane permeability for our inhibitors as well as for Vaborbactam. Since the latter is reported to exploit OmpK35 and OmpK36 porins[2] to cross the outer membrane and access the periplasm, we are currently exploring structural modifications aimed at facilitating porin-mediated uptake.

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. This article is based upon work from COST Action EURESTOP, CA21145, supported by COST (European Cooperation in Science and Technology).

REFERENCES

1. Tsivkovski R., et al.: Antimicrob. Agents Chemother. *64*, 1 (2020).
2. Lomovskaya O., et al.: Antimicrob. Agents Chemother. *61*, 10 (2017).

P-02
NOVEL HOST FACTORS AND AUTOREGULATORY ELEMENTS INFLUENCING HBV PRECORE PROTEIN PROCESSING AND SUBCELLULAR DISTRIBUTION

HELENA ZÁBRANSKÁ, ALEŠ ZÁBRANSKÝ, IVA PICHOVÁ

Institute of Organic Chemistry and Biochemistry, Flemingovo náměstí 2, 160 00 Prague, Czech Republic
helena.zabranska@uochb.cas.cz

Hepatitis B virus (HBV) utilizes the e antigen (HBeAg) to modulate the host innate immunity and establish viral persistence in human hepatocytes. The HBeAg precursor (p25, precore protein) is targeted to the endoplasmic reticulum (ER), where cleavage of the signal peptide (sp) generates the initial processing product, p22. This protein then proceeds through the secretory pathway, undergoing a secondary cleavage event in the Golgi apparatus to yield the secreted form, p17 (HBeAg). Approximately 15–20% of p22 is not secreted; instead, it is retro-translocated to the cytosol and transported into the nucleus. The biological function of this intracellular protein pool remains poorly understood, and the mechanism by which p22 is distributed across various cellular compartments is unclear.

This study aimed to analyze the pathway, cellular partners and regulatory mechanisms by which the precore protein is either secreted or retro-translocated back to the cytosol.

Based on MS data, the Translocon-Associated Protein Complex (TRAP) was identified as a novel host factor interacting with the HBV precore protein. This interaction was then validated by pull-down experiments. The study showed that depletion of individual TRAP subunits changes the subcellular distribution of the precore protein and leads to its degradation. The N-terminal region immediately downstream of the signal peptide was shown to be important for interaction with the TRAP δ subunit.

Additionally, three cysteine residues within the p25 signal peptide were identified as key autoregulatory elements controlling signal peptide cleavage efficiency. Mutations of these residues enhanced sp processing but simultaneously triggered the unfolded protein response and mislocalization of precore protein variants. Comparable mutations in duck HBV precore protein increased glycosylation level, suggesting that this autoregulatory mechanism is conserved among hepadnaviruses.

The research uncovered a novel regulatory mechanism within the HBV precore protein signal peptide that modulates HBeAg maturation and secretion. Additionally, TRAP was identified as a novel interacting partner involved in the precore protein translocation into the ER.

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.

P-03

CLOSE COOPERATION OF THE PURINE *DE NOVO* SYNTHESIS AND SALVAGE PATHWAY IS CRUCIAL FOR *IN VIVO* NUCOLEOTIDE POOL BALANCING IN MYCOBACTERIA

ZDENĚK KNEJZLÍK[a], MATTEO DEDOLA[a], ONDŘEJ BULVAS[a], MICHAL DOLEŽAL[a], DOMINIK REJMAN[a], MARTINA HALMOVÁ[a], JIŘÍ POSPÍŠIL[b], LIBOR KRÁSNÝ[b], IVA PICHOVÁ[a]*

[a] *Institute of Organic Chemistry and Biochemistry of Czech Academy of Scicences, Flemingovo náměstí 542/2 160 00 Praha 6,* [b] *Institute of Microbiology of Czech Academy of Scicences, Vídeňská 1083, 142 00 Praha 4-Krč, Czech Republic*
Zdenek.knejzlik@uochb.cas.cz

The genus Mycobacterium comprices numerous pathogens, including the highly successful species *Mycobacterium tuberculosis* (Mtb), which infects one-third of the human population and caused 1.25 million deaths in 2023 (ref.[1]). The most pressing problem is the rapid emergence of multidrug-resistant and extensively drug-resistant strains that are difficult or impossible to treat, which requires the search for new targets. A novel compound, JNJ-6640, which blocks *de novo* purine biosynthesis (DNPBS) by inhibiting the PurF enzyme, has been shown to be effective against multidrug-resistant *Mycobacterium tuberculosis*[2]. However, the precise mechanism by which purine metabolism is regulated remains to be elucidated.

The model species *Mycobacterium smegmatis* (Msm) was utilised in order to explore metabolome regulation. The utilisation of gene knockout and the compound JNJ-6640 has yielded data that demonstrate the distinctive regulatory patterns of mycobacterial DNPBS compared to other bacterial species. During exponential growth, the synthesis of purine nucleotides via DNPBS exceeds current cellular needs, with the excess being stored as free nucleobases for use during adaptation in the stationary phase. We identified salvaging enzymes, including hypoxanthine-guanine phospho-ribosyltransferase, GMP reductase, and IMP dehydrogenase, which participate in regulation of GTP levels during adaptation of Msm to adverse conditions. We determined the mechanistic basis of allosteric regulation by GTP and propose a general model of purine biosynthesis in mycobacteria.

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.

REFERENCES

1. World Health Organization. Global tuberculosis report 2024 (2024).
2. Lamprecht D. A., Wall R. J., Leemans A., et al.: Nature *644*, 214 (2025).

P-04

ALLOSTERIC REGULATION OF *PSEUDOMONAS AERUGINOSA* INOSINE-5′-MONOPHOSPHATE DEHYDROGENASE BY DIADENOSINE TETRAPHOSPHATE

ONDŘEJ BULVAS, ZDENĚK KNEJZLÍK, ADÉLA MATYÁŠOVÁ, TOMÁŠ KOUBA, IVA PICHOVÁ*

Institute of Organic Chemistry and Biochemistry of the Czech Academy of Sciences, Flemingovo náměstí 2, 166 10 Prague, Czech Republic
ondrej.bulvas@uochb.cas.cz; iva.pichova@uochb.cas.cz

Inosine-5′-monophosphate dehydrogenase (IMPDH) is a crucial purine metabolism enzyme and a promising drug target against bacterial infections. IMPDH catalyzes the NAD-dependent oxidation of inosine-5′-monophosphate (IMP) to xanthosine-5′-monophosphate (XMP), the first committed step in guanine nucleotide biosynthesis. Regulation of IMPDH activity is therefore essential for cell survival. Recent studies revealed complex allosteric regulation of bacterial IMPDH by purine nucleotide effectors, but the way in which purine metabolism is balanced differs between species.

In this project, we describe the allosteric regulation of IMPDH from the human pathogen *Pseudomonas aeruginosa* (*Pa*IMPDH). Using a combination of biochemical and structural biology methods, we show that *Pa*IMPDH is regulated by the bacterial stress-signaling molecule diadenosine tetraphosphate (Ap4A). This effector locks the regulatory domains of *Pa*IMPDH in a way that prevents the activation of the enzyme by the natural activator ATP. Ap4A is an alarmone that accumulates in response to proteotoxic stresses such as oxidative stress, heat shock, or antibiotic treatment, and it influences bacterial survival and virulence. The described allosteric regulation of *Pa*IMPDH thus provides a molecular mechanism by which purine metabolism is reprogrammed under stress conditions. Understanding these mechanistic details may open opportunities for the development of selective inhibitors targeting *Pa*IMPDH.

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.

P-05
SECRETORY CARRIER MEMBRANE PROTEINS MODULATE HEPATITIS B VIRUS INFECTION

KAROLÍNA ŠTAFLOVÁ, ALEŠ ZÁBRANSKÝ, JAN HODEK, IVA PICHOVÁ

Institute of Organic Chemistry and Biochemistry of Czech Academy of Scicences, Flemingovo náměstí 542/2, 160 00 Praha 6, Czech Republic
karolina.staflova@uochb.cas.cz

Approximately two people die each minute from hepatitis B, a viral infection that affects more than 250 million people worldwide and remains a leading cause of cirrhosis and hepatocellular carcinoma. Despite the availability of effective vaccines, current antiviral therapies do not eradicate the virus, largely because of the persistence of covalently closed circular DNA (cccDNA) in infected hepatocytes. Understanding how hepatitis B virus (HBV) exploits host cell pathways is therefore essential for developing new therapeutic strategies.

HBV depends on host membrane trafficking machinery for multiple steps of its life cycle, including viral entry, intracellular trafficking, nucleocapsid delivery to the nucleus, virion assembly at the endoplasmic reticulum, and egress through the secretory pathway. Secretory carrier membrane proteins (SCAMPs) are tetraspanning membrane proteins that mediate intracellular vesicle trafficking, particularly in endocytosis and exocytosis. Given these roles, SCAMPs may influence key processes in HBV infection.

We investigated the contribution of SCAMP1–4, all of which are expressed in hepatocytes, to HBV infection *in vitro*. siRNA-mediated knockdown of SCAMP3 prior to infection in HepG2-NTCP cells and primary human hepatocytes led to a significant reduction in intracellular HBV RNA levels and viral antigen secretion. Notably, this effect was absent in HepG2.2.15 cells harboring integrated HBV genomes, indicating that SCAMP3 acts at an early stage of the HBV life cycle, possibly during viral entry, cccDNA formation, or cccDNA transcription. Silencing of SCAMP4 in HepG2-NTCP cells also reduced intracellular viral RNA levels and HBeAg secretion.

These findings suggest that SCAMP proteins, specifically SCAMP3 and SCAMP4, contribute to HBV replication, likely by modulating membrane trafficking.

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.

P-06
SCREENING FOR NOVEL HOST FACTORS INTERACTING WITH ENTEROVIRAL 3A PROTEINS – ROLE OF CRM1

VLADIMIRA HOROVA, MARTIN KLIMA, EVZEN BOURA

Institute of Organic Chemistry and Biochemistry of Czech Academy of Scicences, Flemingovo náměstí 542/2, 160 00 Praha 6, Czech Republic

Enteroviruses, members of the *Picornaviridae* family, are small, non-enveloped viruses with icosahedral capsids and a single-stranded, positive-sense RNA genome. They represent the most common viral pathogens in humans, causing illnesses ranging from the common cold to severe and sometimes life-threatening diseases such as acute haemorrhagic conjunctivitis or poliomyelitis[1].

To replicate efficiently within host cells, enteroviruses hijack several host factors, including the lipid kinase PI4KB and the adaptor protein ACBD3 (ref.[2,3]). During infection, ACBD3 is recruited to viral replication sites through a direct interaction between its C-terminal GOLD domain and the viral non-structural 3A proteins[4].

In this study, we identify the nuclear export protein CRM1 (Chromosomal Maintenance 1) as another potential host factor for enteroviruses. Using LC-MS/MS screening and co-immunoprecipitation with different enteroviral 3A proteins, we examined its interaction with viral components and assess its contribution to CVB3 replication in HeLa cells.

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.

REFERENCES

1. Tapparel C., Siegrist F., Petty T. J., Kaiser L.: Infect. Genet. Evol. *14*, 282 (2013).
2. Klima M., et al.: Structure *25*, 219 (2017).
3. Chalupska D., Różycki B., Klima M., Boura E.: Protein Sci. *28(12)*, 2073 (2019).
4. Horova V., Lyoo H., Różycki B., Chalupska D., Smola M., Humpolickova J., Strating JRPM, van Kuppeveld FJM, Boura E., Klima M.: PLoS Pathog. *15(8)*, e1007962 (2019).

P-07
DEVELOPMENT OF EBOLA VIRUS AND MARBURG VIRUS METHYLTRANSFERASE INHIBITORS

BARBORA LANDOVA, TOMAS OTAVA, EVZEN BOURA, RADIM NENCKA

Institute of Organic Chemistry and Biochemistry of the Czech Academy of Science, Flemingovo nam. 542/2, 166 10 Prague, Czech Republic
barbora-landova@uochb.cas.cz

Ebola virus disease (EVD) and Marburg virus disease (MVD) are rare but severe illnesses caused by *Filoviridae*. EVD is linked to *Ebola virus* (EBOV), *Sudan virus* (SUDV), and *Bundibugyo virus* (BDBV), while MVD is caused by *Marburg virus* (MARV) and *Ravn virus* (RAVV). Both diseases are clinically similar and characterized by high fatality rates—averaging ~50% for EBOD and reaching up to 88% for MVD—though survival improves with early, intensive care. Since their first recorded outbreaks in 1976 (EBOD) and 1967 (MVD), both have caused multiple epidemics across Africa, with recent MVD cases reported in Rwanda (2024) and Tanzania (2025). Vaccines and therapeutics are approved only for EVD.

Filoviruses rely on the large (L) protein, which has polymerase, capping, and methyltransferase activities essential for RNA synthesis. The methyltransferase, uniquely among *Mononegavirales*, catalyses 2′-O-methylation of internal adenosines, a feature that may contribute to immune evasion[1]. While the SUDV methyltransferase structure has been solved, comparative knowledge of related enzymes is limited[2].

In this study, we generated and tested methyltransferase constructs from SUDV and MARV. Enzymatic activity was assessed, and our in-house compound library was screened using fluorescence assay. Several promising inhibitors were identified, demonstrating conserved enzymatic vulnerabilities across filoviruses. These findings may provide a foundation for the development of broad-spectrum antivirals against Ebola and Marburg viruses.

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.

REFERENCES

1. Baptiste Martin, et. all: Nucleic Acids Research *46*, 7902 (2018).
2. Coralie Valle, et. all: Nucleic Acids Research *49*, 1737 (2021).

P-08
A CONCERT FOR FLUORESCENCE, NMR, AND MS: A TOOLKIT FOR NS5 METHYLTRANSFERASE DRUG DISCOVERY

TOMÁŠ OTAVA[a], MATÚŠ DREXLER[a], DOMINIKA CHALUPSKÁ[a], PETRA KRAFČÍKOVÁ[a], KAREL CHALUPSKÝ[a], VÁCLAV VEVERKA[a,b]*, EVŽEN BOUŘA[a]*, RADIM NENCKA[a]*

[a] Institute of Organic Chemistry and Biochemistry of the Czech Academy of Sciences, Flemingovo náměstí 542/2, 16000, Prague 6, [b] Department of Cell Biology, Faculty of Science, Charles University, Vinicna 7, 12810 Prague, Czech Republic
tomas.otava@uochb.cas.cz,
dominika.chalupska@uochb.cas.cz

Flaviviruses, such as the Dengue virus, represent a major global health threat. The absence of effective agents against these viruses highlights the urgent need for new antiviral strategies. The NS5 methyltransferase (MTase) catalyzes both N-7 and 2′-O methylation reactions. These modifications then stabilize RNA, promote translation, and protect the virus against immune detection. Despite its potential as a therapeutic target, the development of small-molecule NS5 MTase inhibitors has been limited by the lack of complementary and reliable screening assays[1].

Here, we present an integrated workflow combining three orthogonal approaches to support the discovery of NS5 MTase inhibitors. A focused library of thirty *S*-adenosyl-L-homocysteine (SAH) analogs, synthesized by copper-catalyzed click chemistry, was profiled using: (i) fluorescence polarization assay to detect ligand displacement in the SAM-binding site, (ii) NMR titration assay providing residue-level insights into protein–ligand interactions, and (iii) an Echo® MS assay that directly quantifies enzymatic activity.

These three platforms show strong correlations, forming a reliable pipeline in which high-throughput hits can be identified, validated for activity and further characterized at the molecular level. In addition, X-ray crystallography of a NS5-SAH analog complex revealed the structural basis of ligand engagement within the SAM-binding pocket[2].

This work establishes a modular and adaptable screening toolkit that not only supports rational discovery and development of flavivirus MTase inhibitors but also offers a framework applicable to other RNA-capping enzymes.

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.

REFERENCES

1. Pierson T. C., Diamond M. S.: Nat. Microbiol. *5* (6), 796 (2020).
2. Otava T., Drexler M., Chalupská D., et al.: Manuscript submitted.

P-09

C-NUCLEOSIDES AS ANTIVIRAL AGENTS: SYNTHESIS AND BIOLOGICAL EVALUATION OF NOVEL PSEUDONUCLEOBASE DERIVATIVES

TEREZA KÁRNÍKOVÁ[a,c], MICHAL ŠÁLA[a], SIMONA HORKELOVÁ[a], MILAN DEJMEK[a], ELIŠKA PROCHÁZKOVÁ[a], LUDĚK EYER[b], EVŽEN BOUŘA[a], JAN WEBER[a], DANIEL RŮŽEK[b], RADIM NENCKA[a]*

[a] Institute of Organic Chemistry and Biochemistry of the Czech Academy of Sciences, Fleminogovo náměstí 542/2, 160 00 Prague 6, [b] Department of Virology, Veterinary Research Institute, Brno, [c] Department of Organic Chemistry, Faculty of Science, Charles University, Prague 128 00, Czech Republic
nencka@uochb.cas.cz, tereza.karnikova@uochb.cas.cz

Nucleoside analogues represent key components of modern antiviral therapy. However, their therapeutic usability is often limited by rapid degradation *in vivo* and low metabolic stability. C-nucleosides, in which the heteroaromatic part is connected to the sugar moiety through a stable C–C bond, offer enhanced resistance to enzymatic degradation, leading to a prolonged biological half-life and potentially improved efficacy. The clinical relevance of this scaffold is highlighted by approved antiviral remdesivir (authorized for the treatment of severe COVID-19)[1].

In this work, we report the synthesis of a novel series of C-nucleosides incorporating pseudonucleobases substituted at the 5- and 6-positions with various (hetero)aromatic groups. The goal was to expand structural diversity and investigate the effect of these modifications on biological activity.

A small library of these analogues was prepared and evaluated *in vitro* against a panel of RNA viruses, including HIV, SARS-CoV-2, Influenza virus, Coxsackie B3 virus, DENV, HSV and TBEV. Most compounds showed modiocre antiviral activity throughout this panel in cell-based assays. The results nevertheless provide a valuable starting point, but further studies are necessary to better understand the structure -activity relationships and to explore additional modifications that could enhance their therapeutic potential.

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.

REFERENCE

1. Cho A., Saunders O. L., Butler T., Zhang L., Xu J., Vela J. E., Feng J. Y., Ray A. S., Kim C. U.: Bioorg. Med. Chem. Lett. *22*, 2705 (2012).

P-10

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

TOMÁŠ KOTAČKA[a,b]*, MICHAL KRÁĽ[a,b], VERONIKA LIŠČÁKOVÁ[a,b], RÓBERT REIBERGER[a,c], ALEŠ MACHARA[a,c], TROY FEDIRKO, MILAN KOŽÍŠEK[a]

[a] Institute of Organic Chemistry and Biochemistry of the Czech Academy of Sciences, Flemingovo n. 2, 166 10 Prague 6, [b] First Faculty of Medicine, Charles University, Kateřinská 1660/32, 121 08 Prague 2, [c] Faculty of Science, Charles University, Hlavova 8, 128 00 Prague 2, Czech Republic
tomas.kotacka@uochb.cas.cz

Bunyaviricetes represent a group of emerging arboviruses with serious implications for human and animal health. Several members, such as Rift Valley fever virus (RVFV), Crimean-Congo haemorrhagic fever virus (CCHFV), are classified by the World Health Organization (WHO) as pathogens with pandemic potential. These viruses possess a segmented negative-sense RNA genome, with replication and transcription entirely dependent on the viral RNA-dependent RNA polymerase (L-protein). The enzyme employs a cap-snatching mechanism analogous to that of influenza A virus.

Despite their increasing relevance, therapeutic options remain scarce and no vaccines are licensed for human use. Our project aims to develop and characterize small-molecule inhibitors of *Bunyaviricetes* polymerases. To this end, we employ recombinant protein expression in *E. coli* and baculovirus systems, combined with biophysical and structural approaches such as isothermal titration calorimetry and X-ray crystallography.

A particular focus of our work is the endonuclease domain of the L-protein, which is essential for cap-snatching but available high-resolution structures of CCHFV and RVFV endonucleases are missing. We are addressing this knowledge gap by producing and structurally analyzing the RVFV and CCHFV endonuclease domains, and by probing its interactions with small molecules.

Recently, we have identified a promising inhibitor hit and successfully crystallized the apo structure of the RVFV endonuclease domain. These advances lay the foundation for structure-guided antiviral development targeting the replication machinery of *Bunyaviricetes*.

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 – Next Generation EU.

P-11
MOLECULAR INTERACTIONS AND THERAPEUTIC TARGETING OF INFLUENZA A VIRUS POLYMERASE

VERONIKA LIŠČÁKOVÁ[a,b]*, MICHAL KRÁĽ[a,b], TOMÁŠ KOTAČKA[a,b], PAVEL BRÁZDA[a], TOMÁŠ KOUBA[a], MILAN KOŽÍŠEK[a]

[a] Institute of Organic Chemistry and Biochemistry of the CAS, Flemingovo n. 2, 166 10 Prague 6, [b] First Faculty of Medicine, Charles University, Kateřinská 1660/32, 121 08 Prague 2, Czech Republic
veronika.liscakova@uochb.cas.cz

Influenza A virus (IAV), a negative-sense, single-stranded RNA virus from the *Orthomyxoviridae* family, poses a significant threat to global public health. Its RNA-dependent RNA polymerase, composed of the PA, PB1, and PB2 subunits, replicates and transcribes the viral genome within the host cell nucleus. Among its functional domains, the N-terminal endonuclease domain of the PA subunit (NPA) plays a pivotal role in the "cap-snatching" mechanism, in which short, capped RNA fragments from host pre-mRNAs are cleaved and used to prime viral mRNA synthesis. Inhibiting this activity is an attractive antiviral strategy, and recent evidence indicates that certain small molecules, originally developed as HIV integrase inhibitors, can also target the NPA active site. This cross-reactivity arises from similarities in the metal-ion coordination chemistry between the two enzymes. Structural analyses have shown that such compounds can bind within the NPA catalytic pocket, chelating the essential divalent metal ions (Mg^{2+} or Mn^{2+}) and thereby blocking its enzymatic function. Given that several of these inhibitors are already clinically approved, their repurposing for influenza therapy could accelerate the development of effective treatments. In this study, we will assess their inhibitory activity against the NPA endonuclease and determine their binding modes through X-ray crystallography.

Although direct enzymatic inhibition represents a promising approach, viral replication is also critically dependent on interactions between the polymerase complex and host proteins, which can present additional therapeutic targets. For example, the PA-PB1 heterodimer engages Ran-binding protein 5 (RanBP5), a human karyopherin involved in nuclear import, which is essential for the IAV life cycle. Similarly, the PB2 subunit interacts with Janus kinase 1 (JAK1), a central regulator of host immune signaling. While these host factors have been implicated in supporting viral replication, many details of their mechanistic roles remain unresolved. Here, we will investigate these interactions using surface plasmon resonance (SPR) to quantify binding affinities and kinetics, and cryo-electron microscopy to determine high-resolution structural models of the complexes.

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 – Next Generation EU.

P-12
DURING EARLY INFECTION

MARCELA PÁVOVÁ*, JITKA CHALUPOVÁ, ANEŽKA RAJMONOVÁ, ANASTASIA PRIS, ALENA KŘENKOVÁ, JAN WEBER

Institute of Organic Chemistry and Biochemistry of CAS, Flemingovo nám. 2, 166 10 Prague 6, Czech Republic
marcela.pavova@uochb.cas.cz

HIV-1, the causative agent of AIDS, relies on a tightly regulated interplay between viral and host factors during the early stages of infection. The viral capsid protein (CA) orchestrates critical pre-integration events, including core transport, nuclear entry, uncoating, and integration site targeting. Through CA pull-down assays followed by SWATH-MS/MS proteomics, we identified the multifunctional DEAD-box RNA helicases DDX1 and DDX3 as novel CA-interacting host factors during early HIV-1 infection in MT-4 cells. To assess their functional relevance, we performed siRNA-mediated knockdown in Jurkat E6-1 and primary blood mononuclear cells, which led to a significant decrease in HIV-1 replication as assessed by reverse transcriptase activity. Single-round infection assays and time-of-addition studies with small-molecule DDX3 inhibitors further localized their contribution to early post-entry steps. Digital droplet PCR revealed that inhibition of DDX3 compromised vDNA integration, while co-immunoprecipitation confirmed a specific interaction between CA and DDX3 but not DDX1. Importantly, molecular dynamics simulations and mutational analysis were used to map the DDX3–CA interface, revealing key amino acids in both proteins critical for the interaction. These amino acids were replaced by alanine, and the effects of these point mutations on DDX3–CA interaction were assessed by co-immunoprecipitation, confirming their importance. Overall, these data suggest that DDX3 facilitates early replication events preceding vDNA integration, likely by interacting with CA to promote efficient core trafficking and/or uncoating. Moreover, a small-molecule inhibitor of DDX3 significantly suppressed viral replication, underscoring its potential as a therapeutic target. Our findings expand the understanding of host factors involved in early HIV-1 replication and highlight DDX3 as a promising candidate for the development of novel antiviral strategies targeting essential host–virus interactions.

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 – Next Generation EU.

P-13
INVESTIGATING AGPCR EXPRESSION DYNAMICS IN SARS-COV-2 AND INFLUENZA A INFECTION OF LUNG CELLS: A META-ANALYSIS APPROACH

SANDRA IŠLER[a,b], MAREK POSPÍŠIL[a], ONDŘEJ LUKŠAN[a], KATEŘINA BALÁŽOVÁ FALTEJSKOVÁ[a], JAN WEBER[a]*

[a] Institute of Organic Chemistry and Biochemistry of CAS, Flemingovo nám. 2, 166 10, Prague, [b] Department of Genetics and Microbiology, Charles University, Faculty of Sciences, 128 44 Prague, Czech Republic
sandra.isler@uochb.cas.cz

Adhesion G protein-coupled receptors (aGPCRs) represent a unique and understudied family of seven-transmembrane proteins, with many members still classified as orphan receptors due to their unknown functions. While certain aGPCRs have been implicated in neurodevelopment, immune responses, and cancer, their involvement in viral infections remains largely unknown. Recently, we reported a potential role for one member, ADGRD1, in SARS-CoV-2 infection in human lung adenocarcinoma cells (Calu-3)[1].

To broaden our understanding of aGPCR involvement in respiratory viral infections, we performed RNA sequencing analysis of SARS-CoV-2 infected Calu-3 cells and incorporated it into meta-analysis integrating publicly available datasets, focusing specifically on SARS-CoV-2 and Influenza A virus (IAV) infections in lung cell lines. Based on our observations, aGPCR expression exhibits considerable variability influenced by factors such as cell type, multiplicity of infection, culture density, and handling conditions. Therefore, only datasets using untreated and non-transfected cells were included.

We confirmed upregulation of ADGRD1 as a common candidate for SARS-CoV-2 and IAV. Furthermore, common downregulated genes included ADGRC1 and ADGRG6. Additionally, virus-specific significantly changed expression were observed – ADGRE2 and ADGRG1 in SARS-CoV-2 and ADGRF3 and ADGRL2 in IAV. To account for subtle shifts that can significantly impact signaling, we included even aGPCRs with modest expression changes present in at least one third of analyzed datasets.

These findings suggest that aGPCRs may have broader roles in host responses to viral infections than currently recognized, highlighting the need for deeper functional studies.

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.

REFERENCE

1. Žáčková S, Pávová M, Trylčová J, Chalupová J, Priss A, Lukšan O, Weber J.: Cells *13*, 791 (2024). doi: 10.3390/ cells13100791.

P-14
EFFECT OF METHYLTRANSFERASES INHIBITORS ON SARS-CoV-2 INFECTION

MICHALA ZGARBOVÁ[a,b]*, TOMÁŠ OTAVA[a], JAN WEBER[a], RADIM NENCKA[a]

[a] Institut of Organic Chemistry and Biochemistry of the CAS, Flemingovo náměstí 2, 166 10, Prague, [b] Department of Genetics and Microbiology, Faculty of science, Charles University, Viničná 5, 128 00, Prague, Czech Republic
Michala.Zgarbova@uochb.cas.cz

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is the cause of the COVID-19 pandemic. Coronaviruses have the largest genomes of all RNA viruses. Importantly, viral RNA must be protected from the cellular innate immunity.[1] To mimic the host's mRNA cap, SARS-CoV-2 utilizes its own capping enzymes, including two MTases – nsp14 (N7 methylation; cap-0) and nsp16 (2′O methylation; cap-1). Both MTases are SAM-dependent, which makes them a suitable target for small-molecule inhibitors.[2] One of the first agents shown to have activity against coronavirus nsp14 MTase is sinefungin.[3]

Based on the structure of sinefungine, library of MTases inhibitors was created, currently there are more than one thousand structures. To select suitable candidates, we tested whole library in two types of cell cultures (Calu-3 and Vero E6) under two multiplicities of infection. Based on the percentage of SARS-CoV-2 inhibition, we selected 46 hits to determine EC_{50} and CC_{50} values. For futher testing of innate immune response we selected 5 compounds with EC_{50} values below 1 μM.

To observe innate immune response in Calu-3 cells we designed an assay where we can monitor changes in IFN-β, IFN-λ1, and MxA mRNA expression by RT-qPCR and phosphorylation of TBK1 and IRF3 by flow cytometry. These methods allowed us to compare immune activation in the presence or absence of inhibitors during SARS-CoV-2 infection to select candidate molecules for further development.

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.

REFERENCES

1. Krafcikova P., Silhan J., Nencka R., Boura E.: Nat. Commun. *11*, 3717 (2020)
2. Kocek H., Chalupská D., Dejmek M., Dvořáková A., Zgarbová M., Šála M., Chalupský K., Krafčíková P., Otava T., Drexler M., Procházková E., Klepetářová B., Štefek M., Kozic J., Mertlíková-Kaiserová H., Boura E., Weber J., Nencka R.: RSC Med. Chem. *15*, 3469 (2024)
3. Štefek M., Chalupská D., Chalupský K., Zgarbová M., Dvořáková A., Krafčíková P., Shi Ming Li A., Šála M., Dejmek M., Otava T., Chaloupecká E., Kozák J., Kozic J., Vedadi M., Weber J., Mertlíková-Kaiserová H., Nencka R.: ACS Omega *8*, 27410 (2023).

BIOLOGY CENTRE CAS

L-07
SEEING IS BELIEVING: 3D MAPPING OF TICK-BORNE ENCEPHALITIS VIRUS DISTRIBUTION IN MOUSE BRAIN USING TURBOGFP REPORTER VIRUS

MICHAELA BERÁNKOVÁ[a,b,c], **SIMONE LEONI**[d], **JAN HAVIERNIK**[b], **JIŘÍ HOLOUBEK**[a,b,c], **DANIEL RŮŽEK***[a,b,c]

[a] *Department of Experimental Biology, Faculty of Science, Masaryk University, CZ-62500, Brno, Czechia,* [b] *Laboratory of Emerging Viral Diseases, Veterinary Research Institute, CZ-62100, Brno, Czechia,* [c] *Institute of Parasitology, Biology Centre of the Czech Academy of Sciences, CZ-37005, Ceske Budejovice, Czechia,* [d] *Institute for Infectious Diseases, University of Bern, Bern, Switzerland*

Tick-borne encephalitis virus (TBEV) is a neurotropic orthoflavivirus that invades the central nervous system (CNS), causing severe neurological disease and posing a significant public health threat. Traditionally, detecting TBEV in infected cells or animal models relied on labor-intensive secondary methods, preventing real-time observation of infection and limiting studies of viral spread and host responses.

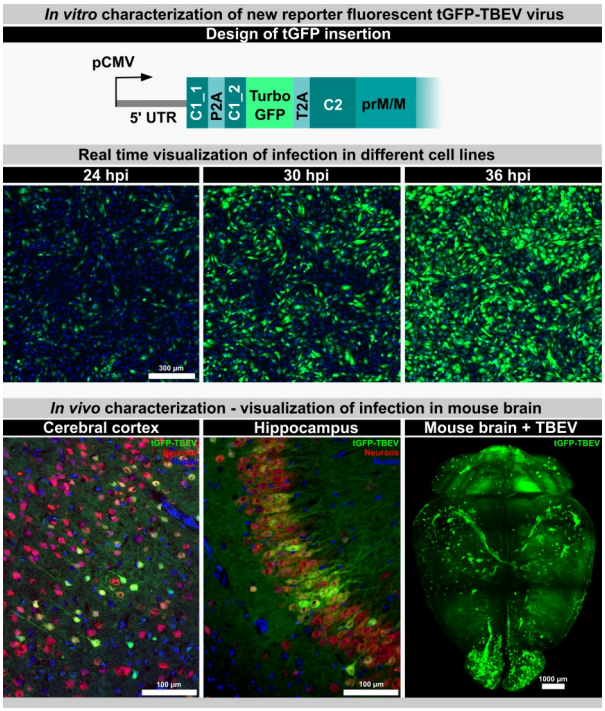
To address these limitations, we engineered a recombinant TBEV virus expressing the TurboGFP (tGFP) reporter fluorescent protein. While a similar approach of using a traceable reporter cloned into the viral genome has been previously used for various flaviviruses, maintaining their stability has been challenging[1]. Our work presents a reporter virus that remains stable for at least 7 passages, producing a strong fluorescent signal into the cytoplasm of infected cells. This enables direct visualization of infection both *in vitro* and *in vivo*, supporting detailed studies of viral replication, tropism, and host-pathogen interactions.

Using this tool, we observed tGFP-TBEV infection *in vitro* across various cell lines in real-time, enabling detailed monitoring of viral replication and spread. We also analyzed viral growth and fluorescence kinetics during infection, providing reliable information about the tGFP-TBEV replication. *Ex vivo* experiments using rat organotypic cerebellar slice cultures offered further insights into TBEV infection within a complex tissue.

For *in vivo* experiments, we infected BALB/c mice with tGFP-TBEV and monitored disease progression to assess the pathogenicity of the reporter virus in comparison with the wild-type TBEV strain. Following infection, mouse brains were collected and processed for both tissue sectioning and advanced imaging approaches.

To further explore the spatial distribution of infection, we combined tissue-clearing techniques with light-sheet microscopy, enabling high-resolution, three-dimensional visualization of viral spread throughout the entire mouse brain. These findings offer a unique perspective on how TBEV spreads within the mouse brain and provides new insights into viral spread and pathology.

The tGFP-TBEV reporter virus also serves as a robust platform for testing antiviral compounds and evaluating their effects on viral replication and spread. Its high stability and easily observed fluorescence make it well suited for diverse experimental approaches.



Scheme 1. **Graphical abstract of the study**

This work was recently published, and comprehensive details can be found in the full study[2]. Future studies will expand on these findings to deepen our understanding of TBEV biology and to support the development of effective antiviral strategies.

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.

REFERENCES

1. Haviernik J., Eyer L., Yoshii K., Kobayashi S., Cerny J., Nougairède A., Driouich J. S., Volf J., Palus M., de Lamballerie X., Gould E. A., Ruzek D.: Antiviral Res. *185*, 104968 (2021).
2. Berankova M., Leoni S., Holoubek J., Haviernik J., Salat J., Grandgirard D., Leib S. L., Ruzek D.: Emerging Microbes Infect. *14*(1), 2542246 (2025).

P-15
BLOOD-BRAIN BARRIER INVOLVEMENT IN TICK-BORNE ENCEPHALITIS VIRUS PATHOGENESIS

MONIKA CIZKOVA[a,b], VERONIKA PRANCLOVA[a,b], ELISKA KOTOUNOVA[a,b], MARIE VANCOVA[a], MARIKA DAVIDKOVA[a], MARKETA DVORAKOVA[a], DANIEL RUZEK[a,c,d], VACLAV HÖNIG[a,c], MARTIN PALUS[a,c,d]

[a] Institute of Parasitology, Biology Centre CAS, Branisovska 1160/31, 370 05 Ceske Budejovice, [b] Faculty of Science, University of South Bohemia (FSci USB), Branisovska 1760, 370 05 Ceske Budejovice, [c] Department of Infectious Disease and Preventive Medicine, Veterinary Research Institute, 621 00 Brno, [d] Department of Experimental Biology, Faculty of Science, Masaryk University, Brno, Czech Republic
palus@paru.cas.cz

Tick-borne encephalitis virus (TBEV) causes severe CNS disease with lasting neurological sequelae. We investigated how non-neuronal neurovascular unit (NVU) cells contribute to blood–brain barrier (BBB) dysfunction and neuroinflammation, and assessed the role of viral protein NS1 as a therapeutic target. Using primary human NVU cells, microglia, *in vitro* BBB models, and a mouse infection model, we examined viral replication, immune responses, barrier integrity, and antibody-based interventions. TBEV infected all NVU cell types with distinct kinetics; pericytes and astrocytes showed immune activation, while microglia sustained long-term infection. TBEV crossed endothelial monolayers transcellularly without disrupting tight junctions, whereas NS1 increased endothelial permeability. Anti-NS1 monoclonal antibodies showed specific and cross-reactive binding and extended survival in mice, but did not fully protect. Our findings reveal that NVU cells facilitate TBEV CNS entry and neuroinflammation without overt BBB breakdown, and identify NS1 as a contributor to endothelial dysfunction and a partial therapeutic target.

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 from the Czech Science Foundation (grant No. 23-08039S). The BC CAS core facility was supported by MEYS CR (LM2023050 Czech-BioImaging and OP VVV CZ.02.1.01/0.0/0.0/18_046/0016045).

UNIVERSITY OF CHEMISTRY AND TECHNOLOGY PRAGUE

L-08

PROBING OF THE NSP14/NSP10 INTERFACE REVEALS A SUSCEPTIBLE ACTIVATION HOTSPOT IN THE SARS-COV-2 EXONUCLEASE

MATĚJ DANDA*[a], TEREZA NEŠPOROVÁ[b], BARBORA CHVÁTALOVÁ[a], DANIELA NEČASOVÁ[a], ONDŘEJ VANĚK[c], MICHAELA RUMLOVÁ[a]

[a] *Department of Biotechnology, University of Chemistry and Technology, Technická 5, 166 28 Prague,* [b] *Institute of Organic Chemistry and Biochemistry, Prague, Flemingovo nám. 542/2, 160 00 Prague,* [c] *Institute of Organic Chemistry and Biochemistry, Prague, Flemingovo nám. 542/2, 160 00 Prague, Czech Republic*
dandam@vscht.cz

The SARS-CoV-2 exoribonuclease (ExoN) is a key component of viral replication, ensuring fidelity by proofreading RNA synthesis. The ExoN represents a complex of non-structural protein (nsp) 14 along with its cofactor nsp10. Intriguingly, nsp14 was also shown to effectively excise nucleoside analogs from the nascent RNA strand. Given this activity and its importance in the coronaviral replication cycle, ExoN has been readily explored as an antiviral target. Despite this, no clinically viable ExoN inhibitors have been identified[1]. Computational studies have proposed phenolic phytochemicals as candidates for ExoN inhibition[2–4], yet their efficacy remains largely unexplored.

In this study, we employed a dual-assay approach to screen 60 phenolic natural products and related compounds for inhibitory activity and binding to the ExoN complex with nano-differential scanning fluorimetry. This selected panel was based on published computational predictions and *in silico* screens. Interestingly, the experimental evaluation revealed only very limited correlation with the computational predictions (Fig. 1). Nevertheless, it also identified shikonin as an inhibitor with an IC50 in the single-digit micromolar range and a potentially distinct mode of action, involving the disruption of nsp14 and nsp10 interaction.

While the direct use of shikonin is unlikely due to high cytotoxicity, we hypothesized that an in-depth analysis of such molecule could pave the way for discovering novel alternatives to classical active-site binders. We thus performed a comprehensive analysis of shikonin using enzyme kinetics, microscale thermophoresis, thermal denaturation methods, chemical crosslinking, and analytical ultracentrifugation, which confirmed the putative mode of action and indicated targeting of nsp10. Subsequently, we utilized hydrogen-deuterium exchange coupled with mass spectrometry (HDX-MS) to probe the ExoN complex and identify the affected region at its subunits. This structural analysis not only confirmed that the complex disruption results from conformational changes in nsp10, particularly in the region that initiates its interaction with nsp14. Together, these results position ExoN as a viable antiviral target and underscore the potential of disrupting cofactor interactions as a novel strategy in coronavirus drug discovery.

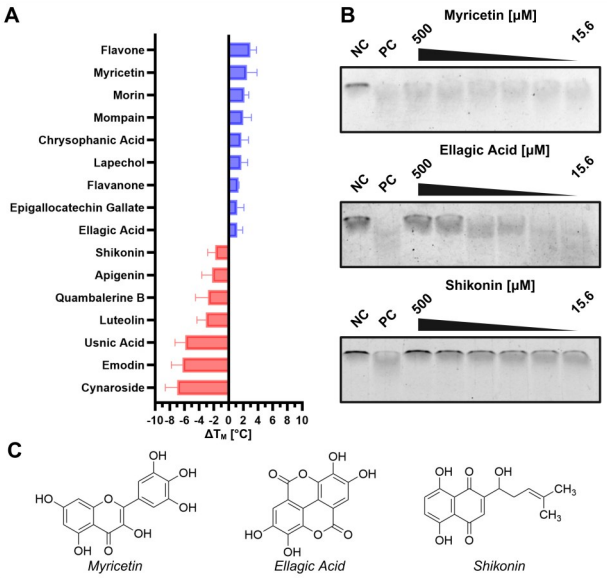


Fig. 1. **Results of thermal shift assay and nuclease activity assay for selected compounds (A) Changes of the first midpoint denaturation temperature (ΔTM) calculated in comparison with the control nsp14:nsp10 mixture (molar ratio = 1:3) in DMSO. The ΔTM values of the compound exhibiting at least 1 °C are presented, involving both destabilizing (red) and stabilizing (blue) molecules. (B) Evaluation of concentration-dependent inhibition of nsp14:nsp10 exonuclease by selected compounds (myricetin, ellagic acid and shikonin). The compounds were added in two-fold dilutions, resulting in final concentrations of 500 nM, 250 nM, 125 nM, 62.5 nM 32.3 nM and 15.6 nM. NC represents a negative control without the enzyme and PC is a positive control reaction in the presence of DMSO. (C) Chemical structures of the selected compounds exhibiting inhibitory potency in (B).**

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.

REFERENCES

1. Tahir M.: Journal of medical virology *93*(7), 4258 (2021).
2. Khater S., Kumar P., et al.: Frontiers in Microbiology *12*, 6476934 (2021).
3. De A., Bhattacharya S., et al.: In Silico Pharmacology *11*, 12 (2023).
4. Chandramouli V.s, Niraj S., et al.: Current Microbiology *78*, 3620 (2021).

P-16
STEROIDAL HORMONES FOR ANTIBACTERIAL THERAPY: DESIGN, SYNTHESIS, AND BIOLOGICAL ASSESSMENT

DANIELA BRDOVÁ[a], BÁRA KŘÍŽKOVSKÁ[a], JAN ŠPAČEK[a], JAN TKADLEC[b], JAN LIPOV[a], EVA KUDOVÁ[c], JITKA VIKTOROVÁ[a]*

[a] Department of Biochemistry and Microbiology, University of Chemistry and Technology Prague, Technická 5, 166 28 Prague, [b] Department of Medical Microbiology, Charles University, 2nd Faculty of Medicine and Motol University Hospital, V Úvalu 84, 150 06 Prague 5, [c] Institute of Organic Chemistry and Biochemistry of the Czech Academy of Sciences, Flemingovo náměstí 2, 166 10 Prague 6, Czech Republic
prokesoj@vscht.cz

The global rise of antibiotic resistance necessitates novel therapeutic strategies to restore the efficacy of existing antibiotics. One promising approach is the inhibition of bacterial efflux pumps, which contribute significantly to multidrug resistance in *Staphylococcus aureus*.

In this study, we evaluated 26 endogenous steroidal hormones and neurosteroids, along with 30 synthetic derivatives, for their ability to modulate bacterial efflux activity. Using a combination of fluorescence-based ethidium bromide accumulation, broth microdilution, and checkerboard assays, we identified several compounds that enhanced antibiotic susceptibility in multidrug-resistant *S. aureus*. A structure-activity relationship (SAR) analysis revealed that specific modifications of the pregnanolone scaffold, particularly the introduction of polar substituents, markedly improved activity.

Transcriptome analysis revealed that the most effective derivative significantly reduced the expression of genes involved in *S. aureus* virulence when administered alone or in combination with antibiotic adjuvant therapy. Cytotoxicity was assessed using the resazurin assay in human PBMCs, and receptor-mediated hormonal activity was evaluated *via* transactivation assays for estrogen, androgen, and progesterone receptors. Importantly, the most active derivatives were non-toxic and lacked measurable endocrine activity, suggesting a favorable pharmacological safety profile.

These findings support the hypothesis that rationally designed steroidal scaffolds, especially pregnanolone-based derivatives, can act as competitive bacterial efflux pump inhibitors. Such compounds may serve as effective antibiotic adjuvants, offering a viable strategy to mitigate efflux-mediated resistance and enhance the therapeutic utility of existing 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.

P-17
EFFECT OF NATURALLY OCCURRING SUBSTITUTION I42V IN SARS-COV-2 NSP14 ON EXORIBONUCLEASE ACTIVITY AND STABILITY

LUCIE HODBOĎOVÁ[a], MATĚJ DANDA[b], MARKÉTA ČASTORALOVÁ[a], MICHAELA RUMLOVÁ[b], TOMÁŠ RUML[a]*

[a] Department of Biochemistry and Microbiology, University of Chemistry and Technology Prague, Technická 3, 166 28 Prague, [b] Department of Biotechnology, University of Chemistry and Technology Prague, Technická 3, 166 28 Prague, Czech Republic
lucie.hodbodova@vscht.cz

SARS-CoV-2 replication-transcription complex (RTC), a complex of non-structural proteins and RNA, is crucial for the replication of SARS-CoV-2, the causative agent of the global pandemic COVID-19. The RNA-dependent RNA polymerase (RdRp), which is responsible for the synthesis of 29.9kb single-stranded genomic RNA, plays a crucial role in this complex. Unlike cellular DNA polymerases, viral RdRp does not contain a high-fidelity exonuclease proofreading domain. High-fidelity replication of the viral genome is mediated by the 3′-to-5′ exoribonuclease (ExoN), which is comprised by the N-terminal part of non-structural protein 14 (nsp14). The formation of the nsp14 complex with another viral protein, nsp10, is essential for ExoN activity. ExoN excises nucleotides including nucleotide-based antivirotics misincorporated by low-fidelity viral RdRp. In the Omicron SARS-CoV-2 variant and its subvariants, including currently circulating variants, an amino acid substitution I42V in nsp14 is conserved. The mutation is situated outside of the active site of nsp14 and also it does not participate in interaction with nsp10 or with RNA substrate. We hypothesize that the mutation does not directly affect the ExoN activity of nsp14, but that it could play a role during the formation of the nsp14/ nsp10 complex.

We expressed, isolated and purified the nsp14 WT protein and its mutant variant nsp14 I42V to compare their properties. Protein production was carried out in the bacterial expression system. This was followed by multi-step isolation and purification by fast protein liquid chromatography according to the protocol available in the laboratory. In order to study the exoribonuclease activity of nsp14, it was also necessary to express, isolate and purify nsp10. The ExoN activity of prepared proteins was verified by activity assay.

Preliminary data of thermal stability show that nsp14 I42V mutant exhibits lower stability than the wild-type protein. The effect was comparable to that obtained through chemical denaturation with urea.

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.

P-18
MASON-PFIZER MONKEY VIRUS RECRUITS DHX15 TO PROMOTE RNA PACKAGING

ANETA PAGÁČOVÁ, KLÁRA BLAHOUTOVÁ, MARINA KAPISHEVA, JAN PRCHAL, TOMÁŠ RUML, MICHAELA RUMLOVÁ

University of Chemistry and Technology, Technická 5, 166 28 Prague, Czech Republic
pagacova@vscht.cz

The DHX15 is an RNA helicase that plays a pivotal role in various steps of cellular RNA metabolism. Its activity and substrate specificity are modulated by protein cofactors, especially those containing glycine-rich sequence known as the G-patch domain (GPD)[1]. While this regulatory mechanism has been well-characterised in the context of cellular processes, recent studies have revealed that certain viruses have evolved to utilise this mechanism for their own benefit. A specific example is Mason-Pfizer monkey virus (M-PMV), a betaretrovirus in which GPD has been identified. The sequence and secondary structure of this viral GPD resemble cellular ones known to activate DHX15 (e. g. NF-kappa-B-repressing factor) suggesting a shared mode of action.

Using PAR-CLIP analysis, constitutive transport element, an RNA element crucial for the export of unspliced viral RNA from the nucleus, and its upstream sequence were identified as potential binding site of DHX15 in M-PMV genome[2]. In order to confirm and also narrow the DHX15 binding site, side-directed mutagenesis of this region was performed and the interaction was studied *in vitro* using microscale thermophoresis. Simultaneously, the impact of the mutations on M-PMV life cycle was investigated.

The overall results confirm the binding site position for DHX15 in the M-PMV genome. Mutagenesis of the binding site led to substantially decreased level of DHX15 in purified viral particles, accompanied by reduced levels of viral RNA.

These findings reveal a previously uncharacterized mechanism by which M-PMV manipulates host RNA helicase activity to optimize its replication strategy. This work not only expands our understanding of host–virus interactions at the molecular level but also opens new avenues for exploring helicase-mediated control of RNA fate in retroviral life cycles.

Acknowledgement
The work was supported by the grant of Specific university research (grant No. A2_FPBT_2025_047) and the project National Institute of Virology and Bacteriology (Programme EXCELES, ID Project No. LX22NPO5103) – Funded by the European Union – NextGenerationEU.

REFERENCES

1. Studer M. K., Ivanovic L., Weber M. E., Marti S., Jonas S.: Proc. Natl. Acad. Sci. U. S. A. *117*(13), 7159 (2020).
2. Dostálková A., Křížová I., Junková P., Racková J., Kapisheva M., Novotný R., Danda M., Zvonařová K., Šinkovec L., Večerková K., Bednářová L., Ruml T., Rumlová M.: Proc. Natl. Acad. Sci. U. S. A. *121*(40), e2407990121 (2024).

MASARYK UNIVERSITY

L-09
STRUCTURES OF ASYMMETRIC PARTICLES OF TBEV PROVIDE INSIGHT INTO FLAVIVIRUS ASSEMBLY AND MATURATION

TIBOR FÜZIK[a], MARIA ANASTASINA[b,c], PETER PAJTINKA[a], AUSRA DOMANSKA[b,c], LAURI I. A. PULKKINEN[b,c], LENKA ŠMERDOVÁ[a], LUCIE NEPOVÍMOVÁ[a], PETRA FORMANOVÁ-POKORNÁ[d], PETRA STRAKOVÁ[d], JIŘÍ NOVÁČEK[a], DANIEL RŮŽEK[d], ROBERT VÁCHA[a], SARAH J. BUTCHER[b,c]*, PAVEL PLEVKA[a]*

[a] Central European Institute of Technology, Masaryk University, Brno, Czech Republic, [b] Faculty of Biological and Environmental Sciences, Molecular and Integrative Biosciences Research Programme, University of Helsinki, Helsinki, Finland, [c] Helsinki Institute of Life Sciences-Institute of Biotechnology, University of Helsinki, Helsinki, Finland, [d] Laboratory of Emerging Viral Infections, Veterinary Research Institute, Brno, Czech Republic

Immature particles of flaviviruses are coated by a membrane decorated by spikes, each formed by three heterodimers of pre-membrane (prM) and envelope (E) proteins[1]. Maturation requires cleavage of prM into pr and M fragments and rearrangement of the coat proteins into a smooth herringbone pattern of M-E heterodimers[2]. Despite the global health impact of flaviviruses, their assembly and maturation are poorly understood[1,2]. Here, we show that most tick-borne encephalitis virus (TBEV) particles are asymmetric and lack subsets of surface heterodimers. Transmembrane and peripheral membrane helices of prM and E induce membrane bending, which is necessary for TBEV budding into the ER membrane. Immature particles of TBEV contain incomplete spikes, providing evidence that their coats assemble directly from prM-E heterodimers. Exposure of TBEV particles to acidic pH in the Golgi complex promotes maturation. The spikes and herringbone patterns in TBEV maturation intermediates are arbitrarily oriented relative to each other rather than being aligned to one icosahedral symmetry. Furthermore, the shapes of the bare membrane areas of TBEV virions and immature particles are different. Therefore, the mature herringbone pattern assembles from a randomly oriented nucleation center by gradually adding M-E heterodimers to its edges as the spikes disassemble and prMs are cleaved. The incompleteness of the protein coats explains how flaviviruses can be neutralized by antibodies binding to parts of E proteins inaccessible at the surface of the spiky and herringbone structures, and opens possibilities for developing antivirals targeting the virus membrane.

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.

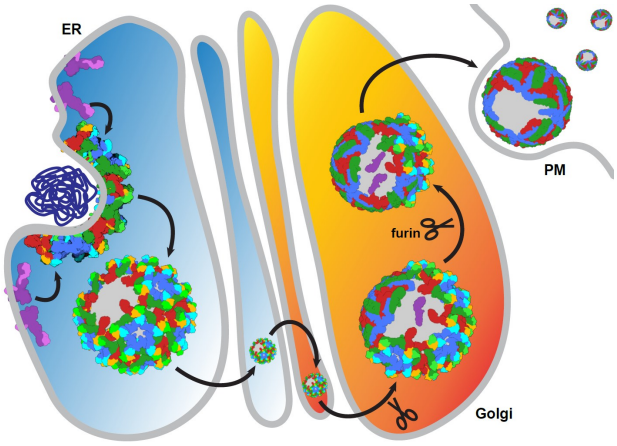


Fig. 1. **Scheme of TBEV assembly and maturation**

REFERENCES

1. Anastasina M., Füzik T., Domanska A., Pulkkinen L. I. A., Šmerdová L., Formanová P. P., et al.: Sci. Adv. *10* (27), eadl1888 (2024).
2. Newton N. D., Hardy J. M., Modhiran N., Hugo L. E., Amarilla A. A., Bibby S., et al.: Sci. Adv. *7*(20), eabe4507 (2021).

L-10
MEMBRANE SHAPE MATTERS: MOLECULAR SIMULATION AND MACHINE LEARNING FOR DESIGNING CURVATURE-SENSING PEPTIDES

PETER PAJTINKA[a], **ROBERT VÁCHA**[a,b,c,*]

[a] *Central European Institute of Technology, Masaryk University, Kamenice 753/5, 625 00 Brno,* [b] *National Centre for Biomolecular Research, Faculty of Science, Masaryk University, Kamenice 5, 625 00 Brno,* [c] *Department of Condensed Matter Physics, Faculty of Science, Masaryk University, Kotlářská 267/2, 611 37 Brno, Czech Republic*
robert.vacha@ceitec.muni.cz

Membrane curvature sensing is a fundamental biophysical mechanism[1] that underlies key cellular processes, including vesicular trafficking, membrane remodeling, and the internalization of transmembrane proteins such as G protein–coupled receptors (GPCRs)[2,3]. Beyond their intrinsic biological relevance, curvature-sensing peptides have also shown translational potential, for example in selectively targeting enveloped viral particles[4] or extracellular vesicles in cancer[5].

Despite their significance, relatively few curvature-sensing peptides have been characterized, and a generalizable design framework remains lacking. Amphipathic helices – a class of α-helical peptides with hydrophobic and hydrophilic faces – have traditionally been considered sensors of positive membrane curvature. We previously showed that they can also recognize negative curvature, broadening their functional scope[6].

Here, we introduce an integrated framework that combines evolutionary optimization with molecular dynamics (Evo-MD)[7] to generate a large-scale in silico library of curvature-sensing peptides. This approach establishes a systematic platform to probe structure-function relationships and identify new design principles.

Guided by these principles, curvature-sensing peptides can be engineered as precision-targeting modules, for example in chimeric constructs with antimicrobial peptides, to achieve controlled delivery of bioactive compounds. By localizing to regions of negative mean curvature, such modules could promote uptake via endocytosis, whereas avoiding these regions may suppress unintended internalization. Our simulations further indicate that curvature-sensing elements can modulate the spatial organization of transmembrane proteins, offering a route to influence membrane-associated signaling.

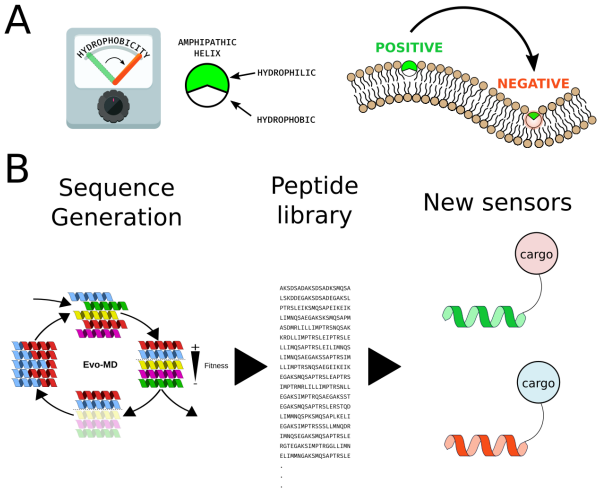


Fig. 1. **Project summary A) Our previous study showed that insertion depth of peptides can alter their curvature preference B) Overview of the follow-up study to design novel curvature sensing peptides**

Together, this work presents a computationally driven strategy for the rational design of curvature-sensing peptides, with broad implications for membrane biology, targeted therapeutics, and synthetic biology.

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 the European Research Council (ERC) under the European Union's Horizon 2020 research and innovation programme (grant agreement No 101001470). Computational resources were provided by the CESNET, CERIT Scientific Cloud, IT4 Innovations National Supercomputing Center by MEYS CR through the e-INFRA CZ (ID:90254).

REFERENCES

1. McMahon H.T., Boucrot E.: J. Cell Sci. *128*, 1065 (2015).
2. Kozlov M. M., Taraska, J. W.: Nat. Rev. Mol. Cell Biol. *24*, 63 (2023).
3. Schmidt, et al.: Sci. Adv. *11*, eadv1499 (2025).
4. Jackman J. A., et al.: Nat. Mater. *17*, 971 (2018).
5. Shin S., et al.: Nat. Mater. *22*, 656 (2023).
6. Pajtinka P., Vácha R.: J. Phys. Chem. Lett. *15*, 175 (2024).
7. van Hilten N., Methorst J., Verwei N., Risselada H. J.: Sci. Adv. *9*, eade8839 (2023).

L-11
MECHANISTIC INSIGHTS INTO E. COLI SLOW RECOVERY FROM GROWTH ARREST

AHMED HASSAN[a], YUKO NAKANO[b], ISAO MASUDA[b], HOWARD GAMPER[b], MATYAS PINKAS[a], GREGOR BLAHA[c], YA-MING HOU[b], GABRIEL DEMO[a]*

[a] Central European Institute of Technology, Masaryk University, Brno, Czech Republic, [b] Department of Biochemistry and Molecular Biology, Thomas Jefferson University, Philadelphia, USA, [c] Department of Biochemistry, University of California, Riverside, California, USA
gabriel.demo@ceitec.muni.cz

Bacterial ribosome assembly is a highly coordinated, multistep process essential for cellular viability[1]. It begins with the transcription, folding, and extensive modification of ribosomal RNA (rRNA), followed by the hierarchical incorporation of ribosomal proteins into pre-ribosomal particles[1,2]. This process is guided by a network of specialised assembly factors, including RNA chaperones, GTPases, rRNA modification enzymes, and quality control proteins, which ensure accurate folding, prevent kinetic traps, and license subunits for translation. Disruption of these factors can stall assembly at specific checkpoints, leading to the accumulation of immature particles, reduced translational capacity, and growth defects[3,4].

In *Escherichia coli*, the small ribosomal subunit assembly factor RimM plays a central role in the late stages of 30S maturation, facilitating the correct positioning of key ribosomal proteins in the head domain[1,2]. Deletion of *rimM* results in slow growth, accumulation of assembly-arrested 30S particles, and impaired translation efficiency[3,4]. Intriguingly, despite these defects, bacterial growth can gradually recover over time[3,4], indicating that compensatory mechanisms can remodel the translation apparatus to bypass the block in canonical assembly. Such adaptation reflects the inherent plasticity of ribosome biogenesis and suggests the existence of alternative maturation pathways that integrate with global translation control.

Here, we show that this adaptation involves coordinated actions of the ribosomal silencing factor RsfS[5] and translation initiation factors. Using high-resolution cryo-electron microscopy, we demonstrate that initiation factors associate with immature 30S subunits, preventing premature subunit joining until 30S assembly is complete. In parallel, RsfS binds to 50S subunits, blocking their association with incompletely matured 30S subunits. Together, these mechanisms safeguard translation fidelity during late stages of ribosome biogenesis (Figure 1). Our results reveal a previously unrecognised layer of quality control in bacterial ribosome maturation, in which ribosome-associated factors act cooperatively to maintain translation under biogenesis stress.

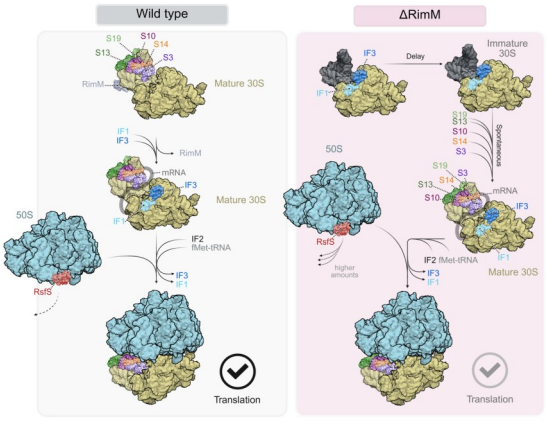


Fig. 1. **Proposed model for 70S assembly and translation recovery.** Left: In wild-type cells, RimM enhances the 30S head ribosomal proteins incorporation and facilitates correct 30S subunit maturation. IF1 and IF3 interact with mature subunits, allowing efficient initiation following subunit joining. Right: 30S head maturation is slowed down, and IF1 and IF3 due to their anti-association activities retain longer on the 30S subunit in ΔrimM strain in order to enable the 30S head maturation, where the ribosomal proteins are incorporated in spontaneous and stochastic manner. RsfS coordinates its anti-association function with IF1 and IF3 in order to avoid premature subunit association.

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.

REFERENCES

1. Davis J. H., Williamson J. R.: Philos. Trans. R. Soc. Lond. B Biol. Sci. *372,* 20160181 (2017).
2. Starosta A. L., et al.: FEMS Microbiol. Rev. *38*, 1172 (2014).
3. Guo Q., et al.: Nucleic Acids Res. *41*, 2609 (2013).
4. Leong V., Kent M., Jomaa A., Ortega J.: RNA *19*, 789 (2013).
5. Khusainov I., et al.: Nat. Commun. *11*, 1656 (2020).

L-12
RECENT ADVANCES IN CULTIVATION OF TREPONEMA PALLIDUM SUBSP. PALLIDUM, THE SYPHILIS SPIROCHETE

JURAJ BOSÁK[a], MATĚJ HRALA[a], FILIP SKOKAN[b], HANA JEDLIČKOVÁ[b], LENKA PAŠTĚKOVÁ[a], PETRA POSPÍŠILOVÁ[a], DAVID ŠMAJS[a]*

[a] Department of Biology, Faculty of Medicine, Masaryk University, Kamenice 5, 625 00 Brno, [b] First Department of Dermatovenerology, St. Anne's University Hospital Brno, Pekařská 53, 602 00 Brno, Czech Republic
dsmajs@med.muni.cz

Treponema pallidum subsp. *pallidum* (*T. pallidum*) is the causative agent of syphilis, a sexually transmitted human disease with over 8 million new cases per year worldwide[1].

For more than a century, *T. pallidum* was considered to be continuously uncultivable *in vitro*. In 2018, Edmondson and colleagues published a technique allowing *in vitro* cultivation of *T. pallidum*. The culture conditions involve the rabbit epithelial feeder cells, a complex liquid medium, and incubation at 34 °C in the microaerobic atmosphere (5% CO_2 and 1.5% O_2). For long-term propagation, the *T. pallidum* is subcultured every 7 days[2]. This method has revolutionized *T. pallidum* research, providing a platform for investigating treponemal physiology[3], antibiotic susceptibility[4–7], and genetic manipulation[8,9]. However, humanization of this culture system is necessary to study the host-pathogen interaction.

Only a handful of treponemal strains have been isolated so far, maintained *in vivo* using passages in rabbits. Recently, we developed an *in vitro* method to isolate *T. pallidum* directly from clinical samples (Figure 1). This way, we isolated six contemporary strains (MU1-MU6) from the Czech population, and characterized their genome. This approach eliminates the need for rabbit propagation, simplifying the isolation of clinically relevant treponemal strains[10].

While rabbit epithelial cells support treponemal multiplication in the original *in vitro* system[2], we now introduce human foreskin fibroblasts as feeder cells for *T. pallidum* cultivation[11]. This new system allows for the continuous cultivation of several treponemal strains, including the contemporary strain MU-6. This represents a significant step toward "humanizing" *T. pallidum* culture (Figure 1).

By developing a rabbit-free method for isolation and cultivation of *T. pallidum*, our research provides a more accessible and clinically relevant model to study syphilis pathogenesis.

Acknowledgment
The project National Institute of Virology and Bacteriology (Programme EXCELES, ID Project No. LX22NPO5103) – Funded by the European Union – Next Generation EU.

REFERENCES

1. Peeling R. W., Mabey D., Kamb M. L., Chen X.-S., Radolf J. D., Benzaken A. S.: Syphilis. Nat. Rev. Dis. Primers *3*, 17073 (2017).
2. Edmondson D. G., Hu B., Norris S. J.: mBio *9*, e01153-18 (2018).
3. Bosák J, Mikalová L, Hrala M, Pospíšilová P, Faldyna M, Šmajs D.: PLoS One *19*, e0304033 (2024).
4. Edmondson D. G., Wormser G. P., Norris S. J.: Antimicrob. Agents Chemother. *6*, e00979-20 (2020).
5. Hayes K. A., Dressler J. M., Norris S. J., Edmondson D. G., Jutras B. L.: Npj Antimicrob. Resist. *1*, e4 (2023).
6. Tantalo L. C., Lieberman N. A. P., Pérez-Mañá C., Suñer C., Vall Mayans M., Ubals M., González-Beiras C., Rodríguez-Gascón A., Canut A., González-Candelas F., Mueller J., Tapia K., Greninger A. L., Giacani L., Mitjà O.: The Lancet Microbe *4*, e994–1004 (2023).
7. Pospíšilová P., Bosák J., Hrala M., Krbková L., Vrbová E., Šmajs D.: Commun. Med. *5(1)*, 224 (2025).
8. Romeis E., Tantalo L., Lieberman N., Phung Q., Greninger A., Giacani L.: PLoS Pathog *17*, e1009612 (2021).
9. Grillová L., Romeis E., Lieberman N. A. P., Tantalo L. C., Xu L. H., Molini B., Trejos A. T., Lacey G., Goulding D., Thomson N. R., Greninger A. L., Giacani L.: Mol. Microbiol. *122*, 455 (2024).
10. Bosák J., Hrala M., Skokan F., Nečas M., Strnadel R., Vrbová E., Pospíšilová P., Jedličková H., Šmajs D.: Clin. Microbiol. Infect. *31(6)*, 1061 (2025).
11. Bosák J., Hrala M., Pospíšilová Bosakova M., Šmajs D.: PLoS Pathog, submitted.

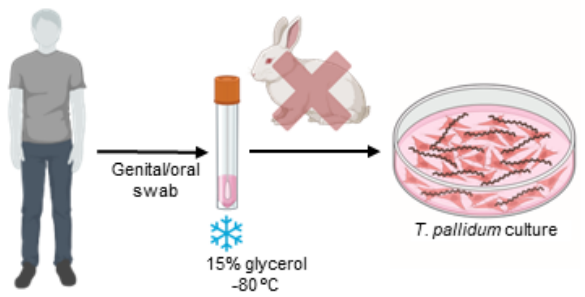


Fig. 1. **Isolation of clinically relevant *T. pallidum* strains**

L-13
VIRAL SATELITES OF *STAPHYLOCOCCUS EPIDERMIDIS*: HOW THEY AFFECT THE BIOLOGY OF BACTERIOPHAGES

ADAM VINCO, TIBOR BOTKA, ELIŠKA KUČEROVÁ, LUCIE KUNTOVÁ, KRISTÍNA ROVŇÁKOVÁ, IVANA MAŠLAŇOVÁ, ROMAN PANTŮČEK*

Department of Experimental Biology, Faculty of Science, Masaryk University, Kamenice 5, 625 00 Brno, Czech Republic
pantucek@sci.muni.cz

Bacteria and bacteriophages are the most widespread entities in the world[1]. Together, they form the basis of the nutrient cycle in ecosystems and are also used in fields such as biotechnology or medicine in the form of phage therapy, where we take advantage of the parasitic nature of phages. Similarly, phages also have their parasites, phage satellites[2]. These usually wait quietly in cells for a productive phage cycle when they hijack the phage machinery for packaging their DNA to capsids composed of phage proteins and, usually at the same time, interfere with phage propagation. Their accessory genes often contain virulence factors, antiphage defence systems or genes of unknown functions. This three-way relationship between bacteria, phages, and phage satellites gives rise to intriguing interactions and the simplicity of transmission, together with their abundance in bacterial genomes, makes phages and their satellites an integral part of bacterial populations.

Typical phage satellites in staphylococci are phage-inducible chromosomal islands (PICIs). We focused our research on *Staphylococcus epidermidis* PICI designated as $SeCI_{SE48}$[3]. The genome structure of $SeCI_{SE48}$ strongly resembles typical PICI, but unlike others, it does not encode any known genes associated with virulence or antibiotic resistance. To better understand its transduction and how it interacts with phages, we inserted an erythromycin marker into its genome, created in-frame deletions in individual $SeCI_{SE48}$ genes, and challenged strains bearing the modified island by phages with different life cycles and morphologies (myovirus and siphovirus).

This led us to the identification of a novel capsid morphogenesis protein (Cmp) responsible for small-headed virion formation, as well as an antiphage system encoded at the 3´ end of the $SeCI_{SE48}$ genome. We implemented various methods (mass spectrometry, cryo-EM, RT-qPCR, gene expression, and long-read Nanopore sequencing) and further analysed Cmp interactions with phage structural proteins, implications for its transduction, and how the antiphage system protects the bacterial population against therapeutic phages (Fig. 1). Sequence data analysis provided us with a detailed picture of the mechanism of packaging and the origin of DNA in virions, depending on the effects of various stressors. Together, these results expand our knowledge of $SeCI_{SE48}$ biology, the interaction of phages with their satellites in general, and how it affects bacterial populations.

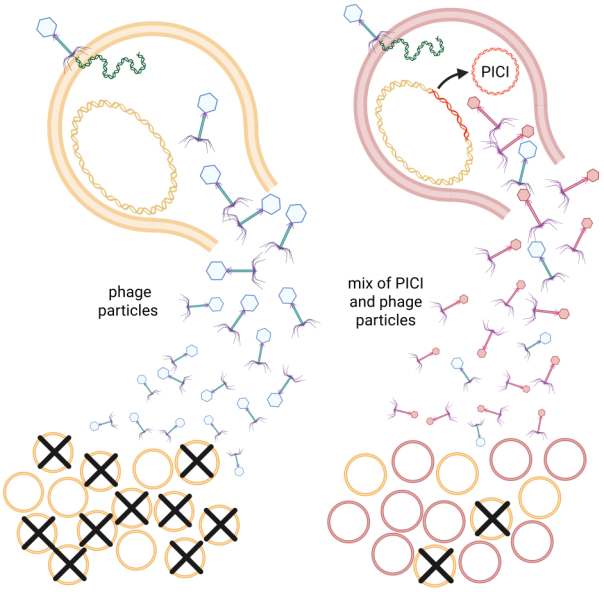


Fig. 1. **Phage-inducible chromosomal island (PICI) protects the bacterial population against phages. *Staphylococcus epidermidis* PICI interference with the bacteriophage life cycle provides resistance for bacteria at the population level and promotes its transduction to new recipients**

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.

REFERENCES

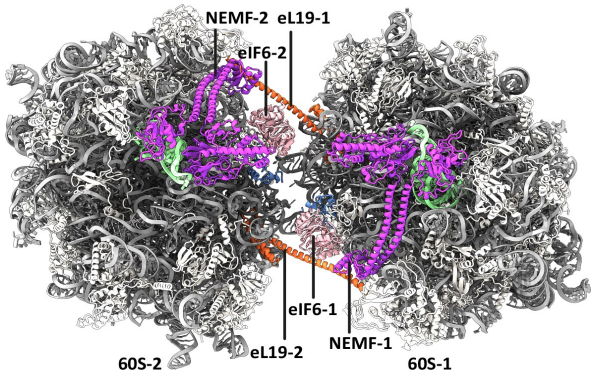
1. Brüssow H., Hendrix R. W.: Cell *108*, 13 (2002).
2. Penadés J. R., Seed K. D., Chen J., Bikard D., Eduardo P. C. Rocha.: Nat. Rev. Microbiol. *23*, 410 (2025).
3. Fišarová L., Botka T., Du X., Mašlaňová I., Bárdy P., Pantůček R., Benešík M., Roudnický P., Winstel V., Larsen J., Rosenstein R., Peschel A., Doškař J.: mSphere *6*, e00223-21 (2021).

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

PETR TĚŠINA*, VITA VIDMAR, MICHAL SÝKORA

CEITEC MU, Kamenice 753/5, 625 00 Brno, Czech Republic
petr.tesina@ceitec.muni.cz

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. **Molecular model of a human RQC complex dimer cryo -EM structure with P-site tRNA, NEMF, eIF6 and eL19 molecules highlighted.** The dimer consists of two 60S ribosomal subunits stabilized in this conformation by interaction of RQC factors and ribosomal proteins.

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.

To provide mechanistic understanding of these processes, we employ cryogenic electron microscopy (cryo-EM). To be able to study translation of defined viral mRNAs and allow for straightforward structural characterization, we are developing a cell free *in vitro* translation system from specifically modified human cells.

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.

REFERENCES

1. Ikeuchi K., et al.: EMBO J. *38*, e100276 (2019).
2. Saito K., et al.: Nature *603*, 503 (2022).
3. Narita M., et al.: Nat. Commun. *13*, 6411 (2022).
4. Wu C. C., et al.: Cell *182*, 404 (2020).
5. Costa-Mattioli M., Walter P.: Science *368*, eaat5314 (2020).

P-19

CRISPR-ASSISTED STRUCTURAL MODIFICATION OF STAPHYLOCOCCAL BACTERIOPHAGES FOR IMAGING AND BIOSENSING APPLICATIONS

HANA ŠIMEČKOVÁ[a,b], PAVOL BÁRDY[a,c], LUCIE KUNTOVÁ[a], ELIŠKA MACHÁČOVÁ[a,d], TIBOR BOTKA[a], JÁN BÍŇOVSKÝ[b,e], JOSEF HOUSER[b,e], ZDENĚK FARKA[a,d], PAVEL PLEVKA[b], ROMAN PANTŮČEK[a], IVANA MAŠLAŇOVÁ[a]

[a] Department of Experimental Biology, Faculty of Science, Masaryk University, Brno, Czech Republic; [b]CEITEC MU, Masaryk University, Brno, Czech Republic; [c]Department of Chemistry, York Structural Biology Laboratory, University of York, York, United Kingdom; [d]Department of Biochemistry, Faculty of Science, Masaryk University, Brno, Czech Republic; [e]National Centre for Biomolecular Research, Faculty of Science, Masaryk University, Brno, Czech Republic
maslanova@mail.muni.cz

Lytic bacteriophages are effective therapeutic agents against antibiotic-resistant bacteria. Furthermore, recent advances in CRISPR-Cas–based genome editing techniques have expanded the possibilities of engineering phages with novel and unique properties. In our study, the polyvalent *Staphylococcus aureus* phage 812h1 was genetically modified by inserting a polyhistidine tag into an exposed loop of the tail sheath protein. An editing strategy combining homologous recombination with CRISPR-Cas10-assisted counter-selection allowed the construction of stable recombinant particles.

The His-phages were specifically recognized by antibodies, and their attachment to bacteria was visualized by fluorescence microscopy. The structural modifications did not impair the biological activity of the phages, and their functionality was further validated using bio-layer interferometry, enzyme-linked immunosorbent assay, and flow cytometry[1]. The engineered phages provide new opportunities for applications in research, diagnostics, environmental monitoring, and the development of advanced biosensing tools.

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.

REFERENCE

1. Šimečková H., Bárdy P., Kuntová L., Macháčová E., Botka T., Bíňovský J., Houser J., Farka Z., Plevka P., Pantůček R., Mašlaňová I.: ACS Synth. Biol. *14*(8), 2979 (2025).

P-20

CORRELATIVE FLUORESCENCE AND CRYO-ELECTRON MICROSCOPY FOR BIOFILM MATRIX ULTRASTRUCTURE ANALYSIS

MICHAELA PROCHÁZKOVÁ[a]*, DMYTRO DZIUBA[b], GREGOR WEISS[c]

[a] CEITEC Masaryk University, Kamenice 5, 62500 Brno, CZ, [b]CNRS, Laboratory of Bioimaging and Pathology, University of Strasbourg, 74 Route du Rhin – CS60024, Illkirch-Graffenstaden cedex, F-67401 Strasbourg, FR, [c]Institute of Medical Microbiology, University of Zurich, Gloriastrasse 28/30, 8006 Zurich, CH
michaela.prochazkova@ceitec.muni.cz

Staphylococcus aureus biofilms pose a significant challenge in clinical settings due to their resilience against antimicrobial treatments[1]. Understanding the ultrastructure of the biofilm extracellular matrix (ECM) is crucial for developing targeted interventions[2]. However, the ECM's hydration-sensitive nature and structural complexity make high-resolution imaging particularly challenging[3].

This study explores a correlative fluorescence and cryo-electron microscopy (cryo-EM) approach to visualize biofilms in near-native conditions. Biofilms are grown directly on EM grids and vitrified using the 'waffle' method to preserve ultrastructure[4]. Fluorescent markers targeting key ECM components – extracellular DNA, polysaccharides, and amyloid-like proteins – are evaluated for cryo-compatibility. We integrate two complementary cryo-EM workflows: volume cryo-focused ion beam scanning electron microscopy (cryo-FIB-SEM) in a slice-and-view mode to assess overall architecture and cryo-lamella preparation for high-resolution cryo-electron tomography.

Preliminary results demonstrate the successful retention of fluorescence signals in vitrified biofilms, enabling targeted high-resolution imaging. Future work will refine labeling strategies, optimize correlative workflows, and apply this approach to study biofilm adaptation under stress conditions. By bridging fluorescence-based biofilm characterization with high-resolution cryo-EM, this study will advance structural insights into biofilm ECM organization and function. This project is currently in the stage of a proposal for the MSCA fellowship.

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.

REFERENCES

1. Kaushik A., et al.: Pathogens *13*(1), 76 (2024).
2. Karygianni L., et al.: Trends Microbiol. *28*(8), 668 (2020).
3. Sauer K., et al.: Nat. Rev. Microbiol. *20*(10), 608 (2022).
4. Kelley K., et al.: Nat. Commun. *13*, 1857 (2022).

P-21
THE ROLE OF SEMI-DISORDERED SCAFFOLD PROTEINS IN PHAGE LUZ19 PROCAPSID ASSEMBLY

ZUZANA CIENIKOVÁ, YULIIA MIRONOVA, ANNA SOBOTKOVÁ, PAVEL PLEVKA*

CEITEC Masaryk University, Kamenice 753/5, 625 00 Brno, Czech Republic
zcienikova@mail.muni.cz

Pseudomonas phage LUZ19 exhibits broad infectivity across clinically relevant *P. aeruginosa* strains. Phage assembly starts from a dodecameric portal complex attached to the host cell membrane. The construction of the capsid shell around the portal is facilitated by scaffold proteins which create a mesh structure located inside the completed immature procapsid. The scaffolding is cleaved and discarded during the genome filling and the accompanying procapsid expansion.

To understand how the scaffold protein of LUZ19, with roughly half of its residues predicted in disordered linkers, establishes the icosahedral shape of the phage capsid and mediates the symmetry mismatch between the capsid and the portal vertex, we conducted a cryo-EM study of the immature LUZ19 phage particle. We show that the immature procapsid is 16% smaller and exhibits a rougher surface compared to the expanded capsid. The particle contains scaffold and inner core proteins alongside portal and capsid proteins. The inner core complex forms a tower structure inside the capsid, composed of three types of proteins stacked over the portal complex. Interestingly, the portal complex is not in direct contact with capsid proteins; instead, C-terminal α-helical domains of scaffold proteins bridge the interaction. Our ongoing research aims to elucidate the intricate interactions between scaffold proteins within the mesh, with particular focus on the functional role of their long, disordered N-termini.

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.

P-22
PROHEAD STRUCTURE OF *STAPHYLOCOCCUS AUREUS* BACTERIOPHAGE PHI812

MARYNA ZLATOHURSKA[a,b,*], MICHAELA PROCHÁZKOVÁ[a], ZUZANA CIENIKOVA[a], TIBOR FÜZIK[a], PAVEL PLEVKA[a]*

[a] *Central European Institute of Technology, Masaryk University, Kamenice 753/5, 625 00 Brno, Czech Republic*
[b] *Zabolotny Institute of Microbiology and Virology of the National Academy of Sciences of Ukraine, Zabolotny str. 154, 03143 Kyiv, Ukraine*
maryna.zlatohurska@ceitec.muni.cz, pavel.plevka@ceitec.muni.cz

Staphylococcus aureus phage phi812 is a promising agent for phage therapy due to its lytic activity against a broad host range, including antibiotic-resistant and biofilm-forming strains. To fill the gaps in knowledge about phi812 head assembly, this work used cryo-electron tomography (cryo-ET) and cryo-electron microscopy (cryo-EM).

Cryo-ET data revealed sequential stages of head assembly in infected cells: membrane-associated cup-like precursors, spherical proheads I, expanded proheads II, and DNA-filled mature heads. Sub-tomogram averaging revealed three distinct structures of phage assembly intermediates, differing in size and surface features. These include prohead I, genome-packaging intermediate of prohead II, and the DNA-filled head, with resolutions of 35, 45, and 31 Å, respectively.

The icosahedral reconstruction of phi812 head assembly intermediates resulted in three prohead II classes that differ in the occupancy of minor capsid proteins. The mature head and prohead II display a similar overall organization of the major capsid proteins. During DNA packaging, the asymmetric unit of prohead II undergoes an inward tilt of ~1.6° around a pivot point at the 5-fold axes, leading to capsid flattening and a slight size contraction in the mature head.

This study provides detailed structural insight into the *in situ* assembly of phage phi812 heads in *S. aureus*. Cryo-ET captured key stages of head maturation. High-resolution cryo-EM revealed that maturation involves radial expansion, followed by compaction and stepwise incorporation of minor capsid proteins.

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.
This project has received funding through the MSCA4Ukraine project, which is funded by the European Union. Views and opinions expressed are however those of the author(s) only and do not necessarily reflect those of the European Union. Neither the European Union nor the MSCA4Ukraine Consortium as a whole nor any individual member institutions of the MSCA4Ukraine Consortium can be held responsible for them.
The authors acknowledge the Cryo-electron Microscopy and Tomography core facility of CEITEC Masaryk University for obtaining the scientific data presented in this work.

P-23
LIPOSOMAL BIONANOCOMPOSITES: ADVANCED BIO-BASED FORMULATION TECHNOLOGY FOR SUSTAINABLE APPLICATIONS

ANHELINA KYRYCHENKO[a,b]*, OLEKSII KOVALENKO[a,b]

[a] *D.K. Zobolotny Institute of Microbiology and Virology, NAS of Ukraine, 154 Acad. Zabolotny Street, D03680 Kyiv, Ukraine,* [b] *Institute of Biochemistry and Biophysics, Polish Academy of Sciences, 5a Pawińskiego, 02-106 Warsaw, Poland*
a.kyrychenko@imv.org.ua

Liposomes are artificial vesicular structures formed by lipid bilayers, capable of encapsulating both hydrophilic and hydrophobic substances in aqueous environments. Widely applied in pharmaceuticals, cosmetics, and food industries for drug delivery, liposomes have recently shown promise in agriculture as eco-friendly carriers of pesticides, fertilizers, and antiviral agents, enabling controlled release and reducing environmental impact.

We have developed a novel technology for producing liposomal bionanocomposites (BNCs) based on biologically active compounds of microbial and fungal origin, specifically rhamnolipids (RhLs) from *Pseudomonas aeruginosa* 301 ONU and polysaccharides (PS) from *Ganoderma lucidum* (Curtis:Fr.) Karst. Optimization of production conditions for RhLs and PS, along with purification technologies, was performed, and the optimal ratio of components was determined to maximize the yield, stability, and biological activity of the resulting nanostructures. The BNCs, organized as artificial glycan–glycolipid complexes, were characterized for their structural, morphological, physicochemical, and biological properties. Their biological activity was evaluated in various *in vitro* and *in vivo* experimental systems, demonstrating potential applications in agriculture as antiviral agents, biostimulants, and inducers of natural plant resistance. Field trials on *Triticum* L. confirmed enhanced crop productivity and improved resistance to cereal viruses.

The developed BNC biotechnology, leveraging hydrophobic-hydrophilic interactions to form supramolecular structures, expands the toolkit for modulating host–pathogen interactions. Understanding these processes will accelerate the development of broad-spectrum prophylactic and therapeutic agents for controlling viral infections in plants, warm-blooded animals, and humans. The biodegradability and low toxicity of BNCs position them as safe and universal tools for both agricultural and biomedical applications.

Acknowledgement
This research was supported by the Polish Academy of Sciences and the US National Academy of Sciences through the Long-term Program of Support for Ukrainian Research Teams (No. PAN.BFB.S.BWZ.407.022.2024). Additional support was provided by the project National Institute of Virology and Bacteriology (Programme EXCELES, ID Project No. LX22NPO5103), funded by the European Union – NextGenerationEU.

P-24
WHOLE-GENOME SEQUENCING AND BIOSYNTHETIC POTENTIAL OF SOIL-DERIVED *STREPTOMYCES* STRAINS ISOLATED FROM WAR-IMPACTED SOILS

MARIIA LOBODA[a]*, IVAN ROMAN[b], ANDRII SYLCHUK[a]

[a] *D.K. Zabolotny Institute of Microbiology and Virology of the National Academy of Sciences of Ukraine, Zabolotny str. 154, 03143 Kyiv, Ukraine,* [b] *Ivan Franko National University of Lviv, Hrushevskoho Street 4, 79005, Lviv, Ukraine*
mariialoboda@imv.org.ua

Streptomyces are valuable sources of natural products. *Streptomyces* sp. Y15, SK4, and SK16 were isolated from soil samples collected in Kryvyi Rih, Ukraine, near the Kryvorizhstal industrial complex, an area affected by missile strike. The soil was characterized by elevated concentrations of heavy metals: Zn(II) (921.5 mg/L), Ni(II) (200.0 mg/L), Cr(IV) (124.4 mg/L), Cu(II) (54.3 mg/L), and Fe(III) (218,4 g/L). Whole-genome sequencing was performed using the Illumina NovaSeq platform. Genome annotation was conducted using DFAST, the RAST, and antiSMASH 8.0. Antifungal activity against *Fusarium* species (was evaluated using a dual-block confrontation assay. Biocontrol potential against opportunistic microorganisms and phytopathogenic bacteria was assessed via a modified dual plate assay. To determine the ability to synthesize bioactive metabolites, LC-MS analysis was performed, and data were processed using Compass DataAnalysis 6.1 software. Phylogenetic analysis revealed that *Streptomyces* sp. Y15 was closely related to *S. youssoufiensis* X4 and *S. guanduensis* 701. *Streptomyces* sp. SK4 exhibited 100% identity with *S. lidicus* ATCC 25470, while *Streptomyces* sp. SK16 displayed close evolutionary ties to *S. apricus* SUN 51, *S. dioscori* QTP243123, *S. tauricus* JCM 4837, and *S. aurantiacus* BCCO 10-394. The genome of the studied strains encoded a diverse array of BGCs, indicating strong biosynthetic potential. Thus, *Streptomyces sp.* Y15 has an ecoded ability to synthesize youssoufene A1, B1, B2, B3, B4 piericidin, echoside A–E, disionitrile antibiotics SF2768, etc. Strain SK4 exhibited strong activity against *F. oxysporum* TR1 (11.00 mm). Strain Y15 showed broad-spectrum antifungal activity, particularly against *F. culmorum* DSM 21733 (11.05 mm). In contrast, SK16 inhibited only *F. oxysporum* DSM 21731 (11.3 mm). The analysis revealed limited bioactivity of the strains against phytopathogenic bacteria; however, they exhibited strong inhibitory effects against *S. aureus* ATCC 25923. In conclusion, the studied *Streptomyces* strains demonstrated diverse biosynthetic capacities and variable antimicrobial profiles highlighting their potential for future biotechnological applications.

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.

P-25
STEPWISE MECHANISM OF IF2α-DRIVEN BACTERIAL TRANSLATION INITIATION

GABRIEL SOARES GUERRA[a], HASAN ZAFAR[a], XUELIANG GE[b], AHMED HASSAN[a], SYLVA BRABENCOVA[a], LUIE SLAMOVA[a], HOWARD GAMPER[c], YA-MING HOU[c], SUPARNA SANYAL[b], GABRIEL DEMO[a]*

[a] Central European Institute of Technology, Masaryk University, Brno, Czech Republic, [b] Department of Cell and Molecular Biology, Uppsala University, Uppsala, Sweden [c] Department of Biochemistry and Molecular Biology, Thomas Jefferson University, Philadelphia, USA
gabriel.soares@ceitec.muni.cz

Bacterial translation initiation is a key regulatory step in gene expression, requiring precise coordination between initiator tRNA recruitment, initiation factors, and ribosomal subunit joining[1,2]. The process begins with the placement of the initiator $tRNA^{fMet}$ into the P-site of the 30S subunit, aided by initiation factor 1 (IF1) in the A-site and initiation factor 2 (IF2), a GTPase, promoting 50S subunit docking to form the 70S initiation complex (IC)[3]. IF2 exists in three isoforms (α, β, γ) in *E. coli*, with the α-form being the most active in promoting initiation[4]. Despite its functional importance, the α-form has never been structurally and mechanistically characterised in the context of the complete initiation pathway. In particular, the influence of its extended N-terminal domain on subunit joining and the molecular sequence linking GTP hydrolysis, phosphate release, and IF2 dissociation remain unresolved.

Here, we use the α-form of *E. coli* IF2 in combination with time-resolved cryo-electron microscopy to fill these gaps. Our results reveal that the IF2 N-terminal domain serves as a stable anchor on the 30S subunit during initiation. We capture distinct conformational states of IF2 following GTP hydrolysis, providing structural snapshots that map the pathway from subunit joining to IF2 release. These findings deliver the first integrated structural framework for IF2α–mediated initiation, offering time-resolved insight into factor dynamics that define the transition from initiation to elongation.

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.

REFERENCES
1. Gualerzi C. O., Pon C. L.: Cell. Mol. Life Sci. *72*, 4341 (2015).
2. Milón P., Rodnina M. V.: Crit. Rev. Biochem. Mol. Biol. *47*, 334 (2012).
3. Kaledhonkar S., Fu Z., et al.: Nature *570*, 400 (2019).
4. Eiler D., Lin J., et al.: Proc. Natl. Acad. Sci. USA *110*, 15662 (2013).

P-26
STRUCTURAL AND MECHANISTIC INSIGHTS INTO REPLICATION OF RIFT VALLEY FEVER VIRUS

AMIYARANJAN DAS[a,b], TOMAS KOTACKA[c], MILAN KOZISEK[c], GABRIEL DEMO[a]*

[a] Central European Institute of Technology, Masaryk University, Brno, [b] National Centre for Biomolecular Research, Masaryk University, Brno, [c] Institute of Organic Chemistry and Biochemistry, Czech Academy of Sciences, Prague, Czech Republic
amiyaranjan.das@ceitec.muni.cz

Rift Valley fever virus (RVFV) is a segmented, zoonotic RNA virus that causes severe disease in both humans and livestock. In newborn animals, mortality rates can approach 100%, while in humans, severe hemorrhagic forms carry fatality rates of up to 50%[1]. Central to RVFV replication and transcription is the viral RNA-dependent RNA polymerase (L-protein), which initiates RNA synthesis through a distinctive prime-and-realign mechanism directed by promoter sequences at the 5′ and 3′ termini of each genomic segment[2,3]. While this strategy is essential for viral propagation, the structural and mechanistic basis for promoter-driven regulation of RVFV replication has remained poorly understood.

Here, we applied single-particle cryo-electron microscopy to visualise the RVFV L-protein engaged with promoter sets during replication. Our structures capture distinct conformational intermediates and reveal how promoter binding modulates the architecture of functional domains, stabilising specific states along the replication pathway. These findings provide a mechanistic framework for understanding promoter-dependent control of viral RNA synthesis and identify structural features of the L-protein that could be targeted for antiviral intervention.

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.

REFERENCES
1. Kieny M. P., Salama P.: Lancet *389*, 2469 (2017).
2. Malet H., Williams H. M., Cusack S., Rosenthal M.: PLoS Pathog. *19*, e1011060 (2023).
3. Shalamova L., et al.: Nat. Commun. *15*, 7602 (2024).

P-27

EVALUTAION OF β-LACTAM ANTIBIOTIC RESISTANCE IN *TREPONEMA PALLIDUM*: AN EXPERIMENTAL APPROACH

PETRA POSPÍŠILOVÁ, JURAJ BOSÁK, MATĚJ HRALA, ELIŠKA VRBOVÁ, DAVID ŠMAJS*

Department of Biology, Faculty of Medicine, Masaryk University, Kamenice 5, 625 00 Brno, Czech Republic
dsmajs@med.muni.cz

Although syphilis diagnosis can be challenging due to variable symptomatology and limitations of serological tests, syphilis treatment has been considered straightforward, based on the decades-long universal susceptibility of TPA to penicillin[1]. Despite the lack of documented clinical evidence of antibiotic resistance, the numbers of reports of penicillin treatment failure are increasing.

Recently we reported a single nucleotide change (A1873G) in the penicillin binding protein gene TP0705 of *Treponema pallidum* subsp. *pallidum* (TPA) that resulted in partial resistance to ceftriaxone and penicillin G *in vitro*[2] representing a risk of TPA strains accumulating additional mutations leading to increased of β-lactam antibiotics resistance. Analysis of available sequence data revealed that contemporary TPA strains harbor 2 additional frequent polymorphisms (G1516A and C1517T) in TP0705 affecting the same amino acid suggesting they could also have influence on β-lactam antibiotic susceptibility of TPA.

During *in vitro* cultivation of type strains representing both clades of TPA in the presence of borderline ceftriaxone concentration experimentally shown to eliminate 95% of bacterial population, 2 different strains resistant to ceftriaxone were isolated. The level of resistance to ceftriaxone was same or bigger than of A1873G mutant proved to be partially resistant to ceftriaxone and penicillin G[3]. The sequencing revealed single nucleotide change in metal-dependent phosporylase TP0073 and peptidase family M23 protein TP0864 in SS14 strain and probable lipoprotein TP0346 and penicillin binding protein TP0760 in DAL-1 strain.

Altogether the presented findings challenge the alarming fact that syphilis management depends solely on antibiotics and our further experimental efforts aim to stress the need to accelerate syphilis vaccine development.

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 from the Czech Science Foundation (Grant No. 23-07021).

REFERENCES

1. Stamm L. V.: Epidemiol. Infect. *143*, 1567 (2015).
2. Pospíšilová P., Bosák J., et al.: Commun. Med. (Lond). *5*, 224 (2025).

P-28

GENOME-WIDE IDENTIFICATION OF GENETIC VARIABILITY WITHIN INFECTING TREPONEMAL POPULATIONS

KLÁRA JANEČKOVÁ, ELIŠKA VRBOVÁ, LENKA MIKALOVÁ, DAVID ŠMAJS*

Department of Biology, Faculty of Medicine, Masaryk University, 625 00 Brno, Czech Republic
dsmajs@med.muni.cz

Bacterium *Treponema pallidum* is a human pathogen. Based on the subspecies, it causes several diseases; *Treponema pallidum* subsp. *pallidum* (*TPA*) is the causative agent of syphilis, *Treponema pallidum* subsp. *pertenue* (*TPE*) is the causative agent of yaws, and *Treponema pallidum* subsp. *endemicum* (*TEN*) is the causative agent of bejel.

During host infection, populations of treponemes are not genetically identical but contain different subpopulations[1]. Previous studies revealed genetic heterogeneity on a genome-wide scale[2,3], but were limited in number of analysed genomes. Other studies mapped the instrastrain heterogeneity in 37 genomes and found more than a hundred variable positions in more than 70 genes. However, these studies were limited by low depth coverage of the sequenced genomes[4], incomplete genome sequences[5], and limited number of available genomic sequences[6].

This study analysed 13 genomes with available finished genome sequences where average depth coverage was higher than 100x to overcome the abovementioned limitations. Moreover, the sequences were analysed in separate parts to allow analysis of paralogous genome regions. Intrastrain heterogeneity sites including variability in homopolymeric tracts were determined and compared between the genomes.

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.

REFERENCES

1. Baseman J. B., Nichols J. C., Rumpp J. W., Hayes N. S.: Infect Immun. *10*, 1062 (1974).
2. Matějková P., Strouhal M., Šmajs D., Norris S. J., Palzkill T., Petrosino J. F., Sodergren E., Norton J. E., Singh J., Richmond T. A., Molla M. N., Albert T. J., Weinstock G. M.: BMC Microbiol. *8*, 76 (2008).
3. Pětrošová H., Pospíšilová P., Strouhal M., Čejková D., Zobaníková M., Mikalová L., Sodergren E., Weinstock G. M., Šmajs D.: PLoS One *8*, e74319 (2013).
4. Čejková D., Strouhal M., Norris S. J., Weinstock G. M., Šmajs D.: PLoS Negl. Trop. Dis. *9*, e0004110 (2015).
5. Pinto M., Borges V., Antelo M., Pinheiro M., Nunes A., Azevedo J., Borrego M. J., Mendonça J., Carpinteiro D., Vieira L., Gomes J. P.: Nat. Microbiol. *2*, 1 (2016).
6. Strouhal M., Mikalová L., Havlíčková P., Tenti P., Čejková D., Rychlík I., Bruisten S., Šmajs D.: PLoS Negl. Trop. Dis. *11*, e0005894 (2017).

P-29
MAPPING THE ESSENTIAL GENOME OF *TREPONEMA PALLIDUM* VIA TRANSPOSON MUTAGENESIS

MATĚJ HRALA, JURAJ BOSÁK, PETR ANDRLA, DAVID ŠMAJS*

Department of Biology, Faculty of Medicine, Masaryk University, Brno, Czech Republic
dsmajs@med.muni.cz

Treponema pallidum subsp. *pallidum* (*T. pallidum*), the etiological agent of syphilis, remains a major public health concern worldwide. For decades, the organism's fastidious growth requirements hindered its continuous *in vitro* propagation and greatly limited the development of genetic tools. Recent advances in cultivation methods[1] have now made genetic manipulation possible[2]. In this work, we aimed to identify genes that are non-essential for *T. pallidum* survival. To achieve this, we applied random transposon mutagenesis, generating insertional mutants at multiple genomic locations. Disruption of specific genes demonstrated that these loci are dispensable for bacterial viability under *in vitro* conditions. Our approach utilized chemocompetence-mediated transformation of two *T. pallidum* strains, DAL-1 and SS14, with a suicide plasmid harboring a transposon. Following transformation, cultures were maintained over multiple *in vitro* passages under continuous kanamycin selection, which enriched for viable mutants. High-throughput Illumina sequencing was then performed to accurately identify and map over thirty transposon insertion sites across the genome. These loci provide insights into the minimal gene set required for *T. pallidum* growth and may help uncover key determinants of treponemal virulence.

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.

REFERENCES
1. Edmondson D. G., Hu B., Norris S. J.: mBio *9*, e01153-18 (2018).
2. Pospíšilová P., Bosák J., Hrala M., Krbková L., Vrbová E., Šmajs D.: Commun. Med. (Lond). *5*, 224 (2025).

P-30
FROM QCM TO CHRONIC WOUND MODEL: EXPLORING PHAGE-ANTIBIOTIC SYNERGY AGAINST BIOFILMS

ELIŠKA KUČEROVÁ[a], RADKA OBOŘILOVÁ[b,c], HANA VAISOCHEROVÁ-LÍSALOVÁ[d], PETR SKLÁDAL[c], ZDENĚK FARKA[b,c], ROMAN PANTŮČEK[a], TIBOR BOTKA[a]*

[a] Department of Experimental Biology, Faculty of Science, Masaryk University, Kamenice 5, 625 00 Brno, [b] Department of Biochemistry, Faculty of Science, Masaryk University, Kamenice 5, 625 00, Brno, [c] Central European Institute of Technology, Masaryk University, Kamenice 5, 625 00, Brno, [d] Institute of Physics of the Czech Academy of Sciences, Na Slovance 1999/2, 182 00, Prague, Czech Republic
tibor.botka@mail.muni.cz

Chronic wound infections are often associated with biofilm-forming opportunistic pathogens, including *Staphylococcus aureus* and *Pseudomonas aeruginosa.* These biofilms are highly tolerant to conventional antibiotic therapy, leading to treatment failures and persistent infections. Bacteriophages represent a promising alternative or complementary approach, as they can target bacteria within biofilms. Importantly, synergistic interactions between bacteriophages and antibiotics may enhance therapeutic efficacy by improving bacterial eradication and preventing resistance emergence. To study such interactions, sensitive methods for real-time monitoring are needed.

Quartz crystal microbalance (QCM) enables the detection of biofilm formation and disruption with high temporal resolution and sensitivity. We applied QCM to monitor biofilm responses to phage–antibiotic combinations and identified conditions permissive for synergy[1]. To validate these findings, we employed an *in vitro* chronic wound model that mimics the complex environment of wound infections. Phage–antibiotic combinations were tested for their ability to reduce biofilm biomass and bacterial survival compared to single treatments.

This approach enables the rational design and optimisation of combined treatments for difficult-to-treat biofilm-associated infections.

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.

REFERENCE
1. Obořilová R., Kučerová E., Botka T., Vaisocherová-Lísalová H., Skládal P., Farka Z.: Sci. Rep. *15*, 3419 (2025).

P-31
COMPARISON OF PHARMACODYNAMIC AND PHARMACOKINETIC ASPECTS IN SYSTEMIC AND LOCAL INFECTION DURING PHAGE THERAPY IN A MURINE MODEL

IVANA MAŠLAŇOVÁ[a]*, LUCIE KUNTOVÁ[a], GABRIELA AMBROŽOVÁ[b], SOŇA SMETANOVÁ[c], EDITA JEKLOVÁ[d], HANA ŠIMĚČKOVÁ[a], ADAM VINCO[a], PETER MAKOVICKÝ[e], BŘETISLAV LIPOVÝ[f], ROMAN PANTŮČEK[a]

[a] Faculty of Science, MUNI, Kamenice 5, 625 00 Brno, [b] Institute of Biophysics of the Czech Academy of Sciences, Královopolská 2590/135, 612 00 Brno, [c] RECETOX, Faculty of Science, MUNI, Kamenice 5, 625 00 Brno, Czechia [d] Veterinary Research Institute, Hudcova 296/70, 621 00 Brno, [e] Faculty of Medicine, University of Ostrava, Syllabova 19, 703 00 Ostrava, [f] Third Faculty of Medicine, Charles University and University Hospital Kralovske Vinohrady, Ruská 87, 100 00 Prague 10, Czech Republic
maslanova@mail.muni.cz

Despite its long history and the urgent need for alternatives to antibiotics, phage therapy has not become a standard medical approach, largely due to limited pharmacological data from preclinical and clinical trials. Key gaps include understanding phage distribution and bacterial targeting. Insufficient knowledge of these aspects can lead to reduced therapeutic efficacy, such as low phage concentration at the site of infection or failure against specific strains.

Our study compares the pharmacological aspects of phage therapy in a murine model of open wound, where systemic *Staphylococcus aureus* infection occurs, and in a murine model of abscess, which simulates local infection. The pharmacological and pharmacokinetic aspects of phage therapy were studied in 170 mice over 5 days using an *S. aureus* abscess infection model. Phages exhibited immunomodulatory activity, as evidenced by changes in the expression of anti-inflammatory and pro-inflammatory markers of animals, assessed by RT-PCR and Western Blotting. The expression of inducible nitric oxide synthase (iNOS), which is a pro-inflammatory marker in the liver, was increased in groups of animals infected with *S. aureus* and decreased in the phage-treated group. Phage kinetics were monitored using qPCR and plaque titration methods throughout the experiments, revealing rapid clearance of bacteriophages from the bloodstream within one-hour post-administration.

This study highlights pharmacological aspects of phage therapy, supporting its broader clinical application.

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 by the *Ministry of Health of the Czech Republic (NU22-05-00042).*

P-32
GENOMIC AND STRUCTURAL CHARACTERIZATION OF NOVEL *MACROCOCCUS* SIPHOVIRUS THAT WILL LIKELY REPRESENT A NEW SUBFAMILY WITHIN CAUDOVIRICETES

IVANA MAŠLAŇOVÁ[a]*, ZUZANA HLAVENKOVÁ[b], TIBOR FÜZIK[b], TIBOR BOTKA[a], KRISTÍNA ROVŇÁKOVÁ[a], LUCIE KUNTOVÁ[a], JIŘÍ NOVÁČEK[b], ROMAN PANTŮČEK[a]

[a] Department of Experimental Biology, Faculty of Science, Masaryk University, Kamenice 5, 625 00 Brno, [b] Central European Institute of Technology, Masaryk University, Kamenice 5, 625 00, Brno, Czech Republic
maslanova@mail.muni.cz

Within the Gram-positive family *Staphylococcaceae*, bacteriophages infecting *Staphylococcus aureus* have been extensively characterized, whereas those associated with the genus *Macrococcus* remain largely unexplored. Here, we characterized phage phi9224 induced from the *Macrococcus psychrotolerans* 19Msa1099 (ref.[1]) genome by mitomycin C.

SDS-PAGE and mass spectrometry were used for the identification of virion proteins. A viral genome was sequenced on the MinION sequencing platform. For the structure determination of phage phi9224, cryo-electron microscopy has been used.

Proviral DNA is integrated into the *rpsD* gene. A full-length viral genome is linear, 38.6 kbp long and encodes 56 predicted genes, including nine virion structure genes. The genome modular structure is typical for *Staphylococcaceae* siphophages. Two integrases are encoded: a site-specific integrase in the lysogeny genomic module and a tyrosine recombinase in the DNA-metabolism module. Database search showed that the phage has no significant similarity with known phage proteins.

The virion consists of a 65 nm icosahedral capsid (2.96 Å resolution) containing the phage genome, a connector between the capsid and the tail, and an approximately 300 nm long tail, which is terminated by a 52 nm long tail tip. The tail structure is composed of the major tail protein forming a hexameric ring arranged into a six-entry helix with a twist of 11.5° and 41.67 nm rise. The major tail protein was reconstructed to 2.90 Å resolution using a helical reconstruction algorithm. The tail tip of this phage is very inhomogeneous and not visible in cryo-EM data. Preliminary experiments showed the role of the tail tip in the infection of bacteria with very thick cell walls.

A novel phage subfamily is proposed based on morphological, phylogenetic, and genomic analyses.

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.

REFERENCE

1. Mašlaňová I., Kovařovic V., Botka T., Švec P., Sedláček I., et al.: Appl. Environ. Microbiol. *91*, e01652-24 (2025).

INSTITUTE OF MICROBIOLOGY OF THE CAS

L-15
THE DERMONECROTIC TOXIN OF BORDETELLA: A HIGHLY TOXIC PROTEIN WITH AN UNKNOWN ROLE IN PERTUSSIS INFECTION

ONDŘEJ STANĚK[a]*, IRENA LINHARTOVA[a], TOMAS VOMASTEK[a], ANICKA KRATOCHVILOVA[b], JANA HOLUBOVÁ[a], ZUZANA NICHTOVA[c], PETR MACEK[c], MILOSLAV KORINEK[d], TEREZA SMEJKALOVA[d], ONDREJ STEPANEK[c], PETER ŠEBO[a]

[a] Institute of Microbiology of the Czech Academy of Sciences, Vídeňská 1083, Prague, 142 20, [b] Institute of Molecular Genetics of the Czech Academy of Sciences, Vídeňská 1083, Prague, 142 20, [c] Biocev - Institute of Molecular Genetics of the Czech Academy of Sciences, Průmyslová 595, Vestec, 252 50, [d] Institute of Physiology of the Czech Academy of Sciences, Vídeňská 1083, Prague, 142 20, Czech Republic
stanek@biomed.cas.cz

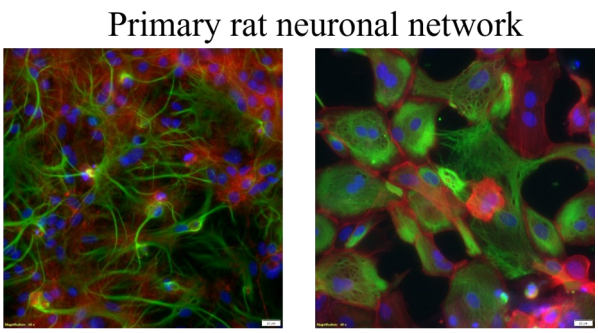
Lysing pathogenic *Bordetella* bacteria release a neurotropic dermonecrotic toxin (DNT), which is endocytosed into host cells and permanently activates RhoA family GTPases by polyamination or deamidation of glutamine residues in their switch II regions (e.g., Gln63 of RhoA).

In *B. bronchiseptica*, DNT facilitates bacterial colonization in the nasal cavity of pigs and inhibits the differentiation of nasal turbinate bone osteoblasts, contributing to atrophic rhinitis. However, the role of DNT in virulence of *B. pertussis* and pathogenesis of whooping cough remains unclear.

Recent studies identified T-type voltage-gated calcium channels (Cav3.1 and Cav3.2) as receptors for DNT. Our findings confirm that DNT interacts with these channels, facilitating calcium entry into cells and enhancing its RhoA polyaminase and deamidase activity. However, we did not observe a direct binding of DNT to cells via these channels, suggesting that additional receptor(s) may be involved.

We developed a method to purify large quantities of lipopolysaccharide (LPS)-free recombinant DNT with high biological activity on sensitive cells, including its fragments and detoxified variants. Contrary to earlier reports, we show that the C-terminal enzymatically active domain specifically binds to sensitive cells, while the N-terminal "binding" domain does not. Our results reveal that even extremely low concentrations of DNT (femtomolar) disrupt the function of primary rat neurons cultured *in vitro*. DNT damages astrocyte protrusions, halting their support for neurons, leading to progressive neuronal death and loss of action potential transmission. Additionally, intravenous administration of as little as 3 ng (18 fmol) of DNT in mice causes weight loss and severe neurological symptoms, ultimately resulting in death. We report significant progress in understanding of the molecular mechanisms underlying DNT's effects at such low concentrations and mapping of its cell-binding domains, shedding light on its potential role in *B. pertussis* pathogenesis.

Despite these findings, unfortunately, we have so far been unable to identify a clear role for DNT in *in vivo* infections using the mouse model. We have attempted to analyze the composition of cellular populations following infection with wild-type bacteria and bacteria with a mutation of the DNT gene, but no significant differences attributable to DNT were observed.



Control cells or
100 pg/ml DNT C1305S

100 pg/ml DNTwt

Low doses of rDNT destroy the neuronal network. Primary rat neuronal cells were isolated from one day old rats. Astrocytes were isolated from the brain cortex and cultured separately for 14 days before hippocampal neurons were isolated and seeded onto astrocytes to form the astrocytes-neuron network (neurons are not stained in the images, only green-labelled astrocytes are visible). The co-culture of neurons + astrocytes was treated with 100 pg/ml of rDNTwt or DNT carrying the mutation in Cys1305 (rDNT_CS, the detoxified version of DNT) as a control for 5 days. rDNTwt caused loss of astrocyte protrusions and consequently astrocytes stopped feeding the neurons, which died and fall apart over time of incubation.

Acknowledgement
This work was supported by the Programme EXCELES No. LX22NPO5103) Funded by the European Union – NextGenerationEU

REFERENCE

1. Staněk O., et al.: Toxins (Basel) *12*, 596 (2020).

L-16
MODE OF ACTION OF SUBLANCIN: A GLYCOSYLATED ANTIMICROBIAL

JIŘÍ POSPÍŠIL[a]*, SÁRA MICHKOVÁ[a], VERONIKA KOČÁRKOVÁ[a], DRAGANA VÍTOVSKÁ[a], MAREK SCHWARZ[a], JAN KEIL[a], ANNA BENEŠOVÁ[b], TEREZA DOLEJŠOVÁ[b], RADOVAN FIŠER[b], DOMINIK REJMAN[c], LIBOR KRÁSNÝ[a]*

[a] *Institute of Microbiology of the Czech Academy of Sciences, Vídeňská 1083, 142 20 Prague,* [b] *Faculty of Science, Charles University, Viničná 5, 128 00 Prague,* [c] *Institute of Organic Chemistry and Biochemistry of the Czech Academy of Sciences, Flemingovo nam. 542/2, 160 00 Prague, Czech Republic*
jiri.pospisil@biomed.cas.cz

In the constant battle for nutrients and space, bacteria are equipped with an arsenal of toxic weapons. Among these belong glycocins, a distinct class of ribosomally synthesized and post-translationally modified antimicrobial peptides that contain covalently attached sugar moieties.

Despite growing interest in this unique group of antimicrobials, the modes of action of glycocins remain largely unclear. Unlike many classical antimicrobial peptides that disrupt membrane integrity through pore formation, glycocins tend to exhibit more specific and often non-lytic activity. Some are thought to interfere with essential intracellular targets or inhibit key metabolic processes, but the precise molecular mechanisms of action remain unknown for all currently characterised glycocins[1].

One of the best-characterised members of the glycocin family is sublancin, produced by *Bacillus subtilis*. Sublancin is a 37-amino-acid long peptide containing a unique S-linked glucose modification on a cysteine residue, along with two disulfide bridges that confer structural stability[2]. Sublancin kills various Gram-positive bacterial species, including clinically relevant pathogens such as *Bacillus cereus*, *Staphylococcus aureus*, and *Streptococcus pyogenes*, making it a promising candidate for development as a novel antibiotic[3]. Previous studies have shown that sublancin activity depends on the phosphotransferase system (PTS)[4]. It has also been shown that sublancin interferes with nucleic acid synthesis – specifically replication and transcription, suggesting that sublancin is translocated into the bacterial cell via the PTS system and that its target(s) are located intracellularly[5].

Another aspect that remains unclear is how the sublancin-producing strain protects itself against its own antimicrobial. It has been shown that protection is conferred by a membrane-bound antitoxin, SunI, although the underlying mechanism by which it provides protection has yet to be elucidated[6].

Advances in our understanding of sublancin action and immunity will be presented and discussed.

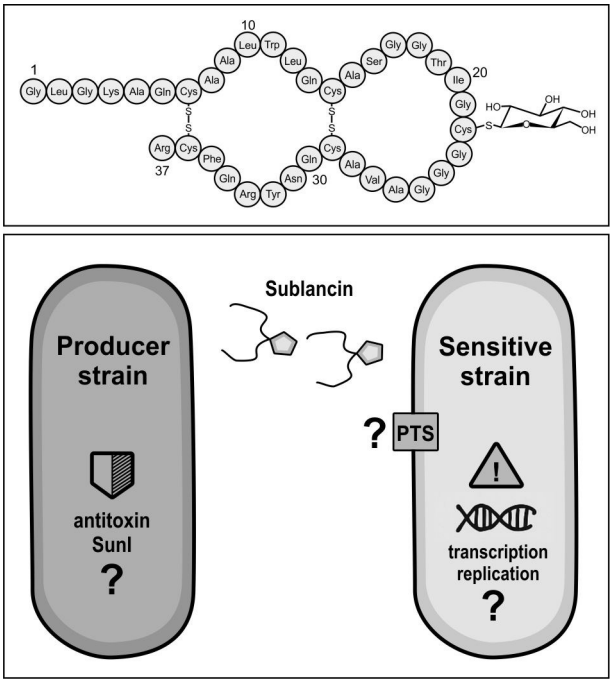


Fig. 1. **Sublancin – current knowledge. Upper panel** – Structure representation of sublancin. **Bottom panel** – A model summarizing all information published to date about sublancin: (i) The producer of sublancin is protected by the SunI antitoxin. (ii) The PTS system of the sensitive strain plays an important role in the effect of sublancin. (iii) Sublancin inhibits nucleic acid synthesis.
Individual aspects that require further investigation are marked with a question mark. The mechanism of action of sublancin has not yet been clarified.

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.

REFERENCES

1. Ahlawat S., Shukla B. N., Singh V., Sharma Y., Choudhary P., Rao A.: Biotechnol. Adv. *75*, 108415 (2024).
2. Oman T. J., Boettcher J. M., Wang H., Okalibe X. N., van der Donk W. A.: Nat. Chem. Biol. *7*(2), 78 (2011).
3. Paik S. H., Chakicherla A., Hansen J. N.: J. Biol. Chem. *273*(36), 23134 (1998).
4. Garcia De Gonzalo C. V., Denham E. L., Mars R. A., Stülke J., van der Donk W. A., van Dijl J. M.: Antimicrob. Agents Chemother. *59*(11), 6844 (2015).
5. Wu C., Biswas S., Garcia De Gonzalo C. V., van der Donk W. A.: ACS Infect. Dis. *5*(3), 454 (2019).
6. Dubois J. Y., Kouwen T. R., Schurich A. K., Reis C. R., Ensing H. T., Trip E. N., Zweers J. C., van Dijl J. M.: Antimicrob. Agents Chemother. *53*(2), 651 (2009).

L-17

NOVEL ERYTHROMYCIN RESISTANCE MECHANISM MEDIATED BY MrmA METHYLTRANSFERASE IN *C. DIFFICILE*

FANNY DEMAY[a], KAREL SKUBNIK[b], VOJTECH KOVAROVIC[a], MICHAELA NOVOTNÁ[a], MARTIN HLAVATY[c], ANNEMIEKE FRIGGEN[c], WEIS K. SMITS[c], MARCELA KRŮTOVÁ[d], GABRIELA BALÍKOVÁ NOVOTNÁ[a]*

[a] *Institute of Microbiology, The Czech Academy of Sciences, BIOCEV, Vestec, Czech Republic,* [b] *CryoElectron Microscopy and Tomography Core Facility, Central European Institute of Technology, Brno, 601 77, Czech Republic,* [c] *Experimental Bacteriology, Leiden University Center for Infectious Diseases, Leiden Medical Center, Leiden, Netherlands,* [d] *Department of Medical Microbiology, Charles University 2nd Faculty of Medicine and Motol University Hospital, Prague, Czech Republic*

Clostridioides difficile is a leading cause of hospital-acquired diarrhea. However, the rise of antimicrobial resistance in epidemic *C. difficile* lineages compromises treatment efficacy and increases the risk of spreading resistance to other pathogens[1].

We have identified a novel macrolide resistance gene, *mrmA* (macrolide resistance methyltransferase A), by comparative genomic analysis of erythromycin-susceptible and resistant *C. difficile* strains[2]. The encoded MrmA, a putative SAM-radical 23S rRNA methyltransferase, shares homology with RlmN and Cfr. RlmN methylates the C2 atom at nucleotide A2503, which controls translational accuracy, whereas Cfr methylates the C8 atom at the same nucleotide, resulting in resistance to phenicols, lincosamides, oxazolidinones, pleuromutilins and streptogramin A, but not to erythromycin[3]. However, heterologous expression of *mrmA* in *E. coli* resulted only in specific resistance to erythromycin and streptogramin B.

We hypothesize that MrmA (Figure 1), similar to RlmN or Cfr, possesses methyltransferase activity but targets a distinct adenine position, disrupting macrolide and streptogramin B binding. Direct 23S rRNA nanopore sequencing and primer extension assays have indeed revealed altered methylation patterns at the 23S rRNA nucleotide A2058 in strains producing MrmA. This nucleotide is typically dimethylated at its N6 atom by Erm-family methyltransferases, conferring broad resistance to macrolides, lincosamides, and streptogramin B antibiotics. Structural basis of MrmA-mediated ribosome modification was determine by Cryo-EM studies and reveal new methylation on C2 of A2058.

This research uncovers a unique resistance mechanism mediated by RlmN-family enzymes in *C. difficile*, providing new insights into their substrate adaptability and the evolution of antibiotic resistance.

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.

REFERENCES

1. Krutova M., Kinross P., Barbut F., Hajdu A., Wilcox M. H., Kuijper E. J., ... & Morris T.: Microbiol. Infect. *24* (5), 469 (2018).
2. Krutova M., Kovarovic V., Brajerova M., Demay F., Zikova J., Prasad S., ... & Balikova Novotna G.: Emerging Microbes Infect. *14*(1), 2482698 (2025).
3. Atkinson G. C., Hansen L. H., Tenson T., Rasmussen A., Kirpekar F., Vester B.: Antimicrob. Agents Chemother. *57*(8), 4019 (2013).
4. Osterman I. A., Dontsova O. A., Sergiev P. V.: Biochemistry (Moscow) *85*, 1335 (2020).

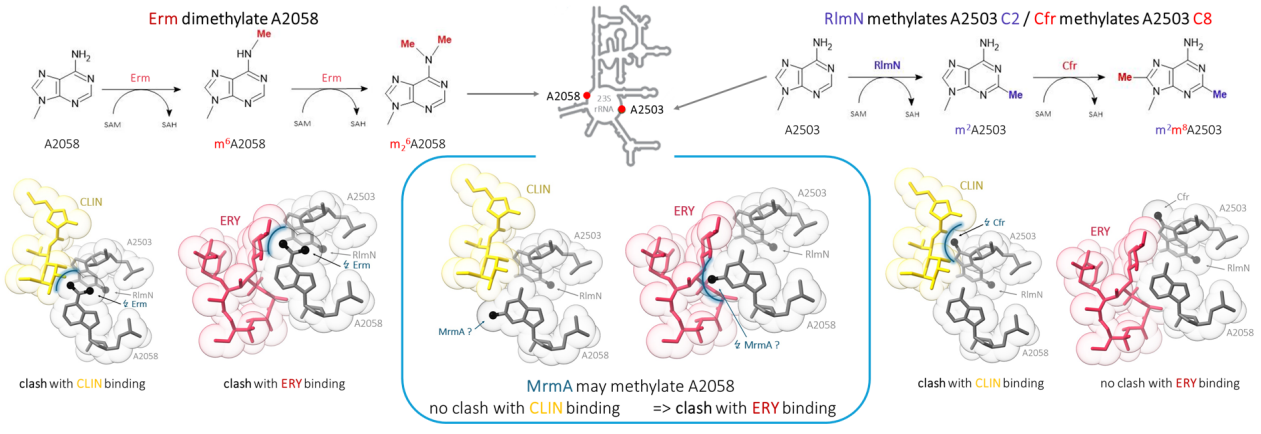


Fig. 1. **Hypothesis of MrmA-mediated methylation**

P-33

UNRAVELLING THE ROLE OF FILAMENTOUS HEMAGGLUTININ IN *BORDETELLA PERTUSSIS* COMPLEMENT EVASION

KEVIN MUNOZ-NAVARRETE[a], KATERINA VOCADLOVA[a], JANA PROSKOVA[a], JANA HOLUBOVA[a], ONDREJ STANEK[a], DAVID JURNECKA[a], LUDMILA BLECHOVA, ELODIE LESNE[b], ANDREW GORRINGE[b], PETER SEBO[a], LADISLAV BUMBA[a]*

[a] Institute of Microbiology of the Czech Academy of Sciences, Vídeňská 1083, 142 20 Prague 4, Czech Republic, [b] UK Health Security Agency, Porton Down, United Kingdom bumba@biomed.cas.cz

Bordetella pertussis is the causative agent of whooping cough (pertussis), a severe respiratory disease that remains the least controlled vaccine-preventable illness. The bacterium produces filamentous hemagglutinin (FhaB), a surface-exposed adhesin essential for bacterial attachment to the airway epithelium and biofilm formation. Additionally, FhaB is thought to have immunomodulatory functions, potentially subverting host immune defences through mechanisms that are not yet fully understood. In this study, we aimed to characterize the molecular mechanisms by which FHA contributes to *B. pertussis* resistance against complement-mediated killing. Using enzyme-linked immunosorbent assays (ELISA) and bio-layer interferometry (BLI), we demonstrated that the processed N-terminal ~220 kDa fragment of FhaB (mature FHA) interacts with the regulatory C4b-binding protein (C4BP), as well as with the C3 and C1q components of the complement system. Deletion of the fhaB gene (Δ*fhaB*) resulted in a significant increase in sensitivity of the deletion mutant to complementmediated killing as compared to the parental strain. Furthermore, flow cytometry revealed that the *ΔfhaB* mutant exhibited enhanced antibody-dependent deposition of complement proteins C3b/iC3b and C5b9 on its surface. These findings provide new insights into the complement evasion strategies of *B. pertussis* and may have implications for the development of targeted therapeutics against whooping cough.

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.

P-34

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

LUDMILA BLECHOVÁ[a,b], JANA HOLUBOVÁ[a], ONDŘEJ STANĚK[a], LADISLAV BUMBA[a], VERONIKA NIEDERLOVÁ[c], ALEŠ NEUWIRTH[c], JURAJ MICHALÍK[c], ANNA MORALES MENDEZ[c], ONDŘEJ ŠTĚPÁNEK[c], PETER ŠEBO*[a]

[a] Institute of Microbiology of the Czech Academy of Sciences, [b] Faculty of Science, Charles University, [c] Institute of Molecular Genetics of the Czech Academy of Sciences, Czech Republic

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 (Holubova et al., 2022, PLoSPathog 18: e1010402). 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.

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.

REFERENCE

1. Holubova J., et al.: PLoS Pathog *18*(4), e1010402 (2022).

P-35
BORDETELLA PERTUSSIS TOXINS DRIVE THE EMERGENCE OF A UNIQUE CD8⁺ T CELL SUBSET IN THE RESPIRATORY TRACT

ANNA KRATOCHVÍLOVÁ[a], JANA HOLUBOVÁ[a], ONDREJ STANEK[b], PETER SEBO[b], ONDREJ STEPANEK[a]*

[a] Institute of Molecular Genetics of the Czech Academy of Sciences, Vídeňská 1083, 142 20 Prague 4, [b] Institute of Microbiology of the Czech Academy of Sciences, Vídeňská 1083, 142 20 Prague 4, Czech Republic
ondrej.stepanek@img.cas.cz

Bordetella pertussis, the causative agent of whooping cough, is known for its ability to manipulate the host immune defense through the action of its enzymatically active adenylate cyclase, dermonecrotic and pertussis toxins (e.g. ACT, DNT and PT). The impact of their action on T cell populations and airway-specific mucosal immunity, however, remains poorly understood. We thus investigated the nasal cavity colonization dynamics of wild-type and toxin mutant strains of *B. pertussis* in a mouse model, focusing on the characterization of T cells in the mucosa of the upper respiratory tract (URT) and in lungs.

Our findings identified a distinct subset of $CD8^+$ T cells with an atypical phenotype that emerged both in the URT and lungs following *B. pertussis* infection. These cells exhibited an unconventional phenotype, marked by the expression of the transcription factor Eomes and checkpoint-inhibition receptors (Tigit and PD-1). Pertussis toxin (PT) activity was strongly implicated in driving this phenotype, while the effects of DNT and ACT action on T cell populations were more moderate. Additionally, we observed reduced T cell infiltration in the nose and lungs after infection with the mutant strain producing an enzymatically inactive PT-toxoid.

The emergence of the "strange" $CD8^+$ T cells following infection with PT+ wild-type bacteria reveals a novel immunomodulatory mechanism by which *B. pertussis* toxin action impacts adaptive immunity. Further studies will focus on the function and antigen specificity of these atypical $CD8^+$ T cells to elucidate their role in the immune response and disease pathology.

These results provide important insights into the interplay between *B. pertussis* toxins and host immunity, with implications for understanding respiratory immune responses and developing improved therapeutic strategies targeting pertussis and other respiratory infections.

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.

P-36
EVEN DISORDER HAS ITS RULES

KLÁRA MIEKSKOVÁ, LIBOR KRÁSNÝ, HANA ŠANDEROVÁ*

Laboratory of Microbial Genetics and Gene Expression, Institute of Microbiology, Czech Academy of Sciences, Vídeňská 1083, 142 20 Prague 4, Czech Republic
sanderova@biomed.cas.cz

Intrinsically disordered proteins (IDPs) do not adopt unique, well-defined three-dimensional structures and can rapidly convert their arrangement. Importance of IDPs in various physiological processes (often regulatory) has been recently recognized and studies by various approaches.

Transcripton is the key process of gene expression. The bacterial RNA polymerase (RNAP) is a mutlisubunit enzyme and in G+ Firmicutes contains also non-essential subunit delta. This protein consists of two domains – structured N-terminal domain and intrinsically disorded C-terminal domain[1,2]. The importance of this subunt for virulence of pathogenic Group B *Streptococcus* and *Staphylococccus aureus* was shown[3,4].

Here, we have performed set of experiments showing the role of the intrinsically disorded domain in regulation of transcription and cell fitness in G+ model organism *Bacillus subtilis* and pathogenic *S. aureus*.

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.

REFERENCES

1. Lopez de Saro F. J., et al.: J. Mol. Biol. *252*, 189 (1995).
2. Kubáň V., et al.: J. Am. Chem. Soc. *141*, 16817 (2019) .
3. Jones A. L., et al.: Mol. Microbiol. *37*(6), 1444 (2000).
4. Weiss A., et al.: Infect. Immun. *82*, 1424 (2014).

P-37
NOVEL TRANSCRIPTION FACTORS IN MYCOBACTERIA

NABAJYOTI BORAH[a], HANA ŠANDEROVÁ[a], TOMÁŠ KOVAL[b], ALENA KŘENKOVÁ[c], MARTIN HUBÁLEK[c], JAN DOHNÁLEK[b], LIBOR KRÁSNÝ[a]*

[a] Institute of Microbiology of the Czech Academy of sciences, Vídeňská 1083, 142 20 Prague, [b] Institute of Biotechnology of the Czech Academy of Sciences, v.v.i., Průmyslová 595, 252 50, Vestec, [c] Institute of Organic Chemistry and Biochemistry of the Czech Academy of Sciences v.v.i., Flemingovo nám. 2, 166 10 Prague 6, Czech Republic
krasny@biomed.cas.cz

Transcription, a fundamental step of gene expression, requires interaction of RNA polymerase (RNAP) with several other transcription factors[1]. While our understanding of bacterial transcription largely derives from model organisms such as *Escherichia coli* and *Bacillus subtilis*, species-specific transcriptional adaptations remain poorly characterized. Mycobacteria, for instance, employ unique transcription factors such as CarD and RbpA- absent in *E. coli* – to modulate their gene expression and adapt to stress conditions[2].

In this study, we aimed to define the RNAP interactome in *Mycobacterium smegmatis* and identify novel transcription factors. To achieve this, we performed pull-down experiments using a FLAG-tagged RNAP strain, followed by mass spectrometry. Several proteins co-purified with RNAP-FLAG, including previously uncharacterized ones. Among these, the two most enriched uncharacterized proteins (hereafter referred to as X1 and X2) were selected for further analysis.

Interestingly, X1 was also detected in the HelD interactome. HelD is a transcription factor that helps dissociate RNAP from stalled transcription complexes and protects RNAP against the antibiotic rifampicin[3]. Our findings suggest a possible formation of a X1-HelD-RNAP complex. Additionally, X2 was successfully purified and structurally characterized using X-ray crystallography, providing a foundation for future mechanistic studies. Ongoing work aims to further characterize the functions of X1 and X2 and their roles in transcription regulation under both basal and stress-induced conditions.

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.

REFERENCES

1. Chen J., Boyaci H., Campbell E. A.: Nat. Rev. Microbiol. *19*(2), 95 (2021).
2. Jensen D., Manzano A. R., Rammohan J., Stallings C. L., Galburt E. A.: Nucleic Acids Res. *47*(13), 6685 (2019).
3. Kovaľ T., Borah N., Sudzinová P., Brezovská B., Šanderová H., Vaňková Hausnerová V., ... Krasny L., Kouba T.: Nat. Commun. *15*(1), 8740 (2024).

P-38
IDENTIFICATION OF A NOVEL RESISTANCE MECHANISM AGAINST D-CYCLOSERINE

VERONIKA KOČÁRKOVÁ, LENKA DEJMALOVÁ, MAREK SCHWARZ, ZDENĚK KAMENÍK, TAMARA BALGOVÁ, HANA ŠANDEROVÁ, LIBOR KRÁSNÝ*

Institute of Microbiology of the Czech Academy of Sciences, Vídeňská 1083, 142 20 Prague, Czech Republic
krasny@biomed.cas.cz

Antibiotic resistance is a major global health challenge. The incidence of infections caused by multidrug-resistant bacteria has been increasing in recent decades, while effective therapeutic options remain limited.

The broad-spectrum antibiotic D-cycloserine (DCS) is used as a second-line treatment for multidrug- and extensively drug-resistant tuberculosis[1–3]. DCS, a structural analog of D-alanine, is naturally produced by *Streptomyces garyphalus* and *Streptomyces lavendulae*[1,3]. It inhibits two essential enzymes of the bacterial cell wall peptidoglycan biosynthetic pathway – alanine racemase and D-alanine:D-alanine ligase[1–3].

Despite its clinical importance for over 60 years, the molecular mechanisms underlying resistance to DCS are not yet fully understood. In most cases, resistance arises from mutations in its primary target genes, *alr* and *ddlA*[2,4]. Additionally, mutations in the *cycA* gene, which encodes the DCS transporter, have been reported to contribute to DCS resistance[5].

Identifying and understanding additional mechanisms of DCS resistance is therefore critical for improving treatment outcomes and may contribute to the development of new therapeutic strategies.

Experiments in our laboratory suggest a novel resistance mechanism against DCS in the model bacterium *Bacillus subtilis*. The molecular basis of this resistance will be presented and discussed.

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.

REFERENCES

1. Batson S., de Chiara C., Majce V., Lloyd A. J., Gobec S., Rea D., Fülöp V., Thoroughgood C. W., Simmons K. J., Dowson C. G., Fishwick C. W. G., de Carvalho L. P. S., Roper D. I.: Nat. Commun. *8*, 1939 (2017).
2. Evangelopoulos D., Prosser G. A., Rodgers A., Dagg B. M., Khatri B., Ho M. M., Gutierrez M. G., Cortes T., de Carvalho L. P. S.: Nat. Commun. *10*, 4177 (2019).
3. de Chiara C., Homšak M., Prosser G. A., Douglas H. L., Garza-Garcia A., Kelly G., Purkiss A. G., Tate E. W., de Carvalho L. P. S.: Nat. Chem. Biol. *16*, 686 (2020).
4. de Chiara C., Prosser G. A., Ogrodowicz R., de Carvalho L. P. S.: ACS Bio Med Chem Au *3*, 233 (2023).
5. Chen J. M., Uplekar S., Gordon S. V., Cole S. T.: PLoS One *7*, e43467 (2012).

P-39
REPROGRAMMING STREPTOMYCES LINCOLNENSIS FOR HYBRID LINCOSAMIDE CELIN PRODUCTION

MARKÉTA KOBĚRSKÁ*, LUCIE KORBOVÁ, LUCIE ZDVOŘÁKOVÁ, DURGA MAHOR, MICHAELA PLECHATÁ, TOMMASO STEFANI, ZDENĚK KAMENÍK, GABRIELA BALÍKOVÁ NOVOTNÁ, JIŘÍ JANATA

Institute of Microbiology AS CR; Biocev, Průmyslová 595, 252 50 Vestec, Czech Republic
koberska@biomed.cas.cz

Lincomycin and celesticetin produced by *Streptomyces* species are lincosamide antibiotics, which share several early biosynthetic steps catalyzed by homologous enzymes. The key determinants of lincosamide antibiotic diversification are the pyridoxal-5′-phosphate (PLP)-dependent enzymes LmbF and CcbF, which direct the biosynthesis towards lincomycin and celesticetin, respectively. Although both enzymes recognize S-glycosyl-L-cysteine substrates, they catalyze distinct transformations that result in different sulfur atom modifications (1,2). Previously, leveraging this enzymatic divergence, a hybrid lincosamide named CELIN with improved antimicrobial activity was synthesized from precursors by *in vitro* combinatorial biosynthesis (3). While *in vitro* experiments highlight the utility of enzyme promiscuity and pathway recombination for antibiotic innovation, *in vivo* studies revealed the greater complexity of whole-pathway combinatorial biosynthesis.

In this poster, we demonstrate that replacement of the key biosynthetic enzyme LmbF with CcbF in *Streptomyces lincolnensis*, together with expression of the entire CELIN biosynthetic pathway, does not directly result in CELIN production. Instead, residual downstream reactions from lincomycin biosynthesis modify the CcbF activity, thereby favoring lincomycin formation. Consequently, we outline the critical biosynthetic steps required to reprogram *S. lincolnensis* into an efficient CELIN producer.

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.

REFERENCES

1. Kamenik Z., Kadlcik S., Radojevic B., Jiraskova P., Kuzma M., Gazak R., ... Janata J.: Chem. Sci. *7*(1), 430 (2016).
2. Mori T., Moriwaki Y., Sakurada K., Lyu S., Kadlcik S., Janata J., Mazumdar A., Koberska M. ... Abe I.: Nat. Chem. *17*(2), 256 (2025).
3. Kadlcik S., Kamenik Z., Vasek D., Nedved M., Janata J.: Chem. Sci. *8*(5), 3349 (2017).

P-40
UNIQUENESS OVER UNIVERSALITY: LS_AP ANTIBIOTICS ARE SEQUENCE-DEPENDENT TRANSLATION INHIBITORS

MICHAELA NOVOTNÁ[a,b], THIBAUD T. RENAULT[c], MÉLANIE GILLARD[c], VOJTĚCH KOVAŘOVIC[a], JULIE POKORNÁ[a], MARKÉTA KOBĚRSKÁ[a], C. AXEL INNIS[c], GABRIELA BALÍKOVÁ NOVOTNÁ[a]*

[a] Institute of Microbiology, The Czech Academy of Sciences, BIOCEV, Vestec, 25250, Czech Republic, [b] Faculty of Science, Department of Genetics and Microbiology, Charles University in Prague, Prague, 12800, Czech Republic, [c] Acides Nucléiques: Régulations Naturelle et Artificielle, UMR 5320, U1212, Bordeaux Biologie Santé, Université de Bordeaux, Centre National de la Recherche Scientifique, Institut National de la Santé et de la Recherche Médicale, Bordeaux, 33000, France
gnovotna@biomed.cas.cz

Lincosamide, streptogramin A, and pleuromutilin (LS_AP) antibiotics inhibit bacterial translation by binding to the catalytic center of the ribosome. However, despite their extensive use in medicine and research as inhibitors of translation initiation, their modes of action have never been studied in detail. Here, we applied ribosome profiling using both the standard Ribo-seq and the recently developed iTP-seq[1] approach to identify peptide-encoding transcripts that promote ribosome stalling in the presence of LS_AP antibiotics. We show that these drugs are not universal inhibitors of ribosome function, as commonly assumed, but instead display distinct, sequence-dependent modes of action dictated by the amino acid sequence of the nascent peptide. This was independently validated by *in vitro* and *in vivo* fluorescence reporter assays. Bacteria exploit this sequence-dependent inhibition to discriminate between antibiotics and to regulate the expression of resistance genes via ribosome-mediated attenuation. Building on these findings, we are now validating how LS_AP antibiotics modulate resistance gene regulation in native systems. Elucidating the mode of action of LS_AP antibiotics provides a conceptual framework for the rational development of next-generation antibiotics capable of overcoming resistance.

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.

REFERENCE

1. Seip B., et al.: Ribosomal stalling landscapes revealed by high-throughput inverse toeprinting of mRNA libraries. Life Sci. Alliance 1.5 (2018).

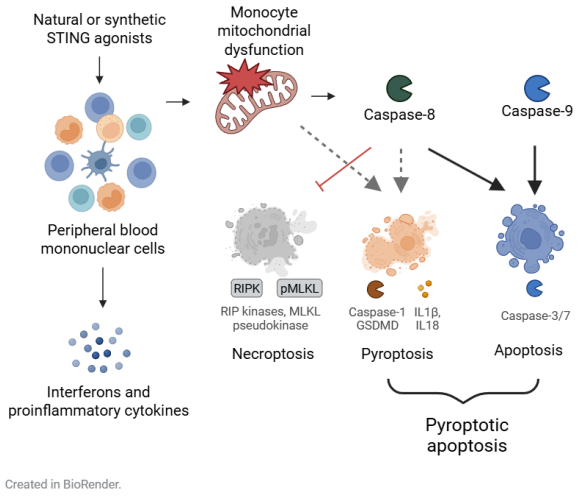
CHARLES UNIVERSITY

L-18
STING AGONISTS INDUCE PYROPTOTIC APOPTOSIS IN HUMAN MONOCYTES

MARKETA PIMKOVA POLIDAROVA[a,b], LYDIE PLECITA-HLAVATA[c], IVAN HIRSCH[a,b], KLARA GRANTZ SASKOVA[a]*, ANDREA BRAZDOVA[a]*

[a] Department of Genetics and Microbiology, Faculty of Science, Charles University, BIOCEV, Vestec, [b] Institute of Organic Chemistry and Biochemistry of the Czech Academy of Sciences, Prague, [c] Laboratory of Pancreatic Islet Research, Institute of Physiology of the Czech Academy of Sciences, Prague, Czech Republic
marketa.polidarova@natur.cuni.cz

Regulated cell death (RCD) represents a key outcome of the cyclic GMP–AMP synthase (cGAS)–stimulator of interferon genes (STING) activation in human monocytes, in addition to its canonical role in sensing cytoplasmic double-stranded DNA and inducing secretion of type I and III interferons and proinflammatory cytokines.[1,2] Pharmacological activation of cGAS–STING by STING agonists triggers a mixed RCD phenotype. Beyond induced cytokine release[3], it combines apoptotic mechanisms (caspase-9, caspase-8, and caspase-3/7 activation) with pyroptotic features (caspase-1 activation, gasdermin-D cleavage, and secretion of mature interleukin-1β and interleukin-18), while necroptosis is actively suppressed through caspase-8-dependent cleavage of receptor-interacting protein kinase 1 (RIPK1)[4]. This process is associated with mitochondrial dysfunction, which precedes caspase activation and likely functions both as a driving mechanism and a downstream consequence of cGAS–STING signaling via the STING–IRF3–BAX axis. Altogether, these findings define a distinc form of RCD in human monocytes referred to as "pyroptotic apoptosis" and provide mechanistic insights into the noncanonical outcomes of cGAS–STING pathway.



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.

REFERENCES

1. Motwani M., Pesiridis S., Fitzgerald K.A.: Nat. Rev. Genet. *20*, 657 (2019).
2. Murthy A. M. V., Robinson N., Kumar S.: Cell Death Differ. *27*, 2989 (2020).
3. Pimkova Polidarova M., Brehova P., Dejmek M., Birkus G., Brazdova A.: ACS Infect. Dis. *8*, 463 (2022).
4. Pimkova Polidarova M., Plecita-Hlavata L., Hirsch I., Grantz Saskova K., Brazdova A.: Cell Death Discov. Accepted in principle 3.9.2025 (2025).

L-19
REVERSE GENETICS AS A KEY TO UNDERSTANDING TOSCANA VIRUS – SAND FLY DYNAMICS

MARKÉTA STEJSKALOVÁ[a], NIKOLA POLANSKÁ[a], MAGDALÉNA JANČAŘOVÁ[a], MAXIME RATINIER[b], PETR VOLF[a]

[a]Charles University, Viničná 7, 128 00 Prague 2, Czech Republic, [b] Universite Claude Bernard Lyon 1, EPHE, Université PSL, Lyon, France
marketa.stejskalova@natur.cuni.cz

The Toscana virus (TOSV), a member of the genus *Phlebovirus* (*Phenuiviridae, Hareavirales*), is a neglected human pathogen that can cause febrile illness, severe infection of the CNS, or remain asymptomatic. It is distributed throughout the Mediterranean area and is transmitted by phlebotomine sand flies. To date, three TOSV lineages (A, B, C) have been identified; however, only two (A, B) have been successfully isolated. Vector competence varies among sand fly species. Although *Phlebotomus tobbi* and *P. sergenti* were found susceptible to TOSV B infection, *P. papatasi* and *Sergentomyia schwetzi* were refractory. Another tested TOSV strain (TOSV A) was found to be incapable of developing in any of the species tested[1].

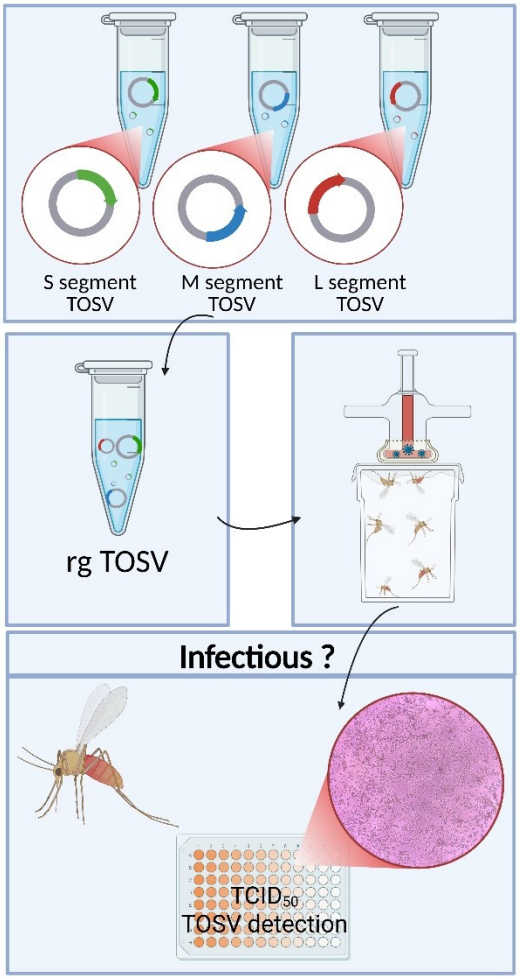
To explore these differences, we used a reverse-genetic system[2,3] to prepare TOSV, which produces viruses with defined RNA genomes and ensures a more uniform inoculum compared to repeatedly passaged isolates. This approach also enables the manipulation of viral genetic information, such as the reassortment of viral RNA segments (L, M, and S) or the introduction of targeted mutations.

We assessed the susceptibility of *P. tobbi* to two reverse genetics-derived TOSV strains: lineage A (rgTOSV A) and lineage B (rgTOSV B). Furthermore, we examined the infectivity of rgTOSV A reassortments carrying one of the RNA segments derived from rgTOSV B.

Our results show that the infection dissemination rate of rgTOSV B in *P. tobbi* is comparable to the wild-type (wt) strain. Unexpectedly, rgTOSV A could establish an infection (infection rate 10%) and disseminate (dissemination rate 15%) through the body of the sand fly to the head and salivary glands, unlike its wild-type counterpart.

Following the successful establishment of rgTOSV production and infectious sand fly feeding, ongoing experiments with rgTOSV A/B reassortments will elucidate which segments (and the proteins they encode) are crucial for sand fly infection.

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.



REFERENCES

1. Jancarova M., Polanska N., Thiesson A., Arnaud F., Stejskalova M., Rehbergerova M., Kohl A., Viginier B., Volf P., Ratinier M.: PLoS Negl. Trop. Dis. *19*, e0013031 (2025).
2. Alexander A. J. T., Confort M. P., Desloire S., Dunlop J. I., Kuchi S., Sreenu V. B., Mair D., Wilkie G. S., Da Silva Filipe A., Brennan B., Ratinier M., Arnaud F., Kohl A.: Viruses *12*, 411 (2020).
3. Thiesson A., Confort M. P., Desloire S., Kohl A., Arnaud F., Ratinier M.: npj Viruses *3*, 28 (2025).

L-20

UNVEILING THE COMPLEXITY OF PML NUCLEAR BODIES: IDENTIFICATION OF NOVEL PML ISOFORMS

KAROLÍNA ANDEROVÁ[a], LENKA HORNÍKOVÁ[a], VOJTĚCH ŠROLLER[a], BORIS RYABCHENKO[a], DALIBOR PÁNEK[b], PROKOPIS C. ANDRIKOPOULOS[c], JIŘÍ ZAHRADNÍK[c], JITKA FORSTOVÁ[a], SANDRA HUERFANO[a]*

[a] Faculty of Science, Charles University, BIOCEV Center, Průmyslová 595, Vestec 25250, [b] Imaging Methods Core Facility, BIOCEV Center, Průmyslová 595, Vestec 25250, [c] First Faculty of Medicine, Charles University, BIOCEV Center, Průmyslová 595, Vestec 25250, Czech Republic
huerfano@natur.cuni.cz

Promyeloctic leukemia protein (PML) provides structural scaffolding within multiprotein, membraneless organelles known as PML nuclear bodies[1]. Originally discovered as a tumor suppressor, PML is associated with diverse cellular regulatory processes and immune signalling. Intriguingly, the activity of PML is context-dependent, varying with intra- and extracellular environment[2,3]. While the broad spectrum of identified interacting partners suggests potential modes of action, the molecular mechanisms underlying the diverse functions of PML nuclear bodies remain incompletely understood. Emerging evidence indicates that individual splicing variants of PML are crucial factors affecting their composition and functions, opening new perspectives in PML nuclear body reseach[4,5].

Considering the limitations of current human *PML*$^{-/-}$ model systems, we aimed to deepen our understanding of the PML nuclear body system in mice, the most accessible homolog for human PML. We analyzed and characterized the full repertoire of PML isoforms expressed in various mouse cell lines and tissues. Building on the original cloning of murine *Pml*, our cDNA sequencing revealed that, in addition to the three previously described isoforms (mPML1–3), mice also express six computationally predicted PML variants (mPMLX1–X6)[6,7]. Furthermore, we identified a novel isoform, mPMLXK, characterized by a unique splicing event within its RBCC motif that disrupts its scaffolding property.

To examine isoform-specific features, we developed and validated a plasmid-based expression system encompassing all ten murine PML isoforms (mPML1–XK). Using diverse experimental approaches, we demonstrated that all isoforms – except mPMLXK – can form PML nuclear bodies *de novo* in cells lacking endogeonous PML, and that these bodies undergo SUMOylation-depedent degradation upon arsenic exposure. In contrast, all ten isoforms exhibit distinct turnover kinetics at endogenous PML nuclear bodies. Notably, molecular dynamics simulations indicated that mPMLXK adopts a stable, three-dimensional conformation, consistent with its integration into pre-formed nuclear bodies, suggesting its potential role as a cytosolic PML isoform.

Altogether, this isoform-resolved murine PML system offers a robust, physiologically relevant platform for dissecting isoforms-specific functions of the PML protein and PML nuclear body-associated processes.

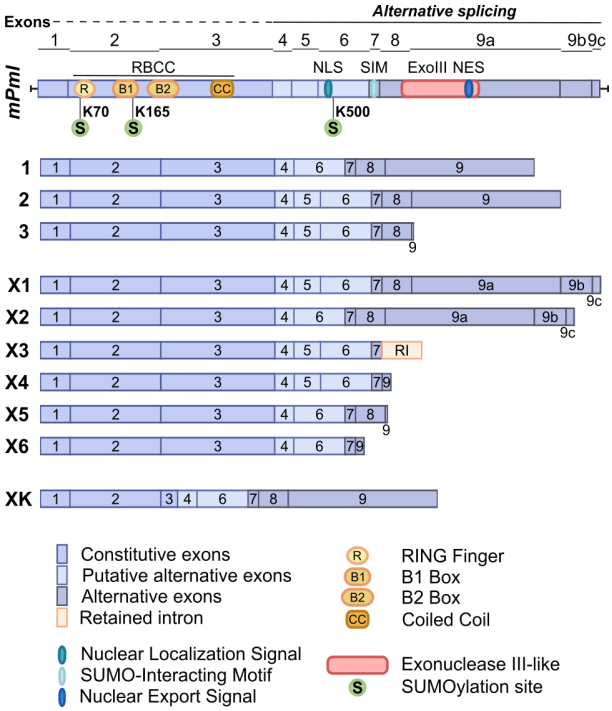


Fig. 1. **Schematic representation of exon organization and domain composition of individual murine PML isoforms**

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.

REFERENCES

1. Ishov A. M., et al.: J. Cell Biol. *147*, 221 (1999).
2. Kakizuka A., et al.: Cell *66*, 663 (1991).
3. Bernardi R., Pandolfi P. P.: Nat. Rev. Mol. Cell Bio. *8*, 1006 (2007).
4. Uggè M., Simoni M., Fracassi C., Bernardi R.: Trends Biochem. Sci. *47*, 609 (2022).
5. Nisole S., Maroui M. A., Mascle X. H., Aubry M., Chelbi-Alix M. K.: Front. Oncol. *3*, 1 (2013).
6. Goddard A. D., et al.: Mamm. Genome *6*, 732 (1995).
7. Condemine W., et al.: Cancer Res. *66*, 6192 (2006).

L-21
INVESTIGATING THE IMPACT OF ADAR1 ON HCV REPLICATION

MARTIN KUBŮ[a], KRISTINA ROUČOVÁ, MARTIN POSPÍŠEK, VÁCLAV VOPÁLENSKÝ

Laboratory f RNA Biochemistry, Department of Genetics and Microbiology, Faculty of Science, Charles University, 128 44 Prague 2 - Nové Město, Czech Republic
kubumart@natur.cuni.cz, vaclav.vopalensky@natur.cuni.cz

The hepatitis C virus (HCV) is a member of the Flaviviridae family. The virus is the causative agent of hepatitis C, a disease that affects tens of millions of people worldwide. New direct-acting antivirals (DAAs) have been shown to be highly effective in treating hepatitis C; however, a preventive vaccine against HCV has not yet been developed. Despite the fact that the HCV genome consists of single-stranded RNA, this RNA forms numerous secondary structures that can act as substrates for RNA-binding proteins of the innate immune system, including adenosine deaminase acting on double-stranded RNA 1 (ADAR1). This enzyme catalyses the conversion of adenosine to inosine, which is recognised by cellular mechanisms as guanine. This, in turn, leads to mutations in the targeted dsRNA molecule.

ADAR1 has been found to express two isoforms: a constitutively expressed nuclear isoform and an interferon-inducible cytoplasmic isoform. The process of ADAR1 editing of dsRNA molecules has been shown to impede the detection of these molecules by cytoplasmic antiviral signalling pathways. These pathways lead to interferon production, and subsequently, to ADAR1 production. It is therefore concluded that ADAR1 functions as a modulator of innate immunity. Previous studies have examined the relationship between HCV and ADAR1, attributing an antiviral function to the ADAR1 enzyme in the context of HCV infection[1–3].

In order to evaluate the impact of ADAR1 on HCV replication, an ADAR1 knockout cell line was generated from Huh7.5 hepatocellular carcinoma cells[1] and HCV was produced using pJFH1-pUC[5].

These cell lines, alongside with wild-type cell line, were used to generate the HCV replication curve and to ascertain their susceptibility to HCV infection. Furthermore, these cells were used for production of infectious viral particles of HCV, with which the same experiments were performed as with the virus produced in wild-type cells.

Our preliminary findings indicate that the knockout of the ADAR1 enzyme does not appear to have a significant effect on the amount of HCV RNA produced. However, it has been observed to affect the susceptibility of Huh7.5 cells to infection by HCV. We also show that HCV appears to adapt over time to an environment lacking the ADAR1 enzyme.

Acknowledgement
This research was supported by the project National Institute of virology and bacteriology (Programme EXCELES, ID Project No. LX22NPO5103) – Funded by the European Union – NextGenerationEU, by The Charles University Grant Agency (GAUK, no 249623) and by the Project P JAC CZ.02.01.01/00/22_008/0004575 RNA for therapy, Co-Funded by the European Union

REFERENCES

1. Taylor D. R., Puig M., Darnell M. E., Mihalik K., Feinstone S. M.: J. Virol. *79*(10), 6291 (2005).
2. Li Y., Masaki T., Shimakami T., Lemon S. M.: J. Virol. *88*(13), 7199 (2014).
3. Pujantell M., Franco S., Galván-Femenía I., Badia R., Castellví M., Garcia-Vidal E., Ballana E.: Antiviral Res. *156*, 116 (2018).
4. Roučová K., Vopálenský V., Mašek T., Del Llano E., Provazník J., Landry J. J., Pospíšek M.: RNA *30*(9), 1164 (2024).
5. Wakita T., Pietschmann T., Kato T., *et al.:* Nat. Med. *11*, 791 (2005).

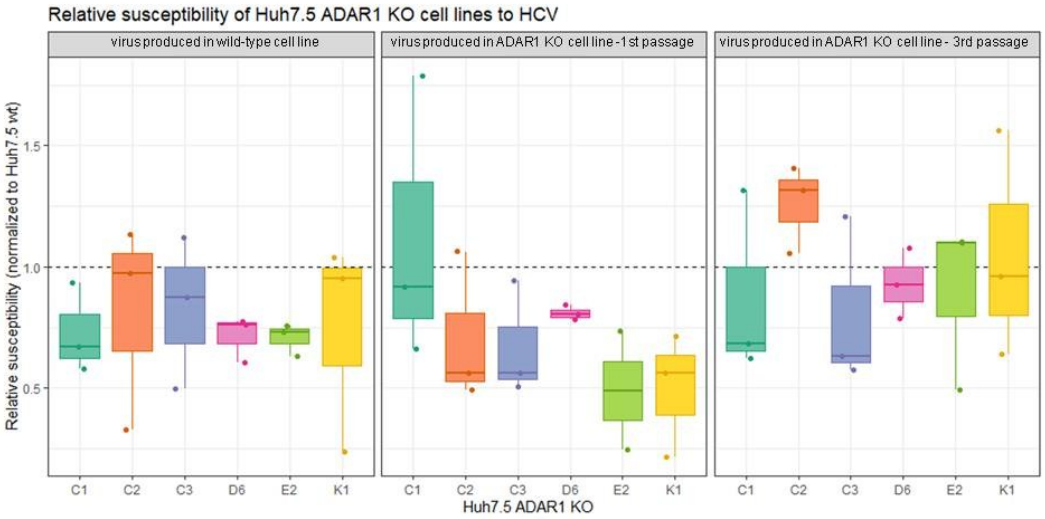


Fig. 1. **Relative susceptibility of Huh7.5 ADAR1 KO clones to infection of HCV produced in wild-type Huh7.5 cell line and Huh7.5 ADAR1 KO cell line (1st and 3rd passage of the virus)**

L-22

THE ROLE OF ASPARTATE BETA-HYDROXYLASE IN TUMORS ASSOCIATED WITH HUMAN PAPILLOMAVIRUSES

MADIHA KANWAL[a]*, JANA ŠMAHELOVÁ[a], SHWETA DILIP JOHARI[a], INGRID POLAKOVÁ[a], BARBORA ŽUCHA[a], MARK OLSEN[b], KATEŘINA KRAUSOVÁ[a], BARBORA POKRÝVKOVÁ[a], CARLOS EDUARDO MADUREIRA TRUFEN[c], JAROSLAV NUNVÁŘ[a], ELIŠKA ŠŤOVÍČKOVÁ[a], MAREK GREGA[d], ONDŘEJ VENCÁLEK[e], VLADIMÍR KOUCKÝ[f], SIMONA MALÉŘOVÁ[f], JAN KLOZAR[f], MURTAZA KHAN KASI[a], RUTH TACHEZY[a], MICHAL ŠMAHEL[a]*

[a] Department of Genetics and Microbiology, Faculty of Science, Charles University, BIOCEV, Průmyslová 595, 252 50 Vestec, Czech Republic, [b] Department of Pharmaceutical Sciences, College of Pharmacy – Glendale, Midwestern University, Glendale, AZ 85308, USA, [c] Czech Centre for Phenogenomics & Laboratory of Transgenic Models of Diseases, Institute of Molecular Genetics of the Czech Academy of Sciences, Prague, Czech Republic, [d] Department of Pathology and Molecular Medicine, Second Faculty of Medicine, Charles University and University Hospital Motol, Prague, Czech Republic, [e] Department of Mathematical Analysis and Applications of Mathematics, Faculty of Science, Palacky University Olomouc, Olomouc, Czech Republic, [f] Department of Otorhinolaryngology and Head and Neck Surgery, First Medical Faculty, Charles University and University Hospital Motol, Prague, Czech Republic
kanwalm@natur.cuni.cz, smahelm@natur.cuni.cz

Aspartate beta-hydroxylase (ASPH) is a transmembrane protein that is localized in the endoplasmic reticulum. It is not produced in most normal cells, but the expression is increased in tumor cells, where it can be localized to the cell surface and increase cell proliferation, migration, and invasion. ASPH stimulates several signaling pathways, including Notch1 and SRC pathways. It also interacts with vimentin to induce epithelial–mesenchymal transition, and with pRb to enhance cell cycle progression. ASPH overexpression is associated with poor prognosis in patients with various cancers. Small molecule inhibitors (SMIs) have been developed to reduce ASPH enzymatic activity[1].

We detected ASPH expression in head and neck squamous cell carcinomas[2]. A proportion of these tumors is associated with infection by human papillomaviruses (HPVs). Since ASPH is a hypoxia-responsive gene and hypoxia often leads to poor treatment response, some markers of hypoxia were also detected. We found increased levels of ASPH and also hypoxia markers in HPV-positive tumors, which can indicate active HPV infection and suggest sensitivity to ASPH inhibition.

Next, we analyzed the effect of ASPH inhibition in tumor cell lines of various origins (from cervical, pharyngeal, and breast cancers)[3]. Cell proliferation, migration, and invasion were reduced in all cell lines, but high heterogeneity was found in the alterations of signaling pathways, particularly canonical and noncanonical Notch1 pathways (Fig. 1).

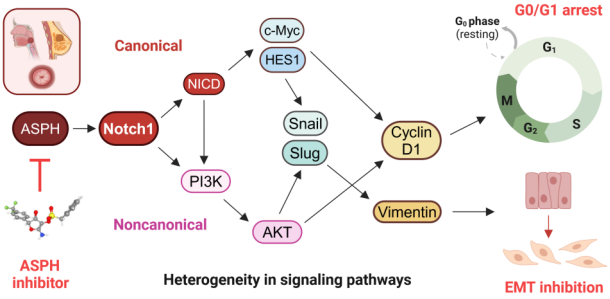


Fig. 1. **The effect of ASPH signaling on the canonical and non-canonical Notch1 pathways.** Created with BioRender.com

To detect new targets of ASPH signaling, we performed transcriptomic analysis in mouse and human tumor cell lines after ASPH inhibition. In mouse cell lines, we found reduced expressions of members of the lymphocyte antigen 6 (Ly6) family, which was also confirmed in human cell lines, including SiHa, HeLa, and CaSki cells infected with HPVs[4]. In human cell lines, we revealed downregulation of the interleukin 7 receptor (*IL7R*) gene. Since overexpression of Ly6 genes and IL7R signaling promote tumor development and progression, these results suggest novel mechanisms of carcinogenesis mediated by ASPH.

Finally, we tested the impact of ASPH inhibition on cancer immunotherapy in the mouse TC-1/A9 model of HPV-induced tumors. ASPH inhibition enhanced T-cell-mediated adaptive immunity induced by DNA vaccination against the HPV16 E7 oncoprotein. Various types of lymphoid and myeloid cells were involved in the stimulated immune response. These results suggest the possibility of combination therapy for human tumors, in which ASPH inhibition could suppress the oncogenic properties of tumor cells while promoting antitumor immunity.

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.

REFERENCES

1. Kanwal M., Smahel M., Olsen M., Smahelova J., Tachezy R.: J. Exp. Clin. Cancer Res. *39*, 163 (2020).
2. Smahelova J., Pokryvkova B., Stovickova E., Grega M., Vencalek O., Smahel M., Koucky V., Malerova S., Klozar J., Tachezy R.: Infect. Agents Cancer *19*, 26 (2024).
3. Kanwal M., Polakova I., Olsen M., Kasi M. K., Tachezy R., Smahel M.: J. Cancer *15*, 3466 (2024).
4. Kanwal M., Smahelova J., Ciharova B., Johari S.D., Nunvar J., Olsen M., Smahel M.: J. Cancer *15*, 1138 (2024).
5. Johari S. D., Krausova K., Zucha B., Madureira Trufen C. E., Polakova I., Olsen M., Smahel M.: Immunotargets Ther. *14*, 697 (2025).

L-23
EMERGING PATTERNS IN RESISTOME OF GRAM-POSITIVE HEALTHCARE-ASSOCIATED PATHOGENS

MARIE BRAJEROVÁ, JAROSLAVA ZÍKOVÁ, MARCELA KRUTOVÁ*, PAVEL DŘEVÍNEK

Department of Medical Microbiology, Charles University, Second Faculty of Medicine and Motol University Hospital, V Úvalu 84, 150 06 Prague 5, Czech Republic.
marcela.krutova@lfmotol.cuni.cz

Antibiotic resistance is a growing threat to public health, leading to higher healthcare costs and increased patient mortality. While a relatively large amount of data is available for Gram-negative bacteria in the Czech Republic, we still have insufficient information on the population structure and resistance of Gram-positive pathogens.

Epidemiology of vancomycin-resistant enterococci in the Czech Republic. In 2022, 165 vancomycin-resistant and 86 vancomycin-susceptible isolates from 20 hospitals across the Czech Republic were collected. Vancomycin resistance was predominantly driven by the *van*A operon carried on pELF2-like plasmids.

Resistance mechanisms to linezolid were found in 10 isolates (23S rDNA_G2576T, *cfrB*, *poxtA*), and 23 isolates were resistant to tigecycline (*tet*(L), *tet*(M), *tet*(S), nucleotide changes in *rpsJ*. In addition, 47 VRE isolates (29.0%) carried at least one recently described resistance mechanism to daptomycin, most frequently the RpoB_S491F mutation, along with RpoB_G482D, RpoB_H486Y, LiaS_T120M, and LiaR_W73C. However, isolates with these mutations displayed minimum inhibitory concentrations (MICs) to daptomycin within the range of those of wild-type strains.

Whole genome sequencing identified close genetic relatedness between resistant and susceptible enterococci.

These findings underscore the emergence and spread of vancomycin-resistant *E. faecium*, which has also acquired resistance mechanisms to last-resort antibiotics.

Newly emerging metronidazole-resistant *Clostridioides difficile* PCR ribotype 955[1]. In response to an alert about a metronidazole-resistant *C. difficile* PCR ribotype (RT) 955 outbreak in England, we investigated its presence in Central Europe.

Among 7,437 isolates from the Czech Republic and Slovakia, no RT955 strains were detected; however, 13 cases were identified in three Polish hospitals between 2021 and 2023. Whole genome sequencing revealed close clonal relatedness to a UK strain (Figure 1) and consistent resistance to multiple antimicrobials, including metronidazole, a drug of choice. Importantly, the metronidazole resistance was detectable only on haem-supplemented media, highlighting limitations of standard susceptibility testing.

These findings suggest the emergence of clonally related, haem-dependent metronidazole-resistant *C. difficile* RT955 in Poland and emphasise the urgent need for coordinated surveillance across Europe[1].

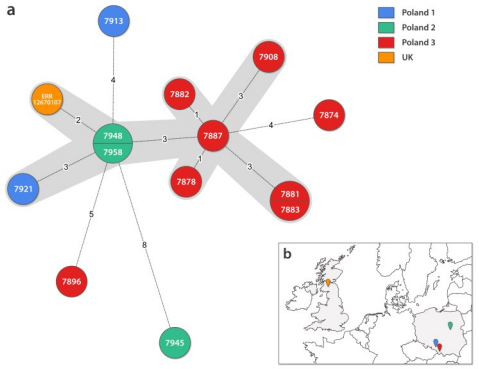


Fig. 1. **Minimum spanning tree of *Clostridioides difficile* ribotype 955 isolates and localisation of hospitals where the cases were identified, Poland, 2021–2023 (n = 13)[1]**

The heme-dependent metronidazole resistance in currently circulating Czech isolates of *Clostridioides difficile.* Although metronidazole is recommended only as a third-line treatment for *C. difficile* infection, it remains widely used in some countries. A recent study suggested that metronidazole resistance could be driven by a single mutation in the promoter of the *nim*B gene ($PnimB^G$) and detected phenotypically on media containing hemin[2].

To assess resistance mechanisms in Czech clinical isolates, 173 strains were tested, including 72 with mutations in the *nim*B promoter and/or coding region.

Resistance was consistently associated with additional *nim*B amino acid substitutions, not with promoter mutations alone. Distinct ribotypes carried unique mutations that variably affected susceptibility, and results differed between Fastidious anaerobe agar batches, complicating resistance detection.

These findings show that heme-dependent metronidazole resistance requires more than promoter mutations and that currently used media may fail to identify resistant strains reliably.

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.

REFERENCES

1. Zikova J., et al.: Euro Surveill. *30*(21), 2400675 (2025).
2. Olaitan A. O., et al.: Nat. Commun. *14*(1), 4130 (2023).

L-24

MONITORING THE ALTERNATIVE PATHWAY OF MEROPENEM DEGRADATION BY OXA-48-TYPE CARBAPENEMASES

VENDULA ŠTUDENTOVÁ[a,b], VERONIKA PAŠKOVÁ[a,b], LUCIA ĎAĎOVSKÁ[a,b], JAROSLAV HRABÁK[a,b]

[a] *Department of Microbiology, Faculty of Medicine, University Hospital in Pilsen, Charles University, Pilsen,* [b] *Biomedical Center, Faculty of Medicine, Charles University, Pilsen, Czech Republic*

Background: Overuse of carbapenems has been associated with an increasing prevalence of resistance, particularly among the *Enterobacterales*, *Pseudomonas* spp., and *Acinetobacter* spp. Resistance is mediated by the action of carbapenemases, which hydrolytically cleave the carbapenem molecule. In the case of OXA-48-type carbapenemases, an alternative pathway of carbapenem (e.g., meropenem) degradation via lactonization has been observed. According to current studies, this alternative degradation pathway is linked to the occurrence of false-negative results in carbapenemase detection methods based on hydrolytic cleavage of carbapenems. The aim of the study was to investigate the occurrence of β-lactone formation in Gram-negative bacteria and to assess the impact of the SOS response on β-lactone production in OXA-48-type carbapenemases.

Materials and Methods: Screening of isolates for carbapenemase production was performed using a meropenem hydrolysis assay by MALDI-TOF MS. The impact of SOS mechanisms on β-lactone formation was investigated in four fully sequenced OXA-48 carbapenemase-producing isolates (*Klebsiella pneumoniae*, *Escherichia coli*, *Citrobacter freundii*, and *Enterobacter cloacae*). For the experiments, Mitomycin C was applied at concentrations of 20, 40, and 60 mg/L, and amikacin was used at subinhibitory levels, as determined by the MIC (25 mg/L and 1 mg/L). Additionally, the effects of EDTA (20 mM), NaCl (150 mM), and pyridine (20 mg/L) on β-lactone formation were evaluated. The effects were monitored by LC-MS using a timsTOF Pro MS instrument (Bruker Daltonics, Germany).

Results: A total of 1,543 bacterial isolates were examined and screened for carbapenemase production. A specific β-lactone signal (m/z 362) was detected in 268 out of 272 OXA-48-type carbapenemase producers. The results further demonstrated that in the presence of both OXA-48 and NDM carbapenemases, β-lactone was not detected; 113 bacterial isolates produced these enzymes. Assessment of the impact of SOS-related mechanisms on β-lactone formation following exposure to Mitomycin C, EDTA, NaCl, and pyridine revealed no observable effect on β-lactone production. In contrast, experiments with amikacin consistently resulted in inhibition of β-lactone formation in the *C. freundii* isolate, whereas β-lactone production remained preserved in the other bacterial species.

Conclusion: Our results confirm that β-lactone formation is a characteristic feature of OXA-48-type carbapenemases as we previously published. The absence of β-lactone in strains producing both OXA-48 and NDM carbapenemases is most likely attributable to the faster kinetics of meropenem degradation by NDM enzymes. The observed inhibition of β-lactone formation in the presence of amikacin in the *C. freundii* isolate warrants further investigation.

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 by the Charles University Research Fund (GAUK) Nr. 280323.

REFERENCES

1. Studentova V., et al.: Sci. Rep. *12*, 22235 (2022).
2. Studentova V., et al.: Int. J. Antimicrob. Agents *63*, 107130 (2024).

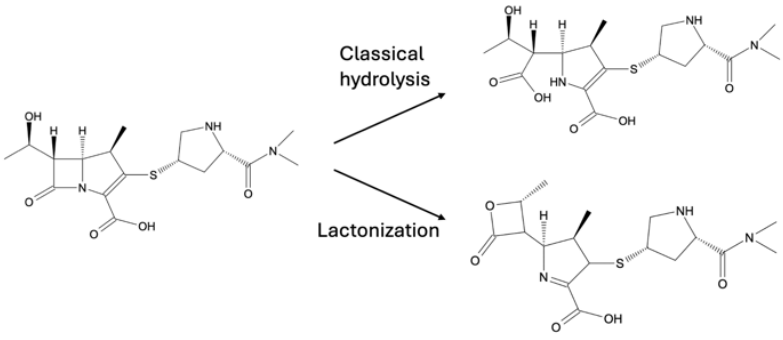


Fig. 1. **Schematic representation of the classical hydrolytic degradation of meropenem compared with the alternative lactonization pathway mediated by OXA-48-type carbapenemases**

L-25
STRUCTURE–ACTIVITY RELATIONSHIPS OF NOVEL PYRIMIDINE DERIVATIVES AS POTENTIAL ANTIMYCOBACTERIAL AGENTS

MARTIN KUFA[a,b], ONDREJ KOVAR[a,b], VLADIMIR FINGER[a,b], JAN KORABECNY[b], MARTIN KRÁTKÝ[a], JAROSLAV ROH[a]

[a] *Charles University, Faculty of Pharmacy in Hradec Kralove, Akademika Heyrovskeho, 1203, 500 03 Hradec Kralove,*
[b] *Biomedical Research Center, University Hospital Hradec Kralove, Sokolska 581, 500 05 Hradec Kralove, Czech Republic*
kufamar@faf.cuni.cz

Tuberculosis (TB), caused by *Mycobacterium tuberculosis* (Mtb), remains the leading cause of death from a single infectious agent, exerting severe health and economic burdens, particularly in Sub-Saharan Africa and South-East Asia. According to the WHO Global TB Report 2024, an estimated 10.8 million people developed TB and 1.25 million died from the disease in 2023, with approximately 400,000 cases of rifampicin- or multidrug-resistant TB.[1] Despite the WHO "End TB Strategy" launched in 2015,[2] progress towards its 2030 targets has been hindered by the COVID-19 pandemic, with only modest declines in incidence and mortality achieved so far. While TB primarily affects the lungs, extrapulmonary forms are common. Targeting the lipid -rich mycobacterial cell wall remains a key therapeutic approach; the essential transport protein MmPL3, involved in mycolic acid synthesis and export, represents a promising drug target whose inhibition disrupts cell-wall formation and leads to bacterial death.[3]

The pyrimidine derivative K1827 with antimyco-bacterial activity was designed by scaffold hopping from previously studied purine derivatives.[4] This structural simplification aimed to improve the physicochemical properties of the resulting compound, particularly its aqueous solubility. K1827 retained potent activity against the nontuberculous mycobacterium *M. kansasii* but lost efficacy against *M. tuberculosis* (Mtb). Through further structural optimization, activity against Mtb was restored to an MIC of 8 μM for K2783 and maintained with only a minor decrease against clinically isolated drug-resistant Mtb strains (Fig. 1).

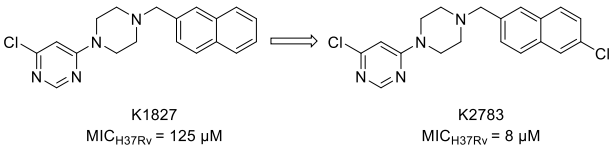


Fig. 1. **Structures and antimycobacterial activities of K1827 and its derivative K2783**

When investigating the mechanism of action, we initially focused on DprE1 – the validated target of the parent purine derivatives.[4] However, the scaffold simplification appeared to alter the mode of action, and current data suggest that inhibition of the essential transporter MmpL3 is the most probable mechanism.

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.

REFERENCES

1. WHO, Global tuberculosis report (2024).
2. WHO, End TB Strategy (2015).
3. Williams J. T., Abramovich R. B.: Microb. Drug Resist. *29*, 5, 190 (2023).
4. Finger V., et al.: Eur. J. Med. Chem. *258*, 115611 (2023).

L-26
OMICRON EMERGED UNDER STRONG AFFINITY SELECTION PRESSURE

RUOJIN TIAN[a], AVIV SHOSHANY[b], MiGUEL PADILLA-BLANCO[a,c], ADAM HRUŠKA[a], ADITI KONAR[a], KATARINA BAXOVA[d], EYAL ZOLER[b], MARTIN MOKREJŠ[a], GIDEON SCHREIBER[b], JIRI ZAHRADNIK[a]

[a] First Faculty of Medicine, Charles University, BIOCEV, Prumyslova st. 595, Vestec 25250, Czechia, [b] Dep. Biomolecular Sciences, Weizmann Institute of Science, Herzl st. 234, Rehovot 7610001, Israel, [c] Viral Immunology Lab, Molecular Biomedicine Department, Margarita Salas Center for Biological Research (CIB-CSIC), Madrid, Spain, [d] Institute of Organic Chemistry and Biochemistry, Czech Academy of Sciences, Flemingovo sq. 542/2, Prague 160 00, Czechia

In vitro protein evolution offers a unique lens to explore how viruses adapt to their hosts. We applied this approach to the SARS-CoV-2 spike receptor-binding domain (RBD), focusing on its interaction with the human ACE2 receptor which is the critical first step of infection. Because many hallmark SARS-CoV-2 mutations map to the RBD, we asked under what cirumstances the directed evolution of ACE2 binding could mirror the virus's natural trajectory and reveal the selective pressures behind it.

To address this, we designed two distinct evolutionary regimes: a low selection stringency system (LSS), in which ACE2 was present at concentrations approximating the wild-type affinity, and a stringent selection system (HSS), where ACE2 was reduced stepwise to sub-nanomolar levels and coupled with a heat challenge to eliminate unstable RBD variants. LSS mimics broad, permissive conditions by retaining a diversity of "reasonable" binders, whereas HSS selects only the most optimal binders. Random mutagenesis-based mimicking of viral evolution of the RBD was iterated through several rounds of selection in both regimes, starting from ancestral Wuhan and multiple variant sequences (Alfa, Beta, BA.1 and BA.2).

The results were striking. Under HSS, evolution rapidly converged on a limited set of high-frequency, non-synonymous mutations that closely match those found in Omicron and its sub-lineages, including N440K, L452R, N460K, S477N, T478K, Q498R, and N501Y[1]. Some later Omicron-associated mutations such as F486P and the "Flip 455–456" motif also emerged. By contrast, LSS produced a broader distribution of mutations, but at low frequencies, without reaching full Omicron-like convergence – even after extended rounds of mutagenesis. Importantly, when Omicron (BA.1/BA.2) was used as the starting point, its defining mutations were preserved with high fidelity under both LSS and HSS, demonstrating the evolutionary stability of the Omicron constellation once established.

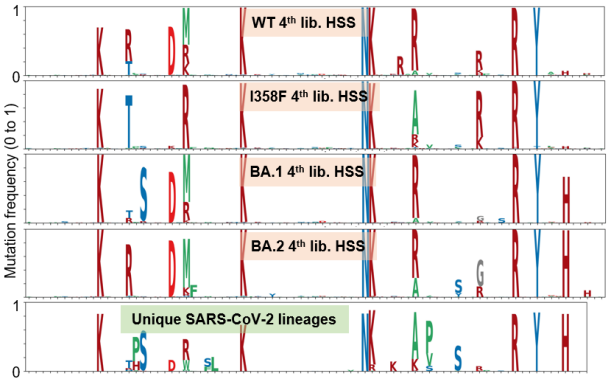


Fig. 1. **Sequence Logo plots showing the frequencies of mutations from HSS libraries in comparison to SARS-CoV-2 evelution represented as sequence logo of mutation frequencies among all Pango designed lineages**

Comparison with global SARS-CoV-2 data and in silico simulations confirmed that HSS conditions mimic real-world evolutionary leaps, while LSS reflects the slower, incremental accumulation of neutral or mildly beneficial mutations. Notably, mutations primarily associated with immune escape[2] (e.g., in the 444–456 and 486–490 regions) were not favored in our *in vitro* system, highlighting that receptor-binding constraints alone can explain much of the viral adaptation, while antibody-driven pressure accounts for the fixation of immune-evasion sites *in vivo*. This separation of pressures clarifies why some mutations that compromise ACE2 binding persist in natural viral evolution: they are maintained through immune evasion advantages.

Together, these findings support a model in which Omicron's abrupt emergence resulted from rare, high-stringency selection events – possibly in immuno-compromised hosts – superimposed on a broader landscape of mild selection pressures and immune evasion. Once Omicron emerged, its optimized binding profile ensured long-term evolutionary stability, positioning it as a "humanized" form of SARS-CoV-2. More broadly, our work highlights the predictive potential of *in vitro* evolution to anticipate adaptive pathways in viral proteins. Applying these principles to other coronaviruses, or even to SARS-CoV-1, reveals convergent solutions to ACE2 binding, pointing toward evolutionary constraints that may limit future zoonotic adaptation.

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.

REFERENCES

1. Dejnirattisai W., Jiandong H., Daming Z., et al.: Cell *185*, 3, 467 (2022).
2. Bdeir N., Lüddecke T., Maaß H., et al.: Nat. Commun. *16*, 809 (2025).

P-41
THE ROLE OF MIRNA-146A IN ANTIVIRAL IMMUNE RESPONSE TO TLR9 STIMULATION IN PERIFERAL BLOOD MONONUCLEAR CELLS

OLENA BEREHOVSKA[a,b], MARKETA PIMKOVA POLIDAROVA[a,b], ANDREA BRAZDOVA[a], IVAN HIRSCH[a], KLARA GRANTZ SASKOVA[a]*

[a] *Faculty of Science, Charles University, Department of Genetics and Microbiology, Biocev, Prumyslova 595, 252 50 Vestec,* [b] *Institute of Organic Chemistry and Biochemistry of the CAS, Flemingovo namesti 542/2, 166 10, Prague, Czech Republic*
berehovo@natur.cuni.cz

Hepatitis B virus (HBV) is a major global health threat, causing acute and chronic liver disease, leading cirrhosis, and hepatocellular carcinoma, with nearly one million deaths each year. Over 250 million people worldwide remain dependent on lifelong treatment with HBV polymerase inhibitors, as pegylated interferon-α (PEG-IFNα) benefits only a minority of patients.

Interferon-α (IFNα) plays a central role in antiviral defence and is primarily produced by plasmacytoid dendritic cells[1] upon Toll-like receptor 9 (TLR9) activation. This pathway is tightly regulated by microRNA-146a (miR-146a), which downregulates the signalling mediator, tumour necrosis factor receptor-associated factor 6[2]. Importantly, miRNAs can act beyond the miRNA-expressing cells, since extracellular vesicles (EVs) can deliver them to recipient immune cells, thereby modulating host antiviral responses.

Hence, we hypothesized that HBV infection can increase EV-associated miR-146a,[3] which is then taken up by immune cells, where it can downregulate antiviral responses. To test this, we exposed peripheral blood mononuclear cells (PBMCs) to conditioned media from HBV-producing and control hepatoma cell lines. While HBV did not directly activate TLR9 in PBMCs, conditioned media from HBV-producing cells selectively impaired responses to TLR9 agonists.

We are currently investigating whether inhibition of miR-146a restores antiviral activity.

Taken together, these findings suggest that miR-146a could serve as promising biomarker of immune dysfunction and a potential therapeutic target in chronic HBV infection.

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.

REFERENCES

1. Collin M., Bigley V.: Immunology. *154*, 3 (2018).
2. Park H., Huang X., Lu Ch., Cairo M. S., Zhou X.: J. Biol. Chem. *290*, 2831 (2015).
3. McKenzie A. J., Hoshino D., Hong N. H., Cha D. J., Franklin J. L., Coffey R. J., Patton J. G., Weaver A. M.: Cell Rep. *15*, 978 (2016).

P-42
STUDIES OF TOSV IN SAND FLIES: KEY MILESTONES

NIKOLA POLANSKÁ[a], MARKÉTA STEJSKALOVÁ[a], MAGDALÉNA JANČÁŘOVÁ[a], MAXIME RATINIER[b], PETR VOLF[a]

[a] *Charles University, Viničná7, Prague 2, 128 00, Czech Republic,* [b] *Universite Claude Bernard Lyon 1, EPHE, Université PSL, Lyon, France*
nikola.polanska@natur.cuni.cz

In recent years, our team has focused on Toscana virus (TOSV), a neglected pathogen that can be asymptomatic, cause febrile illness, or rarely severe CNS infection. TOSV is transmitted by Mediterranean sand flies, with *Phlebotomus perniciosus* and *P. perfiliewi* confirmed as vectors. However, the virus is also present in regions where these species are not widely distributed. We identified *P. tobbi* and *P. sergenti* as additional susceptible species[1].

Besides viruses, sand flies also transmit *Leishmania*, causing leishmaniasis, which co-circulates in the same geographical area as TOSV[2]. Their co-occurrence in the same vector may influence both pathogens and their transmission and epidemiology[3]. We demonstrated that in *P. tobbi* coinfected with *L. infantum* and TOSV, viral infection rates were significantly lower than in flies infected with TOSV alone.

Using the newly developed TOSV reverse genetic system[4,5] (rgTOSV), we further studied the role of individual TOSV RNA segments in sand fly infectivity.

All these findings advance our understanding of TOSV circulation, its potential spread across Europe, and its interactions with other important human pathogens.

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.

REFERENCES

1. Jancarova M., Polanska N., Thiesson A., Arnaud F., Stejskalova M., Rehbergerova M., Kohl A., Viginier B., Volf P., Ratinier M.: PLoS Negl. Trop. Dis. *19*, 5, e0013031 (2025).
2. Moriconi M., Rugna G., Calzolari M., Bellini R., Albieri A., Angelini P., Cagarelli R., Landini M. P., Charrel R., Varani S.: PLoS Negl. Trop. Dis. *11*, 8, e0005660 (2017).
3. Ayhan N., Prudhomme J., Laroche L., Bañuls A. L., Charrel R. N.: Microorganisms *8*, 1, 114 (2020).
4. Alexander A. J. T., Confort M. P., Desloire S., Dunlop J. I., Kuchi S., Sreenu V. B., Mair D., Wilkie G. S., Da Silva Filipe A., Brennan B., Ratinier M., Arnaud F., Kohl A.: Viruses *12*, 4, 411 (2020).
5. Thiesson A., Confort M. P., Desloire S., Kohl A., Arnaud F., Ratinier M.: npj Viruses *3*, 28 (2025).

P-43
TRAF6 DRIVES PARALLEL INNATE IMMUNE RESPONSES VIA TLR4 AND CGAS-STING DURING MPYV INFECTION

JUI BISWAS[a], BORIS RYABCHENKO[a], LENKA HORNIKOVA[a], VACLAV JANOVEC[a,b], KATEŘINA BRUŠTÍKOVÁ[a], MILAN DEJMEK[b], CZECH JAUCE[a], SANDRA HUERFANO[a]*

[a] Faculty of Science, Charles University, BIOCEV Center, Průmyslová 595, 252 50 Vestec, [b] Institute of Organic Chemistry and Biochemistry AS CR, v.v.i., Flemingovo nam. 2., 166 10 Prague 6, Czech Republic
huerfano@natur.cuni.cz

Polyomaviruses are small DNA viruses linked to a range of human diseases, including cancer. The murine polyomavirus (MPyV) serves as a well-established model for exploring the biology of human polyomaviruses. MPyV activates two key innate immune pathways: toll-like receptor 4 (TLR4), which promotes a cancer-associated fibroblast-like phenotype and enhances cell invasiveness, and the cGAS-STING (cyclic GMP–AMP synthase–stimulator of interferon genes) pathway, which triggers robust interferon (IFN) responses[1,2].

In this study, we investigated how these two pathways interact during MPyV infection, focusing on TRAF6 – a known E3 ubiquitin ligase adaptor in TLR4 signaling that has recently been implicated in modulating the cGAS-STING pathway. Using mouse fibroblasts lacking either TRAF6 or cGAS, along with a combination of agonists and inhibitors, we found that TRAF6 is essential for NF-κB activation. This activation drives the expression of pro-inflammatory cytokines IL-6 and CXCL10, supports a tumor-promoting microenvironment, and boosts IFN-β production.

Interestingly, we also discovered that TRAF6 enhances cGAS activity during MPyV infection, leading to increased production of the second messenger cGAMP. Supporting this crosstalk, TRAF6 was shown to bind to GAS droplets containing DNA when cells were stimulated with synthetic DNA carrying the minimal cGAS recognition motif.

Together, our findings uncover a previously unrecognized, dual, and non-redundant role for TRAF6 in coordinating both antiviral and tumor-associated immune responses. They also point to a broader regulatory function for TRAF6 within the cGAS–STING pathway – an area that warrants further exploration.

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.

REFERENCES

1. Janovec V., et al.: Cancers (Basel). *13*, 2076 (2021).
2. Ryabchenko B., et al.: FEBS J. *288*, 5964 (2021).

P-44
DECODING INTERFERON REGULATION IN BKPYV-INFECTED BLADDER ENDOTHELIAL CELLS

ALEXEY LOVTSOV, SANDRA HUÉRFANO*

Faculty of Science, Charles University, BIOCEV Center, Průmyslová 595, 252 50 Vestec, Czech Republic
huerfano@natur.cuni.cz

BK polyomavirus (BKPyV) infects approximately 80% of the human population, typically establishing persistence during childhood by replicating at low levels in the urinary tract. Low-level activation of innate immune responses appears to support both viral control and long-term persistence. In immunocompromised individuals, BKPyV can cause serious complications such as BK virus-associated nephropathy.

Given our previous findings that reservoir cells in the urinary tract – human bladder microvascular endothelial cells (HBMVECs) – can mount a moderate interferon (IFN) response via the cGAS-STING pathway in response to BKPyV infection, we aim to better understand the molecular mechanisms underlying the modulation of this response, which results in low levels of IFN production.

We hypothesize that agnoprotein, a viral protein with membrane-binding properties, may interfere with STING signaling, which depends on intact endoplasmic reticulum (ER) membranes. Using confocal microscopy, we observed that agnoprotein localizes to various cellular membranes, including mitochondria, ER, and extracellular and intracellular vesicles near the cell periphery. Furthermore, we noted pronounced remodeling of ER and mitochondrial membranes, suggesting a potential role for agnoprotein in altering cellular architecture and immune signaling.

Based on the recently described role of lipid droplets (LDs) in modulating IFN responses, we examined LD production and found that HBMVECs exhibit strong early induction of LDs following BKPyV infection. Notably, agnoprotein was bound to LD membranes, suggesting a potential functional interaction. We also analyzed LD production in renal proximal tubular epithelial cells (RPTECs) – the primary target during viral reactivation and known to be immunologically unresponsive to BKPyV. Strikingly, LD production in RPTECs was delayed and limited, indicating a possible interplay between LD dynamics and IFN signaling.

Our ongoing research focuses on generating agnoprotein mutants with reduced hydrophobicity or null variants, and on standardizing conditions to either enhance or inhibit LD production. These efforts aim to clarify how agnoprotein–membrane interactions and LD formation influence innate immune responses.

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.

P-45
UNCONVENTIONAL mRNA PROCESSING AND CAP-INDEPENDENT TRANSLATION IN YEAST LINEAR PLASMIDS

VÁCLAV VOPÁLENSKÝ*, MICHAL SÝKORA, TOMÁŠ MAŠEK, MARTIN POSPÍŠEK*

Department of Genetics and Microbiology, Faculty of Science, Charles University, Viničná 5, 128 00 Prague, Czech Republic
vaclav.vopalensky@natur.cuni.cz
martin.pospisek@natur.cuni.cz

Linear plasmids have been identified in various yeast species, notably the *Kluyveromyces lactis* pGKL1/2 plasmids, which are cytoplasmic and serve as models for this class. These plasmids carry terminal proteins, compact genomes encoding 15 genes – including a killer toxin, RNA and DNA polymerases, a helicase, and a putative capping enzyme – though most gene functions remain unverified. We analyzed pGKL mRNAs using 5' and 3' RACE and hDcp2 decapping assays. Despite encoding a predicted capping enzyme, only a subset of plasmid transcripts are 5'-capped, and none are 3'-polyadenylated. Surprisingly, most pGKL-derived mRNAs begin with short, non-templated poly(A) sequences and lack 5' caps. To test the role of the poly(A)-binding protein (Pab1) in this process, we constructed a *K. lactis* strain with deletions in PAB1 and PBP1. These deletions had no effect on plasmid stability, 5' UTRs, or toxin production. Furthermore, several pGKL transcripts fail to bind the cap-binding translation factor eIF4E *in vitro*, unlike host mRNAs. Killer toxin expression from native pGKL mRNAs persists in eIF4E-depleted strains, while expression from a Pol II-driven construct ceases under the same conditions, indicating cap-independent translation. Notably, the pGKL-encoded RNA polymerase and capping enzyme resemble those of vaccinia virus, as do the plasmid mRNAs' 5'-terminal poly(A) tracts, analogous to intermediate/late vaccinia transcripts. These findings suggest a convergent strategy for cap-independent gene expression in yeast linear plasmids and poxviruses.

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 OP JAC CZ.02.01.01 /00/22_008/0004575 RNA for therapy.

REFERENCES

1. Sykora M., Pospisek M., Novak J., Mrvova S., Krasny L., Vopalensky V.: PLoS Pathog *14*(10), e1007377 (2018).
2. Vopalensky V., Sykora M., Masek T., Pospisek M.: Front. Microbiol. *10*, 2366 (2019).

P-46
CELL-FREE HPV DNA AS A BIOMARKER FOR THE TREATMENT RESPONSE MONITORING IN OROPHARYNGEAL CANCER PATIENTS IN THE CZECH REPUBLIC

JANA SMAHELOVA[a], ZUZANA VOJTECHOVA[a], PETR MILT[a], MARTINA SALAKOVA[a], RADKA LOHYNSKA[b], MICHAL LACEK[b], ALES COCEK[c], ZUZANA KRATKA[c], RUTH TACHEZY[a]

[a] Department of Genetics and Microbiology, Faculty of Science, BIOCEV, Charles University, Prague, [b] Clinic of Oncology of the 1st Faculty of Medicine Charles University and Thomayer University Hospital, Prague, [c] Department of Otorhinolaryngology and Head and Neck Surgery, Thomayer University Hospital, Prague, Czech Republic

Cell-free tumor DNA (cfDNA) in liquid biopsies is a promising diagnostic and prognostic biomarker for cancers, including human papillomavirus (HPV)-driven oropharyngeal carcinoma (OPC). Monitoring cfHPV DNA dynamics in plasma or saliva during follow-up could enable personalized treatment approaches and improve patient outcomes.

Plasma and saliva samples were collected from p16-positive OPC patients at multiple time points: before treatment initiation (day 0), early post-treatment (days 1 and 7), at the start and end of radiotherapy, and every three months thereafter. Twenty patients received surgery followed by radiotherapy, one patient had surgery only, twenty-eight underwent chemoradiotherapy, and two received palliative treatment for metastatic disease. To date, plasma samples at enrollment were analyzed from 43 patients, and follow-up plasma samples from 29 patients. Paired plasma and saliva samples at enrollment were collected from 31 patients. Levels of cfHPV DNA were quantified using digital droplet PCR (ddPCR). Additionally, tumor tissues were tested for HPV E6 mRNA expression to confirm active viral infection and determine HPV type specificity.

Active HPV16 infection was confirmed in 92% of tumor tissues. Among 141 plasma samples from HPV16-positive patients, cfHPV DNA levels declined significantly immediately after surgery/treatment initiation, reaching undetectable levels in long-term follow-up (median: 13.4 months; range 4.5-27.2 months). Of the two patients with disease progression, one tested positive for cfHPV DNA at two consecutive visits prior to recurrence. Two patients with metastatic disease never achieved undetectable levels of cfHPV DNA. The agreement between plasma and saliva results was 90.3% ($p = 0.0044$)

Plasma cfHPV DNA levels correlate with treatment response, becoming undetectable in remission but persisting in cases of recurrence or poor response. Saliva shows promise as a non-invasive alternative for monitoring.

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.

P-47
THE ROLE OF β-2-MICROGLOBULIN IN TUMOR DEVELOPMENT

BARBORA ŽUCHA, INGRID POLÁKOVÁ, ADRIANNA PIATAKOVÁ, MICHAL ŠMAHEL

Department of Genetics and Microbiology, Faculty of Science, Charles University, BIOCEV, Průmyslová 595, 252 50 Vestec, Czech Republic
smahelm@natur.cuni.cz

The tumor microenvironment (TME) is a dynamic ecosystem where cancer cells interact with the immune system to either promote or inhibit tumor progression. A crucial aspect of this interaction involves the major histocompatibility complex class I (MHC-I) molecules on the surface of tumor cells. To analyze the role of this MHC-I expression, we use the TC-1 mouse model of tumors induced by human papillomaviruses (HPVs). In our previous study, we identified functional differences between tumor-associated macrophages in TC-1/A9-induced tumors with reversibly reduced MHC-I expression and TC-1/dB2m tumors with irreversible MHC-I downregulation, which is mediated by knocking out the *B2m* gene encoding the light MHC-I chain β-2-microglobulin (B2m)[1]. To extend our research, we developed a new TC-1/ dH2 cell line with deactivated *H2-D* and *H2-K* genes that code for the MHC-I heavy chains. Comparing the TC-1/dB2m and TC-1/dH2 cell lines and tumors may reveal an intratumoral role of B2m that can function as a soluble growth factor in TME, and its deactivation can be responsible for the reduced proliferation of TC-1/dB2m cells and the decreased growth of TC-1/dB2m-induced tumors, compared to TC-1 cells and tumors, respectively[2]. However, we found that TC-1/dH2 cell proliferation was reduced similarly to that of TC-1/dB2m cells. Furthermore, TC-1/dH2 tumors resemble TC-1/dB2m tumors in terms of growth, resistance to immunotherapy against the HPV16 E7 oncoprotein, immune cell infiltration, and macrophage polarization. Therefore, we could not confirm that *B2m* deactivation contributes to changes in the properties of TC-1/dB2m cells and tumors by a mechanism other than MHC-I downregulation. Single-cell RNA sequencing could uncover potential subtle differences in immune cells infiltrating TC-1/dB2m and TC-1/dH2 tumors.

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.

REFERENCES
1. Piatakova A., Polakova I., Smahelova J., Johari S. D., Nunvar J., Smahel M.: Cancers *13*, 3057 (2021).
2. Lhotakova K., Grzelak A., Polakova I., Vackova J., Smahel M.: Oncol. Rep. *42*, 2826 (2019).

P-48
THE HEME-DEPENDENT METRONIDAZOLE RESISTANCE IN CURRENTLY CIRCULATING CZECH ISOLATES OF *CLOSTRIDIOIDES DIFFICILE*

JAROSLAVA ZÍKOVÁ, PAVEL DŘEVÍNEK, MARCELA KRUTOVÁ*

Department of Medical Microbiology, Charles University, Second Faculty of Medicine and Motol University Hospital, V Úvalu 84, 150 06 Prague 5, Czech Republic
marcela.krutova@lfmotol.cuni.cz

Metronidazole, though recommended only as a third-line option for the treatment of *Clostridioides difficile* infection[1] (CDI), is still frequently used in some countries. A recent study suggested that metronidazole resistance could be driven by a single mutation in the promoter of the *nim*B gene ($PnimB^G$), but phenotypically detected only on media containing hemin[2].

To investigate the occurrence of this type of resistance in currently circulating Czech clinical *C. difficile* isolates, we analysed 173 clinical isolates, including those with mutations in the *nim*B gene promoter and/or the *nim*B gene (n=72) and wild-type strains (n=101). Antimicrobial susceptibility testing to metronidazole was performed on Chocolate agar, Brain Heart Infusion agar supplemented with 5 mg/L of hemin and several batches of Fastidious anaerobe agar.

We found the reported promoter mutation in epidemic ribotypes 001, 027, and 176, but resistance was consistently linked to additional amino acid substitutions in the *nim*B gene, rather than the promoter mutation alone. Other ribotypes carried unique mutations with variable effects on susceptibility. Moreover, testing results differed between Fastidious anaerobe agar batches.

Overall, our findings indicate that heme-dependent metronidazole resistance cannot be explained by promoter mutations alone but requires additional *nim*B amino acid substitutions. Importantly, the Fastidious anaerobe agar recommended for antimicrobial susceptibility testing in anaerobes cannot reliably detect this type of resistance.

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.

REFERENCES
1. van Prehn J., et al.: Clin. Microbiol. Infect. *27*, S1-S21 (2021).
2. Olaitan A. O., et al.: Nat. Commun. *14*, 4130 (2023).

P-49
FOSFOMYCIN RESISTANCE IN ENTEROBACTERALES: A ONE-YEAR SURVEILLANCE IN NOTHERN ITALY

KATERINA CHUDEJOVA[a,b], VITTORIA MATTIONI MARCHETTI[c], MARIA SOFIA CALTAGIRONE[d], ANTONELLA REZZANI[d], ANTONELLA NAVARRA[d], JAROSLAV HRABAK[a,b], IBRAHIM BITAR[a,b]

[a] Department of Microbiology, Faculty of Medicine, University Hospital in Pilsen, Charles University, Pilsen, Czech Republic, [b] Biomedical Center, Faculty of Medicine, Charles University, Pilsen, Czech Republic, [c] S.C.C.D.P. Department, Microbiology Unit, University of Pavia, Pavia, Italy, [d] Microbiology Unit, I.R.C.C.S. Maugeri Institute, Pavia, Italy

Background: Fosfomycin (FOS) is an effective option for treating severe infections caused by multidrug-resistant pathogens. National surveillance of FOSR profiles in *Enterobacterales* is still rare in Italy. Thus, the present study aims to investigate FOSR in *Enterobacterales* strains in Northern Italy.

Materials and Methods: Species identification and FOSR detection were assessed by MicroScan WalkAway (Beckman Coulter). FOS MICs were confirmed by agar-dilution method (ADM; based on EUCAST 2024), FosA production by PPF Test, and transporter impairments with Carbon source growth test (CSG). PCR was used on FosA-producing strains to check for the presence of *fosA*-like genes. The *fosA* genes were horizontally transferred by conjugation. Whole-genome sequencing (WGS) of selected strains was conducted using the NovaSeq 6000 (Illumina). Sequences were analyzed for the presence of mutations in transporters, and their functional effect was evaluated through SIFT (https://sift.bii.a-star.edu.sg).

Results: From December 2022 to December 2023, a total of 2,886 *Enterobacterales* strains were collected (n=2018 *E. coli*, n=157 *Citrobacter* spp., n=711 *Proteus* spp.) from eight clinical settings within the Istituti Clinici Scientifici Maugeri IRCCS Spa, Italy. Among these 136 strains showed resistance profiles to FOS. These included 38 *E. coli*, 91 *Proteus mirabilis*, six *P. vulgaris*, and one *C. amalonaticus* from different specimens. The ADM investigations confirmed the FOSR for 82.3% (112/136). WGS pointed out the mutations E448K and L297F in GlpT and E350Q in UhpT as main FOSR mechanisms among *E. coli*, while F315S and deleted portion in GlpT for *P. mirabilis*.

Conclusion: Here, we report the first wide epidemiology on FOSR among *Enterobacterales* in Italy. Despite the low FOSR rate (4.7%), this study highlights the Northern Italian circulation of *Enterobacterales* with different mechanisms for FOSR.

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 by the Czech Health Research Council (NU23J-09-00067) financed by Ministry of Health of the Czech Republic.

P-50
HOW OMICRON MUTATIONS ALTER LINOLEIC ACID BINDING AND CONFORMATIONAL STABILITY OF THE SARS-COV-2 SPIKE

PROKOPIS C. ANDRIKOPOULOS, ADITI KONAR, RUOJIN TIAN, JIRI ZAHRADNIK*

Charles University, 1st Faculty of Medicine, BIOCEV, Průmyslová 595, CZ-252 50 Vestec, Czech Republic
jiri.zahradnik@lf1.cuni.cz

SARS-CoV-2 is undergoing continuous evolution, with mutations that enhance its ability to escape immune detection and improve infectivity. A key region under strong selective pressure is the Receptor Binding Domain (RBD) of the spike protein, which is essential for host cell entry and a primary target of neutralizing antibodies. Our research aims to investigate the evolutionary dynamics shaping the RBD by mimicking natural evolutionary forces in an *in vitro* setting.

Complementing the experimental approach, our primary computational focus is the investigation of the fatty acid (FA) binding site located within the Receptor Binding Domain (RBD) of the SARS-CoV-2 spike protein. When populated by linoleic acid (LA), the FA site stabilizes the spike in a locked, less infectious conformation[1]. However, it has been proposed that mutations characteristic of the Omicron variants, in conjunction with mildly acidic conditions (pH ~4.5), disrupt this stabilization. To test this hypothesis, we follow a two-pronged computational strategy:

1) Firstly, we evaluate the impact of single Omicron mutations on LA binding by determining the ΔΔG values of mutations via the Thermodynamic Integration (TI) methodology[2]. Additionally, mutations with minimal impact on LA binding are classified as possible contributors to the immune evasion mechanism of the virus. A total of 16 mutations are investigated and the dependence of the LA binding strength on the pH is explored.

2) Secondly, large scale dynamics (>600 ns) are performed on the whole SARS-COV-2 spike of the wild-type (WT) and Omicron variant models. Through dynamic non-equilibrium dynamics[3], the differential response to LA dissociation, between the WT and the Omicron variants, is recorded and scrutinized providing valuable insight into the allostery between regions of the spike and the LA site.

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.

REFERENCES

1. Toelzer C., Gupta K., Yadav S. K. N., Borucu U., Davidson A. D., et al.: Science *370*, 725 (2020).
2. Kaus J., Pierce L. C. T., Walker R. C., McCammon J. A.: J. Chem. Theory Comput. *9*, 4131 (2013).
3. Oliveira A. S. F., Ciccotti G., Haider G. S., Mulholland A. J.: Eur. Phys. J. B *94*, 144 (2021).

P-51
MUTATION_SCATTER_PLOT: TACKLING CODON USAGE ANALYSIS FROM A DIFFERENT ANGLE

MARTIN MOKREJŠ, JIŘÍ ZAHRADNÍK*

First Faculty of Medicine, Charles University, BIOCEV, Prumyslova st. 595, Vestec 25250, Czech Republic
jiri.zahradnik@lf1.cuni.cz

Codon usage is commonly used in phylogenetics to unleash evolution of protein-coding regions, in particular, as the ratio between synonymous and non-synonymous changes. We wanted to study multiple sequence alignment of the Covid19 S protein in a vast set of raw NGS reads and determine which codons out of the theoretical 64 are used in every amino acid position of the encoded protein. To our surprise we could not find a tool to achieve that. Polishing the multiple sequence alignment spanning dozens of millions of entries is not possible with conventional tools although mostly just some gaps would need to be introduced here and there. The major obstacle is introducing padding gaps into the reference sequence to facilitate recognition of INSertion events in the sample reads. First, we developed rather simple program calculate_codon_frequencies.py to count the codons occurring in three columns of the DNA alignment while moving along the reference sequence and keeping ribosome reading-frame of the CDS region and output TSV files with their frequencies. Alternatively, the same tool can provide frequencies of the encoded amino acid residues. Second, we developed mutation_scatter_plot.py to display the frequencies as scatter plots with interactive bubbles upon mouse hover(). The changes can be color-coded according to e.g. physicochemical properties of the amino acid residues (PAM matrices) or their evolutionary conservation (BLOSUM matrices) or any other color-palette. We prefer the BLOSUM62 as the default. However, such efforts are a bit naïve as the weights for each amino acid are not within the same minimum-maximum range and thus are not directly comparable. The software is available at https://github.com/host-patho-evo/mutation_scatter_plot .

Acknowledgement
The work was in part supported by the project National Institute of Virology and Bacteriology (Programme EXCELES, ID Project No. LX22NPO5103) – Funded by the European Union – NextGenerationEU and by Czech Science Foundation Grant No. 25-17643M: „Unveiling Divergence and Convergence Points in Coronavirus Evolution for Host Receptor Recognition“.

REFERENCES

1. Zahradník J., Nunvář J., Schreiber G.: Front. Cell Infect. Microbiol. *12*, 748948 (2022).
2. Zahradník J. and 17 co-authors: Nat. Microbiol. *6*, 1188 (2021).
3. Zahradník J. and 6 co-authors: ACS Synth. Biol. *10*, 3445 (2021).

PALACKÝ UNIVERSITY OLOMOUC

L-27
DIAGNOSIS OF MICROBIAL INFECTIONS USING RADIOLABELLED SIDEROPHORES

KATEŘINA DVOŘÁKOVÁ BENDOVÁ[a], BARBORA NEUŽILOVÁ[a], KATARÍNA HAJDUOVÁ[a], PATRIK MLYNÁRČIK[b], KRISTÝNA KRASULOVÁ[a], CLEMENS DECRISTOFORO[c], GIANPAOLO DI SANTO[c], ZBYNĚK NOVÝ[a,d,e], MILOŠ PETŘÍK[a,d,e]*

[a] *Institute of Molecular and Translational Medicine, Faculty of Medicine and Dentistry, Palacky University, Hněvotínská 5, Olomouc 779 00, Czech Republic,* [b] *Department of Microbiology, Faculty of Medicine and Dentistry, Palacky University, Hněvotínská 3, Olomouc 775 15, Czech Republic,* [c] *Department of Nuclear Medicine, Medical University Innsbruck, Innsbruck, Austria* [d] *Laboratory of Experimental Medicine, University Hospital, 779 00, Olomouc, Czech Republic,* [e] *Czech Advanced Technology and Research Institute, Palacký University, 779 00, Olomouc, Czech Republic*
katerina.dvorakovabendova@upol.cz

Bacterial infections are becoming an increasing threat as antimicrobial resistance continues to rise. This problem is particularly challenging in healthcare settings, where rapid and accurate diagnosis is essential to prevent pathogens from spreading within the clinical environment[1]. However, conventional diagnostic approaches often lack the specificity or sensitivity required to reliably detect active bacterial infection.

We propose the use of radiolabelled siderophores for the non-invasive detection of bacterial infections through positron emission tomography (PET). Siderophores are low-molecular weight chelators produced by microorganisms, actively imported into bacterial cells through specific transporters. By substituting the naturally bound iron in siderophores with the positron emitter gallium-68, siderophores can be detected using nuclear medicine techniques, as was demonstrated in previous studies[2].

We focused our studies on clinically relevant bacterial pathogens associated with limited therapeutic and diagnostic options in healthcare settings, such as *Burkholderia cepacia* complex (BCC), *Acinetobacter baumannii* (AB) and *Klebsiella pneumoniae* (KP). We evaluated several gallium-68 labelled siderophores through series of *in vitro* experiments, including assessment of radiochemical purity, stability, and bacterial uptake. Promising candidates were subsequently tested in *in vivo* PET/CT imaging studies in both healthy and infected animal models to identify the most suitable tracers for infection imaging (Fig. 1A).

Another part of the project focused on developing siderophore derivates, investigated their pharmacological properties and bacterial specificity. Building on previous research[3], we broadened the spectrum of bacterial species and further studied the potential usability of these compounds in both *in vitro* and different *in vivo* models of infection (Fig. 1B).

In addition, a phase I/IIa clinical study was conducted to assess the safety and diagnostic performance of [^{68}Ga]Ga-deferoxamine B in seven patients with bacterial joint or vascular infections. The compound showed no safety concerns and demonstrated rapid and stable accumulation at the site of infection, although blood clearance was slightly slower than observed in mice. The study is currently being finished, and the results are being evaluated (Fig. 1C).

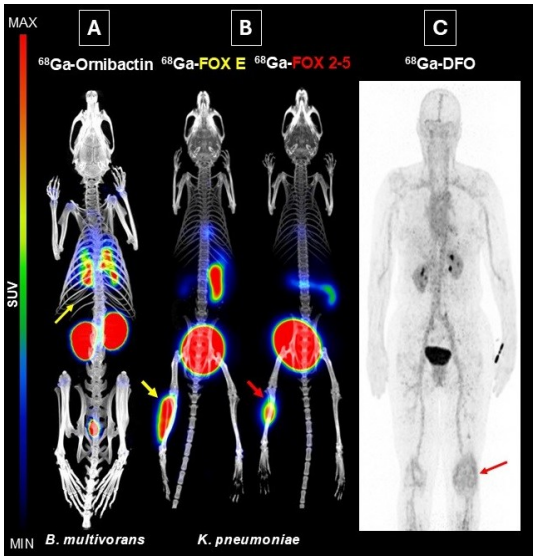


Fig. 1. **Examples of results from PET/CT imaging of biodistribution with gallium-68 labelled siderophores in (A) rat with pulmonary infection induced by *B. multivorans;* (B) mice with myositis induced by *Klebsiella pneumoniae*, with different amount of derivate uptake at the site of infection; (C) a patient with a total knee endoprosthesis with unidentified infection.** Coloured arrows indicate the site of infection.

Our results demonstrate the ability of radiolabelled siderophores to combine bacterial specificity with favourable pharmacokinetic properties, enabling accurate detection of infection *in vivo*. The findings show that gallium-68 labelled siderophores are perspective as PET tracers for imaging bacterial infections, which might provide a foundation for the development of novel imaging agents that could complement existing diagnostic methods.

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.

REFERENCES

1. Suetens C., Latour K., Kärki T., Ricchizzi E., Kinross P., Moro M. L., Jans B., Hopkins S., Hansen S., Lyytikäinen O., Reilly J., Deptula A., Zingg W., Plachouras D., Monnet D. L.: Euro Surveill. *23*, 46 (2018).
2. Petrik M., Umlaufova E., Raclavsky V., Palyzova A., Havlicek V., Pfister J., Mair C., Novy Z., Popper M., Hajduch M., Decristoforo C.: Eur. J. Nucl. Med. Mol. Imaging. *48*, 372 (2021).
3. Mular A., Hubmann I., Petrik M., Bendova K., Neuzilova B., Aguiar M., Caballero P., Shanzer A., Kozłowski H., Haas H., Decristoforo C., Gumienna-Kontecka E.: J. Med. Chem. *14*, 67 (2024).

L-28
GRAPHENE-INDUCED RESISTANCE

VERONIKA ŽĎÁRSKÁ[a], VENDULA PUDOVÁ[a], RENÁTA VEČEŘOVÁ[b], PAVLA KUČOVÁ[b], MILAN KOLÁŘ[a,b], DAVID PANÁČEK[c]

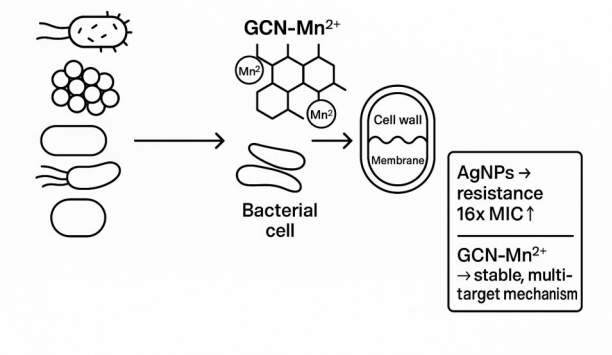
[a] Faculty of Medicin and Dentistry, Palacky University in Olomouc , Hněvotínská 976/3 779 00 Olomouc, [b] The University Hospital Olomouc, Zdravotníků 248/7, 779 00 Olomouc, [c] Czech Advanced Technology and Research Institute (CATRIN), Šlechtitelů 241/27, 783 71, Olomouc – Holice, Czech Republic
veronika.zdarska@upol.cz

Rising antibiotic resistance is one of the most serious problems of our time. The increasing resistance of bacteria to so-called "last-resort" antibiotics may return us to the pre-antibiotic era. For this reason, a substantial portion of current research is focused on the development of new antimicrobial agents. Among the promising candidates are highly active (bio)molecules and nanomaterials, in our case, graphene-based nanomaterials (NGA-Mn). These graphene particles functionalized with manganese cations exhibited strong antibacterial properties against a broad spectrum of multidrug-resistant bacteria, similar to AgNPs, while no bacterial resistance developed even after repeated passaging (in contrast to AgNPs).

The aim was to determine the possible induction of resistance at the phenotypic and genotypic levels in bacteria repeatedly exposed to NGA-Mn. Resistance induction was carried out using the broth microdilution method in microtiter plates through repeated exposure (30 cycles) to subinhibitory concentrations of NGA-Mn. The following bacterial strains were tested: susceptible strains *E. coli* and *S. aureus*, and resistant strains *A. baumannii*, *S. aureus*, *E. faecalis*, *E. coli*, and *K. pneumoniae*. DNA and RNA were isolated from these strains, subsequently subjected to sequencing and further analysis.

According to our results, resistance induction upon exposure to NGA-Mn was not demonstrated in either susceptible or multidrug-resistant strains at the phenotypic level. Genomic analysis revealed only minor changes in the form of point mutations, which did not indicate the development of resistance.

In our study, we did not detect resistance induction in the selected bacterial strains after 30 cycles of exposure; therefore, NGA-Mn appears to be a compound with promising broad-spectrum activity and persistence against the development of bacterial resistance.



Scheme 1. **Graphene-Mn^{2+} interaction with bacterial cells and comparison with AgNPs**

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, IGA_LF_2025_022 a TECHSCALE (No. CZ.02.01.01/00/22_008/0004587)

REFERENCES

1. Kolář M., Urbánek K., Látal T.: Int. J. Antimicrob. Agents *17*(5), 357 (2001).
2. Panáček A., Kvítek L., Smékalová M., Večeřová R., Kolář M., Röderová M., et al.: Nat. Nanotechnol. *13*, 65 (2018).
3. Panáček D., Belza J., Hochvaldová L., Baďura Z., Zoppellaro G., Šrejber M., Malina T., Šedajová V., Paloncýová M., Langer R., Zdražil L., Zeng J., Li L., Zhao E., Chen Z., Xiong Z., Li R., Panáček A., Večeřová R., Kučová P., Kolář M., Otyepka M., Bakandritsos A., Zbořil R.: Adv. Mater. *36*(50), e2410652 (2024).

P-52
PRECLINICAL PET/MRI MONITORING OF PHAGE THERAPY OF STAPHYLOCOCCUS AUREUS INFECTION USING ^{68}Ga-RADIOLABELLED SIDEROPHORE

KATARÍNA HAJDUOVÁ[a], BARBORA NEUŽILOVÁ[a], MARTIN BENEŠÍK[b], ROSTISLAV HALOUZKA[b], MILOŠ PETŘÍK[a], MAREK MOŠA[b], MARTIN STAROSTKA[b], ZBYNĚK NOVÝ[a]

[a]Institute of Molecular and Translational Medicine, Faculty of Medicine and Dentistry, Palacký University, 779 00, Olomouc, [b]FAGOFARMA s.r.o. Londýnská 730/59, 120 00 Praha, Czech Republic

Infections with *Staphylococcus aureus* are a major clinical concern, especially with increasing antibiotic resistance. Radiolabelled siderophores such as ^{68}Ga-desferrioxamine (^{68}Ga-DFO) allow non-invasive infection imaging by exploiting bacterial iron acquisition. We investigated the use of bacteriophage MB403 in bacteriophage therapy by ^{68}Ga-DFO PET/MRI in a murine dorsal wound infection model. Mice were inoculated with three different bacterial doses (n = 6 per dose). Within each group, three animals received local bacteriophage treatment at the infection site 24 h post-inoculation, while three controls were treated with PBS. Imaging was performed on a 3T PET/MRI scanner. ^{68}Ga-DFO showed specific uptake at infection sites, enabling visualization of bacterial burden across the tested doses. The effect of single dose phage therapy at various doses (from 10^5 to 10^{10} CFU) was tested on this infection model, where only the highest dose (10^{10} CFU) lead to complete eradication of *S. aureus* in studied lesions. Future studies will assess repeated systemic administration, which may improve therapeutic efficacy. This approach could contribute to the development of phage therapy as an alternative to conventional antibiotics.

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. This work was also supported by Technology Agency of the Czech Republic (project number FW08010029).

P-53
ARE PUTATIVE BETA-LACTAMASES A FUTURE THREAT TO GLOBAL HEALTH?

PATRIK MLYNÁRČIK, VERONIKA ŽĎÁRSKÁ, MILAN KOLÁŘ

Department of Microbiology, Faculty of Medicine and Dentistry, Palacky University Olomouc, Hněvotínská 3, 775 15 Olomouc, Czech Republic
patrik.mlynarcik@upol.cz

Beta-lactamases are key drivers of antimicrobial resistance. Building on our previous identification of 2,340 candidate beta-lactamases across 673 bacterial genera, we focused on 129 putative enzymes sharing 70–98.5% identity with known beta-lactamases.

We meticulously analyzed these enzymes across 102 genera and 13 phylogenetic classes from environmental, animal, and human sources. Conserved catalytic motifs, genomic context, and AI-based substrate predictions were used to assess functional potential. We also evaluated associations with mobile genetic elements (MGEs) and phylogenetic placement.

All four beta-lactamase classes (A–D) were represented, with several genes linked to MGEs, suggesting high mobility and a potential for immediate and widespread horizontal transfer. Using a composite prioritization framework, we identified multiple high-priority loci spanning diverse bacterial hosts and regions. Often flanked by MGEs within ±10 kb, these loci represent strong candidates for sentinel screening and further validation.

While not claiming biochemical validation, our research presents a bias-aware, reproducible early-warning framework. This framework is designed to prioritize putative beta-lactamases for One Health surveillance. It underscores the global distribution and mobility of resistance genes and, importantly, the need for continuous, proactive monitoring and validation efforts.

Acknowledgement
This work was supported by the IGA_LF_2025_022 project and the project National Institute of Virology and Bacteriology (Programme EXCELES, ID Project No. LX22NPO5103) – Funded by the European Union – NextGenerationEU.

INSTITUTE OF MOLECULAR GENETICS OF THE CAS

L-29
LOCATION, LOCATION, LOCATION! DOES IT MATTER? PROBING THE GENOME FUNCTIONALITY WITH RETROVIRUSES

DALIBOR MIKLÍK, JAKUB KAŇKA, MARTINA SLAVKOVÁ, DANIEL ELLEDER, JIŘÍ HEJNAR*

Institute of Molecular Genetics of the Czech Academy of Sciences, Vídeňská 1083, 142 20, Prague 4, Czech Republic
hejnar@img.cas.cz

Retroviruses are major pathogens that cause lifelong infections in humans and in economically important animals such as poultry, cattle, and sheep. A hallmark of the retroviral replication cycle is the insertion of the viral genome into the host cell's genome. Once integrated, the invading retroviral genome, a provirus, becomes an integral part of the host genome, with its expression fully dependent on the cellular machinery. This feature makes retroviral infections effectively incurable. On the other hand, this ability makes retroviruses useful tools for gene therapy and transgenesis.

Activity of individual proviruses often varies from high expression to completely silent persistence. Understanding the mechanisms of provirus silencing is essential for tackling latent infections, while deciphering the mechanisms of silencing-resistant conditions is crucial for the design and application of gene-transfer techniques. Epigenomic environment at the site of integration is an obvious suspect in the expression variability. A key question is how the genomic context of the provirus influences its expression. Our previous comparative studies have revealed that different retroviruses exhibit distinct sensitivities to their local epigenetic environment[1]. Importantly, the common feature is that silencing-resistant proviruses are enriched at active regulatory elements such as promoters and enhancers (Scheme 1), adding to evidence that the target site environment strongly influences the stability of retroviral expression.

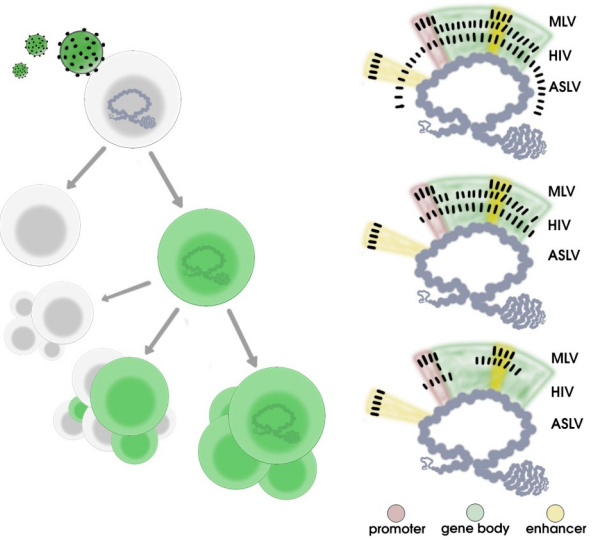
Building on these findings, our recent projects addressed two key questions:

1) Can retroviruses establish stable expression away from host genes and regulatory elements?
2) Are proviruses silenced early after integration briefly active?

Our efforts to resolve these questions revealed that retroviral and non-retroviral promoters can maintain long-term gene expression even in lamina-associated domains (LADs)[2] – genomic regions traditionally considered repressive to transcription. Our results highlight not only the strength of some promoters, but, most importantly, feature LADs as potential safe genomic "landing pads" for therapeutic gene delivery.

To explore the latent reservoir, we developed a novel detection system exploring the post-integration history of silent yet transcription-competent proviruses. Utilizing the system, we reveal a functionally distinct population of silent proviruses with as-yet undefined properties.

Together, our findings establish a novel strategy for identifying safe genomic targets for therapeutic gene insertion and introduce a novel method to uncover a previously unrecognized class of silent proviruses. These advances demonstrate that studying retroviral integration sites not only deepens our understanding of retroviral pathogenesis but also paves the way for safer and more effective applications of both viral and non-viral vectors in gene therapy and biotechnology.



Scheme 1. **A schematic depiction of variegation of proviral expression and selection of expression-permissive integration sites.** Cells expressing a provirus are marked in green. The right part of the scheme depicts target chromatin with various functional parts marked in different colors. The distribution of integration sites of proviruses at different stages of selection is represented by black lines. MLV, murine leukemia virus; HIV, human immunodeficiency virus; ALSV, avian sarcoma and leukosis virus.

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.

REFERENCES

1. Miklík D., Šenigl F., Hejnar J.: Viruses *10* (3), 116 (2018).
2. Miklík D., Slavková M., Kučerová D., Mekadim C., Mrázek J., Hejnar, J.: Viruses *16* (10), 1518 (2024).

L-30

MANIPULATING EFFECTOR AND MEMORY FATES IN CD8+ T CELLS: A NOVEL TPAM TRANSITIONAL STATE, T-CELL SISTERS AND THE ROLE OF TGF-β

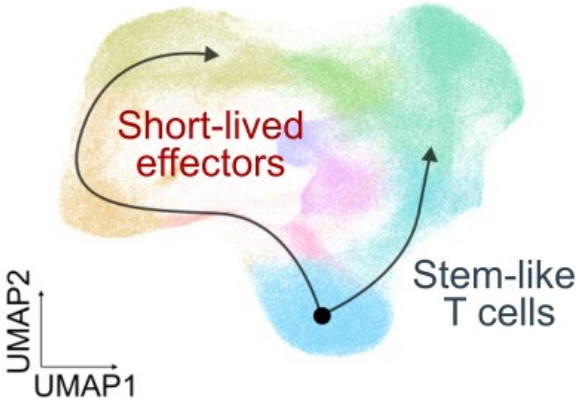
VERONIKA NIEDERLOVA, VERONIKA CIMERMANOVA, JACHYM ANTONIN HARWOOD, CARLY SPRAGUE, ALES DROBEK, ALES NEUWIRTH, JURAJ MICHALIK, ONDREJ STEPANEK*

Laboratory of Adaptive Immunity, Institute of Molecular Genetics of the Czech Academy of Sciences, Prague, Czech Republic
veronika.niederlova@img.cas.cz, ondrej.stepanek@img.cas.cz

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 developing effective anti-cancer therapies and vaccines, the molecular mechanisms governing SLEC vs. memory cell commitment remain unclear.

To address this gap, we built a comprehensive single-cell multiomics atlas of CD8+ T cells, profiling them across multiple steady-state and infection conditions, including various pathogens and time points. Alongside conventional types of CD8+ T cells, this approach revealed a novel transient state in effector differentiation dependent on the polyamine biosynthesis pathway and hence named “Tpam”.

To study Tpam cells *in vivo*, we developed an inducible OT-I TCR mouse model (TCR-switch), in which T cells initially develop with a polyclonal TCR repertoire but, upon induction, switch to express an OVA-reactive TCR. Using this system, we demonstrated that Tpam is required for the emergence of SLECs *in vivo*.



Scheme 1. **UMAP representation of the integrated single-cell RNA sequencing atlas of CD8+ T cell differentiation**

From our atlas, we also uncovered key regulatory factors of effector vs. memory cell differentiation, including *Myc, Odc1, Satb1,* and *Zbtb20*, whose CRISPR-mediated knockouts significantly skewed T-cell fate. Moreover, single-cell RNA and ATAC multiome sequencing of the earliest T-cell divisions during infection revealed a pivotal requirement for TGF-β signaling in regulating Tpam and effector cell development. Finally, spatial transcriptomics demonstrated Tpam state emergence in tissue, dependent on interactions with cDC1 dendritic cells.

A key unanswered question in the effector vs. memory cell differentiation is, what is the role of asymmetric cell division, i.e. the first division that a T cell makes after forming the immunological synapse with an antigen presenting cell. This phenomenon has never been observed *in vivo*, due to lack of suitable models and rare frequency of such events. To address this longstanding question, we used the TCR-switch mouse model. In this model, OVA-reactive T cells keep their endogenous TCR beta chain, which can be used as a cell barcode to allow tracking their fate using scRNAseq with VDJ immune profiling. We analyzed T-cell “sisters” in the first division after activation using scRNAseq and showed that their transcriptomic signature is surprisingly similar. Thus, in contrast to previous *in vitro* studies, we excluded asymmetric division as a cell fate determining mechanism *in vivo*.

Collectively, our findings indicate that effector vs. memory fate is determined remarkably early after T-cell activation, involves a newly defined Tpam transitional state, and can be manipulated through targeted genetic interventions – offering new avenues to optimize T cell-based immunotherapies.

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.

REFERENCES

1. Verbist K. C., Guy C. S., Milasta S., Liedmann S., Kamiński M. M., Wang R., Green D. R.: Nature *532* (7599), 389 (2016).
2. Jobin K., Seetharama D., Rüttger L., Fenton C., Kharybina E., Wirsching A., ... & Kastenmüller W.: Science *388*(6743), eadq1405 (2025).

P-54
HOW DO VIROPHAGES INTEGRATE THEIR DNA INTO THE PROTIST GENOME?

ANNA KOSLOVÁ[a]*, JAKUB RYBA[a], MATTHIAS FISCHER[b]

[a] Institute of Molecular Genetics, Czech Academy of Sciences, Vídeňská 1083, Prague 4, 14220, Czech Republic,
[b] Max Planck Institute for Marine Microbiology, Celsiusstr. 1, Bremen, D-28359, Germany
anna.koslova@img.cas.cz

Virophages are small dsDNA viruses that parasitize lytic giant viruses of the phylum *Nucleocytoviricota*. Although endogenous sequences of virophages and related viruses have been found in many protist genomes, few viruses have been isolated in culture. The ability to integrate viral DNA into the host cell genome has only been demonstrated experimentally for the virophage mavirus, which provides a unique model system to study the integration of DNA viruses in eukaryotes.

Mavirus replicates in the marine protist *Cafeteria burkhardae* in the presence of the giant Cafeteria roenbergensis virus (CroV). Mavirus can enter the host cell independently of CroV, and in the absence of CroV, mavirus DNA integrates into the host cell nucleus. Mavirus encodes a retroviral integrase, MaV-INT, which is likely responsible for DNA insertion into the *C. burkhardae* genome. Our goal is to characterize a molecular mechanism of mavirus DNA integration into the host genome. Our preliminary data suggest that, unlike retroviral integrases, the MaV-INT alone is not sufficient to catalyze DNA integration *in vitro*. Structural predictions indicate that MaV-INT requires interaction with a terminal protein, which is located at the 5' end of the mavirus gDNA. In addition, MaV-INT contains a chromodomain that may be responsible for specific targeting of mavirus DNA integration. To test this hypothesis, a library of mavirus integration sites in *C. burkhardae* was generated. We are currently analyzing whether the detected integration sites share common genomic or transcriptional features.

Overall, the results of our study will shed first light on virophage DNA integration.

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.

P-55
PREDICTING THE PAST: EVOLUTIONARY INSIGHTS INTO SYNCYTIN-2 FUNCTION

LUKÁŠ JECH, MARTIN TRÁVNÍČEK, KRYŠTOF ŠTAFL, JIŘÍ HEJNAR, KATEŘINA TREJBALOVÁ*

Institute of Molecular Genetics of the Czech Academy of Sciences, Vídeňská 1083, 124 00 Prague 4, Czech Republic
National Institute of Virology and Bacteriology, Czech Republic
lukas.jech@img.cas.cz, katerina.trejbalova@img.cas.cz

Envelope glycoprotein of the human endogenous retrovirus Syncytin-2 expressed in placental tissue is essential for placental formation and maintenance during pregnancy. Its fusogenic activity mediates the fusion of adjacent cellular membranes, leading to syncytium formation. These multinucleated cells provide the physiological basis for efficient exchange of respiratory gases, nutrients, and waste products between mother and fetus. Impaired placental function is associated with multiple pregnancy complications that may have severe consequences for both mother and child. Composed of multiple protein motifs, Syncytin-2 is a fine-tuned fusogen in which even subtle changes in amino-acid sequence can have dramatic consequences for functionality.

The aim of this project is to uncover critical functional regions of Syncytin-2 with the help of ancestral sequence prediction. Because Syncytin-2 copies are present in genomes of Old World and New World monkeys, we were able to predict an ancestral sequence. Recapitulating Syncytin-2's past allowed us to focus on specific motifs that changed over millions of years. In addition to highly conserved regions, several motifs show stable changes that may tune fusogenic ability and influence Syncytin-2 maturation. As the reconstructed ancestral Syncytin-2 displays clear cell-fusion activity, it will be examined further to characterize effects of these changes in more detail.

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.

P-56
PREDICTION OF CD4+ VS. CD8+ T CELLS FROM MURINE SINGLE-CELL TCR SEQUENCE DATA USING MACHINE LEARNING APPROACHES

JURAJ MICHALIK, BELA CHARVATOVA, VERONIKA NIEDERLOVA, ONDREJ STEPANEK*

Laboratory of Adaptive Immunity, Institute of Molecular Genetics of the Czech Academy of Sciences, Prague, Czech Republic
juraj.michalik@img.cas.cz, ondrej.stepanek@img.cas.cz

The surge in available single-cell data, coupled with recent advances in machine learning, has paved the way for studies that were previously difficult to conduct, including those involving T cell receptor (TCR) sequences. However, despite recent advances the significant breakthrough in predicting the specificity of a particular TCRs from its amino acid sequence has not yet been achieved.

Here, we present our current results on predicting T cell fate – whether a cell becomes CD4+ or CD8+ – from TCR sequences alone using machine learning, an intermediate step towards the aforementioned goal. After assembling a data set of approximately 55,000 unique murine TCR sequences containing both α and β chains, we analyzed it to identify distinguishing features between CD4+ and CD8+ T cells. We then numerically encoded the gathered data and applied various machine learning and deep learning methods, including neural networks. Our current best model, Gradient Boosting, achieves a prediction accuracy of 74% when tested with 10-fold cross-validation. These results indicate that machine learning can extract patterns from TCR sequences that allow for prediction of CD4+ vs. CD8+ T-cell fate to a certain degree. However, existing theories and the expansion of specific T-cell types in monoclonal mice, such as OT-I, suggest that the accuracy of such methods can be further improved. Moving forward, we aim to enhance the performance of our best-performing model through better parameterization and explore new models based on transformer neural networks. Additionally, to facilitate feature extraction by machine learning, we plan to refine our data encoding approach, which currently relies on dividing sequences into k-mers.

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.

P-57
SINGLE CELL PROFILING OF NASAL MUCOSA LYMPHOCYTES IN COVALESCENT PERTUSSIS PATIENTS

ANNA MORALES MENDEZ[a], VERONIKA NIEDERLOVA[a], JURAJ MICHALIK[a], ALES NEUWIRTH[a], SARKA KNOBLOCHOVA[b], ONDREJ VLADYKA[c], LUDMILA BLECHOVA[b], ADAM KLOCPERK[c], PETER SEBO[b], ONDREJ STEPANEK[a]

[a] Institute of Molecular Genetics of the Czech Academy of Sciences, Prague, [b] Institute of Microbiology of the Czech Academy of Sciences, Prague, [c] Department of Immunology, 2nd Faculty of Medicine, Charles University and University Hospital in Motol, Prague, Czech Republic
anna.kratochvilova@img.cas.cz

Bordetella pertussis, the causative agent of pertussis, continues to pose a significant public health challenge despite the widespread use of acellular vaccines[1]. In 2024, the Czech Republic reported an epidemic of pertussis, with over 37,000 cases confirmed. The early stages of *B. pertussis* infection are characterized by colonization of the nasal epithelium, where the bacterium initially interacts with the host immune system. The adaptive immune system of the nasal mucosa may play an important role in protection against pertussis, preventing both severe pulmonary disease and transmission of the pathogen to others[2,3]. Recent advances in single-cell transcriptomics have enabled high-resolution profiling of mucosal immune responses directly from nasal swab samples. In our study, we performed single-cell RNA sequencing on lymphocytes from nasal swabs of pertussis convalescent patients and healthy individuals with no history of the disease. To contextualize our findings, we also incorporated publicly available single-cell transcriptomics datasets of nasal mucosal lymphocytes from COVID-19 patients. We observed a decrease in the proportion of CD8 T cells expressing Killer-cell Immunoglobulin-like Receptors (KIR) in convalescent pertussis patients compared to healthy controls. These cells are characterized by an innate-like phenotype and were more abundant in the inferior nasal turbinate than in the nasopharynx. Additionally, several Toll-like receptor genes and interferon response genes were downregulated in patients. These findings offer insight into immune responses at a key mucosal barrier and may support the development of more effective pertussis vaccines.

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.

REFERENCES

1. Domenech de Cellès M., Rohani P.: Nat. Rev. Microbiol. *22*(11), 722 (2024).
2. Rice T. F., Diavatopoulos D. A., Guo Y., et al.: EBioMedicine *72*, 103612 (2021).
3. Schmitt P., Borkner L., Jazayeri S. D., McCarthy K. N., Mills K. H.: Curr. Opin. Immunol. *84*, 102355 (2023).

CONTENTS

INSTITUTE OF ORGANIC CHEMISTRY AND BIOCHEMISTRY OF THE CAS

P-11	*V. Liščáková*, M. Král', T. Kotačka, P. Brázda, T. Kouba, M. Kožíšek*	MOLECULAR INTERACTIONS AND THERAPEUTIC TARGETING OF INFLUENZA A VIRUS POLYMERASE
P-12	*M. Pávová*, J. Chalupová, A. Rajmonová, A. Pris, A. Křenková, J. Weber*	DURING EARLY INFECTION
P-13	*S. Išler, M. Pospíšil, O. Lukšan, K. Balážová Faltejsková, J. Weber**	INVESTIGATING AGPCR EXPRESSION DYNAMICS IN SARS-COV-2 AND INFLUENZA A INFECTION OF LUNG CELLS: A META-ANALYSIS APPROACH
P-14	*M. Zgarbová*, T. Otava, J. Weber, R. Nencka*	EFFECT OF METHYLTRANSFERASES INHIBITORS ON SARS-COV-2 INFECTION

BIOLOGY CENTRE CAS

L-07	*M. Beránková, S. Leoni, J. Haviernik, J. Holoubek, D. Růžek**	SEEING IS BELIEVING: 3D MAPPING OF TICK-BORNE ENCEPHALITIS VIRUS DISTRIBUTION IN MOUSE BRAIN USING TURBOGFP REPORTER VIRUS
P-15	*M. Cizkova, V. Pranclova, E. Kotounova, M. Vancova, M. Davidkova, M. Dvorakova, D. Ruzek, V. Hönig, M. Palus*	BLOOD-BRAIN BARRIER INVOLVEMENT IN TICK-BORNE ENCEPHALITIS VIRUS PATHOGENESIS

UNIVERSITY OF CHEMISTRY AND TECHNOLOGY PRAGUE

L-08	*M. Danda*, T. Nešporová, B. Chvátalová, D. Nečasová, O. Vaněk, M. Rumlová*	PROBING OF THE NSP14/NSP10 INTERFACE REVEALS A SUSCEPTIBLE ACTIVATION HOTSPOT IN THE SARS-COV-2 EXONUCLEASE
P-16	*D. Brdová, B. Křížkovská, J. Špaček, J. Tkadlec, J. Lipov, E. Kudová, J. Viktorová**	STEROIDAL HORMONES FOR ANTIBACTERIAL THERAPY: DESIGN, SYNTHESIS, AND BIOLOGICAL ASSESSMENT
P-17	*L. Hodboďová, M. Danda, M. Častorálová, M. Rumlová, T. Ruml**	EFFECT OF NATURALLY OCCURRING SUBSTITUTION I42V IN SARS-COV-2 NSP14 ON EXORIBONUCLEASE ACTIVITY AND STABILITY
P-18	*A. Pagáčová, K. Blahoutová, M. Kapisheva, J. Prchal, T. Ruml, M. Rumlová*	MASON-PFIZER MONKEY VIRUS RECRUITS DHX15 TO PROMOTE RNA PACKAGING

MASARYK UNIVERSITY

L-09	*T. Füzik, M. Anastasina, P. Pajtinka, A. Domanska, L. I. A. Pulkkinen, L. Šmerdová, L. Nepovímová, P. Formanová-Pokorná, P. Straková, J. Nováček, D. Růžek, R. Vácha, S. J. Butcher*, P. Plevka**	STRUCTURES OF ASYMMETRIC PARTICLES OF TBEV PROVIDE INSIGHT INTO FLAVIVIRUS ASSEMBLY AND MATURATION
L-10	*P. Pajtinka, R. Vácha**	MEMBRANE SHAPE MATTERS: MOLECULAR SIMULATION AND MACHINE LEARNING FOR DESIGNING CURVATURE-SENSING PEPTIDES

L-11	*A. Hassan, Y. Nakano, I. Masuda, H. Gamper, M. Pinkas, G. Blaha, Ya-Ming Hou, G. Demo**	MECHANISTIC INSIGHTS INTO E. COLI SLOW RECOVERY FROM GROWTH ARREST
L-12	*J. Bosák, M. Hrala, F. Skokan, H. Jedličková, L. Paštěková, P. Pospíšilová, D. Šmajs**	RECENT ADVANCES IN CULTIVATION OF TREPONEMA PALLIDUM SUBSP. PALLIDUM, THE SYPHILIS SPIROCHETE
L-13	*A. Vinco, T. Botka, E. Kučerová, L. Kuntová, K. Rovňáková, I. Mašlaňová, R. Pantůček**	VIRAL SATELITES OF *STAPHYLOCOCCUS EPIDERMIDIS*: HOW THEY AFFECT THE BIOLOGY OF BACTERIOPHAGES
L-14	*P. Těšina*, V. Vidmar, M. Sýkora*	TRANSLATION CONTROL AND CO-TRANSLATIONAL PROCESSES IN HEALTH AND DISEASE
P-19	*H. Šimečková, P. Bárdy, L. Kuntová, E. Macháčová, T. Botka, J. Bíňovský, J. House, Z. Farka, P. Plevka, R. Pantůček, I. Mašlaňová*	CRISPR-ASSISTED STRUCTURAL MODIFICATION OF STAPHYLOCOCCAL BACTERIOPHAGES FOR IMAGING AND BIOSENSING APPLICATIONS
P-20	*M. Procházková*, D. Dziuba, G. Weiss*	CORRELATIVE FLUORESCENCE AND CRYO-ELECTRON MICROSCOPY FOR BIOFILM MATRIX ULTRASTRUCTURE ANALYSIS
P-21	*Z. Cieniková, Yu. Mironova, A. Sobotková, P. Plevka**	THE ROLE OF SEMI-DISORDERED SCAFFOLD PROTEINS IN PHAGE LUZ19 PROCAPSID ASSEMBLY
P-22	*M. Zlatohurska*, M. Procházková, Z. Cienikova, T. Füzik, P. Plevka**	PROHEAD STRUCTURE OF *STAPHYLOCOCCUS AUREUS* BACTERIOPHAGE PHI812
P-23	*A. Kyrychenko*, O. Kovalenko*	LIPOSOMAL BIONANOCOMPOSITES: ADVANCED BIO-BASED FORMULATION TECHNOLOGY FOR SUSTAINABLE APPLICATIONS
P-24	*M. Loboda*, I. Roman, A. Sylchuk*	WHOLE-GENOME SEQUENCING AND BIOSYNTHETIC POTENTIAL OF SOIL-DERIVED *STREPTOMYCES* STRAINS ISOLATED FROM WAR-IMPACTED SOILS
P-25	*G. S. Guerra, H. Zafar, X. Ge, A. Hassan, S. Brabencova, L. Slamova, H. Gamper, Y.-M. Hou, S. Sanyal, G. Demo**	STEPWISE MECHANISM OF IF2α-DRIVEN BACTERIAL TRANSLATION INITIATION
P-26	*A. Das, T. Kotacka, M. Kozisek, G. Demo**	STRUCTURAL AND MECHANISTIC INSIGHTS INTO REPLICATION OF RIFT VALLEY FEVER VIRUS
P-27	*P. Pospíšilová, J. Bosák, M. Hrala, E. Vrbová, D. Šmajs**	EVALUTAION OF β-LACTAM ANTIBIOTIC RESISTANCE IN *TREPONEMA PALLIDUM*: AN EXPERIMENTAL APPROACH
P-28	*K. Janečková, E. Vrbová, L. Mikalová, D. Šmajs**	GENOME-WIDE IDENTIFICATION OF GENETIC VARIABILITY WITHIN INFECTING TREPONEMAL POPULATIONS
P-29	*M. Hrala, J. Bosák, P. Andrla, D. Šmajs**	MAPPING THE ESSENTIAL GENOME OF *TREPONEMA PALLIDUM* VIA TRANSPOSON MUTAGENESIS
P-30	*E. Kučerová, R. Obořilová, H. Vaisocherová-Lísalová, P. Skládal, Z. Farka, R. Pantůček, T. Botka**	FROM QCM TO CHRONIC WOUND MODEL: EXPLORING PHAGE-ANTIBIOTIC SYNERGY AGAINST BIOFILMS
P-31	*I. Mašlaňová*, L. Kuntová, G. Ambrožová, S. Smetanová, E. Jeklová, H. Šiměčková, A. Vinco, P. Makovický, B. Lipový, R. Pantůček*	COMPARISON OF PHARMACODYNAMIC AND PHARMACOKINETIC ASPECTS IN SYSTEMIC AND LOCAL INFECTION DURING PHAGE THERAPY IN A MURINE MODEL
P-32	*I. Mašlaňová*, Z. Hlavenková, T. Füzik, T. Botka, K. Rovňáková, L. Kuntová, J. Nováček, R. Pantůček*	GENOMIC AND STRUCTURAL CHARACTERIZATION OF NOVEL *MACROCOCCUS* SIPHOVIRUS THAT WILL LIKELY REPRESENT A NEW SUBFAMILY WITHIN CAUDOVIRICETES

INSTITUTE OF MICROBIOLOGY OF THE CAS

L-15	*O. Staněk*, I. Linhartova, T. Vomastek, A. Kratochvilova, J. Holubová, Z. Nichtova, P. Macek, M. Korinek, T. Smejkalova, O. Stepanek, P. Šebo*	THE DERMONECROTIC TOXIN OF BORDETELLA: A HIGHLY TOXIC PROTEIN WITH AN UNKNOWN ROLE IN PERTUSSIS INFECTION
L-16	*J. Pospíšil*, S. Michková, V. Kočárková, D. Vítovská, M. Schwarz, J. Keil, A. Benešová, T. Dolejšová, R. Fišer, D. Rejman, L. Krásný**	MODE OF ACTION OF SUBLANCIN: A GLYCOSYLATED ANTIMICROBIAL
L-17	*F. Demay, K. Skubnik, V. Kovařovic, M. Novotná, M. Hlavaty, A. Friggen, W. K. Smits, M. Krůtová, G. Balíková Novotná**	NOVEL ERYTHROMYCIN RESISTANCE MECHANISM MEDIATED BY MRMA METHYLTRANSFERASE IN *C. DIFFICILE*
P-33	*K. Munoz-Navarrete, K. Vocadlova, J. Proskova, J. Holubova, O. Stanek, D. Jurnecka, L. Blechova, E. Lesne, A. Gorringe, P. Sebo, L. Bumba**	UNRAVELLING THE ROLE OF FILAMENTOUS HEMAGGLUTININ IN *BORDETELLA PERTUSSIS* COMPLEMENT EVASION
P-34	*L. Blechová, J. Holubová, O. Staněk, L. Bumba, V. Niederlová, A. Neuwirth, J. Michalík, A. Morales Mendez, O. Štěpánek, P. Šebo**	DECIPHERING THE EARLY INNATE IMMUNE RESPONSE OF NASAL MUCOSA TO BORDETELLA PERTUSSIS INFECTION
P-35	*A. Kratochvílová, J. Holubová, O. Stanek, P. Sebo, O. Stepanek**	*BORDETELLA PERTUSSIS* TOXINS DRIVE THE EMERGENCE OF A UNIQUE $CD8^+$ T CELL SUBSET IN THE RESPIRATORY TRACT
P-36	*K. Miekská, L. Krásný, H. Šanderová**	EVEN DISORDER HAS ITS RULES
P-37	*N. Borah, H. Šanderová, T. Koval, A. Křenková, M. Hubálek, J. Dohnálek, L. Krásný**	NOVEL TRANSCRIPTION FACTORS IN MYCOBACTERIA
P-38	*V. Kočárková, L. Dejmalová, M. Schwarz, Z. Kameník, T. Balgová, H. Šanderová, L. Krásný**	IDENTIFICATION OF A NOVEL RESISTANCE MECHANISM AGAINST D-CYCLOSERINE
P-39	*M. Koběrská*, L. Korbová, L. Zdvořáková, D. Mahor, M. Plechatá, T. Stefani, Z. Kameník, G. Balíková Novotná, J. Janata*	REPROGRAMMING STREPTOMYCES LINCOLNENSIS FOR HYBRID LINCOSAMIDE CELIN PRODUCTION
P-40	*M. Novotná, T. T. Renault, M. Gillard, V. Kovařovic, J. Pokorná, M. Koběrská, C. A. Innis, G. Balíková Novotná**	UNIQUENESS OVER UNIVERSALITY: LS_AP ANTIBIOTICS ARE SEQUENCE-DEPENDENT TRANSLATION INHIBITORS

CHARLES UNIVERSITY

L-18	*M. Pimkova Polidarova, L. Plecita-Hlavata, I. Hirsch, K. Grantz Saskova*, A. Brazdova**	STING AGONISTS INDUCE PYROPTOTIC APOPTOSIS IN HUMAN MONOCYTES
L-19	*M. Stejskalová, N. Polanská, M. Jančářová, M. Ratinier, P. Volf*	REVERSE GENETICS AS A KEY TO UNDERSTANDING TOSCANA VIRUS – SAND FLY DYNAMICS

L-20	*K. Anderová, L. Horníková, V. Šroller, B. Ryabchenko, D. Pánek, P. C. Andrikopoulos, J. Zahradník, J. Forstová, S. Huerfano**	UNVEILING THE COMPLEXITY OF PML NUCLEAR BODIES: IDENTIFICATION OF NOVEL PML ISOFORMS
L-21	*M. Kubů, K. Roučová, M. Pospíšek, V. Vopálenský*	INVESTIGATING THE IMPACT OF ADAR1 ON HCV REPLICATION
L-22	*M. Kanwal*, J. Šmahelová, S. D. Johari, I. Polaková, B. Žucha, M. Olsen, K. Krausová, B. Pokrývková, C. E. M. Trufen, J. Nunvář, E. Šťovíčková, M. Grega, O. Vencálek, V. Koucký, S. Maléřová, J. Klozar, M. K. Kasi, R. Tachezy, M. Šmahel**	THE ROLE OF ASPARTATE BETA-HYDROXYLASE IN TUMORS ASSOCIATED WITH HUMAN PAPILLOMAVIRUSES
L-23	*M. Brajerová, J. Zíková, M. Krůtová*, P. Dřevínek*	EMERGING PATTERNS IN RESISTOME OF GRAM-POSITIVE HEALTHCARE-ASSOCIATED PATHOGENS
L-24	*V. Študentová, V. Pašková, L. Ďaďovská, J. Hrabák*	MONITORING THE ALTERNATIVE PATHWAY OF MEROPENEM DEGRADATION BY OXA-48-TYPE CARBAPENEMASES
L-25	*M. Kufa, O. Kovar, V. Finger, J. Korabecny, M. Krátký, J. Roh*	STRUCTURE–ACTIVITY RELATIONSHIPS OF NOVEL PYRIMIDINE DERIVATIVES AS POTENTIAL ANTIMYCOBACTERIAL AGENTS
L-26	*R. Tian, A. Shoshany, M. Padilla-Blanco, A. Hruška, A. Konar, K. Baxova, E. Zoler, M. Mokrejš, G. Schreiber, J. Zahradnik*	OMICRON EMERGED UNDER STRONG AFFINITY SELECTION PRESSURE
P-41	*O. Berehovska, M. Pimkova Polidarova, A. Brazdova, I. Hirsch, K. Grantz Saskova**	THE ROLE OF MIRNA-146A IN ANTIVIRAL IMMUNE RESPONSE TO TLR9 STIMULATION IN PERIFERAL BLOOD MONONUCLEAR CELLS
P-42	*N. Polanská, M. Stejskalová, M. Jančářová, M. Ratinier, P. Volf*	STUDIES OF TOSV IN SAND FLIES: KEY MILESTONES
P-43	*J. Biswas, B. Ryabchenko, L. Hornikova, V. Janovec, K. Bruštíková, M. Dejmek, C. Jauce, S. Huerfano**	TRAF6 DRIVES PARALLEL INNATE IMMUNE RESPONSES VIA TLR4 AND CGAS-STING DURING MPYV INFECTION
P-44	*A. Lovtsov, S. Huérfano**	DECODING INTERFERON REGULATION IN BKPYV-INFECTED BLADDER ENDOTHELIAL CELLS
P-45	*V. Vopálenský*, M. Sýkora, T. Mašek, M. Pospíšek**	UNCONVENTIONAL mRNA PROCESSING AND CAP-INDEPENDENT TRANSLATION IN YEAST LINEAR PLASMIDS
P-46	*J. Smahelova, Z. Vojtechova, P. Milt, M. Salakova, R. Lohynska, M. Lacek, A. Cocek, Z. Kratka, R. Tachezy*	CELL-FREE HPV DNA AS A BIOMARKER FOR THE TREATMENT RESPONSE MONITORING IN OROPHARYNGEAL CANCER PATIENTS IN THE CZECH REPUBLIC
P-47	*B. Žucha, I. Poláková, A. Piataková, M. Šmahel*	THE ROLE OF β-2-MICROGLOBULIN IN TUMOR DEVELOPMENT
P-48	*J. Zíková, P. Dřevínek, M. Krůtová**	THE HEME-DEPENDENT METRONIDAZOLE RESISTANCE IN CURRENTLY CIRCULATING CZECH ISOLATES OF *CLOSTRIDIOIDES DIFFICILE*
P-49	*K. Chudejova, V. M. Marchetti, M. S. Caltagirone, A. Rezzani, A. Navarra, J. Hrabak, I. Bitar*	FOSFOMYCIN RESISTANCE IN ENTEROBACTERALES: A ONE-YEAR SURVEILLANCE IN NOTHERN ITALY
P-50	*P. C. Andrikopoulos, A. Konar, R. Tian, J. Zahradnik**	HOW OMICRON MUTATIONS ALTER LINOLEIC ACID BINDING AND CONFORMATIONAL STABILITY OF THE SARS-COV-2 SPIKE
P-51	*M. Mokrejš, J. Zahradník**	MUTATION_SCATTER_PLOT: TACKLING CODON USAGE ANALYSIS FROM A DIFFERENT ANGLE

PALACKÝ UNIVERSITY OLOMOUC

L-27	*K. Dvořáková Bendová, B. Neužilová, K. Hajduová, P. Mlynárčik, K. Krasulová, C. Decristoforo, G. Di Santo, Z. Nový, M. Petřík**	DIAGNOSIS OF MICROBIAL INFECTIONS USING RADIOLABELLED SIDEROPHORES
L-28	*V. Žďárská, V. Pudová, R. Večeřová, P. Kučová, M. Kolář, D. Panáček*	GRAPHENE-INDUCED RESISTANCE
P-52	*K. Hajduová, B. Neužilová, M. Benešík, R. Halouzka, M. Petřík, M. Moša, M. Starostka, Z. Nový*	PRECLINICAL PET/MRI MONITORING OF PHAGE THERAPY OF STAPHYLOCOCCUS AUREUS INFECTION USING ^{68}Ga-RADIOLABELLED SIDEROPHORE
P-53	*P. Mlynárčik, V. Žďárská, M. Kolář*	ARE PUTATIVE BETA-LACTAMASES A FUTURE THREAT TO GLOBAL HEALTH?

INSTITUTE OF MOLECULAR GENETICS OF THE CAS

L-29	*D. Miklík, J. Kaňka, M. Slavková, D. Elleder, J. Hejnar**	LOCATION, LOCATION, LOCATION! DOES IT MATTER? PROBING THE GENOME FUNCTIONALITY WITH RETROVIRUSES
L-30	*V. Niederlova, V. Cimermanova, J. A. Harwood, C. Sprague, A. Drobek, A. Neuwirth, J. Michalik, O. Stepanek**	MANIPULATING EFFECTOR AND MEMORY FATES IN CD8+ T CELLS: A NOVEL TPAM TRANSITIONAL STATE, T-CELL SISTERS AND THE ROLE OF TGF-β
P-54	*A. Koslová*, J. Ryba, M. Fischer*	HOW DO VIROPHAGES INTEGRATE THEIR DNA INTO THE PROTIST GENOME?
P-55	*L. Jech, M. Trávníček, K. Štafl, J. Hejnar, K. Trejbalová**	PREDICTING THE PAST: EVOLUTIONARY INSIGHTS IN-TO SYNCYTIN-2 FUNCTION
P-56	*J. Michalik, B. Charvatova, V. Niederlova, O. Stepanek**	PREDICTION OF CD4+ VS. CD8+ T CELLS FROM MURINE SINGLE-CELL TCR SEQUENCE DATA USING MACHINE LEARNING APPROACHES
P-57	*A. Morales Mendez, V. Niederlova, J. Michalik, A. Neuwirth, S. Knoblochova, O. Vladyka, L. Blechova, A. Klocperk, P. Sebo, O. Stepanek*	SINGLE CELL PROFILING OF NASAL MUCOSA LYMPHOCYTES IN COVALESCENT PERTUSSIS PATIENTS

AUTHOR INDEX

CONTENTS

CZECH CHEMICAL SOCIETY SYMPOSIUM SERIES • ročník/volume 23 (2025), čís./no. 4 • ISSN 2336-7202 (Print), ISSN 2336-7210 (On-line) • ISSN 2336-7229 (CD-ROM) • evidenční číslo MK ČR E 21999 • Vydává Česká společnost chemická jako časopis Asociace českých chemických společností ve spolupráci s VŠCHT Praha, s ČSPCH a ÚOCHB AV ČR za finanční podpory Rady vědeckých společností ČR, Akademie věd ČR, Nadace Český literární fond a kolektivních členů ČSCH • IČO 444715 • Published by the Czech Chemical Society • VEDOUCÍ REDAKTOR/EDITOR-IN-CHIEF: V. Vyskočil • REDAKTOŘI/ EDITORS: J. Barek, E. Benešová, P. Drašar, P. Holý, P. Chuchvalec, M. Jurášek, Z. Kolská, B. Kratochvíl, J. Masák, J. Podešva, P. Šmejkal, F. Švec; Webové stránky: P. Drašar • TECHNICKÁ REDAKTORKA/EDITORIAL ASSISTANT: R. Řápková • Redakce čísla (ISSUE EDITOR) Š. Šímová, T. Divíšek • ADRESA PRO ZASÍLÁNÍ PŘÍSPĚVKŮ/MANUSCRIPTS IN CZECH, SLOVAK OR ENGLISH CAN BE SENT TO: Chemické listy, Novotného lávka 5, 116 68 Praha 1; tel./phone +420 221 082 370, e-mail: chem.listy@csvts.cz • PLNÁ VERZE NA INTERNETU/FULL VERSION ON URL: http://www.ccsss.cz • TISK: TG TISK s.r.o., 5. května 1010, 563 01 Lanškroun • SAZBA, ZLOM: ČSCH, Chemické listy • Užití tohoto díla se řídí mezinárodní licencí Creative Commons Attribution License 4.0 • Cena výtisku 180 Kč • Pokyny pro autory najdete na http://www.ccsss.cz , zkratky časopisů podle Chemical Abstract Service Source Index (viz http://cassi.cas.org/search.jsp) • Molekulární námět na obálce: Vladimír Palivec • Dáno do tisku 15.10.2025.