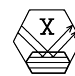

Posters

P1

NOVEL INHIBITORS OF THE METHYLTRANSFERASE METTL3-METTL14 COMPLEX

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N6-methyladenosine (m^6A) is the most prevalent and reversible co-transcriptional modification of mammalian RNA. This modification regulates the fate of m^6A-containing RNA via translation, splicing and degradation. In human, the m^6A modification is catalyzed by a heterodimer methyltransferase complex, which includes methyltransferase-like 3 (METTL3) and 14 (METTL14) so called writers. Demethylation of m^6A is on the other hand mediated by two demethylases also called erasers: AlkB homologue 5 (ALKBH5) and fat mass obesity-associated protein (FTO). Upregulation of METTL3-METTL14 complex has been recently linked to aberrant gene expression and protein synthesis, leading to developmental defects and cancer progression.

METTL3 primarily functions as catalytically active subunit, containing co-factor S-adenosylmethionine (SAM) binding pocket while METTL14 serves as an RNA-binding platform. Here we present crystal structure of this complex with a high-resolution (1.8 Å) view of the catalytic site of METTL3 occupied by a co-factor competitive inhibitor PD-2082. This inhibitor is structurally based on previously reported STM2457 currently used for the research of acute myeloid leukaemia. This preliminary crystal structure provides a valuable structural insights for further rational design of the METTL3 inhibitors as potential drugs.

P2

ALPHAFOLD-DRIVEN COARSE GRAINED DYNAMICS

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AlphaFold 2.x is a useful tool for anyone working in structural biology or related fields. AlphaFold is great at predicting structures of proteins together with confidence scores. Maybe less well-known feature is that AlphaFold can be used also to predict probability distributions of distances of all pairs of amino acid residues in the predicted structure. We use this AlphaFold output to infer the intramolecular potentials acting on the protein of interest. Such information combined with coarse-graining of the molecule can be used to generate pseudo-trajectories of the protein, predicting the dynamics, of the secondary structures, multi-domain interaction or evaluating flexibility of intrinsically disordered parts of the structure. We show how this approach works on several systems.

This work was supported by Czech Science Foundation (22-29667S) and by COST Action ML4NGP, CA21160, . Computational resources were provided by the e-INFRA CZ project (ID:90254), supported by the Ministry of Education, Youth and Sports of the Czech Republic.

P3

COMPARATIVE ANALYSIS OF DNA AND RNA HYDRATION PATTERNS: INSIGHTS FROM CRYSTALLOGRAPHIC DATA

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Understanding the hydration dynamics of nucleic acids is crucial for elucidating their structural stability and functional roles. Building upon our previous work on protein [1,2,3] and DNA hydration [4,5,6], we recently extended our analysis to include RNA, providing a comparative study of hydration patterns between the two nucleic acid types.

Expanding our dataset to encompass RNA crystal structures, we have conducted a detailed examination of hydration around selected conformational classes common for both DNA and RNA. By leveraging a multi-step approach, we analyzed hydration patterns around dinucleotide fragments extracted from a non-redundant set of high resolution crystallographic structures. Our dataset includes 2,727 DNA chains and now incorporates 206 RNA chains, allowing for a comprehensive investigation.

Utilizing Fourier averaging techniques, we computed water probability density distributions around dinucleotides, discerning hydration sites and revealing the nuanced interplay between water molecules and nucleic acid structure. By comparing hydration profiles between DNA and RNA, we aim to elucidate similarities and differences in their hydration landscapes.

This poster presentation will showcase our comparative analysis of DNA and RNA hydration, highlighting key findings and discussing the implications for understanding nucleic acid hydration dynamics. Our results offer valuable insights into the role of hydration in modulating nucleic acid structure and function.

Visitors to the poster can access our data and visualizations online at watlas.datmos.org/watna, facilitating further exploration and analysis of nucleic acid hydration patterns.

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P4

BIOPHYSICAL AND BINDING CHARACTERIZATION OF RHESUS CMV UL144 AS A PROMISING MODULATOR OF THE T-LYMPHOCYTE CD160 PATHWAYS

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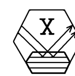
Viral immunomodulatory glycoprotein UL144 isolated from *Rhesus Cytomegalovirus* (RhCMV) is homologous to human *Cytomegalovirus* (HCMV) UL144 and highly orthologous to human tumour necrosis factor receptor HVEM (TNFR/SF14, herpesvirus entry mediator). Endogenous HVEM functions in bi-molecular switch to regulate the host's immune response depending on which ligand is currently binding [1]. Despite the high structural similarities between UL144 viral glycoproteins and the HVEM, they do not share the same binding properties therefore could modulate the immune response differently. An example is the activation of the inhibitory pathway of T-lymphocytes through the coinhibitory molecule CD160 (the natural killer cell–activating receptor). This molecular network is quite well described, but the engagement of CD160 by UL144 has not been satisfactorily studied yet [2]. We are currently focusing on RhCMV UL144, which predicted to interact with both human and the rhesus CD160 with a low affinity [3]. Such evidence could represent the evolutionary divergence between viral species

To fully understand the molecular basis of the interactions between human CD160 and RhCMV UL144, we cre-



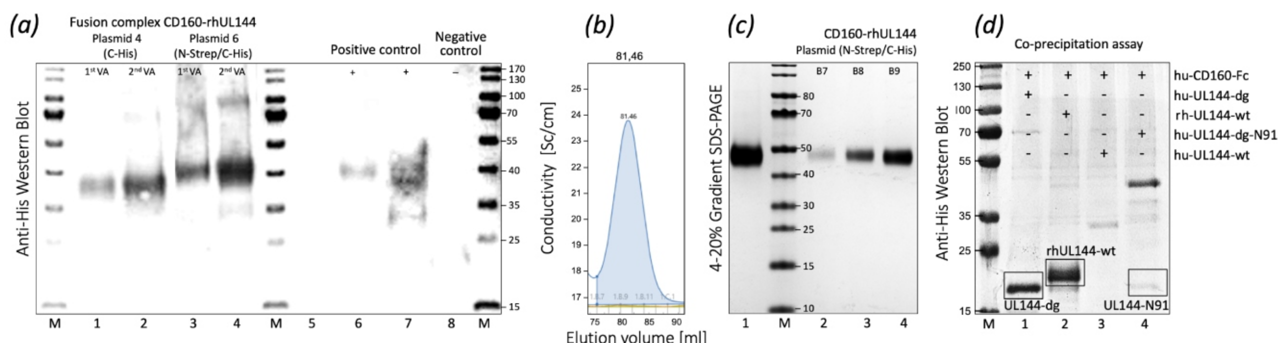


Figure 1. Expression, purification, and binding characteristics of huCD160-rhCMVUL144 fusioncomplex monitored by Western blot (a), FPLC (b), SDS-PAGE (c) and co-precipitation assay (d).

ated their covalently linked protein complex via a baculovirus-mediated expression system in *Spodoptera frugipherda* (BV-Sf9) insect cells. The plasmid pAcGP 67A was used as a transfer vector, into which the synthetically prepared gene for the fusion complex was cloned in the multi-cloning side. Other variants of CD160 and UL144 alone (e.g., UL144-WT, UL144-DG, UL144-N91, CD160-Fc) were similarly prepared and expressed in BV-Sf9. By performing the standard WB with HRPconjugated antibody interacting with the protein's affinity tags we clearly identified the expressed proteins. The protein complex huCD160–rhCMV UL144 was further analyzed by gel filtration chromatography and SDS-PAGE methods and the molecular size of the complex was confirmed (Fig. 1). Furthermore, the molecular mass for UL144 variants were determined by MS. To determine the stabilization characteristics of the purified proteins, the melting temperature (T_m) was estimated biophysically by using the Nanotemper device. The obtained T_m was lower in glycosylation mutants, and onset of unfolding temperature. We also specified the quality of these samples with further measurements of polydispersity index, hydrodynamic radius, and turbidity. In addition, we attempted to confirm the binding capacity of RhCMV UL144 by the co-immunoprecipitation assay where the binding to CD160 was observed. Moreover, all formed complexes and individual proteins were attempted to crystallize by vapor-diffusion technique in a sitting-drop using commercially available crystallization screens. All obtained data will be further analyzed and optimized.

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Financial supports provided by the Slovak Research and Development Agency (APVV-19-0376) and the Scientific Grant Agency of the Slovak Republic (VEGA-02/0026/22 and VEGA-02/0060/21) are gratefully acknowledged..

P5

RTA - REPLICATION AND TRANSCRIPTION ACTIVATOR OF EPSTEIN-BARR VIRUS: FUNCTIONAL AND STRUCTURAL IMPLICATIONS FOR NOVEL ANTIVIRAL STRATEGY

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The Epstein-Barr virus (EBV) is one of the most common human viruses that infects more than 90% of the world population during their lifetime. EBV causes 200,000 cancer cases per year and is associated with various premalignant lymphoproliferative diseases, including Hodgkin's lymphoma, gastric cancer, and nasopharyngeal carcinoma [1]. Besides cancer, infectious mononucleosis and multiple sclerosis are linked to EBV [2, 3].

The Replication and transcription activator (Rta) can activate the lytic phase of EBV from its latency and is therefore essential for the EBV life cycle. As a transcription regulator, Rta binds to Rta Response Element (RRE) localized on viral DNA and transactivates a series of lytic genes, including the viral lytic gene PAN [4]. Rta has not been structurally characterized yet, and no direct homologies were identified compared to other known DNA binding or dimerization motifs [5].

Here, we present an interdisciplinary study of Rta function. We characterized the biophysical properties of the DNA binding domain of Rta and its oligomerization, which is crucial for DNA binding. Moreover, we described the structural features of the DNA binding domain of Rta. We determined the binding affinity of Rta towards a specific DNA sequence containing the RRE motif. In parallel with

an *in vitro* study, we characterized Rta in a human cancer cell line, where we investigated Rta nuclear localization and its sequestration from nucleoli.

Rta targeting with small molecules presents a new potential approach in the fight against EBV-associated diseases. Thus, a detailed understanding of the Rta structure and oligomeric state is critical for future rational anti-EBV drug design.

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P6

REVEALING THE FUNCTION OF THE C-TERMINAL PART OF MYCOBACTERIAL INOSINE-5'-MONOPHOSPHATE DEHYDROGENASE

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Mycobacterial inosine-5'-monophosphate dehydrogenase (IMPDH) is a key enzyme involved in purine metabolism, catalysing the conversion of inosine-5'-monophosphate (IMP) to xanthosine-5'-monophosphate (XMP), a rate-limiting step in guanine nucleotide biosynthesis. Because of its essential role in nucleotide synthesis, IMPDH has been studied as a potential target for antimicrobial drug development.

Mycobacterial IMPDH exhibits cooperative enzyme kinetics characterised by allosteric interactions between protomers within the tetrameric and/or octameric IMPDH assembly. However, the molecular mechanism of these inter-chain interactions is poorly understood. The C-terminus plays a crucial role in enzyme activity, forming direct interactions with the active site of neighbouring protomers and, as such, may play a role in the observed cooperativity. Previous structural studies have been limited by the absence of the C-terminus in many resolved structures without bound substrates.

In this study, we aimed to elucidate the molecular basis of IMPDH cooperativity by investigating the role of the C-terminus. Structural analysis of mycobacterial IMPDH using cryo-EM revealed barrel-like densities within the core of the octameric assembly. Computational modelling using AlphaFold2 generated structural models of C-terminal peptides, revealing an unexpected antiparallel beta sheet barrel-like assembly that correlates well with the observed density features. Experimental validation by site-directed mutagenesis targeting specific C-terminal residues resulted in changes in the kinetic parameters of the enzyme, providing crucial mechanistic insights into IMPDH cooperativity.

Our findings not only contribute to a deeper understanding of IMPDH function-structure relationships but also have potential implications for the development of IMPDH-targeting inhibitors.

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

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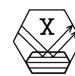

P7

INSIGHTS INTO THE ALZHEIMER'S DISEASE SPECIFIC PRE-AGGREGATION CONFORMATION OF MONOMERIC TAU PROTEINS USING DC11 ANTIBODY

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A key yet unresolved question of the pathogenesis of Alzheimer's disease (AD) and other tauopathies is the cause and the mechanism of the transition from the unstructured monomeric tau protein to the insoluble filaments deposited in the brain tissue. In the physiological state, tau protein exists as a conformational ensemble of interconverting structures and on the scale of transition from monomeric through oligomeric and filamentous species we can observe conformations reacting with specific antibodies, mainly with DC11, which is able to specifically discriminate between tau proteins isolated from healthy brain and tau proteins isolated from the brain of AD patient. The antibody recognizes also the recombinant truncated tau proteins up to the shortest fragment tau321-391 [1].

It was found that conformational antibodies DC11 and MN423 have catalytic pro-aggregatory effects in tau aggregation assay, whereas the antibody DC8E8 has inhibitory effects on tau filament formation [2]. This may imply possible mechanism of induction of pathological tau conformation, in which the antibody prepared against pathological tau imprints the pathological conformation into the physiological tau proteins in solution and therefore speeds up the tau aggregation. The information about conformatial epitopes of these antibodies are therefore of high significance.

To further uncover the binding mode of the conformational antibody DC11, we have performed NMR epitope mapping using ^{13}C, ^{15}N labelled tau321-391 and tau297-391 (dGAE) and recombinantly prepared Fab fragment of DC11 antibody. The overlay of HSQC spectra showed the region of tau between residues 370-390 to be affected by the binding of DC11, i.e. its C-terminal region. However, previous studies suggest the importance of region 321-325 for the interaction of tau with DC11 antibody. We have further characterized the influence of DC11 Fab binding on the non-epitope tau residues using NMR relaxation measurements of ^{15}N labelled tau dGAE.

The results highlight the importance of the R' region of tau, that was recently shown to be important also for tau interaction with microtubules [3]. This sequence forms the interface of rigid filament core and flanking fuzzy C terminal segment in solved tauopathy filaments.

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P8

BIOPHYSICAL TECHNIQUES AT CENTRE OF MOLECULAR STRUCTURE OF BIOCEV

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The biophysical research facility is a part of the Centre of molecular structure of Institute of Biotechnology. Facility is a shared resource that provides an access to instruments, technologies, expert consultation and training to researchers.

For the determination of size, molecular mass, structure and stability of biomolecules, study of conformational changes and thermodynamics of temperature transitions are currently available: mass photometry (Two MP mass photometer) circular dichroism spectroscopy (Chirascan Plus CD spectrometer), spectrophotometry (Specord 50 Plus UV/Vis spectrophotometer), Fourier-transform infrared spectrometry (Vertex 70v spectrometer), fluorescence spectrometry (photoluminescence spectrometer FLS1000), differential scanning fluorescence (Prometheus NT.48), multiangle dynamic light scattering (Zetasizer Ultra), microplate reader (Tecan), differential scanning calorimetry (Microcal VP-DSC). Isothermal titration calorimetry (Microcal iTC200 and PEAQ-ITC), microscale thermophoresis (Monolith NT.115 and NT.LabelFree), surface plasmon resonance (ProteOn XPR36) and bio-layer

Interferometry (OCTET R8) techniques are available for the characterization of biomolecular interactions.

Facility is a member of Instruct-ERIC, Czech Infrastructure for Integrative Structural Biology (CIISB) and Molecular-Scale Biophysics Research Infrastructure (MOSBRI).

All relevant information is on the web pages: https://www.ibt.cas.cz/cs/servisni-pracoviste/centrum-molekularni-struktury/, https://www.ciisb.org/open-access/core-facilities.

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P9

COMPUTER SIMULATIONS OF THE BACTERIAL RIBOSOME USING COARSE-GRAINED MODELS

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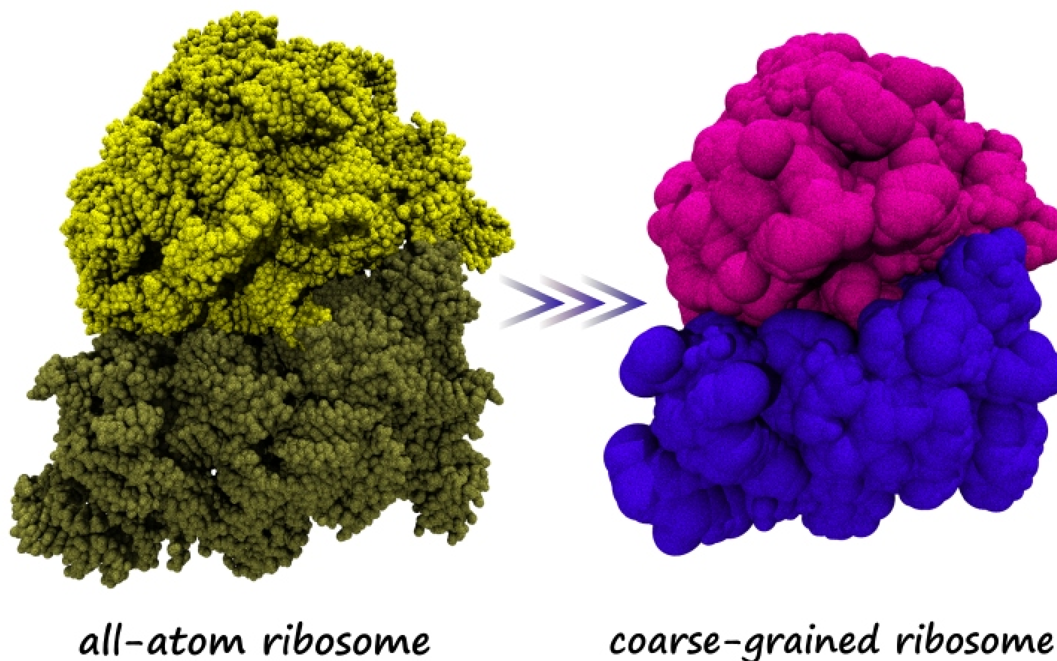
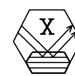
Molecular dynamics simulations serve as valuable tools for investigating biomolecules and biomolecular complexes such as the ribosome. However, the sheer scale of the ribosome presents significant computational challenges for all-atom simulations. Coarse-grained (CG) models offer a solution by replacing groups of atoms with a single bead. This reduces the number of degrees of freedom of the system and, in turn, allows longer simulation times, enabling the study of slower biochemical processes. In this study, we delve into refining the parameters for a coarse-grained representation of the bacterial ribosome. Through a series of CG computer simulations, we scrutinize the so-called elastic networks, pivotal for maintaining tertiary structure of proteins and RNA in CG systems. Our findings demonstrate that coarse-grained simulations yield reasonable structure of the ribsosome across various elastic network parameter values as compared with experimental structural models and all-atom simulations. Moreover, we refine parameter values to better capture the fine dynamics of the ribosome. These results establish a robust framework for our future ribosome studies and also shed light on specific inquiries regarding the nuances of the dynamics that can be studied further by variation of CG simulation parameters.





P10

COMBINING OF HYBRID QM/MM SIMULATIONS WITH MACHINE LEARNED POTENTIALS

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The accurate prediction of the affinity between proteins and other biomolecules or small molecules is crucial in the drug design process or protein engineering. Nowadays, a number of computational methods is available to perform these predictions with sufficient quality for systems that can be correctly described by classical force fields. However, the parameterization of force fields for drugs and drug candidates (ligands) is still not straightforward and it is a common source of inaccuracies. This project is focused on the development of a novel method for free energy calculations. We will combine a new quantum mechanical/molecular mechanical (QM/MM) method (Buffer region neural network, BuRNN) [1] with alchemical free energy calculations. The resulting method should be able to perform alchemical free energy calculations at the QM level of theory.

In my poster, I will focus on the development of the BuRNN approach. The basic principle of the method and its performance on our test system (methanol in water) will be presented. The results will be compared with the traditional QM/MM schemes. In the future, we plan to further develop BuRNN to include the protein environment. This will allow us to describe protein-ligand interactions with QM accuracy.

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P10

TALE OF TAILS; STRUCTURAL COMPARISON OF THE RNA POLYMERASE -SUBUNITS

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The -subunit of prokaryotic RNA polymerase is an integral part of the transcription machinery of many Gram-positive bacteria from the phylum *Firmicutes*. It was demonstrated that it plays critical role in the regulation of the stress responses, environmental adaptation, sporulation and virulence of several important pathogens, such as *Staphylococcus aureus* and S*treptococcus* spp. [1,2]. It plays different roles in transcription; its part in the transcription termination and the RNA polymerase complex recycling was recently well described [3]. On the other hand, the exact mechanism of function during the transcription initiation are still not fully understood.

In the past, our group was able to solve the structure of ä-subunit from *Bacillus subtilis*. The ~20 kDa protein is comprised of a structured N-terminal domain (NTD) and a negatively charged and poorly conserved C-terminal domain (CTD), with an exception of the well-conserved lysine tract at the beginning of the CTD. We previously demonstrated, that the negatively charged CTD transiently interacts with the lysines, leading to a more compacted structure of the -subunit [4]. However, some of the *Firmicutes*, such as *S. aureus*, are missing the lysine tract which is instead located on the RNA polymerase core.

In this poster, we designed purification protocols to obtain pure samples of both proteins and their respective mutants (devoid or with lysine tract included) from *B. subtilis* and *S. aureus*. We then compared their structural properties by means of circular dichroism spectroscopy (CD), small-angle X-ray scattering (SAXS) and nuclear magnetic resonance spectroscopy (NMR) to probe the importance of the lysine tract for the global structure of δ -subunit.

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P12

DECIPHERING THE ROLE OF BSCX AND BSCY SUBUNITS IN THE BORDETELLA TYPE 3 SECRETION SYSTEM

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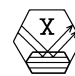
The type 3 secretion system (T3SS), also known as an injectosome, is a multisubunit protein-export apparatus that enables the delivery of bacterial effector proteins directly from bacterial cytosol into the cytosol of the host cells. Structural organization of the injectosomes is highly homologous among the Gram-negative bacteria, but some additional regulatory and structural components are species specific. In *Bordetella pertussis*, the causative agent of whooping cough, as well as in *Bordetella bronchiseptica*, which primarily causes respiratory infections of rodents, these include small protein subunits, BscX and BscY, of which the structure and function remain unknown. Here, we present the solution structure of the BscX-BscY heterodimer determined by nuclear magnetic resonance spectroscopy and demonstrate that both BscX and BscY subunits are critical for the proper function of the T3SS apparatus. The structure shows that BscY adopts a six helical fold, which is wrapped by BscX consisting of the long N-terminal unstructured region with three helices at the C-terminus of the protein. In vitro pull-down assays revealed that the BscX-BscY heterodimer directly interacts with the cytosolic domain of the BcrD protein, suggesting that the heterodimer might regulate the interaction of the inner membrane export apparatus with the cytoplasmic sorting platform. Even though both subunits appear to be tightly connected within the complex, the BscX subunit was found to be secreted out of the bacterial cells, demonstrating certain dynamics of the T3SS apparatus during the secretion of the effectors. Moreover, deletion of the individual *bscX* and *bscY* genes, or the removal of the N-terminal twelve residues of BscX rendered the bacterial mutant non-cytotoxic against the HeLa cells, indicating that the structural integrity of the BscX and BscY subunits is essential for the proper function of the *Bordetella* T3SS apparatus.



P13

UNLOCKING STING'S DEFENSES: FLUORINATED CYCLIC DINUCLEOTIDES RESISTANT TO VIRAL POXINS REVEALED BY CRYSTALLOGRAPHY

Martin Klima[#], Milan Dejmek[#], Vojtech Duchoslav[#], Andrea Eisenreichova, Michal Sala, Karel Chalupsky, Dominika Chalupska, Barbora Novotná, Gabriel Birkuš, Radim Nencka[#], Evzen Boura[#]

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The cGAS-STING pathway plays a vital role in innate immunity by detecting cytoplasmic DNA and providing defense against specific cancers, viruses, and bacteria. In this study, we synthesized fluorinated carbocyclic cGAMP analogs, MD1203 and MD1202D (referred to as MDs), with the aim of improving their stability and enhancing their affinity for STING. Our findings demonstrate that these compounds exhibit significant activity against STING. Despite their unique chemical modifications compared to the conventional cyclic dinucleotides (CDNs), analysis through crystallography revealed a binding mode with STING consistent with that of CDNs. Notably, MDs showed resistance to cleavage by viral poxin nucleases, and the poxin bound to MDs adopted an unliganded-like conformation. Furthermore, the complex formed between MDs and poxin exhibited a distinct conformation from that of cGAMP bound to poxin, closely resembling their conformation when bound to STING. In summary, the development of MD1203 and MD1202D highlights their potential as potent activators of STING, possessing remarkable stability against degradation by poxin—a crucial characteristic for the future development of antiviral agents.

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P14

USE OF A “HELPER PROTEIN” TO IMPROVE CRYO-EM RECONSTRUCTIONS – A CASE OF LA JOLLA AND MOTTS MILL VIRUSES

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Reconstructions of biomolecular structures using cryo-EM nowadays routinely reach sub-4Å resolutions. New algorithms in cryo-EM reconstruction softwares are able to correct some of the aberrations caused by imperfections in microscope setting, further improving the resolution. Since the aberration estimation is map quality dependent, higher resolution maps enable more precise corrections. However, for samples that do not reach ~sub-4Å resolutions, or samples with only very few particles per micrograph the correction is inaccurate. Here we present a case of two viruses, La Jolla (LJV) and Motts Mill virus (MMV), co-purified from *Drosophila suzukii*. Both viruses have non-enveloped icosahedral capsids with diameter ~300 Å enclosing +ssRNA genomes. From one dataset we were able to reconstruct virions of MMV and LJV and empty capsid of LJV with respective resolutions 2.7 Å, 3.0 Å and 3.3 Å. Applying aberration estimates from CTF refinements of MMV improved the resolutions of both, full and empty LJV. We propose that addition of a “helper protein” such as apoferritin to such sample would improve the resolutions even further.

P15

STRUCTURAL CHARACTERISATION OF TAU PROTEIN FILAMENTS

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Tau protein is an intrinsically disordered protein expressed predominantly in the central nervous system. It has countless functions in neurons, namely microtubule regulation, signal transduction, fast axonal transport regulation, and others.

Tau filaments are aggregated polymers of misregulated tau protein, present in the neurons of people suffering from neurodegenerative diseases, namely Alzheimer's disease. Aggregation and accumulation of tau is toxic to the neurons, but the underlying mechanisms of tau pathology are not understood well [1].

The structure of tau filaments remained unknown until recently. Advances in helical reconstruction finally allowed reconstruction of tau filaments from different diseases, and it was revealed, that the structure of tau filaments differs between neurodegenerative diseases [2].

Previously, anionic co-factors were used to induce tau filament assembly *in vitro*. Heparin-induced tau filaments, commonly used for the study of aggregation inhibitors, were found to have a completely different structure than that of pathological tau filaments [3]. Due to that, new *in vitro* models of tau filaments replicating pathological tau filaments are needed to study the mechanism of aggregation and its inhibition.

Our goal is to understand what are the factors causing and influencing tau aggregation and to prepare recombinant *in vitro* tau filaments replicating the known disease folds. We have prepared *in vitro* tau protein filaments from the longest isoform of tau (2N4R) without the use of aggregation co-factors. Filaments were prepared in different buffer conditions, as we were interested in impact of the solvent ions on morphology of the filament. Prepared filaments were studied using atomic force microscopy, negative stain electron microscopy, and cryo-electron micro-scopy. Here we present our preliminary results of the ongoing structural analysis of the filaments, and discuss the morphological differences of prepared filaments.

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This work has received funding from European Union Excellence Hub program (101087124). We acknowledge CEITEC CryoEM CF and Nanobiotechnology CF of CIISB, Instruct-CZ Centre, supported by MEYS CR (LM2023042)) and European Regional Development Fund-Project „UP CIISB“ (No. CZ.02.1.01/0.0/0.0/18_046/0015974).

P16

FLUCTUATIONS IN METABOLIC CONTENT AFFECT THE INTEGRITY OF TAU ENVELOPES

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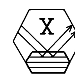
Neuronal microtubule-associated protein (MAP) tau is implicated in neurodegenerative diseases termed tauopathies. Tau can assemble into a cohesive layer termed microtubule envelope, which protects the microtubule surface against microtubule-severing enzymes. In neurodegeneration, tau aggregates presumably via dissociation from microtubules, followed by microtubule degradation. Mechanisms underlying these processes are, however, unclear. Here, we combine *in vitro* reconstitution and molecular modeling to describe how metabolites, which are implicated in neurodegenerative processes, regulate the tau envelope formation and integrity. Our results suggest that the dynamics and protective function of tau envelopes are sensitive to neurodegeneration-related metabolites, and their fluctuations can lead to disintegration of microtubules.



P17

THE STRUCTURE OF BACTERIOPHAGE phi9224

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Bacteriophage phi9224 represents a newly discovered member of the Siphoviridae family, infecting Gram-positive *Macrococcus* bacteria. Mass spectrometry analysis identified nine virion proteins, revealing a genomic modular structure typical of *Staphylococcaceae siphophages*. However, distinct features such as 31-bp terminal inverted repeats and a short 5'-overhang with variable sequence suggest a unique mechanism of replication and packaging.

The structure of this phage has been determined using single particle cryo-electron microscopy to a resolution of about 3 Å. The bacteriophage virion consists of a 65 nm icosahedral capsid (2.96 Å) 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 hexametric ring arranged into six-entry helix with a twist of 11.5° and 41.67nm rise. The major tail protein was reconstructed to 2.90Å resolution using helical reconstruction algorithm as implemented in CryoSparc [1]. The tail tip of this phase is very inhomogeneous and not visible in cryo-EM data. We show that phi9224 infects Gram-positive *Macrococcus* bacteria *in vitro* and we have performed cryo-focused ion beam micromachining (cryo-FIBM) of the phages attached to the bacteria to obtain structural insight into the infection process and the tail tip structure.

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P18

STRUCTURAL VARIABILITY OF PEPTIDE DEFORMYLASE

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The first enzyme that bacterial proteins encounter after their birth is the peptide deformylase (PDF). PDF binds to the ribosome surface and removes the formyl group from the N terminus of the nascent protein, which emerges from the ribosomal exit tunnel. Based upon structure and sequence similarity, PDFs are divided into Type I, II, and III, with Type I being further divided into subgroups IA and IB. Type I PDFs feature a C-terminal -helix that serves as the connection point between the PDF's catalytic domain and the ribosome's surface. Conversely, Type II PDFs exhibit an intrinsically disordered C-terminal region and the mechanism by which Type II PDFs bind to the ribosome is unknown. Due to sequence divergence of Type III in otherwise conserved motifs in Type I and II, Type III PDFs are suggested to be inactive. In our study, we investigate the folding behaviour of the C-terminal region by conducting all-atom molecular dynamics simulations of PDFs derived from various organisms. Our findings reveal significant differences in the conformational ensembles of the C-termini between Type I and Type II PDFs. We quantified the secondary structure propensities of the simulated systems and found notable differences as well. The catalytic domain of PDF plays a pivotal role in shaping the C-terminal fragments. Our results shed light on the potential binding mode of PDF to the ribosomal surface.

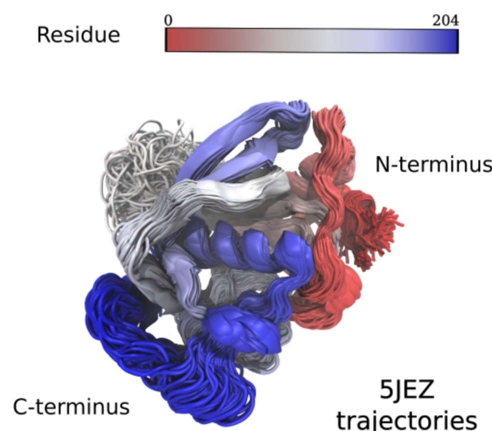

Figure 1. Peptide deformylase 5JEZ

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P19

ENZYMATIC REVELATIONS OF ABASIC SITE INTERSTRAND CROSSLINKS REPAIR FACILITATED BY NEIL3

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Covalent DNA interstrand crosslinks (ICLs) are toxic DNA damage lesions that block the replication machinery that can cause a genomic instability. Abasic site ICLs (Ap-ICLs) are formed spontaneously when an abasic site forms a covalent bond with a base located in the opposite DNA strand.

A recently discovered pathway known to repair Ap-ICL is named after the DNA glycosylase responsible for removing Ap-ICL. NEIL3 is recruited to Ap-ICL through ubiquitylation of DNA helicase, a component of the DNA replication complex. The NEIL3 glycosylase feature multiple zinc-finger domains, binds to damaged DNA, facilitating its catalytic function. However, the precise molecular mechanism of NEIL3 glycosylase in the repair process remains elusive.

Through biochemical experiment we elucidate the detailed biochemical nature of the covalent intermediate formed between NEIL3 and Ap-ICL. This work also highlights importance of methionine removal process of NEIL3 catalytic domain to get its true catalytic activity.

We establish that the previously published catalytic mutant K82A, while not involved in catalysis, plays an essential role in DNA binding. Overall, our insights significantly contribute to our understanding of the mechanism underlying the recognition and removal of Ap-ICL by NEIL3 glycosylase during DNA replication.

*For more experimental and structural data see poster of Barbora Landová.

P20

CALCIUM-MEDIATED REGULATION OF THE HUMAN UBIQUITIN LIGASE Nedd4-2

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Nedd4-2 (neuronal precursor cell-expressed developmentally down-regulated 4-2) ubiquitin ligase is one member among the nine human HECT E3 ubiquitin ligases. It is the last enzyme of the ubiquitination cascade and directly transfers ubiquitin molecules to its various substrates, therefore designating them for either endocytosis or proteasomal degradation. In accordance with its ubiquitous nature, Nedd4-2 has a variety of targets. This explains the necessity of studying its regulation since any disfunction disrupts signalling pathways and leads to the development of different pathophysiological conditions – mostly known and described is Liddle syndrome (form of hypertension resulting from electrolytic imbalance). Like all the members of HECT family, Nedd4-2 also contains three distinct domains: N-terminal C2 domain, 4 WW domains and a catalytic HECT domain. Up until now, several mechanisms of Nedd4-2 regulation were described in literature. Most notable are: autoinhibition (C2 binds to HECT) [1], regulation by calcium ions [2] and interaction with other binding partners such as the 14-3-3 dimer (associates with phosphorylated residues surrounding the WW2 domain and blocks interaction with substrates) [3, 4]. To understand how calcium ions influence this ubiquitin ligase, we must focus our attention on the C2 domain, a calcium-binding domain known to bind to membranes. It is hypothesized that its role in the ubiquitination reaction is to localize the enzyme, specifically its HECT domain near the membranous substrates (e.g., ion channels) and to be the cause of stopping the autoinhibition [2].

To determine the effect and specific binding of calcium ions to Nedd4-2, we performed two functional assays in the presence and absence of calcium: liposome-binding assay (to describe the interaction with membranes) and ubiquitination assay with fluorescently labelled ubiquitin (to describe the activity of Nedd4-2). We also wanted to see the specific interaction between the C2 domain and calcium, so we crystallized it and solved the structure. Lastly, we performed analytical ultracentrifugation to demonstrate how calcium ions influence the interaction between Nedd4-2 and 14-3-3 dimer.

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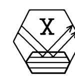

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P21

TOWARDS UNLOCKING THE POTENTIAL OF STAPHYLOKINASE FOR ISCHEMIC STROKE TREATMENT VIA A STRUCTURE-BASED PROTEIN ENGINEERING

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Stroke, the second leading global cause of death, leaves up to 50% of its survivors disabled, emphasizing the critical need for improved treatment. Alteplase, the primary thrombolytic drug for ischemic stroke faces clinical limitations, like high production costs, short half-life, inadequate recanalization, and the risk of haemorrhage due to low fibrin specificity.

Therefore, staphylokinase (SAK), a cost-effective protein encoded by *Staphylococcus aureus,* emerges as a potential alternative. Despite its promise, immunogenicity and affinity issues with its binding partner pose a significant drawback. To fill this gap, we apply protein engineering methods to overcome the limitations of SAK. Our kinetic studies reveal that optimizing the binding affinity towards plasmin could enhance the efficiency of staphylokinase up to ten thousand times [1, 2], and its immunogenicity can be mitigated by site-targeted mutagenesis as demonstrated by multiple non-immunogenic variants with no inferiority to alteplase in clinical trials [3].

In this project, we aim to visualize the macromolecular complexes formed between SAK and its protein partners (plasmin and plasminogen) through an integrative structural biology approach. We have successfully produced recombinant proteins, carried out biochemical characterization and found first crystallization hits. Preliminary electron microscopy imaging has also been conducted. These experiments represent pivotal steps towards capturing near-atomic resolution structural snapshots that should provide crucial insights into the SAK action, guiding protein engineering efforts towards its enhanced safety and efficacy. Moreover, the combination of cryo-electron microscopy and X-ray crystallography will unveil the structural nuances of non-immunogenic SAK variants to illuminate the mitigated immunogenicity and will be critical to engineering the next-wave of SAK-based thrombolytics.

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P22

STRUCTURAL INSIGHT INTO DOMAIN ARCHITECTURE AND REGULATION OF Nedd4-2 E3 UBIQUITIN LIGASE

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Neuronal precursor cell expressed developmentally down-regulated 4 ligase (Nedd4-2) is a member of the HECT family E3 ubiquitin ligases, pivotal in the regulation of various ion channels, membrane receptors, and tumor suppressors. It catalyzes the transfer of ubiquitin from E2 ligase to specific targets, marking them for proteasomal degradation or endocytosis, thereby modulating numerous signaling pathways. Dysregulation of Nedd4-2 has been implicated in hypertension, kidney diseases, and tumor development, highlighting its potential as a therapeutic target. Nedd4-2 regulatory mechanisms involve autoinhibition, calcium binding, modulation of substrate specificity, and phosphorylation, which results in the binding of 14-3-3 proteins and subsequent changes in conformations [1-4]. However, the structural mechanisms of these regulatory processes remain only poorly understood. Here, we present an integrative structural analysis that combines the cryoEM structure of Nedd4-2 with small-angle X-ray scattering (SAXS) data of the Nedd4-2/14-3-3 complex. This approach unveils the spatial organization of different domains within Nedd4-2 and offers insights into the architecture of the complex formed with 14-3-3 proteins. Understanding the structural intricacies of Nedd4-2 and its interaction with 14-3-3 not only sheds light on its regulatory mechanisms but also offer a new opportunities for targeted interventions aimed at restoring its function in pathological conditions.

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P23

HOW DOES PHOSPHORYLATION AFFECT INTERACTION BETWEEN 14-3-3 AND TAU PROTEINS?

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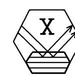
Phosphorylation is a post-translational modification that affects structure, function, and interactions of proteins. 14-3-3 protein, an abundant human regulatory protein, in non-phosphorylated state exists as a dimer [1]. However, after phosphorylation at Ser58 (pS58), it monomerizes and changes its properties [2, 3]. Hyperphosphorylation of Tau protein, a microtubule-associated protein, causes detachment of Tau from microtubules in neurons and leads to neurodegeneration [4]. Hyperphosphorylated Tau aggregates into neurofibrillary tangles (NFTs) - one of the hallmarks of Alzheimer's disease (AD). As 14-3-3 proteins were found colocalized in the NFTs [5], their interconnection with Tau in AD needs to be comprehended.

In our study, we aimed to compare interaction properties of dimeric 14-3-3 WT and monomeric 14-3-3 pS58 with respect to Tau protein. The interaction with Tau protein phosphorylated by protein kinase A (PKA) was inspected from various points of view. The binding affinity, stoichiometry, and interacting residues were studied using native-PAGE, chemical cross-linking, tandem MS, and NMR spectroscopy. We revealed that phosphorylation of 14-3-3 at Ser58 decreases its affinity to Tau protein and changes binding stoichiometry. Both NMR and cross-link-



ing results suggested that Tau is in contact with 14-3-3 proteins via the proline-rich domain and microtubule-binding domain. Moreover, cross-linking data showed that not only the binding channel of 14-3-3 protein is responsible for Tau binding, but also the outer 14-3-3 protein surface and exposed dimeric interface of monomeric 14-3-3 pS58 are involved. In summary, we provide novel insight into the 14-3-3 +Tau interaction and its regulation by phosphorylation of both partners.

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P24

STUDY OF THE RELATIONSHIP BETWEEN EMBRYO QUALITY, SPEED OF DEVELOPMENT AND CLINICAL PREGNANCY IN PGT CYCLES

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Introduction: Aneuploidy is one genetic factor leading to the failure of embryo implantation. At present, the collection of embryo cells for PGT is carried out according to the speed of embryo development on either the 5th or 6th day of blastulation. This study aimed to investigate the relationship between the day of embryo blastulation and the ploidy status of embryo, to select embryos with the most likelihood of being euploid in a noninvasive way.

Methods: This retrospective study recruited women undergoing preimplantation genetic testing (PGT) for aneuploidy with trophectoderm biopsy, first ovarian stimulation cycles only. First Faculty of Medicine and General Teaching Hospital patients were included in the retrospective study in cycles with PGT (83 PGT A, 28 PGT M, 14 PGT - SR). Embryos were cultivated 5-6 days, average age of patients was 37 years. For biopsy we used good quality blastocyst with good expansion of blastocele cavity and the integrity of both the inner cell mass and trophectoderm cells. Just embryos of A type: EB 4AA, 4AB, 4BA, 5AA, 5AB, 5BA, 6AA, 6AB, 6BA [1] and embryos of B type: EB 4BB, 3AB, 3BB [1] were PGT evaluated.

Results: 250 blastocysts from PGT cycles were evaluated. The euploid rate (number of euploid embryos divided by sum of all PGT tested embryos) of day 5 A-blastocysts was significantly higher than that of day 6 A blastocysts, either from the first ovarian stimulation cycles only: 39% (46/119) vs 28% (16/57) . In the case of B blastocysts the effect was even stronger : euploid rate of day 5 B embryos was higher than that of day 6 : 29% (8/28) vs 13% (6/46), either from the first ovarian stimulation cycles. The euploid rate of day 5 was higher than that of day 6 blastocysts, either from the first ovarian stimulation cycles only: 35% (62/176) vs 28% (14/74). The euploid rate of A blastocysts was higher than that of B blastocysts: 37% (54/147) vs 21% (22/103).

Conclusion(s): The euploid rate of A-blastocysts was significantly higher than that of B blastocysts. This effect was stronger for 6 th day blastocysts. So, if we for any reason apply non PGT cycles it can be hepful to determine the categories of blastocysts (A, B), so that on the 5th and especially the 6th day, we can select a significantly more suitable embryo (A) for transfer.

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This work was supported by project of the First Faculty of Medicine and General Teaching Hospital.

P25

ELUCIDATION OF ENZYMATIC MECHANISMS AND STRUCTURE OF NTAYA NS5 ENZYME

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Flaviviruses (family *Flaviviridae*) belong to single-stranded positive sense RNA viruses. They are able to cause human infections such as encephalitis, acute flaccid paralysis etc., therefore effective treatment strategies are urgently needed [1]. Contemporary flaviviral antiviral therapies are based on nucleotide and nucleoside derivatives which target the viral polymerase. It follows that revealing the structure of the polymerase is essential for rational drug design [2]. Our main focus is on the non-structural protein 5 (NS5) of different flaviviruses since it harbors RNA polymerase activity, guanyltransferase and methyltransferase activity which are essential for efficient viral replication. Importantly, NS5 protein possesses highly conserved drug targets shared among flaviviruses [3].

Our man goal is to prepare catalytically active methyltransferase and RNA-dependent RNA polymerase from Ntaya virus for testing potent inhibitors and to obtain a crystal structure with selected inhibitors.

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This research was funded by the project the National Institute Virology and Bacteriology (Programme EXCELES, Project No. LX22NPO5103) - Funded by the European Union - Next Generation EU and the Grant Agency of Charles University (Grant No. 408422).

P26

e-INFRA CZ INTERACTIVE AND FAIR: BEYOND QSUB COMPUTING

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For decades, resources provided by large computing and data infrastructures have been accessed by non-interactive batch job submission and data storage on shared file-systems. There are good reasons to retain this approach — it still offers the optimal performance and the best utilization of the precious resources. On the other hand, it suffers from limited user comfort and support for recently emerging requirements of reproducible science.

Therefore, e-INFRA CZ provides and fully supports alternative computing environments:

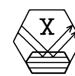
Jupyter notebooks have gained attention in recent years. The user's work is recorded as a sequence of computational steps (``cells''), which may include simple calculations and data processing, visualization of intermediate results, and also spawning extensive calculations which allocate further resources (CPU, GPU, memory) dynamically.

At the technical level, those calculations are containerized applications running in the same Kubernetes environment.

We provide easy-to-adopt and extend examples with molecular dynamics simulation and refer to examples of complex workflows (molecular dynamics combined with machine learning, *ab initio* corrections of molecular mechanics forcefields).

With the use of widgets libraries, experimental Jupyter notebooks can evolve continuously into full-featured GUIs. Currently, we provide such interfaces for Alphafold/Omegafold/Esmfold and a pilot for the molecular dynamics hub; others may emerge according to the community requirements.

Galaxy is a widely supported community framework where thousands of pre-canned tools (originating in genomics/proteomics/metabolomics but not limited to those anymore) are composed into workflows. We provide general-purpose usegalaxy.cz installation, which mirrors the reference usegalaxy.eu set of tools but provides considerably larger resources to e-INFRA CZ users and a few specialized ones for specific communities (Repeat Explorer, UMSA).



The OnDemand platform provides a similar environment for launching graphical applications.

We also provide cloud-native access through Kubernetes, enabling researchers to execute large-scale containerized computations seamlessly.

Both Jupyter notebooks and Galaxy address the reproducibility requirement (“R” from FAIR) natively by recording the history of user’s calculations, as well as interoperability (“I”) to some extent (support of data format conversions, etc.).

e-INFRA CZ gradually develops tools to address findability (“F”, e.g. pilot of semi-automated metadata provisioning for molecular dynamics calculations). Accessibility (“A”) is the principal goal of the current development of the National repository platform.

This complex approach positions our e-INFRA CZ infrastructure at the forefront of empowering researchers through the synergy of advanced computing and innovative data storage services.

P27

RESOLVING PROTEIN DYNAMICS WITH STOKES AND ANTI-STOKES RAMAN CORRELATION MICROSCOPY

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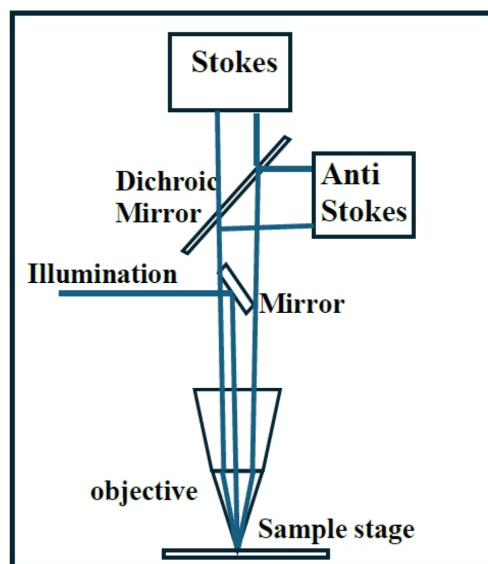
With the advent of lasers, Raman spectroscopy has been used as a valuable tool in vibrational spectroscopy for investigating the properties of matter. When the scattering is inelastic, with the transfer of energy between the photon and molecule, either the molecule gains energy from the photon, leading to the Stokes scattering or the molecule loses energy to the photon, leading to the anti-stokes scattering. Quantum mechanically Stokes and Anti-Stokes are equally likely processes. However, Anti-Stokes scattering requires vibration level to be populated. This population is directly dependent on energy in the given vibration mode. In equilibrium, this energy comes from the temperature but during the reaction, the vibration population can be a direct consequence of the previous reaction. For this reason, an asymmetry between Stokes and anti-Stokes scattering potentially provides valuable information about the (bio)chemical reaction mechanism, especially if it can be recorded with high temporal resolution.

The anti-stokes to stokes intensity ratio as a function of temperatures is

$$\frac{I_{as}}{I_s} \quad \left(\frac{v_1 \quad v_s}{v_1 \quad v_s}\right)^3 \exp(\quad hv_s / kT)$$

where v_s is the frequency of the vibrational mode, v_1 is the laser frequency, T is the sample temperature, k is the Boltzmann constant, I_{as}, I_s are the anti-stokes and stokes intensities.

We are proposing an imaging set up, based on simultaneous measurement of Stokes and anti-Stokes Raman scattering. Such set up should deliver contrast related to specific underlying reactions and enhance resistance to fluorescence background that is notorious in Raman microscopy.



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P28

NEIL3-ssDNA STRUCTURE INSIGHTS INTO REPAIRING ABASIC SITE INTERSTRAND CROSSLINKS

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Genomic DNA undergoes spontaneous base loss and deamination, followed by base removal. These are the leading causes for abasic site formation. The open-ring form of abasic ribose contains reactive aldehyde group that readily interacts with amines, occasionally leading to the formation of Abasic Site DNA interstrand crosslinks, known as Ap-ICL.

DNA crosslinks are blocking DNA replication and may result in cell cycle arrest, cell death, or cancer. Various repair processes have evolved to combat the deleterious effects. Replisome arrest triggers a ubiquitylation of replicative helicases recruiting the NEIL3 glycosylase for the repair of Ap-ICL. However, the molecular mechanisms of recognition and removal of Ap-ICL by this atypical DNA glycosylase remain elusive.

In this study, we present the crystal structure of the glycosylase domain of NEIL3 bound to single-stranded DNA (ssDNA) and a set of biophysical experiments aimed at providing a more comprehensive understanding of the process of Ap-ICL removal. Additionally, we characterize the resulting product of Ap-ICL removal as a 3'-phosphate. Our findings also demonstrate that the reduction of the Shiff-base of the Ap-ICL either impedes/does not affect its removal. Collectively, our data shed light on the molecular details of how NEIL3 glycosylase binds to and prefers ssDNA, rendering it a unique member of the Fpg/Nei family.

*For detailed experimental data see poster of Andrea Hušková.

P29

TOWARDS THE STRUCTURE OF STEAROYL-ACYL CARRIER PROTEIN COMPLEX WITH FATTY ACYL

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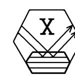
Stearoyl-acyl carrier protein desaturase (SAD) is a diiron cluster-containing enzyme that catalyzes the specific introduction of double bonds into fatty acyl chains. The core of SAD, consisting of four helices, is structurally similar to other diiron-containing enzymes such as peroxidase, rubrerythrin, and methane monooxygenase (MMO). Our previous study of SAD in *Ricinus communis* mapped the contribution of specific mutated residues to the change in product specificity from desaturation to hydroxylation. These mutations in the SAD protein were based on the MMO protein, for which the hydroxylation reaction mechanism is described. *Ricinus communis* SAD is a soluble enzyme, and its structure is known even in complex with the acyl carrier protein. However, the orientation of fatty acyls in the active site of the SAD, the impact of fatty acids on protein structure, and the precise desaturation reaction mechanism remain elusive.

In this study we have co-crystallized SAD from *Ricinus communis* with modified substrates, to obtain a protein-ligand complex that reveals the geometry of the substrate in the active site. To obtain crystals of a protein-ligand complex, the stearoyl-CoA substrate was modified by the addition of an oxime or keto group with chelating properties. Co-crystallization with the modified substrates led to the successful determination of the 3D protein-ligand structure using X-ray crystallography. The newly obtained structure represents the first soluble SAD structure with electron density for the fatty acyl chain in the protein structure. This crystallographic data will be used for the precise simulation of the reaction mechanism using the QM/MM approach, thus contributing to the knowledge of the reaction mechanism.



P30

CHARACTERIZATION OF *DE NOVO* MISSENSE VARIANTS IN INDIVIDUALS WITH NEURODEVELOPMENT DISORDERS AND THEIR IMPACT ON THE STRUCTURE AND FUNCTION OF THE PROTEIN SHP2

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SHP2 is a protein-tyrosine phosphatase encoded by the gene *PTPN11*. It is a positive regulator of RAS-MAPK signalling pathway, which is important for cell division. Deregulation of the pathway can lead to cancer or severe neurodevelopmental disorders (NDD) [1]. Therefore, it needs to be tightly controlled. SHP2 controls RAS-MAPK by dephosphorylating its inhibitor Sprouty1 [2]. Most disease-causing variants in *PTPN11* are gain of function, which means, that SHP2 more efficiently dephosphorylates its substrates [3]. My work focuses on the novel, uncharacterized variants identified in individuals with NDD. These variants are missense and result in single amino acid substitutions.

SHP2 is composed of three domains: N-SH2 domain, C-SH2 domain and PTP-domain (catalytic domain) and can be present in two conformations – inactive state and active state. In the inactive state, N-SH2 domain interacts with the PTP-domain and disables its dephosphorylation activity. This process is called autoinhibition. To become active, a binding protein needs to provide tyrosine phosphates to the N-SH2 and C-SH2 domains. This stops the autoinhibition and the PTP-domain is accessible and can dephosphorylate its substrates [3]. My aim is to investigate the impact of the novel missense variants on the autoinhibition process with the use of molecular dynamics. The results will help to elucidate their pathogenicity and their effect on molecular function. The gained knowledge will be utilized for the selection of the most effective SHP2-targeting drugs from existing libraries or for design of new drugs that are specific to these variants.

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This work is supported by the Czech Grant Agency (grant no. 23-07810S) and by the ERC-CZ program of the Ministry of Education, Youth and Sports (grant no. LL2322).

P31

STRUCTURAL INVESTIGATION OF THE 40S PARTICLE

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Heterogeneous nuclear ribonucleoproteins (hnRNPs) are a family of RNA-binding proteins abundant in a nucleus. They are known to coat the pre-mRNA and to be involved in many aspects of nucleic acid metabolism, like mRNA stabilization, alternative splicing, regulation of transcription and translation, RNA export, and degradation [1]. The early studies (in the 1970s) have shown, that in nuclei lysed without the RNase inhibitors, a large fraction of pre-mRNA was in a defined protein-RNA complex sedimenting at 40S [2]. It was shown that the core of this particle is formed by hnRNP C1/C2, hnRNP A1/B2, and hnRNP A2/B1 [1]. It has led to an intriguing suggestion, that the 40S hnRNP particle could represent a counterpart to the DNA nucleosome [3].

Our goal is to describe the biogenesis of the 40S particle, provide a structural description of the 40S particle using cryo-electron microscopy (cryo-EM), and visualize it *in situ.* We have established TRex-293 lines expressing FLAG-tagged hnRNPC1/C2 proteins and were able to pull down the 40S particles. We have prepared negatively stained grids with the particle and are now optimizing the preparation of vitrified grids for the cryo-EM to structurally characterize these particles using single particle analysis. To study ribonuclosome in intact cells we have prepared thin lamellae by cryo-FIB/SEM and collected the data using cryo-EM.

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P32

PREPARATION OF BIOLOGICAL SAMPLES FOR CRYO-ELECTRON MICROSCOPY USING THE HPF “WAFFLE” METHOD

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Cryo-electron microscopy (cryo-EM) has emerged as a pivotal technique in structural biology, offering unparalleled insights into the architecture of macromolecules at near-atomic resolution.

A crucial requirement for acquisition and collection of high quality data is properly vitrified and highly concentrated specimen. However, sample preparation still presents challenges in thicker specimens as bigger cells or cellular clusters, those are frozen by conventional plunge freezing method and may suffer with improper vitrification. Another problems could be low concentration or inadequate distribution of sample on electron microscopy grid or preferred orientation of the specific sample [1].

Here, we focus on the recently introduced “Waffle” method [1] and show it potential for preparation of various types of sample used in cryo-EM.

The waffle method is based on sample vitrification within the thickness of the TEM grid bars and it combines plunge freezing on the electron microscopy grid with a technique of high pressure freezing, that provides an advantage of proper vitrification of specimens thicker than 15 m.

Thus, a 20-30um thick layer is prepared which needs to be further processed by cryo-focused ion beam micromaching (cryo-FIBM) to final thickness ~200nm before cryo-EM imaging.

We show benefits and limitations of the waffle method for vitrification of purified proteins, protein crystals, bacterial cell suspensions and eukaryotic cells.

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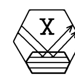

P33

STRUCTURAL CHARACTERIZATION OF SELECTIVE HDAC6 INHIBITOR MARBOSTAT-100 WITH IMPROVED STABILITY

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Histone deacetylase 6 (HDAC6) is a zinc-dependent hydrolase that plays a critical role in numerous (patho) physiological processes and thus serves as a target of therapeutic interventions in cancers, and neurological and inflammatory diseases. Many HDAC6-specific inhibitors exist that are used as research reagents or are in clinical trials, yet these might have limited specificity and suboptimal pharmacokinetics that is translated into suboptimal therapeutic efficacy. Mahboobi group recently reported Marbostat-100 as a highly selective and potent HDAC6 inhibitor [1] built on the Tubastain A (TubA) scaffold [2]. However, pre-clinical *in vivo* studies indicated limited plasma stability as its major liability. To assist further development of Marbostat-100, we solved a crystal structure of HDAC6 in a complex with SS-294, a Marbostat-100 derivative with improved pharmacokinetic properties. Our data detail structural features of SS-294 that are critical for efficient interactions with the enzyme and the can be further optimized for improved potency, stability, and selectivity.

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P34

RESOLVING THE EARLY INFECTION STAGES OF THE VIRUSES FROM THE ORDER PICORNAVIRALES *IN VIVO*

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For all viruses, the initial stages of infection predetermine the success of the entire infection process. The crucial initial steps include receptor binding, cell entry, and genome delivery. In our research, we focus on the cell entry and genome delivery of viruses from the family Picornavirales. To infect the host, picornaviruses utilize the endocytic machinery of the cell. Previously, it was demonstrated that virions undergo structural changes triggered by low pH, receptor binding, or a combination of both. These changes have been attributed to genome delivery into the cell cytoplasm. However, the detailed mechanism was not well understood. In our research, using cryo-electron microscopy methods, we demonstrate how picornaviruses invade the cell and release their RNA *in vivo*.

P35

VISUALIZATION OF TAU PATHOLOGY USING *IN SITU* CRYO-ET

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Misfolded tau proteins undergo pathological alterations, aggregating into fibrils that subsequently form neurofibrillary tangles, the hallmark of neurodegeneration in tauopathies, including Alzheimer's disease (AD). The accumulation of misfolded tau in neurons disrupts cellular physiology, leading to neuronal death and the propagation of tau misfolding throughout the brain. The presence of pathological tau protein in the brain also affects microglia, which play a crucial role in the uptake and clearance of tau aggregates, on the other hand, microglia activity causes chronic neuroinflammation which further damages neurons. Despite advancements in understanding tau pathology, the relationships between initial tau misfolding, fibril formation, the propagation of pathology across connected neurons, and subsequent cytotoxicity on the level of individual neurons and microglia remain unclear.

We employ *in situ* cryo-ET to investigate the ultrastructural aspects of the uptake of AD tau fibrils by neuroblastoma and microglia. Our focus is on morphological information about tau neurofibrils and their interactions within neurons, with an emphasis on the cytoskeleton, mitochondria, and degradation machinery. Regarding microglia, we concentrate on AD tau fibril phagocytosis and degradation.

In this poster, we present our *in situ* visualization workflow and showcase preliminary cryo-ET data of AD-tau fibril intake by neuroblastoma and microglia.

This work has received funding from the Czech Science Foundation (22-15175I). We acknowledge CF CryoEM of CIISB, Instruct-CZ Centre, supported by MEYS CR (LM2023042)) and European Regional Development Fund-Project „UP CIISB“ (No. CZ.02.1.01/0.0/0.0/18_046/0015974).

P36

MUTATIONAL ANALYSIS ON VIRAL HCMV UL141 PROTEIN TO SPECIFY THE BINDING SITE OF NOVEL ANTAGONIST THAT BLOCKS TRAIL-R2 BINDING

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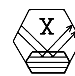
The human cytomegalovirus (HCMV) worldwide seroprevalence is estimated to 83% in the general population [1-2]. Usually, it is controlled by a vigorous immune response so that infections are asymptomatic, or symptoms are mild. However, if the immune system is compromised, HCMV can replicate to high levels and cause serious end organ disease [3]. Although HCMV represents a complex target, the rounds of iterative studies could potentially bring this important and under-recognized human pathogen under control.

Over four receptor-binding patches (RBP) of the glycosylated viral protein UL141 is capable of binding to human TRAIL death receptor 2 (TRAIL-R2) [4] and trigger the NK signalling pathway to benefit viral fitness [5-7]. Hence, it is rational to inhibit the RBP activity of the UL141 protein by blocking the RBP interaction with TRAIL-R2, which makes the UL141 a potential target for designing and developing antiviral agents. In this study, the molecular features of the UL141 of HCMV are highlighted, such as the structure, functions, and interactions of the UL141 and TRAIL-R2. Furthermore, the development of glycomimetic structures by computational design and biochemical testing is reported.

The aim is to develop the short peptide or synthetic compound (UL141 antagonist) based on our recent crystal structure and computational design that would specifically bind viral UL141 to block receptor binding thus prevent the viral action. This is relevant, as the UL141 is also the most abundant HCMV protein on plasma membrane and it is also a component of the virion. Based on our computational screening of iminosugars the 'hit' structure was selected. We test a small library of synthetized compounds (potential UL141 antagonists) that would block the receptor binding *in vitro*, on the cell or virion surface. Series of compounds that have been tested are of glycomimetics structures consisting of various saccharide units linked with non-saccharide.

In particular, non-ionic glycolipids, 'click'-conjugates or iminosugars. The ELISA-like TMB assay has been used in combination with dynabeads coating to test whether the compound could block the TRAIL-R2 binding. Five most promising compounds have proven the ability to block UL141/TRAIL-R2 complex formation. SPR kinetics anal-



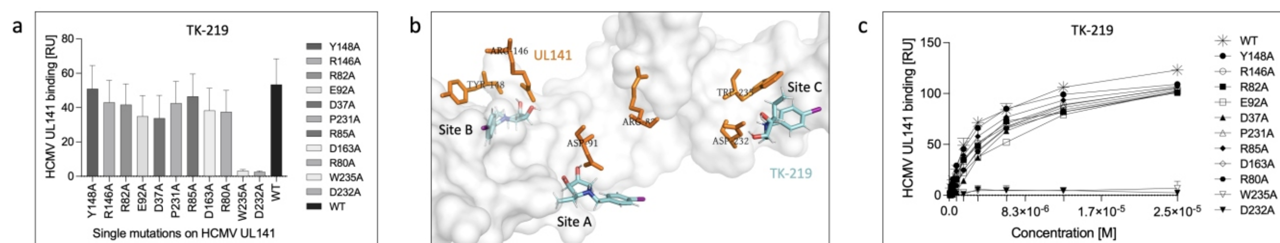


Figure 1. Three potential binding sites were identified by molecular docking on UL141 surface (b). Mutational analysis revealed the specific binding site of novel antagonist TK-219 (a). SPR binding analysis (c) showed two UL141 mutants that lost the ability to block the TRAIL-R2 binding.

ysis was then used to determine the binding constants (KD). The affinities to UL141 were determined in low micromolar scale. Three potential binding sites were revealed by molecular docking on UL141 surface (Fig. 1b). Next, mutational analysis on UL141 protein (Fig. 1a) has revealed the specify binding site of novel antagonist TK-219. Subsequent SPR binding analysis (Fig. 1c) showed two UL141 mutants (W235A and D232A) lost the ability to block the TRAIL-R2 binding. The successful compounds will be further optimized by using *in silico* methods to target epitope on viral glycoprotein UL141 derived from our structural analysis and will be tested *in vivo* for HCMV inhibition.

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P37

VISUALISING ENTEROVIRUS 71 GENOME REPLICATION *IN SITU*

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Enterovirus 71 (EV71), a member of the *Picornaviridae* family, poses a significant threat as a causative agent of hand-foot-and-mouth disease (HFMD), particularly affecting young children. The infection can escalate into severe neurological complications, with mortality rates reaching up to 19% [1]. Amidst escalating outbreaks in China and the Asia-Pacific, urgent research efforts are imperative to combat this pathogen. While existing vaccines target specific serotypes, their efficacy lacks cross-protection evidence, prompting exploration into targeted antiviral therapies [1, 2]. Aiming at distinct stages of the viral life cycle remains a promising albeit challenging strategy, owing to gaps in understanding EV71's replication cycle.

The primary aim of this project is to delve into the lesser-explored dimensions of the enterovirus RNA replication, particularly focusing on its interplay with genome packaging, and viral assembly. A crucial step towards understanding the molecular intricacies of the replication process involves the establishment of a fluorescent reporter system for viral replication sites. These reporters will play a pivotal role in guiding subsequent stages of research, including correlative light and electron microscopy, facilitating precise lamella preparation, and aiding in the collection of data for cryo-electron tomography.

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P38

EVOLUTIONARY STUDY OF HISTONE DEACETYLASE 11

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Histone deacetylase 11 (HDAC11) is the most recently identified member of HDAC family that currently attracts the interest as a target in medicinal treatments of cancer and immune-related disorders. Moreover, potential applications of HDAC11 inhibition in the food industry are intensively studied. However, current understanding of its physiological role, enzymatic activity and 3D structure remains limited. This lack of knowledge impedes progress in the development of highly specific reagents for targeting the enzyme. To gain a closer look at HDAC11 function and structure, our study expanded to include HDAC11 orthologs. Specific sequence motifs were engaged to search for and identify HDAC11 variants across the entire spectrum of life. Phylogenetic analysis revealed the existence of two distinct isoforms of HDAC11. We selected number of HDAC11 variants from various phylogenetic clades to create a representative set for further biochemical characterization, thus shedding light on the evolution of HDAC11 substrate specificity. The data suggested significant diversity of substrate specificity between identified HDAC11 isoforms. Furthermore, X-ray analysis of an ancestral ortholog provided detailed insights into structural features of HDAC11. This data holds promise for enhancing our understanding of HDAC11 function and facilitating the development of inhibitors tailored to specific isoforms and organisms.

P39

CLINCELIN – OUR NEW WEAPON TO COMBAT ANTIBIOTIC-RESISTANT BACTERIAL PATHOGENS

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The increasing antibiotic resistance among bacterial pathogens means that the therapeutics currently used against infectious diseases are ineffective. Using a combinatorial biosynthesis approach and additional chemical modifications, we have developed a novel chimeric antibiotic called clincelin, whose core structurally combines two naturally occurring lincosamide antibiotics, celesticetin and lincomycin [1]. Our extensive *in vivo* activity testing shows that clincelin has significantly higher antibacterial activity compared to the original natural compounds and that it retains antibacterial activity even against resistant strains, including strains expressing the highly efficient ribosomal methyltransferase Erm. Subsequent cryo-EM structural analysis revealed that the drug can bind to a staphylococcal ribosome in two different binding modes to overcome this resistance mechanism. Our data contribute to the further development of novel lincosamide antibiotics aiming for introduction to clinical use.

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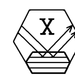

P40

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

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SARS-CoV-2, the causative agent of the COVID-19 pandemic, emerged in Wuhan (China) at the end of December 2019. Since then, the virus has evolved profoundly with many variants. Globally, SARS-CoV-2 has infected almost 775 million people, causing more than 7 million reported deaths. Although SARS-CoV-2 has posed major health and economic challenges, its control has clearly improved during the more than four years of the pandemic. However, the chance that a new coronavirus, with relatively similar characteristics to the SARS-CoV-2, emerges in the future remains high. For this reason, we have selected four coronaviruses closely related to SARS-CoV-2 (BANAL-52, BANAL-236, Guangdong-1 and GX-P5L), as well as the SARS-CoV-1, which caused several outbreaks between 2002 and 2004 in the human population. As both SARS-CoVs, the first three of these coronaviruses were found in bats, its most plausible original host, whereas GX-P5L was detected in pangolins, a suspected intermediate host. All these coronaviruses interact with the angiotensin-converting enzyme 2 (ACE2), the cell host receptor, through the receptor binding domain (RBD) of their spike (S) proteins. For this reason, RBDs were expressed in the yeast surface using a yeast surface display technique and, with the help of flow cytometry (FC), the affinities between the RBDs of these other five coronaviruses and the ACE2 receptor were estimated and compared with the one of SARS-CoV-2 RBD. Furthermore, five consecutive rounds of error-prone mutagenesis to generate libraries and select the highest affinity clones by fluorescent-activated cell sorting (FACS) were conducted. Finally, we used Sanger and Next-Generation Sequencing to reveal these increasing affinity RBD mutations, which should be taken into consideration in case these coronaviruses infect humans in the future.

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

P41

CORE FACILITY DEVOTED TO CRYSTALLIZATION OF PROTEINS AND NUCLEIC ACIDS, CENTRE OF MOLECULAR STRUCTURE, IBT CAS

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The Centre of Molecular Structure in IBT CAS (BIOCEV, Vestec, Czech Republic) is the complex of scientific core facilities focused on Structural Biology. Among the others, it includes a core facility, devoted entirely to the crystallization of biomacromolecules and their complexes. Diffraction analysis of single crystals is one of the main approaches in structural biology and this facility is equipped with all instruments necessary for crystallization experiments. The equipment includes robots that automatically set up high-throughput crystallization experiments in plates, as well as with crystallization hotels, that can monitor and screen the crystallization process using several techniques. One of the latest improvements to this facility is the SONICC instrument (Formulatrix comp.). It is connected to the RI1000 crystallization hotel and enables screening of experiments using SHG (Second Harmonic Generation) and UV-TPEF (Ultraviolet Two-Photon Excited Fluorescence). This allows the detection of microcrystals and even nanocrystals, which is essential for various methodical approaches.

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P42

THE COMPARISON OF 14-3-3 HOMO- AND HETERODIMERS KINETICS

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14-3-3 is a family of highly conserved and ubiquitously expressed eukaryotic proteins. 14-3-3 proteins have regulatory function and act in multiple cellular processes such as cell cycle, signal transduction or cell death. For the proper function of this rigid and highly helical protein, its dimeric state is essential [1]. Mammalian 14-3-3 proteins have 7 known isoforms named and , which can form both homo- and heterodimers. These isoforms differ in expression levels in various tissues, thus in their total concentrations in these tissues [2].

Most studies of 14-3-3 proteins to date have been focused on homodimers and not heterodimers. However, in a mixture of multiple isoforms in addition to homodimers, heterodimers are inevitably formed. Therefore, an accurate description of the kinetic parameters of individual dimerizations and thus the ability to quantify the populations of homo- and heterodimers is desired and could facilitate future work with heterodimers.

In previous years our group designed FRET assay to quantitatively determine 14-3-3 dimerization parameters, namely rate and dissociation constants. Our colleagues already characterized homodimerization of isoforms and heterodimerization of and as well as with phosphorylated at Ser58 [3-5]. In this project, we have further extended the knowledge to homodimerization of isoform and heterodimerizations of and . The main aim of this study is to compare the properties of chosen homo- and heterodimerizations and to show that heterodimers potentially account for a significant portion of the various dimer populations in tissues. We also propose the usage of a mathematical model that can, based on known kinetic parameters and given concentrations of individual isoforms, determine the concentration of all possible variants of dimers and monomers in a mixture.

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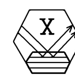

P43

PRODUCTION OF MONOCLONAL ANTIBODIES AND THEIR CO-CRYSTALLIZATION WITH INTRINSICALLY DISORDERED PROTEIN TAU

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The lack of therapeutics to treat Alzheimer's disease (AD) and its increasing worldwide prevalence are showcasing the shortage of insight into the pathogenesis of the disease (Alzheimer's Association report, 2023). Tau protein is responsible for the formation of insoluble neurofibrillary tangles, what is one of the main driver of the pathogenesis in AD. The study of its structure is limited by the fact that tau protein belongs to the family of intrinsically disordered proteins (IDPs). Moreover, tau protein is disordered almost in its full length and its conformation is depending on the binding with its binding partners.

Crystallization of proteins is one of the standard methods to obtain information about the structure of proteins. However, crystallization of IDPs is often unachievable with the standard methods and requires alternative approaches. Therefore, we utilise co-crystallization of tau protein with monoclonal antibodies, specifically its Fab (fragment antigen-binding) (fig. 1) (Skrabana et al., 2012, Sevcik et al., 2007). Monoclonal antibodies MN423 and DC11 recognize AD conformation of tau protein, therefore, their complexes with tau may enable to discover epitopes and structural motifs important for tau aggregation in tangles (Skrabana et al., 2012).

Main condition for successful crystallization is the production of Fabs in high quality as well as quantity. We developed CHO cells protein expression system consisting in universal cloning vector pCMV-3UTR and optimised polyethyleneimine DNA transfection, yielding up to 100 mg proteins per litre of growth medium (Meskova et al., 2023). To optimise the production yield of Fabs even further, we investigated the efficiency of production in different densities of cell cultures. Our results show that high-density cells are potentially more effective (fig. 2). We also performed transient Fab expression from pCMV-3UTR in HEK293(EBNA) cells. HEK cells are human cells and thus proteins produced in HEK cells are the most comparable to human, most importantly mimicking human post-translation modifications.

We were able to obtain crystals of tau-Fab protein complexes with antibodies to discover tau structural motifs, presumably important in tau aggregation (fig. 1). Results obtained from structural analysis of tau protein will be applicable in the design of AD targeting therapeutics as well as for the development of AD-specific vaccine, both leading to improved management of the disease.

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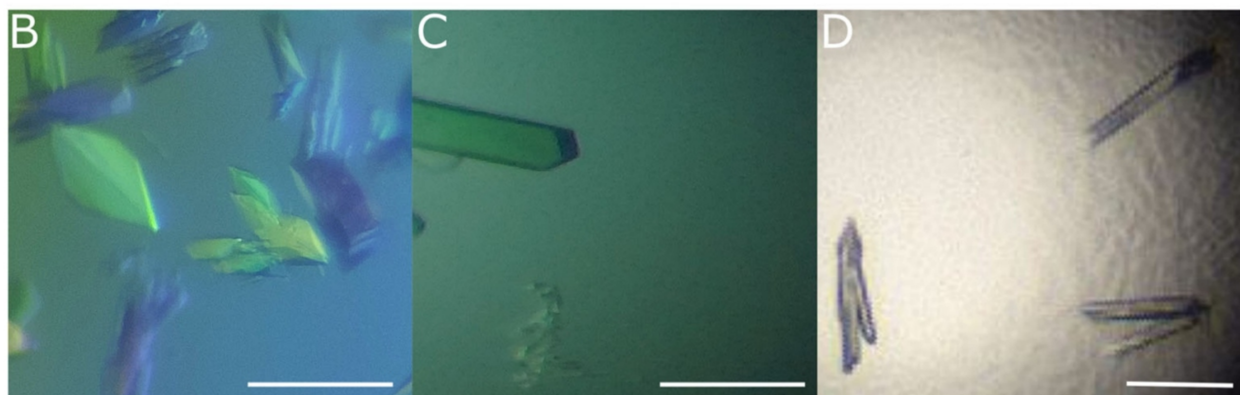

Figure 1. Co-crystallization of intrinsically disordered protein tau with Fabs: B) protein crystals of DC11, C) protein crystals of MN423Fab and dGAE tau isoform (complex), D) protein crystals of DC11Fab, DC25Fab and dGAE tau isoform (complex) (Meskova et al., 2023).

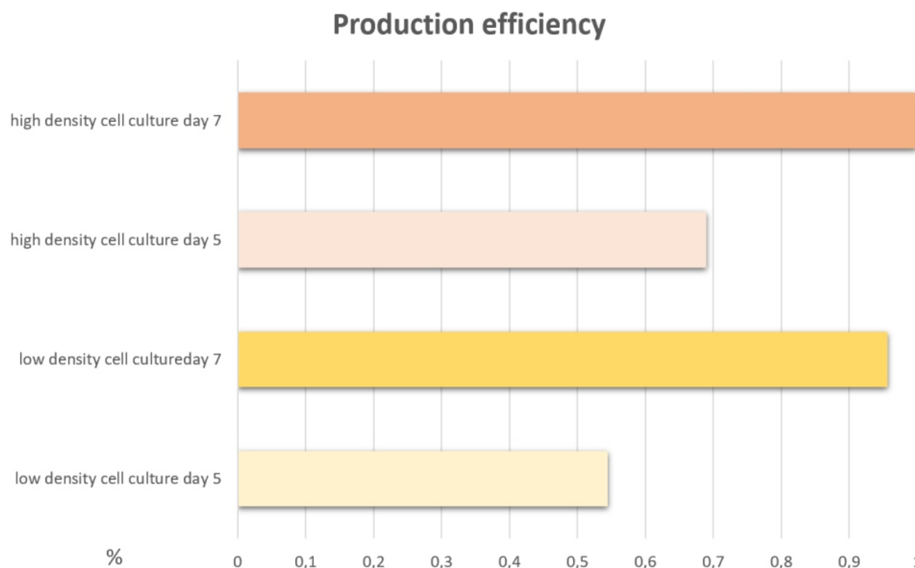

Figure 2. Comparison of production of monoclonal antibodies in CHO cells in high density (20x10^6 cells per ml of culture) and low density (5x10^6 cells per ml of culture) cell cultures.

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P44

PLUM POX VIRUS STRUCTURE AND BASIS FOR STRUCTURAL VARIABILITY OF ITS VIRUS-LIKE PARTICLES

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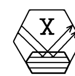
Plum Pox Virus (PPV) is a positive sense single stranded RNA virus (+)ssRNA which is an important plant pathogen. PPV infection, also known as Sharka, affects the production of stone fruits, especially those from Prunus genus. Although these viruses are not directly dangerous to humans, they annually cause large economic and agriculture losses. We present an electron cryo-microscopy (cryo-EM) structure of the PPV virion at 2.8A resolution and determine interaction network among the viral coat protein (CP) which are critical for viral stability and structural plasticity. We show that PPV CP self-assembles into the flexible helical filaments structurally similar to the virus, which contrasts with previously reported Potato Virus Y (PVY) VLPs composed of stacks of octameric discs. In addition, we have determined near-atomic cryo-EM structures of VLPs formed CPs truncated at C-terminus which all have formed VLP filaments with a virus-like organization.



P45

PROTEIN PRODUCTION FACILITY AT THE CENTRE OF MOLECULAR STRUCTURE

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The Protein Production core facility (CF) at the Centre of Molecular Structure (CMS) offers comprehensive protein production services, covering every step from DNA to the purified protein. These include gene cloning into expression vectors, site-directed mutagenesis, and heterologous expression in *Escherichia coli* expression systems, followed by protein purification.

Our cloning services include both traditional cloning using restriction enzymes and restriction-free (RF) methodologies. You can provide us with your templates, or we can order them from external companies. Subsequently, we can deliver and test a number of our plasmids. Furthermore, we perform small-scale expression and solubility tests using various *E. coli* strains under different conditions. Finally, we offer large-scale production and purification of target proteins.

In protein purification, we employ a range of affinity techniques such as Strep-Tactin XT, and immobilized metal chelate affinity chromatography (IMAC), both on FPLC or in gravity flow setups. Chromatography techniques include ion-exchange chromatography and size exclusion chromatography using Superdex columns (75 or 200, 10/300 increase, or HiLoad 16/600). You can request customization to our standardized protocols or provide your established protocols or implementation.

Moreover, we are expanding our services by introducing eukaryotic production during this year. We will provide protein production using human embryonic kidney cells (HEK) and Sf9 insect cells as an alternative to prokaryotic expression in *E. coli*.

The Biocev Protein Production core facility is a part of CMS operated by the Institute of Biotechnology, Czech Academy of Sciences. The Centre of Molecular Structure is supported by: Czech Infrastructure for Integrative Structural Biology (CIISB), Instruct-CZ Centre of Instruct-ERIC EU consortium, funded by MEYS CR infrastructure project LM2023042). European Regional Development Fund-Project „UP CIISB" (No. CZ.02.1.01/0.0/0.0/18_046/0015974).

P46

CRYO-EM REVEALS RECQ5'S REGULATORY ROLE IN RNAPII-MEDIATED TRANSCRIPTION

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In eukaryotic nuclei, the synthesis of mRNA is carried out by RNA polymerase II (RNAPII), a crucial enzyme in transcriptional processes. RECQ5 helicase, a general elongation factor, associates with RNAPII and controls its movement along genes. Despite its significance, the precise mechanism through which RECQ5 regulates RNAPII movement remained unknown.

Here, we present the details of the interaction between RECQ5 and RNAPII determined by cryo-electron microscopy. Single-particle analysis with a resolution of 3 Å revealed near-atomic level details of the interaction between the RECQ5 helix and RNAPII DNA. Additionally, cryo-EM imaging showed that the RNAPII with RECQ5 complex formed large, spherical objects resembling condensates. Correlative light electron microscopy confirmed the presence of both fluorescently labeled RNAPII and RECQ5 within these objects. Subtomogram averaging revealed the organization of condensates, further enhancing our understanding of their functional assembly. The subtomogram averaging model of RNAPII with RECQ5 reached a resolution of 7 Å, confirming the observed interaction between the RECQ5 helix and RNAPII DNA across analytical methods.

Our study emphasizes RECQ5's crucial role in modulating transcriptional processes by using the brake-helix to regulate RNAPII movement along genes. Additionally, we discovered that condensates contain tens to hundreds of RNAPII molecules with RECQ5. The study provides valuable insights into how transcriptional machinery functions in eukaryotic nuclei.

P47

UNVEILING THE COUPLING OF TRANSCRIPTION AND TRANSLATION IN BACTERIA

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The fundamental central dogma of molecular biology, underscores a two-step process in the expression of genetic information. Initially, RNA polymerase (RNAP) orchestrates the transcription of mRNAs, followed by the translation of mRNAs into polypeptides by fully assembled ribosomes. In eukaryotic cells, these processes are compartmentalized by the nucleic envelope. However, in bacteria these pivotal biological events can occur simultaneously in what is known as Coupled Transcription and Translation (CTT) [1]. This mechanism of genetic information flow was structurally described by single particle cryo-EM as a synergistic process, with RNAP and ribosomes directly interacting [2-3], and assisted by various transcription factors (TFs) such as NusG or NusA [4-5]. Moreover, recent work using the genome-reduced human pathogen *M. pneumoniae* described the in-cell architecture of the CTT at sub-nanometer resolution [6].

Our research is focused on investigating CTT within the prototype bacteria *E. coli*, specifically examining its role in the elongation phase of translation. Here, the elongation factor Tu (EF-Tu), in conjunction with the corresponding tRNA and GTP, interacts with the ribosome and initiates the rotation of the ribosome. The ribosome rotation in combination with the intricate interplay between the ribosome and the RNAP in the context of CTT, contribute to the simultaneous movement of the whole complex. Our primary goal is to unravel the structural intricacies of how this sophisticated molecular machinery operates synergistically during elongation. Despite the challenges, our preliminary data provide a foundation for future investigations into CTTs across different organisms [7].

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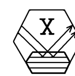

P48

CRYO-EM REVEALS THE STRUCTURE AND INFECTION MECHANISM OF PHAGE LUZ19

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Infections caused by antibiotic-resistant bacteria are a growing health concern. As antibiotic treatments become less effective, alternative approaches are explored. One of the promising methods is phage therapy. However, our understanding of phage–bacterium interactions is limited, and phage therapy is currently considered an experimental treatment.

We use cryo-electron microscopy to study the structure of bacteriophage LUZ19 and the process of LUZ19 infection of PAO1, a clinically relevant strain of *P. aeruginosa*. The virion particle is composed of an icosahedral capsid and a tail decorated by six long tail fibres. The capsid is built from major capsid, head cement, and flexible head decoration proteins, and encloses a 43.5 kbp-long dsDNA genome and an inner core complex. The symmetry mismatch between the capsid and the tail is mediated by a dodecameric portal complex which occupies one vertex of the capsid. The portal complex interacts with the adaptor dodecamer of the tail through its “stem” helices which interlock with the adaptor C-termini. LUZ19 tail extends to a hexameric tail nozzle which is decorated with six flexible tail fibres. The nozzle and the tail fibres mediate the attachment of phage LUZ19 to the type IV pili of PAO1. The contraction of the pili carries the phages to the cell surface, where they irreversibly attach and infect the host cell.

Studying phage LUZ19’s structure and its infection mechanism aims to deepen our understanding of phage-bacterium interaction.

Cryo-electron Microscopy and Tomography core facility (CEMCOF) of CEITEC Masaryk University is gratefully acknowledged for obtaining the scientific data presented in this poster.

P49

ANALYSIS AND ACCELERATION OF MOLECULAR SIMULATIONS BY TIME-LAGGED tSNE

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tSNE (t-distributed Stochastic Neighbour Embedding) is a popular method used to analyse data from single-cell gene expression measurements, RNAseq, flow cytometry and other experiments providing high-dimensional data. It can be also used to analyse structures sampled by molecular dynamics simulations. We developed a variant of tSNE called time-lagged tSNE. Structures sampled by molecular dynamics simulations are first superimposed to a reference structure to remove translational and rotational motions. Next, they are analysed by a variant of independent component analysis. This analysis correlates coordinates of a molecular system with time-lagged coordinates. This emphasizes slow motions and suppresses fast motions. Finally, tSNE is applied on the output.

The result is a 2D map of conformation of a molecular system. For simulations of Trp-cage mini-protein folding and unfolding we obtained a plot with a central cluster corresponding to the unfolded structure. Folded structure as well as other long-lived structures were located as peripheral clusters surrounding the unfolded state. Unlike standard tSNE, this representation captures not only structural differences between states, but also kinetics.

We see a great potential of time-lagged tSNE in acceleration of molecular simulations. We used a method called metadynamics to drive conformational changes along the 2D map from time-lagged tSNE. For this purpose it was necessary to modify time-lagged tSNE to make it possible to calculate time-lagged tSNE coordinates on the fly and to convert forces acting on time-lagged tSNE coordinates into forces acting on individual atoms. We solved this problem by an application of an artificial neural network in parametric time-lagged tSNE.

We successfully applied this method on folding of the Trp-cage mini-protein.

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P50

NEW COMPLEXES OF THE BACTERIAL TRANSCRIPTION SYSTEM

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RNA polymerase, RNAP, is an enzyme complex responsible for synthesizing RNA molecules. RNAP copies RNA based on a DNA template during the process of transcription, a fundamental operation conserved from bacteria to humans.

Identifying specific interactions between RNAP and other proteins can lead to the discovery of new therapeutic targets for antibiotic development that are more effective and less prone to bacterial resistance. Additionally, understanding the mechanisms of resistance, which can arise from mutations altering interactions between RNA polymerase and other proteins, allows us to better address the problem of antibiotic resistance and develop new strategies to combat it [1, 2]. Finally, elucidating the role of RNAP and its protein complexes contributes to comprehensive understanding of bacterial biology.

Here, we discovered that a protein involved in protein translation that binds to RNAP, potentially linking the two processes in a not yet identified manner. Currently, we are characterizing the interactions between this protein and RNAP by biophysical and structural biology methods (cryo-EM, small-angle X-ray scattering, homologous modelling, etc.). In parallel, this interaction is being probed functionally by biochemical approaches.

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This work was supported by CSF (23-06295S), AS CR (86652036), MEYS (LM2023042), ERDF (CZ.02.1.01/0.0/0.0/18_046/0015974), and by support of Biocev CMS – core facilities Biophysical Methods, Structural Mass Spectrometry, Crystallization of Proteins and Nucleic Acids, Diffraction Techniques and of CEITEC, Masaryk University Cryo-electron microscopy and tomography core facility (CEMCOF), all of CIISB, part of Instruct-ERIC.

P51

CF DIFFRACTION TECHNIQUES IN CENTRE OF MOLECULAR STRUCTURE: EMPLOYING HIGH-END X-RAY TECHNOLOGIES FOR LABORATORY STRUCTURAL BIOLOGY

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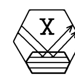
The Centre of Molecular Structure (CMS) provides services and access to state-of-art instruments, which cover a wide range of techniques required by not only structural biologists. CMS operates as part of the Czech Infrastructure for Integrative Structural Biology (CIISB), and European infrastructures Instruct-ERIC and MOSBRI. CMS is organized in 5 core facilities: CF Protein Production, CF Biophysics, CF Crystallization of Proteins and Nucleic Acids, CF Diffraction Techniques, and CF Structural Mass Spectrometry.

CF Diffraction Techniques employs two laboratory X-ray instruments equipped with high flux MetalJet X-ray sources: a single crystal diffractometer D8 Venture (Bruker) and a small angle X-ray scattering instrument SAXSpoint 2.0 (Anton Paar). The configurations of both instruments represent the top tier of possibilities of laboratory instrumentation. Apart from standard applications, the instruments are also extended for advanced experiments: the diffractometer is equipped with the stage for in-situ crystal diffraction, X-ray flourscence detector and crystal dehydration, SAXS is equipped with in-situ UV-Vis spectroscopy and a liquid chromatography system for SEC-SAXS. The setups enable easy access and fast turn-around of samples under different conditions, but also collection of high quality end-state data without further need for synchrotron data collection in many cases. CF Diffraction Techniques provides services in synergy with the other CFs on-site, therefore scientific questions can be quickly answered as they emerge from the experiments.

The Centre of Molecular Structure is supported by: MEYS CR (LM2023042); project Czech Infrastructure for Inte-



grative Structural Biology for Human Health (CZ.02.1.01/0.0/0.0/16_013/0001776) from the ERDF; UP CIISB (CZ.02.1.01/0.0/0.0/18_046/0015974), ELIBIO (CZ.02.1.01/0.0/0.0/15_003/0000447), and MOSBRI from EU Horizon 2020 (No. 101004806).

P52

MOLECULAR DYNAMICS SIMULATIONS OF SINGLE AMINO-ACID SUBSTITUTION IN BRAF:MEK1 COMPLEX

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RAS-MAPK pathway is an important signaling pathway, that regulates mitosis, metabolism, motility, survival, apoptosis, and differentiation. Defects in RAS-MAPK signaling can lead to cancer or severe neurodevelopmental disorders. We are focusing on two proteins in this pathway, BRAF and MEK1. Both of them are kinases and MEK1 is a direct substrate of BRAF [1].

We perform molecular dynamics (MD) simulations of eight single amino-acid substitutions in BRAF. They are caused by genetic missense variants that were found in individuals with neurodevelopmental disorders and their pathogenicity and effect on BRAF function is not known. We are simulating the effect of each substitution on ATP binding and BRAF kinase activity in the complex of BRAF:MEK1 (PDB id: 4MNE) with ATP (Figure 1). As a reference, we use a well-characterized V600E substitution that increases BRAF activity [2]. We expect to see differences in ATP processing and phosphorylation speed.

Our study may decipher how these mutations affect BRAF function. The gained knowledge can be used in the development of BRAF inhibitors, which can block BRAF kinase activity and suppress signaling in the RAS-MAPK pathway. The knowledge can also be implemented in diagnostics, and in choosing the right treatment approach and predicting treatment effectiveness [3].

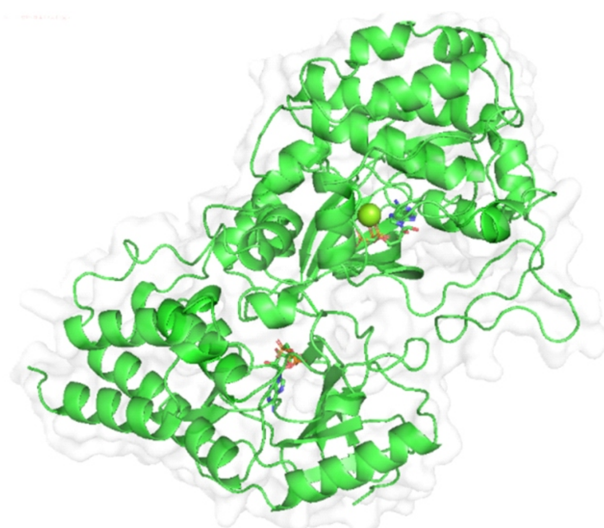

Figure 1. Complex BRAF:MEK1 with ATP

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P53

STRUCTURAL BASIS FOR POSTFUSION-SPECIFIC BINDING TO RESPIRATORY SYNCYTIAL VIRUS F PROTEIN BY THE ANTIGENIC SITE-I ANTIBODY 131-2a

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The Respiratory Syncytial Virus (RSV) Fusion (F) protein is a major target of antiviral antibodies following natural infection and vaccination and responsible for mediating fusion between the viral envelope and the host membrane. The fusion process is driven by a large-scale conformational change in F, switching irreversibly from the metastable prefusion state to the stable postfusion conformation. Previous research has identified six distinct antigenic sites in RSV-F, termed sites Ø, I, II, III, IV, and V. Of these, only antigenic site I is fully specific to the postfusion conformation of F. A monoclonal antibody 131-2a that targets postfusion F specifically has been widely employed as a research tool to probe for postfusion F and to define antigenic site I in serological studies, yet the sequence and epitope of the antibody remained unknown.

We used mass spectrometry-based *de novo* sequencing to reverse engineer 131-2a. Reverse engineered 131-2a was then used to investigate 131-2a epitope and to define antigenic site I by single particle cryo-electron microscopy. This elucidated the structural basis for the antibody binding to the postfusion RSV-F.

P54

EXPLORING THE STRUCTURES OF THE 20S PROTEASOME AND THEIR SMALL MOLECULE INHIBITORS USING CRYO-EM

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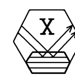
Proteasomes play a key role in maintaining protein homeostasis in mammalian cells and protozoan parasites alike. Human parasites such as *Trichomonas vaginalis* (Tv) with their 20S proteasomes are recognized as viable drug targets. In insect cells we have recombinantly expressed of all fourteen proteins of the Tv20S proteasome, containing seven α and seven subunits with coexpression of the Ump-1 proteasome chaperone. The purified Tv20S was successfully enzymatically active, and subsequent enzymatic assays have revieal ed that Tv20S is a viable target for inhibition by the natural product marizomib (MZB) and peptide inhibitor carmaphycin-17 (CP-17). We conducted Negative stain analysis and Cryo-electron microscopy (cryo-EM) and elucidated two Tv20S cryo-EM structures one with MZB and the other with CP-17 inhibitors. The final resolutions was 2.6 Å, and 2.86 Å respectively. Overall this study outlines the binding details of MZB and CP-17 and provides valuable insights into the possible targetting of Tv20S over human proteasome. The data unveil promising avenues for exploiting these binding sites in future drug design and developments.



P55

STRUCTURAL BASIS FOR RNA-CAP RECOGNITION AND METHYLATION BY THE mpox METHYLTRANSFERASE VP39

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

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P56

CRYOGENIC ELECTRON MICROSCOPY CORE FACILITY AT IOCB PRAGUE

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We are the newly established Cryogenic Electron Microscopy (cryo-EM) Core Facility at the Institute of Organic Chemistry and Biochemistry (IOCB) in Prague. Our specialized cryo-EM team offers a comprehensive range of services, from sample preparation and characterization data collection, and single particle analysis (SPA) conducted on High-Performance Computing (HPC) systems. In close cooperation with other groups, we can ensure atomic-model building service for the structures we analyze and quantum mechanics/molecular mechanics (QM/MM) molecular dynamics (MD) simulations.

Cryo-EM allows visualization of bound small-molecule ligands in the macromolecules at high resolution. These new structures provide beneficial insights into the molecular mechanisms of complex biochemical processes and a profound impact on drug discovery, defining the binding modes and mechanisms of action.

Our proficiency extends beyond cryo-EM, encompassing techniques at ambient temperatures and 3D electron diffraction. The Cryo-EM group is equipped with an inhouse screening microscope and collaborates with CEITEC in Brno to access highresolution instrumentation. Data analysis is performed in collaboration with a high-performance computing (HPC) facility. The cluster consists of ~250 CPU nodes and ~40 GPU nodes.

Our facility will be equipped with state-of-the-art instrumentation (300kV Krios G4 and 200kV Glacios) for single particle analysis and 3D electron diffraction. Our goal is to establish a cutting-edge cryo-EM infrastructure that will meet the needs of IOCB users and potential external collaborators.

P57

ANALYSIS AND SAMPLING OF MOLECULAR SIMULATION WITH EXTENDED AUTOENCODER ARCHITECTURES

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Nowadays molecular dynamics (MD) has become a standard tool to investigate molecular systems. By simulating the dynamic behavior of molecules, it facilitates the exploration of stability, conformational changes and a plenty of other properties essential for understanding molecular functions. However, the application of MD is affected by the large computational costs. It computes steps that must be in order of femtoseconds, to assure numerical stability, for a time scale long enough to likely catch some or rarely occurred processes. There are numerous techniques to enhance simulations to optimize the sampling of the slow motions, one of those is metadynamics. It operates by biasing the Hamiltonian of the system, encouraging it to cross barriers and explore configurations that might otherwise be inaccessible or challenging to sample, in a reasonable amount of time. However, to make metadynamics successful, the scientist has to select the so-called "collective variables'' which are functions of Cartesian coordinates. Designing good collective variables is not a trivial task and it relies on the knowledge of the system and experience of the scientist. Machine learning and artificial neural networks have shown incredible power in supporting the exploration of conformational phase space.

Hereby we show the involvement of two methods based on the control and optimization of the latent space. The developed neural network exhibits the ability to analyze simulation data and to derive optimal combinations of internal coordinates to be used as CVs. The power and the efficiency of the presented approach are demonstrated on Trp-cage foldings.

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P58

MASS SPECTROMETRY AS A TOOL FOR PROTEIN STRUCTURE ANALYSIS

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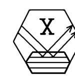
Structural mass spectrometry (MS3D) is a fast-growing field of analytical chemistry that provides an impressive array of structural information about protein topology, conformational dynamics and protein-protein/ligand interaction. To gain this kind of information, well-established techniques are used in CMS, encompassing hydrogen-deuterium exchange (HDX), chemical cross-linking and native mass spectrometry. Importantly, all of these techniques are compatible with membrane proteins. The structural mass spectrometry core facility is equipped with state-of-the-art instrumentation such as 15T FT-ICR, timsToF SCP, timsToF Pro, automation system for HDX and UPLC or nanoUPLC systems. Beyond MS3D, the core facility offers other MS-based proteomic approaches providing protein identification, quantification, precise intact protein molecular mass determination, and characterization of various protein posttranslational modifications (phosphorylation, glycosylation, acetylation, biotinylation, etc.), or disulfide bond linkages assignment. The inherent advantages of these MS-based approaches are their sensitivity, low sample consumption and complementarity to other biophysical techniques.

CIISB, Instruct-CZ Centre of Instruct-ERIC EU consortium, funded by MEYS CR infrastructure project LM2023042 and European Regional Development Fund-Project „UP CIISB" (No. CZ.02.1.01/0.0/0.0/18_046/0015974), is gratefully acknowledged for the financial support of the Structural mass spectrometry core facility.



P59

INSIGHTS INTO MOLECULAR INTERACTIONS BETWEEN nsp14 AND nsp10 IN SARS-CoV-2 REPLICATION

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Coronaviruses are large RNA viruses, with genomes of over 30 kb in some species. They cause a range of diseases in birds, mammals, and humans. While most human coronaviruses lead to mild respiratory infections, in the last two decades, we saw two major epidemics and a global pandemic, mainly caused by the virus SARS-CoV-2, causing severe respiratory illnesses and fatalities. Within the coronavirus genome, there is a gene encoding a non-structural protein 14 (nsp14), which possesses 3'-5'exonuclease and methyltransferase enzymatic activities. The exonuclease nsp14 participates in the repair of misincorporated nucleotides during viral genome replication, and its presence is exceptionally rare among RNA viruses. The exonuclease activity of nsp14 is significantly enhanced by the binding of another viral protein, nsp10, which lacks enzymatic activity, but acts as a critical cofactor for several enzymatically active coronavirus nsps. Both nsp14 and nsp10 are highly conserved and have similar sequences among the various coronavirus species.

To characterize the exonuclease activity of nsp14 and its interactions with nsp10, mutant versions of the nsp14 and nsp10 proteins were created using PCR mutagenesis. Both the wild-type and mutant versions of nsp14 and nsp10, as well as a truncated version of nsp10, were produced in a bacterial expression system utilizing *E. coli* bacteria. The exonuclease activity of each nsp14 variant was observed in vitro using activity assays with ssRNA, dsRNA, and dsRNA, with mismatched base pairs as the substrates in the presence or absence of nsp10 variants. The results of this study provide insights into the functioning of nsp14 exonuclease and its interactions with nsp10, which may serve as a basis for more detailed characterization in future research.

This research was funded by the project the National Institute Virology and Bacteriology (Programme EXCELES; Project No. LX22NPO5103) and by the European Union, Next Generation EU. RVO: 61388963 is also acknowledged.

P60

ACCURATE MULTI-SCALE COMPUTATIONS OF BINDING FREE ENERGIES IN INSULIN/INSULIN RECEPTOR COMPLEX

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Quantitative characterization of protein-protein binding across extensive flexible interfaces is a daunting task because of the and the lack of computational tools for reliable evaluation of all the types of non-covalent interactions. Herein, we capitalize on the recent developments in semiempirical quantum mechanical (SQM) methods and present a hierarchical computational protocol which entails molecular dynamics (MD), fragmentation and virtual glycine scan calculations and apply it to insulin/insulin receptor (IR) binding which had been thoroughly studied both, biochemical and recently also structurally by use of cryo-EM. Comparison of SQM and molecular mechanical (MM) interaction energies to reference DFT-D3 calculations showed a perfect agreement for small dimers with the exception of S…O contacts for which MM failed. For efficient identification of interaction "hotspots", the use of SQM interaction energies on MM optimized snapshots from MD exhibited an excellent agreement with the experimental data. The developed protocol is general and can thus be used for quantification of interactions across other flexible protein-protein interfaces.

P61

QM/MM REACTION SIMULATIONS OF ALPHA-(1,3)-FUCOSYLTRANSFERASE OF *HELICOBACTER PYLORI*

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Helicobacter pylori is a severe human pathogen associated with many gastrointestinal disorders, such as chronic gastric inflammation, ulcers, and cancer. Due to the rise of antibiotic resistance, its treatment has become increasingly more challenging, accelerating the search for novel antibacterial agents [1]. In this study, we focus on *H. pylori* alpha-(1,3)-fucosyltransferase FutA, an essential enzyme in the biosynthesis of Lewis antigens, which camouflages the bacteria from the host's immune system. Thus, FutA represents an attractive pharmacological target for developing novel transition state inhibitors [2].

The exact reaction mechanism of FutA is currently unknown. The X-ray crystal structure of FutA has been solved with the donor substrate only [2]. Moreover, the active site is wide open, suggesting a large conformational change must occur upon the acceptor binding. Employing molecular modelling, we have found several closed enzyme conformations containing both the donor and acceptor ligands in an orientation suitable for the reaction. We started with AlphaFold2 to obtain a suitable model for docking simulations. Using simulated annealing with carefully designed distance restraints for docking, we positioned both substrates in their respective binding sites. Next, we simulated a large conformational change upon the binding of the substrates, confirming the hypothesis that the enzyme must undergo a significant conformational change before the reaction occurs [2].

Here, we will report the results of QM/MM simulations employing steered molecular dynamics (SMD) for the free energy calculations. These simulations aim to validate the suitability of our pre-reaction complexes for the reaction simulations. We investigated the effects of the QM region size, the impact of chosen collective variables, and the level of QM theory on calculated reaction profiles.

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